Anthrax vaccine does not affect semen parameters, embryo quality, or pregnancy outcome in couples with a vaccinated male military service member

Anthrax vaccination has been used in an effort to prevent infection should anthrax be used as a biological weapon, and widespread use has been considered in the event of another anthrax attack on American soil, but the long-term impact of anthrax vaccination on reproductive outcome is unknown. We found that exposure to the anthrax vaccine by males who were undergoing assisted reproduction did not negatively impact semen parameters, fertilization rate, embryo quality, or clinical pregnancy rates. (Fertil Steril® 2005;83:480–3. © 2005 by American Society for Reproductive Medicine.)

The United States developed and licensed a human anthrax vaccine in 1970 (1). Although this vaccine was originally provided for textile mill workers and certain veterinarians or laboratory personnel, the potential threat of weaponized anthrax use in the Persian Gulf War prompted the vaccination of approximately 150,000 soldiers starting in 1991 (2). In March 1998, the U.S. Department of Defense launched an initiative to vaccinate all service-members by 2004. It is estimated that 500,000 service-members have been vaccinated (3).

In the anthrax attacks of October/November 2001 on American civilians, there were 18 confirmed cases of inhalational anthrax, and five deaths despite treatment (4). Given the estimated over 80% mortality rate associated with untreated inhalational anthrax (5) and the increased threat of anthrax use in both conventional settings and terrorist plots by rogue nations and terrorist groups, anthrax vaccinations were provided to military service-members despite few large-scale clinical trials to assess potential side effects of vaccination. There is increasing concern that vaccination of predominantly reproductive-aged service-members may result in deleterious effects on fertility or fetal health (6). There are many examples of vaccines that adversely influence fertility (7–9), but current recommendations only prohibit pregnant service-members from anthrax vaccination (10).

For those at risk of anthrax exposure, it can be argued that the threat of mortality outweighs the threat of serious reaction. This argument was used to justify vaccination in military service-members. However, the long-term effects of vaccination against anthrax are poorly understood, and if population-wide vaccination is considered to protect U.S. citizens from future anthrax attacks, it will be critical to understand unexpected long-term morbidity of vaccination, including adverse effects on reproduction. Walter Reed Army Medical Center's Assisted Reproductive Technologies program is ideally suited to perform such an assessment. All patients are either active duty military or have an active duty sponsor. As a result, the likelihood of anthrax vaccination is greater in this group than in any other potential study group. Furthermore, each couple is intensively monitored to assess reproductive parameters in preparation for assisted reproduction, and their gametes/embryos are carefully monitored throughout the conception and implantation periods.

In this study, we evaluated the impact of anthrax vaccination on sperm parameters and clinical pregnancy rates among couples in which the man had or had not been exposed to anthrax vaccination. Given current prohibitions on accessing the anthrax vaccine database, we queried each patient about exposure to any fraction of the anthrax vaccine; those who had been exposed were treated as "exposed," and those with no exposure to the anthrax vaccine were "unexposed." We assessed parameters including sperm concentration, motility, morphology, need for intracytoplasmic sperm injection (ICSI), high-grade embryo transfer (ET), blastocyst transfer, and clinical pregnancy.

The Walter Reed Army Medical Center Assisted Reproductive Technologies program serves the U.S. armed forces and provides assisted reproduction services. To be eligible for care in this program, the man needs to either be active duty military or be sponsored by his wife who is active duty in the military. After obtaining institutional review board approval, we collected data from the male partners at the time of oocyte and sperm retrieval about exposure to anthrax vaccination. From October

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Reprint requests: William H. Catherino, M.D., Ph.D., Department of Obstetrics and Gynecology, Uniformed Services University of the Health Sciences, Building A, Room 3078, 4301 Jones Bridge Road, Bethesda, Maryland 20814-4799 (FAX: 301-295-6774; E-mail: wcatherino@usuhs.mil).

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TABLE 1

Patient and fertility characteristics.

Characteristic	Anthrax vaccine exposure	No anthrax vaccine exposure
Number	254	791
Age (y)	34.8 ± 5.0	35.9 ± 6.5
Couple diagnosis		
Anovulation	17 (6.7%)	51 (6.4%)
Endometriosis	27 (10.7%)	71 (9.0%)
Male factor[a]	49 (19.4%)	223 (28.2%)
Tubal factor[a]	122 (48.0%)	278 (35.2%)
Unexplained	38 (15.1%)	168 (21.2%)
Other	1 (0.4%)	0 (0%)
Mean semen concentration (million/mL)	74.2 ± 48.3	77.5 ± 54.9
Total motile (million)	81.5 ± 50.5	81.7 ± 56.8
Morphology		
<4%	6.6%	7.5%
5–14%	68.3%	62.5%
>14%	25.1%	30.0%
ICSI (%)	85 (33.5%)	274 (34.7%)
Normal fertilization per number of mature oocytes	71.0%	72.2%
High-grade day 3 embryo transferred	163 (64.2%)	501 (63.3%)
Day-5 embryo transferred[b]	53 (20.9%)	115 (14.5%)
Clinical pregnancy	126 (49.6%)	363 (45.9%)

[a] $P<.01$ by chi-square analysis with Bonferroni correction.
[b] $P=.0507$ by chi-square analysis with Bonferroni correction.

Catherino. Male gametes unharmed by anthrax vaccine. Fertil Steril 2005.

1999 to December 2003, we assessed the impact of the changes in male gametes due to anthrax vaccine exposure with women who had an adequate stimulation experience. We evaluated all data retrospectively. The sample size was determined by the interval of study and not by power analysis, and included 254 males who stated that they had received anthrax vaccination and 791 males who denied vaccination.

All men underwent semen analysis as described above, using Kruger's strict criteria (11). On the day of oocyte retrieval, men provided a semen sample via a medically determined method (masturbation, microsurgical epididymal sperm aspiration, or testicular sperm aspiration). If the spermatozoa concentration was adequate, they were capacitated and combined with oocytes at a ratio of 100,000:1. If concentrations were inadequate, ICSI was performed. All women underwent pituitary desensitization followed by controlled ovarian hyperstimulation, as previously described elsewhere (12). The clinical pregnancy rate was defined as the observation of an intrauterine fetal pole with cardiac activity at 6 to 7 weeks' gestation. Data on age, semen parameters, fertilization, requirement for ICSI, embryos transferred, and clinical pregnancy rates were recorded.

Two sample Student's *t*-test or chi-square test were used to compare if there was a statistically significant difference between men exposed to anthrax vaccination and those who were unexposed for a continuous or categorical outcome, if applicable. If a statistical significance existed, multiple comparisons were evaluated with Bonferroni adjustment. A significance level of 5% was set for each statistical test. The SAS statistical software package (SAS Institute, Inc., Cary, NC) was used to perform the statistical analysis.

To evaluate the impact of anthrax vaccination on the male fertility, we compared characteristics of the men, the couples, the fertilization, and the outcome of fertilization (Table 1). We found that there was no difference in mean age between the exposed and unexposed groups. However, tubal factor infertility was a more common diagnosis in the exposed group (48.0% vs. 35.2%), and the diagnosis of male factor infertility was less common (19.4% vs. 28.2%). These differences reached statistical significance. There was no statistical difference in the percentage of anovulatory, endometriosis, or unexplained infertility diagnoses between the two groups. There was no statistically significant difference between exposed and unexposed men in these standard criteria: mean concentration, total motile

concentration per sample, or morphology. In addition, there was no statistically significant difference in the requirement for ICSI or the percentage of two pronuclei embryos that were produced when the gametes were combined. Finally, exposed men had a trend toward more blastocysts (20.9% vs. 14.5%, $P=.0507$), but there were no statistically significant differences in clinical pregnancy rates. These results suggest that anthrax vaccination did not statistically alter fertility characteristics in the men who were undergoing assisted reproduction.

For men treated at Walter Reed who were part of couples undergoing assisted reproduction, anthrax vaccination did not appear to worsen reproductive parameters. Men exposed to anthrax vaccination were not more likely to undergo ICSI, and embryo quality was no worse when compared with those men who had not been exposed.

Although we have access to the largest population of patients who have been exposed to anthrax vaccination, it could be argued that anthrax vaccination may have a small detrimental effect on clinical pregnancy rates that could only be identified by sampling a larger patient population. It is preferable to perform an a priori power calculation before performing an experiment, so we were limited by the inability to control which patients had received anthrax vaccinations and which patients were receiving infertility care (two distinct but overlapping populations). To assess the level of confidence we could have in our results, we therefore performed a post hoc power analysis to determine the number of patients needed to detect a clinically relevant decrement in clinical pregnancy rate. To identify a 5% decrease in clinical pregnancy rate with a power of 80% and an alpha of 0.05 given the data that we have collected for this study, we would need to evaluate 1,605 patients who have received anthrax vaccination and 1,605 patients who have not. To confirm the 2% difference in clinical pregnancy rates between exposed and unexposed men, we would need to recruit over 10,000 patients per group. Given the limits of patient accrual (couples undergoing ET who have a male service-member who received anthrax vaccination), our results suggest that anthrax vaccination of men does not alter semen parameters, fertilization, or clinical pregnancy rates.

Although our study suggested no clear adverse effect of anthrax vaccination upon male gametes, there were several limitations. It would be optimal to perform such a study in a randomized, prospective, double-blind design, but such a study would not be feasible because of the constraints of sample size (as demonstrated by post hoc analysis) and randomization of vaccine exposure as well as infertility treatment. A retrospective cohort analysis is limited by multiple biases, but our results do provide some insight into the impact of anthrax vaccination on fertility parameters in male service-members. We also depended on self-reporting of anthrax vaccine exposure. It is possible that some men denied anthrax vaccine exposure when, in fact, they had been exposed, or vice-versa. This possibility could have confounded the control group, although it would be unlikely to change the final results. In such a case, it would be expected that relative risk ultimately would approach unity if admitted anthrax exposure was unrelated to actual exposure. To examine the impact of confirmed vaccine exposure with fertility parameters, in the future we plan to obtain data from the vaccine database on both vaccine exposure and number of injections of vaccine. In addition, we plan to assess the impact of anthrax vaccination on female reproductive parameters.

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William H. Catherino, M.D., Ph.D.[a,b,c]
Andrew Levi, M.D.[a,b,c]
Tzu-Cheg Kao, Ph.D.[d]
Mark P. Leondires, M.D.[b]
Jeffrey McKeeby, M.D.[b]
James H. Segars, M.D.[a,b,c]

[a] *Uniformed Services University of the Health Sciences Department of Obstetrics and Gynecology;* [b] *Combined Federal Program in Reproductive Endocrinology;* [c] *Pediatric and Reproductive Endocrinology Branch, National Institute of Child Health and Human Development, National Institutes of Health; and* [d] *Uniformed Services University of the Health Sciences Department of Biostatistics, Bethesda, Maryland*

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