

Developmental and reproductive safety evaluation of AV7909 anthrax vaccine candidate in rats

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Abstract

The AV7909 vaccine, consists of the Anthrax Vaccine Adsorbed (AVA) bulk drug substance and the immunostimulatory Toll-like receptor 9 agonist oligodeoxynucleotide adjuvant CPG 7909. The purpose of this research was to evaluate the potential maternal, reproductive, and developmental toxicity of AV7909 in rats to support licensure for use in women of childbearing potential. Groups of first generation (F₀) female Sprague Dawley rats were dosed by intramuscular injection with water for injection, adjuvant or AV7909 at a volume of 0.5 ml/dose. Each rat received three vaccinations: 14 days prior to start of the mating period, on the first day of the mating period and on gestation day (GD) 7. There was no maternal mortality. Body weights, weight gain, and food consumption were comparable between groups. Findings in F₀ females were limited to transient injection site edema and nodules consistent with immunostimulatory effects of the vaccine and adjuvant. Administration of AV7909 did not affect mating, fertility, pregnancy, embryo-fetal viability, growth, or morphologic development, parturition, maternal care of offspring or postnatal survival, growth, or development. There was no evidence of systemic inflammation in pregnant rats, based on evaluation of serum concentrations of the acute phase proteins alpha-2-macroglobulin and alpha-1-acid glycoprotein on GD 21. Anthrax lethal toxin-neutralizing antibodies were detected in AV7909-vaccinated F₀ females. The antibodies were also detected in the sera of fetuses and F₁ pups. Exposure of the fetuses and pups to maternally derived anthrax lethal toxin-neutralizing antibodies was not associated with developmental toxicity.

KEYWORDS

anthrax vaccine, AV7909, developmental toxicity, rat, reproductive toxicity

1 | INTRODUCTION

Anthrax is an infectious disease caused by the *Bacillus anthracis* bacteria. Infection can be highly lethal and poses a major biological threat. The current recommended

postexposure prophylaxis (PEP) for anthrax includes administration of the commercial BioThrax (Anthrax Vaccine Adsorbed, AVA) vaccine (CDC, 2010). The AV7909 vaccine candidate is being developed as an alternative to BioThrax for PEP in the general population as it provides an

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enhanced immune response and requires fewer dose administrations to achieve protective immunity than BioThrax (Hopkins et al., 2016; Minang et al., 2014). AV7909 and BioThrax contain the same bulk drug substance AVA and adjuvanted with oligodeoxynucleotide (ODN) CPG 7909, an immunostimulatory Toll-like receptor (TLR) 9 agonist.

Clinical evaluations of AV7909 in adult populations have shown that the vaccine is safe and well-tolerated (Hopkins et al., 2013; Hopkins et al., 2016; Rynkiewicz et al., 2011). The nonclinical safety and efficacy of anthrax vaccines has been demonstrated in several animal studies (Ionin et al., 2013; Savransky et al., 2017). Vaccination with rPA7909, a recombinant protective antigen anthrax vaccine candidate adjuvanted with CPG 7909, produced robust immune activation in adult rodents with no systemic toxicity observed after the full human dose of vaccine was administered (Savransky, Lacy, Ionin, Skiadopoulou, & Shearer, 2019). BioThrax vaccination of female rabbits twice prior to mating and once during gestation did not produce any reproductive or developmental toxicity while generating a robust immune response and antibody transfer to fetuses and pups (Franco, Lewis, Morseth, Simon, & Waytes, 2009). According to an analysis, inadvertent anthrax vaccination during pregnancy did not find significant associations between vaccination with BioThrax during pregnancy and birth defects risk in female military service members (Conlin, Sevick, Gumbs, Khodr, & Bukowinski, 2017).

A reproductive and developmental toxicity study is necessary to support the safety assessment of any vaccine intended for use in women of childbearing potential because many pregnancies are unintended and there is a high likelihood of inadvertent exposure of pregnant women and the embryo/fetus to the vaccine (U.S. Food and Drug Administration, 2006). AV7909 is intended for PEP in the general population, which includes women of childbearing potential. Therefore, a nonclinical safety study of AV7909 in pregnant animals was warranted. The study, conducted according to Good Laboratory Practices (U.S. Food and Drug Administration, 1987), covered developmental stages A through E of the ICH Guideline on Detection of Toxicity to Reproduction for Medicinal Products (U.S. Food and Drug Administration, 2005) and followed the FDA guidance for testing of vaccines for reproductive and developmental toxicity (U.S. Food and Drug Administration, 2006).

2 | MATERIALS AND METHODS

2.1 | Test and control articles

The AV7909 engineering batch was manufactured by Emergent BioSolutions Inc. (Lansing, Michigan). The

CPG 7909 adjuvant was obtained from Nitto Denko Avecia Inc. (Milford, Massachusetts). The aluminum hydroxide adjuvant Alhydrogel was purchased from InvivoGen (Toulouse, France). Sodium chloride (0.9%) for injection and sterile water for injection were purchased from Baxter (Deerfield, Illinois) and Hospira (Lake Forest, Illinois), respectively.

An adjuvant formulation was prepared by combining CPG 7909 and Alhydrogel at final measured concentrations of 0.48 mg/ml of bound CPG 7909 (unbound = 0 mg/ml) and 1.3 mg/ml aluminum in 0.85% sodium chloride.

2.2 | Animals

Sprague Dawley Crl:CD (SD) rats were obtained from Charles River (Raleigh, North Carolina). F₀ males and females were nonsiblings and F₀ females were virgin. Females were approximately 10 weeks of age at the start of the study and males used for breeding were 12 weeks of age at the start of the mating period.

General procedures for animal care and housing met current recommendations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International and requirements of the "Guide for Care and Use of Laboratory Animals" (NRC, 2011). Certified feed (Purina Certified Rodent Diet, LabDiet 5002) and water from the municipal water supply (West Jefferson, Ohio) were provided ad libitum. The study design was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC).

F₀ females were assigned to dose groups by stratified randomization of body weights. Animals were individually housed, except during cohabitation of mating pairs, in polycarbonate cages with hardwood bedding (Sani-Chips, P.J. Murphy Forest Products Corporation, Montville, New Jersey). Litters shared their dam's cage until postnatal day (PND) 21. Animals were quarantined for 7 days and acclimated to the laboratory environment prior to initiation of dosing.

2.3 | Experimental design

A schematic of the experimental design is shown in Figure 1. Females (44/group) were assigned to one of three dose groups: control (water for injection), adjuvant (CPG 7909/Alhydrogel in saline), or test article (AV7909). Half of the F₀ females in each group were randomly assigned to laparohysterectomy on GD 21 and half were assigned to natural delivery and allowed to rear offspring until PND 21. Animals were dosed 14 days prior to start

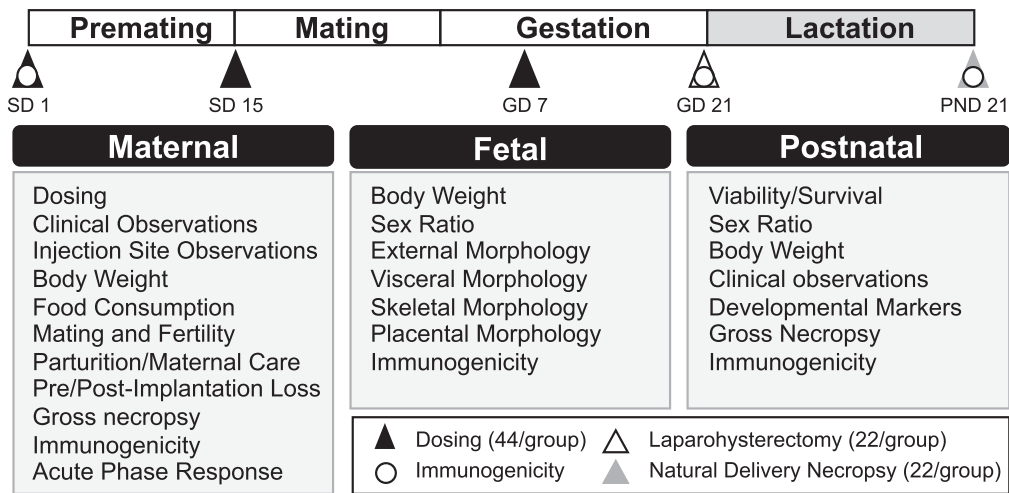


FIGURE 1 Experimental design

of cohabitation (Study Day [SD] 1), on the day of cohabitation (SD 15), and on gestation day (GD) 7. Administration of two doses 2 weeks apart during the premating period was based on a previous study in rats (Savransky et al., 2019). The additional dose on GD 7 was included to ensure high antibody titers and provide exposure to the test article during organogenesis.

AV7909 was administered at the full human dose of 0.5 ml by intramuscular (IM) injection; as were the control article and adjuvant. A single dose level of AV7909 was deemed sufficient to assess safety since the 0.5 ml dose in a rat greatly exceeds (>200 times) the human dose (based on a 70 kg human). The dose volume of 0.5 ml was divided into three injections: 0.2 ml into each thigh, and 0.1 ml alternated between left and right thighs on the different injection days. The thighs were shaved and marked with indelible ink for clear visualization of injection sites.

2.4 | In-life observations and procedures

After a 2-week premating period, each F₀ female was continually cohabited on a 1:1 basis in the cage of a randomly selected male for up to 2 weeks and examined once daily for evidence of mating (copulation plug in situ or sperm in vaginal lavage). On the day mating was confirmed (GD 0), the female was transferred back to individual cage housing.

Observations for moribundity and mortality were performed twice daily during premating, gestation, and lactation. Clinical observations (including maternal care or abnormal maternal behavior) were performed once daily during premating, gestation, and lactation, or twice daily on days of dose administration (predose and 1–2 hr postdose). Dams in the process of delivering were not disturbed.

The injection site area was observed for erythema (redness) and edema (swelling) prior to and 1–2 hr after injection. Any erythema/edema was monitored daily until resolved or for up to 1 week after injection. Body weights and food consumption were recorded throughout the study.

Beginning on GD 21, natural delivery cohort females were checked at least twice daily for signs of parturition. The day all pups within the litter were delivered was defined as PND 0. Each pup was examined on PND 0 through 4, and on PND 7, 10, 14, and 21 for clinical observations (including any external developmental abnormalities), viability, and sex. On PND 4, litters were reduced to a maximum of 8 pups per litter (4 pups/sex, when possible; randomly selected). Litters of 8 offspring or fewer were not reduced. The pups selected to be culled were terminated and disposed of without further examination. Live pups were individually weighed on PND 1, 4, 7, 10, 14, and 21. Each pup was evaluated once daily for achievement of surface righting, incisor eruption, eye opening, and auditory startle (using a clicker), and on PND 21 for pupil constriction.

2.5 | Necropsy and laparohysterectomy

All dams and PND 21 pups were terminated by CO₂ inhalation. Moribund pups were euthanized by administration of sodium pentobarbital. A gross necropsy was performed on dams in the laparohysterectomy cohort on GD 21, dams, and pups in the natural delivery cohort on PND 21, and pups found dead or euthanized due to moribund condition during the lactation period. The necropsy included examination of the external surface of the body and all orifices; the cranial, thoracic, abdominal and pelvic cavities, and their contents. The necropsy also included a cross-section of the kidneys (dams and pups)

and internal examination of the heart (pups). Former implantation sites were enumerated in dams in the natural delivery cohort.

The laparohysterectomy on GD 21 included weighing the gravid uterus and enumerating corpora lutea and implantations (live and dead fetuses, early, and late resorptions). Uteri with no visible implantation sites were placed in 10% aqueous ammonium sulfide to detect early implantation loss. Each fetus was weighed, gender determined, and fetus examined for external abnormalities and palatal closure. The placenta and amniotic fluid were examined for gross abnormalities. Live fetuses were terminated by oral administration of sodium pentobarbital. For each litter, the first fetus and every other fetus thereafter (approximately half of the fetuses) were examined for visceral abnormalities by fresh tissue dissection (Staples, 1974; Stuckhardt & Poppe, 1984); the heads of these fetuses were examined by a free-hand sectioning technique (Astroff et al., 2002; Barrow & Taylor, 1969). Fetuses selected for skeletal examination (fetuses not selected for visceral and head exams) were processed and stained with alizarin red S and alcian blue with some modification of the procedure previously described (Tyl & Marr, 1996) and examined for bone and cartilage anomalies. Fetal observations were recorded using current terminology guidelines (Makris et al., 2009). Fetal morphological examinations were not performed under blinded conditions.

2.6 | Blood collection and serological analyses

Blood was collected from F_0 females prior to the first dose administration for baseline antibody levels and on GD 21 for antibody levels and acute phase response. Blood was also collected on GD 21 from all viable fetuses and on PND 21 from one randomly selected F_1 pup per sex/litter for antibody levels.

Blood was collected from unanesthetized F_0 females from the jugular vein. Fetuses were administered an oral overdose of sodium pentobarbital and blood collected from an incision over the area of the carotid artery/arteries. Trunk blood was collected from pups. Blood was processed to serum and stored on dry ice, or in a freezer at approximately -80°C until analysis. Serum from fetuses and pups was pooled by litter.

Anthrax toxin-neutralizing antibodies (TNA) in serum were measured using the TNA assay, a cell-based cytotoxicity assay that measures the functional ability of serum to neutralize *B. anthracis* lethal toxin (Li et al., 2008). The acute phase proteins alpha-2-macroglobulin

(A2M) and alpha-1-acid glycoprotein (AGP) levels were evaluated in serum using the MILLIPLEX MAP Rat Vascular Injury Magnetic Bead Panel 3 kit obtained from EMD Millipore Corp. (Burlington, Massachusetts) and qualified prior to use.

2.7 | Statistical analysis

Descriptive statistics (group mean and standard deviation where appropriate) were calculated for each group based on the litter as the experimental unit; therefore, the proportion of affected fetuses/pups per litter and the litter mean were calculated for all litter parameters.

All appropriate quantitative in-life data were analyzed by analysis of variance (ANOVA) or analysis of covariance (ANCOVA). Data that met criteria ($p > .01$) for normality (Shapiro, 1965) and homogeneity of variances (Levene, 1960) was analyzed using parametric ANOVA or ANCOVA methods (Guenther, 1964; Snedecor & Cochran, 1967); otherwise data was analyzed using the Kruskal–Wallis nonparametric ANOVA or ANCOVA procedures (Conover, 1980). Litter size was included as a possible confounding variable in the ANCOVA analysis of fetal and pup weights. The mean percent affected fetuses per litter was calculated for each fetal observation and was analyzed using the Kruskal–Wallis nonparametric procedure (Conover, 1980). If the ANOVA or ANCOVA was significant ($p < .05$), pairwise comparisons of the test article and adjuvant groups to the control group were performed using the Dunnett–Hsu test (Dunnett & Tamhane, 1991; Hsu, 1992), or the nonparametric Wilcoxon test (Conover, 1980) as appropriate.

Females that were not gravid were excluded from statistics for gestation body weights, body weight changes, food consumption, and AGP and A2M concentrations. Fertility index, mating index, gestation index, litter survival index, immunogenicity data, and AGP and A2M concentrations are reported but were not analyzed statistically.

3 | RESULTS

There was no mortality among F_0 females. AV7909-related clinical observations in F_0 females were limited to a small mass (<5 mm) in the hindlimb in one female in the vaccine group during gestation and lactation (GD 6 through PND 21). The palpable mass correlated with the presence of a nodule at the injection site at necropsy on PND 21. One female in the adjuvant group had similar small palpable masses during gestation and

lactation; however, there were no correlating nodules at necropsy. These masses were likely a local reaction due to the injection of the adjuvant and AV7909 into the hindlimb.

AV7909-related injection site edema was observed 24 hr postinjection in all F₀ females in the AV7909 group after the first and second injections and in 34 F₀ females after the third injection. The edema was predominantly scored as very slight to slight and was no longer present in most animals 7 days after injection. The edema severity and duration were similar in the adjuvant and AV7909 groups.

AV7909-related postmortem observations were limited to nodule(s) in the injection site area in 1, 8, and 15 animals in the control, adjuvant and AV7909 groups, respectively. One female in the AV7909 group that had nodules also had an ulcer in the injection site area. The higher incidence of nodules in the AV7909 group compared with the control and adjuvant group indicates local reaction to the test article, but the presence of nodules in the control and adjuvant group suggests other vaccine components and/or the injection procedure may have been contributing factors. The nodules/ulcer were observed in the animals necropsied 2 weeks after the last injection but not in the animals necropsied 5 weeks after the last injection, except for one animal.

There were no effects of AV7909 on body weights (Figure 2) or food consumption (data not shown) during pre mating, gestation or lactation in F₀ females. AV7909 administration did not affect reproductive parameters (Table 1), postnatal body weights and developmental landmarks (Figure 3), or body weight gain (data not shown). There were no fetal morphologic abnormalities related to AV7909; findings occurred at low or comparable incidences in the control, adjuvant, and vaccine groups (Table 2). No gross findings related to AV7909 were observed in the placenta or amniotic fluid. Postnatally, there were no AV7909-related clinical observations (data not shown) or postmortem observations in offspring (Table 2).

Anthrax lethal toxin-neutralizing antibodies were detected in F₀ females and their fetuses at the end of gestation (GD 21) and in F₁ pups at the end of lactation (PND 21) in the AV7909 group (Figure 4). Toxin-neutralizing antibodies were not detectable in the control, adjuvant or AV7909 groups prior to dosing (F₀ females), or in the control and adjuvant groups on GD 21 (F₀ females and their fetuses) or PND 21 (F₁ pups).

Serum concentrations of the acute phase proteins were comparable in F₀ females on GD 21 in the control, adjuvant and AV7909 groups (Figure 5).

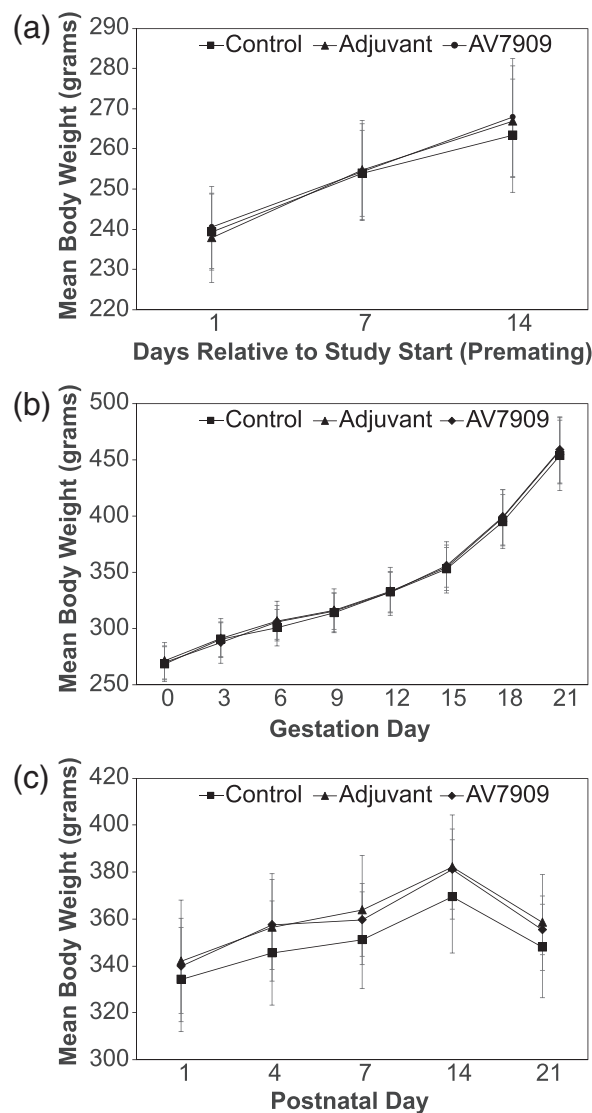


FIGURE 2 Body weights in F₀ females during pre mating (a), gestation (b), and lactation (c). Data are presented as mean and standard deviation. Adjusted body weight (GD 21 body weight minus gravid uterus weight) was 345.1, 345.8, and 350.7 g in the control, adjuvant, and AV7909 groups, respectively. There were no statistically significant differences compared with control ($p < .05$)

4 | DISCUSSION

This nonclinical reproductive and developmental toxicity study was conducted to support the safety assessment of AV7909 in the event of inadvertent exposures pregnant women and the embryo/fetus to the vaccine. The rat was selected for this study because it is the widely accepted and commonly used rodent species for the assessment of developmental and reproductive toxicity. The rabbit, the most commonly used nonrodent species for developmental and reproductive toxicity studies, was

TABLE 1 Reproductive parameters

	Control	Adjuvant	AV7909
Number cohabited	44	44	44
Mating index (%)	100	100	100
Precoital interval (days)	2.9 (2.2) ^a	2.6 (2.1)	2.4 (1.2)
Fertility index (%)	97.7	100	97.7
Laparohysterectomy cohort (GD 21)			
Number of pregnancies	21	22	21
Number with total resorption	0	0	0
Corpora lutea	15.8 (2.9)	15.2 (1.8)	15.5 (2.2)
Implantation sites	15.0 (3.6)	14.9 (2.0)	14.8 (2.5)
Live fetuses	14.5 (3.6)	14.3 (2.2)	14.1 (2.4)
Dead fetuses	0	0	0
Early resorptions	0.5 (0.6)	0.5 (0.7)	0.7 (0.7)
Late resorptions	0.00	0.00	0.05 (0.22)
Preimplantation loss (%)	6.8 (16.0)	2.5 (3.9)	5.5 (6.2)
Postimplantation loss (%)	3.2 (4.0)	3.8 (4.7)	4.8 (5.0)
Sex ratio (% males)	55.0 (18.4)	44.8 (14.7)	51.5 (16.4)
Natural delivery cohort (PND 0–21)			
Number of pregnancies	22	22	22
Gestation length (days)	21.9 (0.3)	21.8 (0.4)	22.0 (0.4)
Viable litters on PND 0	22	22	22
Live litters on PND 21	22	22	22
Live birth (%)	99.0 (2.7)	99.3 (2.3)	98.1 (5.1)
Survival PND 0–4 (%)	99.2 (2.7)	98.1 (4.2)	97.5 (4.0)
Survival PND 4–21 (%)	99.4 (2.7)	100.0 (0.0)	100.0 (0.0)
Sex ratio	0.51 (0.12)	0.55 (0.10)	0.52 (0.15)
Total pups, PND 0	14.3 (2.4)	14.3 (2.0)	13.9 (2.9)
Live pups, PND 0	14.1 (2.4)	14.2 (2.1)	13.6 (3.0)
Live pups, PND 4	14.0 (2.4)	14.0 (2.1)	13.3 (2.9)
Live pups, PND 4 post-cull	8.0 (0.0)	8.0 (0.0)	7.8 (0.9)
Live pups, PND 21	8.0 (0.2)	8.0 (0.0)	7.8 (0.9)
Implantation sites	15.2 (2.3)	15.0 (1.8)	15.0 (3.2)
Postimplantation loss (%)	6.4 (5.8)	5.2 (5.9)	6.7 (6.4)

Notes: There were no statistically significant differences from control ($p < .05$).

^aData are presented as the mean of parameters calculated on a per litter basis. Standard deviation is presented in parentheses.

not acceptable for this study because it does not respond strongly to CPG adjuvants such as CPG 7909 (Rankin et al., 2001).

The current study has shown that the AV7909 anthrax vaccine administered at the full human dose twice prior to mating and once during gestation does not elicit maternal, reproductive, or developmental toxicity in rats. This study adds to the developmental and reproductive toxicity data obtained with BioThrax (Conlin et al., 2017; Franco et al., 2009). The current

study also shows that the adjuvant CPG 7909 does not produce any reproductive or developmental toxicity in pregnant rats or their offspring, which is consistent with a previous study in rats (Destexhe et al., 2015).

There was no evidence of systemic inflammation in pregnant rats, based on evaluation of A2M and AGP on GD 21, 2 weeks after the last of three administrations of AV7909. Serum AGP and A2M are sensitive biomarkers of an acute phase response in rodents (Cray et al., 2009). Therefore, reversibility of the acute phase response is

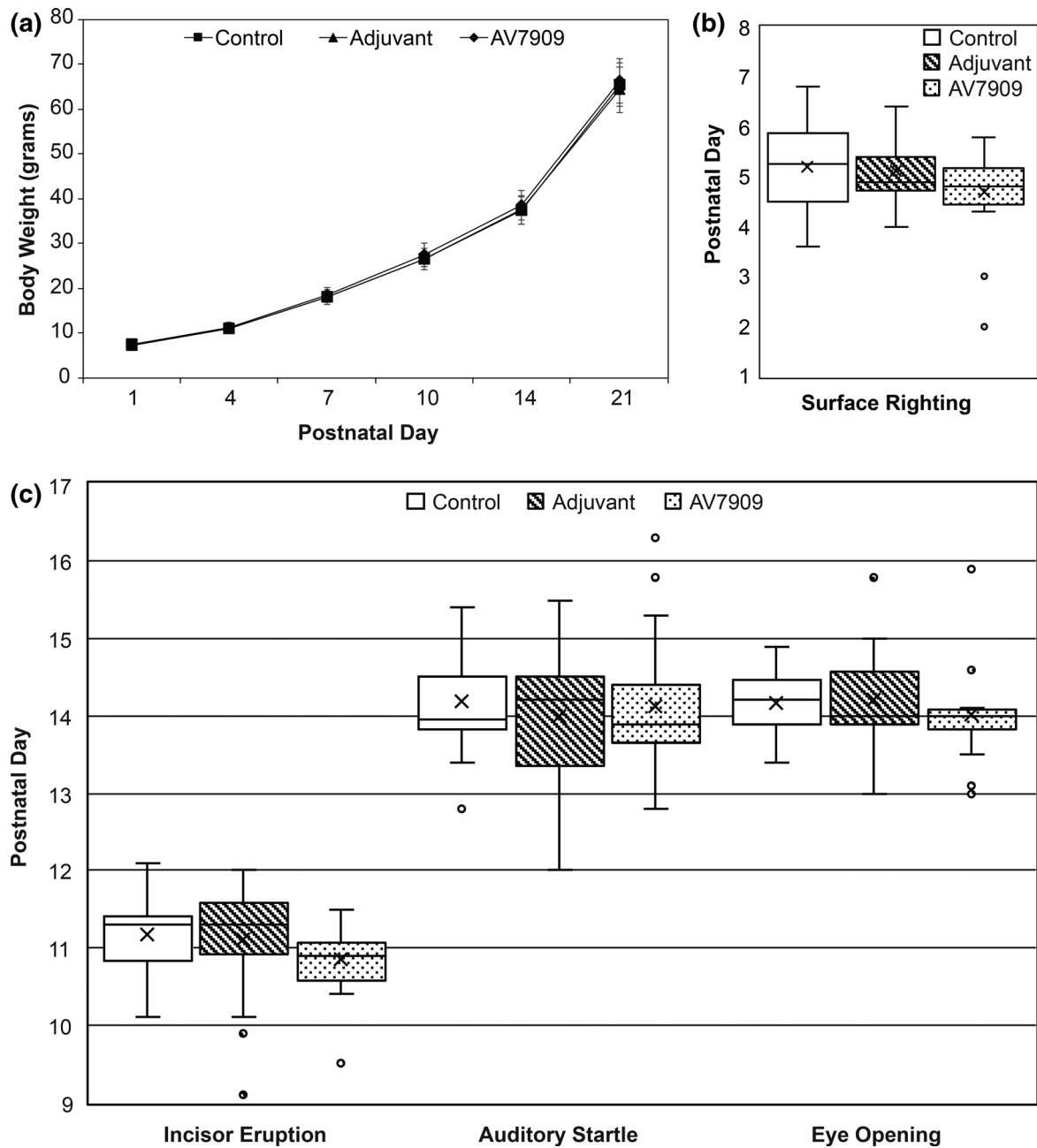


FIGURE 3 F_1 offspring body weights (a) and developmental landmark achievement (b and c) during the postnatal period. Data are presented as mean of litter means and standard deviation (a) or as mean, median, maximum, minimum, and first and third quartile, with outlier data points shown (b, c). There were no statistically significant differences compared with control ($p < .05$). All pups had a positive bilateral pupil response on PND 21 (data not shown)

likely the reason why A2M and AGP were not elevated on GD 21 in the current study.

Nodules were observed at the injection site in the AV7909 group indicating local reaction to the IM injection of AV7909. The nodules were also observed in the control and adjuvant groups albeit at a lower incidence than in the AV7909 group. The presence of nodules in the control and adjuvant group suggests that other formulation components and/or the injection procedure

may have been contributing factors. The nodules and, in one instance, an ulcer were observed 2 weeks but not 5 weeks after the last injection, except in one animal. This observation suggests that the nodules were largely resolved between 2 and 5 weeks after the last injection. Similar nodules associated with IM injection of rPA7909 and CPG 7909, consisting of microscopic inflammation of the skeletal muscle and/or subcutis, have been described previously (Savransky et al., 2019).

TABLE 2 Offspring observations

	Control	Adjuvant	AV7909
Laparohysterectomy cohort (GD 21)			
Fetal weight (g)	5.59 (0.38) ^a	5.45 (0.36)	5.55 (0.28)
Number examined (external/placental)	305 (21) ^b	315 (22)	296 (21)
Number examined (visceral/head)	156 (21)	162 (22)	154 (21)
Number examined (skeletal)	149 (21)	153 (22)	142 (21)
Malformations			
Eyes—small	–	1 (1) [0.6] ^b	–
Heart—interventricular septal defect	7 (6) [4.7]	4 (2) [2.1]	1 (1) [0.6]
Rib, 13th—absent	–	1 (1) [0.6]	–
Ribs—fused	–	1 (1) [0.6] ^c	–
Rib, 13th—short	–	1 (1) [0.6]	–
Variations			
Humerus/femur, incomplete ossification	–	–	1 (1) [1.0] ^d
Hyoid—unossified	–	–	1 (1) [0.7]
Ischium/pubis—incomplete ossification	–	1 (1) [0.6] ^d	4 (2) [3.7] ^{d,e}
Rib—incomplete ossification	2 (2) [1.3] ^{e,d}	2 (2) [1.3] ^{e,f}	2 (2) [1.7] ^{e,f}
Rib—supernumerary, rudimentary, cervical	1 (1) [0.6]	–	–
Rib—supernumerary, rudimentary, thoracolumbar	12 (10) [8.6]	8 (5) [5.4]	6 (4) [3.9]
Rib—supernumerary, full, thoracolumbar	–	–	1 (1) [0.6] ^g
Rib—wavy	1 (1) [0.6] ^d	2 (2) [1.3] ^{e,f}	3 (1) [2.4] ^f
Skull—isolated ossification site	–	–	1 (1) [0.7]
Skull—incomplete ossification	–	1 (1) [0.6] ^d	2 (1) [1.9] ^{d,e}
Skull—supernumerary ossification site	–	1 (1) [0.6]	–
Vertebrae, presacral—greater than 26	–	–	1 (1) [0.6] ^h
Vertebral centrum—split and/or fused cartilage	–	3 (3) [2.0]	–
Vertebral arch—incomplete ossification	–	1 (1) [0.6] ^d	3 (1) [2.9] ^{d,e}
Vertebral arch—misaligned	–	–	1 (1) [0.6] ^g
Vertebral centrum—incomplete ossification	5 (3) [2.9]	9 (7) [5.9]	5 (3) [3.4] ^h
Natural delivery cohort			
Number examined	180 (22) ^b	180 (22)	178 (22)
Heart—interventricular septal defect	–	1 (1) [0.5] ^b	1 (1) [0.5]
Limb—hyperflexion	1 (1) [0.5]	–	–
Renal pelvis—dilated	7 (4) [3.9]	6 (4) [3.3]	3 (1) [1.7]

Notes: There were no statistically significant differences from control ($p < .05$).

Notes: ^{d–h} Findings that occurred in the same fetus are indicated by the same superscript letter: d, e, f, g, or h (except for supernumerary rib).

^aData are presented as mean (standard deviation).

^bData are presented as the number of fetuses/pups and litters examined or affected. The number of litters is presented in parentheses. The mean percent fetuses affected per litter is presented in brackets.

^cOther associated findings in this fetus included misaligned/incompletely ossified thoracic centra, misshapen thoracic arches, intercostal rib, misaligned sternebrae, misshapen costal cartilage.

Anthrax lethal toxin-neutralizing antibodies were detected in AV7909-vaccinated F₀ females. As expected, based on the ability of maternally derived antibodies to

cross the placenta and transfer via the mother's milk, the antibodies were also detected in the sera of fetuses and pups. Exposure of the fetuses and pups to maternally

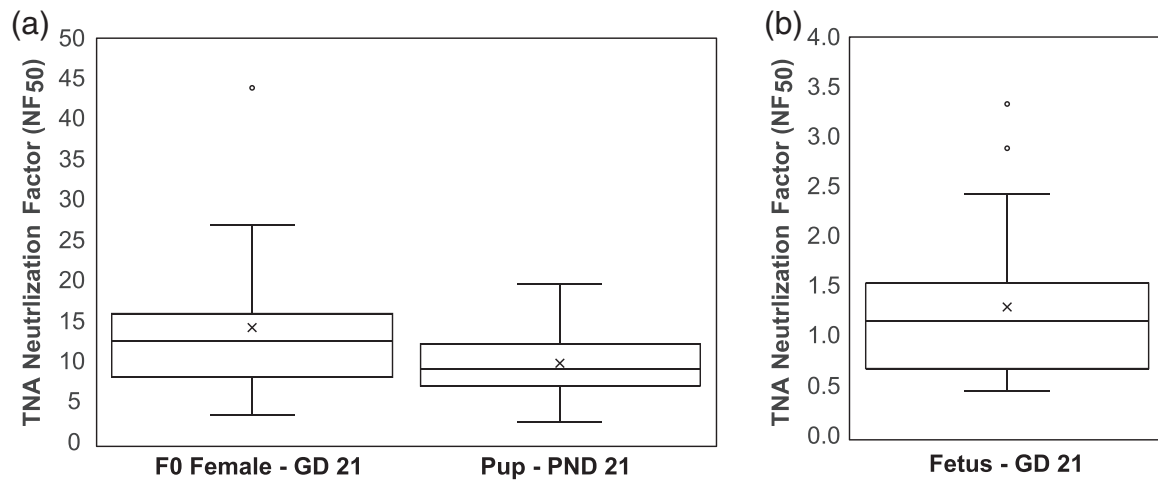


FIGURE 4 Anthrax lethal toxin-neutralizing antibodies in the AV7909 group. Data are presented as mean, median, maximum, minimum, and first and third quartile, with outlier data points shown. Mean (standard deviation) NF_{50} was 14.4 (8.8), 10.0 (4.4), and 1.3 in F_0 females, F_1 pups, and F_1 fetuses, respectively. Antibodies were not detected in the control or adjuvant groups

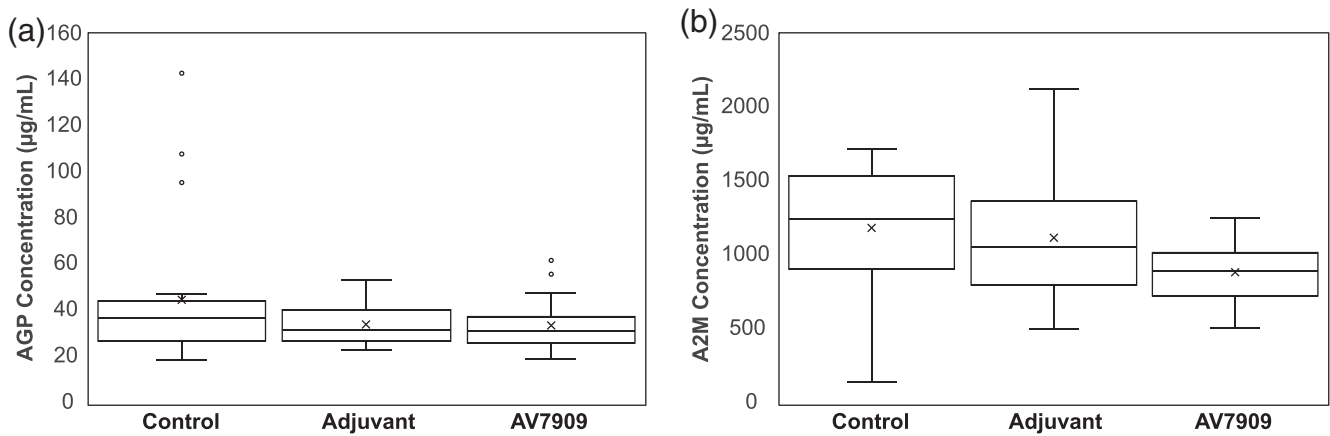


FIGURE 5 Serum concentration of AGP (a) and A2M (b) in F_0 females on gestation day 21. Data are presented as mean, median, maximum, minimum, and first and third quartile, with outlier data points shown. The mean (standard deviation) for AGP was 45 (32), 34 (9), and 33 (11) $\mu\text{g}/\text{mL}$ in the control, adjuvant, and AV7909 groups, respectively. The mean (standard deviation) for A2M was 1,189 (419), 1,121 (403), and 884 (196) $\mu\text{g}/\text{mL}$, respectively. AGP, alpha-1-acid glycoprotein; A2M, alpha-2-macroglobulin

derived anthrax lethal toxin-neutralizing antibodies was not associated with developmental toxicity. Antibody levels in F_1 pups on PND 21 were similar to those in dams suggesting good lactational transfer and little degradation over the 3-week lactation period. Antibodies were also detected in fetuses on GD 21, indicating transplacental transfer, although levels were on average 11 times lower than levels in dams. In the previous rabbit study with BioThrax, relative antibody levels were 1.4–2 times higher in fetuses and 6–7 times lower in kits compared with maternal levels (Franco et al., 2009). These differences in relative antibody levels between the current study and the previous study with BioThrax, could be potentially attributed to species differences. It must also

be noted that it is not possible to directly compare the absolute antibody levels in pregnant animals in the current study with those in the previous study due to differences in the analytical methods used to quantify antibody levels.

In summary, administration of the AV7909 vaccine candidate by IM injection to female Sprague Dawley Crl:CD (SD) rats 14 days prior to cohabitation, on the day of cohabitation (SD 15) and on GD 7 did not produce any developmental or reproductive toxicity. There was no effect on mating, fertility, pregnancy, parturition, maternal care of offspring, or prenatal or postnatal offspring viability, survival, growth, or development.

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CONFLICTS OF INTEREST

BB, JS, JR, BI, and VS are current or former employees of Emergent BioSolutions, Inc. EM, JB and AS are employees of Battelle, a Contract Research Organization where safety studies have been performed on a contractual basis for Emergent BioSolutions, Inc.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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