

# Responses of pregnant ewes and young lambs to ovalbumin immunization, antiovalbumin antibody transfer to lambs, and temporal changes in antiovalbumin antibody<sup>1,2</sup>

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**ABSTRACT:** Factors affecting the decay of maternally derived IgG and ability of neonatal lambs to produce protective amounts of their own IgG are not well understood. Thus, we conducted 3 experiments to quantify the 1) response of pregnant ewes to ovalbumin immunization, 2) antiovalbumin antibody (OV-IgG) transfer to lambs, 3) changes over time in OV-IgG in lambs, and 4) response of young lambs to ovalbumin immunization. In Exp. 1, ewes ( $n = 10/\text{group}$ ) either received control (adjuvant + saline) or ovalbumin (ovalbumin + adjuvant + saline) injections at  $\approx 42$  and 14 d prepartum. Ovalbumin increased ( $P < 0.001$ ) ewe serum and colostrum OV-IgG. Serum OV-IgG was greater ( $P < 0.0001$ ) in lambs from ovalbumin-treated than in lambs from control ewes. In Exp. 2, lambs ( $n = 20/\text{group}$ ), which were from ewes that had received ovalbumin prepartum, were given either control or ovalbumin injections on d 1 and 15 of age. From d 1 to 15, maternally derived OV-IgG was less ( $P < 0.04$ ) in ovalbumin-treated than in control lambs. After d 15, OV-IgG was greater ( $P < 0.001$ ) in ovalbumin-treated than in control lambs. In Exp. 3, lambs ( $n = 20/\text{group}$ ), which were from ewes naïve to ovalbumin, received 1

of 4 treatments: 1) d-1 + d-15 control injections; 2) d-1 + d-15 ovalbumin; 3) d-28 + d-42 control; and 4) d-28 + d-42 ovalbumin. In d-1 + d-15 ovalbumin lambs, OV-IgG increased ( $P < 0.001$ ) from d 7 to 21 after treatment and then decreased ( $P < 0.004$ ) after d 28. In d-28 + d-42 ovalbumin lambs, OV-IgG increased ( $P < 0.001$ ) steadily until d 21 after treatment and then stabilized after d 21. At  $\approx 159$  d of age, lambs in each group received injections consistent with their original type. After the d-159 treatment, ovalbumin injection increased ( $P < 0.0001$ ) OV-IgG, and the injection type  $\times$  time interaction was significant ( $P < 0.0001$ ). In d-28 + d-42 ovalbumin lambs, OV-IgG just before the d-159 injections was greater ( $P < 0.006$ ) than that in the other groups. In this study, late pregnant ewes produced OV-IgG after ovalbumin injections and then transferred OV-IgG to lambs via colostrum. Ovalbumin treatment of young lambs reduced circulating maternally derived OV-IgG, but it also induced an immune response in the lambs. Overall, our results support recommendations to vaccinate ewes against common pathogens during late pregnancy and to ensure that lambs receive adequate colostrum soon after birth.

**Key words:** antibody, lambs, immunization, passive transfer, sheep

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## INTRODUCTION

Lamb wellbeing depends on transfer of immunoglobulins (i.e., IgG), via colostrum, from ewes to newborn lambs (Hunter et al., 1977; Nowak and Poindron, 2006; Massimini et al., 2006). In fact, IgG concentrations in lamb serum are directly related to the transfer of maternal IgG to lambs, and the risk of lamb mortality decreased as neonatal IgG increased (Hunter et al., 1977; Sawyer et al., 1977; Berggren-Thomas et al., 1987; Gilbert et al., 1988; Christley et al., 2003). Perhaps the most effective method for enhancing lamb serum IgG is to immunize late-pregnant ewes against common pathogens, such as the various *Clostridium* species (ASIA, 2015).

Maternally derived IgG is critical because lambs are born with almost no IgG of their own (Halliday, 1971; Sawyer et al., 1977). Moreover, newborn lambs do not have a fully competent immune system and cannot produce ample IgG for several weeks (Hunter et al., 1977; Sawyer et al., 1977; Tizard, 1996; de la Rosa et al., 1997; Nowak and Poindron, 2006). Maternally derived IgG provide passive immunity to common pathogens, but these IgG soon decay to such a degree that they can no longer control disease-causing organisms (Tizard, 1996; Nowak and Poindron, 2006). After the loss of passive immunity, lambs must actively produce their own immunoglobulins that are capable of controlling pathogens.

The production, transfer, and uptake of maternal IgG to lambs are fairly well understood. However, factors that affect the decay of maternally derived IgG and the ability of lambs to produce protective amounts of their own immunoglobulins are not well understood. Thus, we conducted 3 experiments to quantify the 1) response of pregnant ewes to ovalbumin immunization, 2) antiovalbumin antibody (OV-IgG) transfer to lambs, 3) changes over time in OV-IgG in lambs, and 4) response of young lambs to ovalbumin immunization. Data from of this study have been described at a sectional meeting of the American Society of Animal Science (Lewis et al., 2017).

## MATERIALS AND METHODS

### *Animal and Related Procedures*

The USDA, Agricultural Research Service, U.S. Sheep Experiment Station (USSES) Institutional Animal Care and Use Committee reviewed and approved all of the husbandry practices and animal-related methods that were used for this research. Except for control and ovalbumin injections, blood samples, and time of turn out on pasture, ewes and lambs in this study were managed according to standard USSES procedures (for details, see Leeds et al., 2012). Ewes were vaccinated annually in February, which was during late pregnancy, against

clostridial diseases and in October or November, during the breeding season, against *Campylobacter* spp. and *Corynebacterium pseudotuberculosis*.

Ovalbumin is a glycoprotein from egg whites and is used as a common reference protein in immunization experiments. Sheep, for example, have not been naturally immunized against ovalbumin. Thus, OV-IgG must be produced in response to a defined treatment, and not to a pathogen found in sheep production environments.

In all experiments, ovalbumin injections contained 12 mg of ovalbumin (> 90% pure; Sigma-Aldrich Corp., St. Louis, MO), 1 mL of aluminum hydroxide gel as an adjuvant (Alhydrogel; Accurate Chemical and Scientific Corp., Westbury, NY), and 1 mL of sterile isotonic saline (Mousel et al., 2008). Control injections contained 1 mL of Alhydrogel and 1 mL of sterile isotonic saline. All injections were subcutaneous on the neck of each animal and with a syringe and needle, rather than with the standard USSES pneumatic, needle-free method (Mousel et al., 2008). Needles were changed between animals. Syringes used for ovalbumin were not used for control injections, and vice versa.

All lambing was spontaneous, rather than induced, and ewes lambed outdoors. Within approximately 30 min after parturition, each ewe and her lamb(s) were moved indoors into individual bonding pens (i.e., lambing jugs). Experienced personnel monitored all newborn lambs and confirmed that each lamb consumed colostrum from its dam, but the volume consumed was not quantified. For Exp. 1 and 2, a 10-mL colostrum sample was collected from each ewe and stored at  $-20^{\circ}\text{C}$  until OV-IgG was quantified.

All blood samples were collected from a jugular vein, using BD Vacutainer SST Plus Blood Collection Tubes (Becton, Dickinson and Co., Franklin Lakes, NJ). A new needle was used for each sample. Samples were allowed to clot at  $4^{\circ}\text{C}$  and stored for a minimum of 2 h at  $4^{\circ}\text{C}$ . Serum was collected after centrifugation at  $1781 \times g$  (3,000 rpm) and  $4^{\circ}\text{C}$ . Serum was stored at  $-20^{\circ}\text{C}$  until OV-IgG was quantified.

### *Experiment 1: Ewe Antiovalbumin IgG Production and Transfer to Lambs*

On d 0 of the experiment, which was approximately 42 d before the anticipated day of lambing, white-faced ewes received either a primary control or ovalbumin injection ( $n = 10$  ewes/group). On d 28 ( $\approx 14$  d prepartum), each ewe received a secondary control or ovalbumin injection.

Blood samples (10 mL) were collected from each ewe at 7-d intervals from d 0 through approximately d 84 of the experiment. The d-0 and d-28 blood samples were

collected just before the control and ovalbumin injections were administered. On the day after lambing, which was  $46 \pm 5$  d after d 0 of the experiment, an additional 10-mL blood sample was collected from each ewe, and a 5-mL blood sample was collected from each lamb. For lambs, this sample was collected on d 1 of life. Additional 5-mL blood samples were collected from each lamb at weekly intervals until the lambs were 42 of age.

### ***Experiment 2: Antiovalbumin IgG after Ovalbumin Injection into Neonatal Lambs***

Pregnant white-faced ewes ( $n = 40$ ) were inoculated against ovalbumin. Ewes that were part of Exp. 1 were not used for Exp. 2. Primary and secondary immunizations were given at approximately 42 d and 21 d prepartum, respectively. The day after lambing was equivalent to  $45 \pm 3$  d after the primary immunization.

Neonatal lambs ( $n = 20$ /group) were assigned to 2 treatment groups. On d 1 and d 15 of age (day of birth = d 0), lambs received either control or ovalbumin injections. Blood samples (15 mL) were collected just before each injection and at weekly intervals until the lambs were approximately 35 d of age.

### ***Experiment 3: Antibody Response of Lambs Immunized in an Early Period of Life and Again in a Period after Weaning***

Injection type (control vs. ovalbumin) and injection schedule (d 1 and d 15 of age vs. d 28 and d 42 of age; day of birth = d 0) were main effects in an experiment with a  $2 \times 2$  factorial arrangement of treatments. The treatment groups were 1) control injection on d 1 + control injection on d 15; 2) ovalbumin injection on d 1 + ovalbumin injection on d 15; 3) control injection on d 28 + control injection on d 42; and 4) ovalbumin injection on d 28 + ovalbumin injection on d 42. Treatments were randomized in blocks and assigned to white-faced ewe lambs ( $n = 20$ /treatment group) right after they were born. In addition, at an average age of 159 d, which was soon after weaning, lambs received either a control or an ovalbumin injection that was consistent with their original injection type.

To prevent the transfer of maternal OV-IgG to lambs, ewes that produced lambs for this experiment had not been immunized against ovalbumin or used in Exp. 1 or 2. However, blood samples were collected from the ewes and analyzed to determine whether these restrictions had been met.

Blood samples (15 mL) were collected from each lamb before the d-1 and d-15 injections and before the d-28 and d-42 injections. Additional 15-mL blood samples were collected weekly for 5 wk. Blood samples (15 mL)

were also collected immediately before the postweaning injections and at weekly intervals for the next 4 wk.

### ***Measurement of Antiovalbumin Antibody***

Antiovalbumin IgG was quantified with an ELISA, as described previously (Mousel et al., 2008). Colostrum samples were thawed and centrifuged multiple times to remove fat, and then skim-colostrum samples were used in the assay. Serum and skim-colostrum samples were diluted 1:100 in PBS (Product 79383, Sigma-Aldrich Corp.), and then 100  $\mu$ L of diluted sample was used in the assay. Data are expressed as optical density units (odu). The within and between assay CV for serum samples were 6 and 11%, respectively, and for colostrum samples were 9 and 14%, respectively.

### ***Statistical Analyses***

The GLM procedures in SAS (SAS Inst. Inc., Cary, NC) were used to determine the effects of ovalbumin treatment (Exp. 1 and 2) and time after treatment (Exp. 2) on colostrum OV-IgG. Procedures in SAS Proc Mixed were used to analyze data with repeated measures (i.e., serum OV-IgG). Least-squares means and pooled standard errors were reported. Using models comparable to those used in Proc Mixed, Proc GLM was used to generate the estimates of variance that were used to calculate pooled standard errors.

For Exp. 1 and 2, the models included terms for treatment (control vs. ovalbumin), day of sampling, and the treatment  $\times$  day interaction. Day was classified as repeated, and animal within treatment was the subject. Alpha level was set as  $\leq 0.05$ .

For Exp. 3, period of treatment (early in life and after weaning) was confounded with age and initial treatments, and the experiment was not designed to avoid this confounding. Thus, the data were sorted and analyzed within period to evaluate treatment effects early in life and then after weaning. The models for these analyses included terms for injection schedule (d 1 and d 15 of age vs. d 28 and d 42 of age), injection type (control vs. ovalbumin), day of sampling, and all interactions. Day was classified as repeated, and animal within injection schedule  $\times$  injection type was the subject. Alpha level was set as  $\leq 0.05$ .

## **RESULTS**

### ***Antiovalbumin Assay Background***

We considered apparent OV-IgG, which averaged 0.83 odu and ranged from 0.68 to 1.10 odu, in sheep that had not received either ovalbumin injections or

colostrum containing OV-IgG to be assay background. Apparent OV-IgG represents the effects of assay reagents and nonspecific binding of serum components to ovalbumin on odu measurements, although blocking proteins were used to minimize nonspecific binding.

### Experiment 1: Ewe Antiovalbumin IgG Production and Transfer to Lambs

**Ewes:** Ovalbumin injections increased ( $P < 0.001$ ) serum OV-IgG (control, 1.06 odu, vs. ovalbumin treated, 1.34 odu; pooled SE = 0.007 odu), and serum values changed with time after primary immunization, peaking approximately 10 d before parturition (ovalbumin treatment  $\times$  day interaction,  $P < 0.001$ ; Fig. 1). Also, ovalbumin injections increased ( $P < 0.001$ ) colostrum OV-IgG (control, 0.98 odu, vs. ovalbumin treated, 1.47 odu; pooled SE = 0.008 odu).

**Lambs:** Serum OV-IgG was greater ( $P < 0.0001$ ) in lambs from ovalbumin-treated ewes (1.48 odu) than it was in lambs from control ewes (0.69 odu; pooled SE = 0.024 odu). The day and the treatment  $\times$  day interaction were not significant ( $P = 0.34$  and  $0.64$ , respectively; Fig. 2).

### Experiment 2: Antiovalbumin IgG after Ovalbumin Injection into Neonatal Lambs

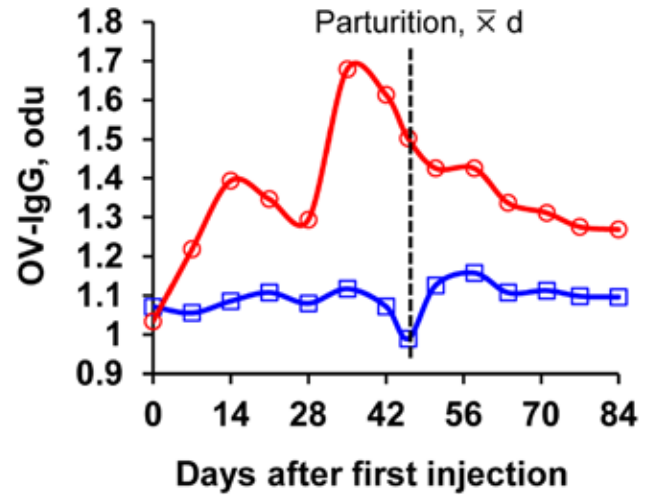
**Ewes:** Serum OV-IgG averaged 1.14 odu (pooled SE = 0.017 odu). Values increased ( $P < 0.0001$ ) over time after the primary immunization (d 0, 0.82 odu; d 21, 1.21 odu; and d 45, 1.37, odu). Colostrum OV-IgG averaged 1.38 odu (pooled SE = 0.022 odu).

**Lambs:** The main effect of ovalbumin treatment did not affect serum OV-IgG (control, 1.19 odu, vs. ovalbumin, 1.23 odu; Pooled SE = 0.009 odu). However, the treatment  $\times$  day-of-age interaction was significant ( $P < 0.0001$ ). From d 1 to d 15, OV-IgG was less ( $P < 0.04$ ) in ovalbumin-treated than in control lambs (Fig. 3). After d 15, OV-IgG was greater ( $P < 0.001$ ) in ovalbumin-treated than in control lambs (Fig. 3).

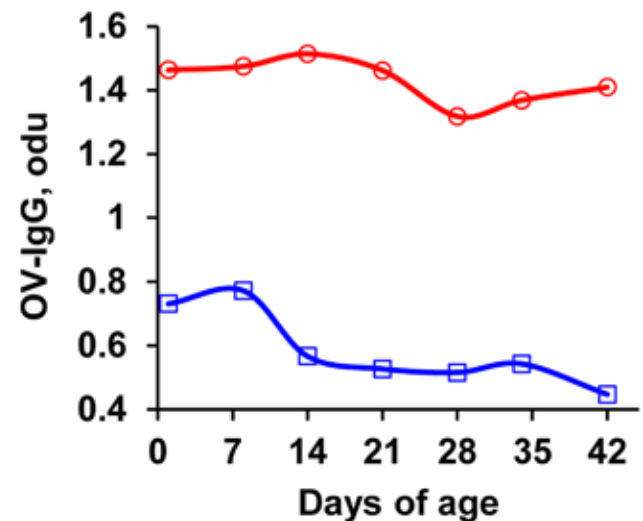
### Experiment 3: Antibody Response of Lambs Immunized in an Early Period of Life and Again in a Period after Weaning

**Ewes:** Apparent serum OV-IgG averaged 0.85 odu (pooled SE = 0.026 odu), and there was no effect of treatment assignment on the values. Thus, a critical requirement for this experiment was met.

**Lambs:** During the early period of life, injection type (control, 0.67 odu, vs. ovalbumin, 1.14 odu;  $P < 0.0001$ ), but not injection schedule (d 1 and d 15, 0.92 odu, vs. d 28 and d 42 of age, 0.89 odu;  $P = 0.59$ ), affected OV-IgG

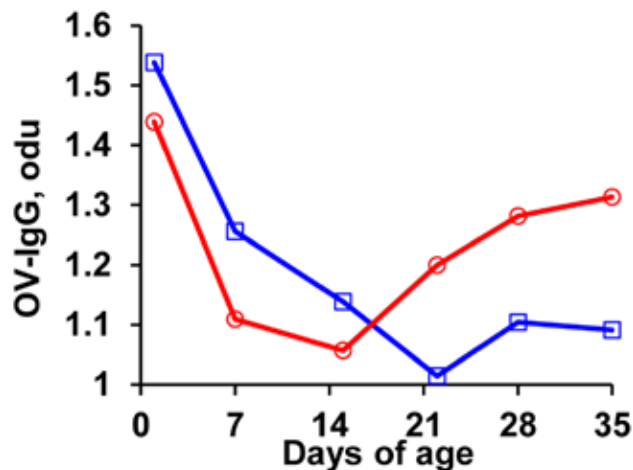


**Figure 1.** Experiment 1: On d 0 and 28 of the experiment (i.e.,  $\approx 42$  d and  $\approx 14$  d prepartum, respectively), ewes ( $n = 10$ /group) received a subcutaneous injection of either a control (open squares; 1 mL of adjuvant + 1 mL of sterile isotonic saline) or ovalbumin preparation (open circles; 12 mg of ovalbumin + 1 mL of aluminum hydroxide gel adjuvant + 1 mL of sterile isotonic saline). Ewes lambed  $45 \pm 5$  d (vertical dashed line) after d 0 of the experiment. Antiovalbumin IgG (OV-IgG) was measured in weekly serum samples from jugular blood. Serum from each sample was diluted 1:100 in PBS; 100  $\mu$ L of diluted sample was assayed; and data were expressed as optical density units (odu). Ovalbumin injections increased ( $P < 0.001$ ) serum OV-IgG, and serum OV-IgG changed with time after the first injection (ovalbumin treatment  $\times$  day interaction,  $P < 0.001$ ). Values are least-squares means, with a pooled SE of 0.007 odu.



**Figure 2.** Experiment 1: Lambs ( $n = 66$ ) were from ewes ( $n = 10$ /group) that had received either control (open squares) or ovalbumin (open circles) injections before lambing. Beginning the day after lambing, jugular blood was collected weekly from each lamb for antiovalbumin IgG (OV-IgG) quantification. Serum from each sample was diluted 1:100 in PBS; 100  $\mu$ L of diluted sample was assayed; and data were expressed as optical density units (odu). Throughout the sampling period, serum OV-IgG was greater ( $P < 0.0001$ ) in lambs from ovalbumin-treated ewes than it was in lambs from control ewes. Values are least-squares means, with a pooled SE of 0.024 odu.

(pooled SE = 0.01 odu), and the injection type  $\times$  injection schedule interaction was not significant ( $P = 0.84$ ). Antiovalbumin IgG changed ( $P < 0.0001$ ) with time after

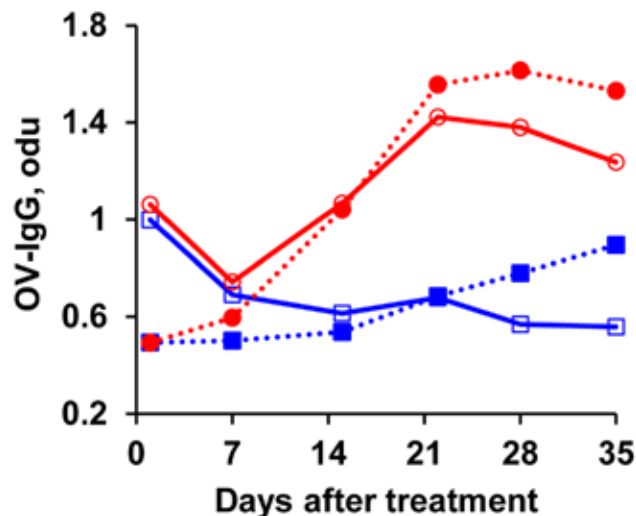


**Figure 3.** Experiment 2: Lambs were from ewes ( $n = 40$ ) that had been inoculated against ovalbumin during the last 6 wk of pregnancy. On d 1 and 15 of age, lambs ( $n = 20$ /group) received a subcutaneous injection of either a control (open squares; 1 mL of adjuvant + 1 mL of sterile isotonic saline) or ovalbumin preparation (open circles; 12 mg of ovalbumin + 1 mL of aluminum hydroxide gel adjuvant + 1 mL of sterile isotonic saline). Jugular blood was collected just before each injection and at weekly intervals until the lambs were approximately 36 d of age, and antiovalbumin IgG (OV-IgG) was quantified. Serum from each sample was diluted 1:100 in PBS; 100  $\mu$ L of diluted sample was assayed; and data were expressed as optical density units (odu). Ovalbumin treatment did not affect mean serum OV-IgG, but the treatment  $\times$  age interaction was significant ( $P < 0.0001$ ). Antiovalbumin IgG was less ( $P < 0.04$ ) in ovalbumin-treated than in control lambs from d 1 to d 15. After d 15, OV-IgG was greater ( $P < 0.001$ ) in ovalbumin-treated than in control lambs. Values are least-squares means, with a pooled SE of 0.009 odu.

injection (Fig. 4), and the injection type  $\times$  time and injection schedule  $\times$  time interactions were significant ( $P < 0.0001$ ). The injection type  $\times$  injection schedule  $\times$  time interaction was not significant ( $P = 0.34$ ).

In lambs that received ovalbumin injections on d 1 and d 15 of age, OV-IgG increased ( $P < 0.001$ ) from d 7 to d 21 after ovalbumin treatment, but values decreased ( $P < 0.004$ ) after d 28 (Fig. 4). In lambs that received injections of ovalbumin on d 28 and d 42 of age, OV-IgG increased ( $P < 0.001$ ) steadily until d 21 after treatment and then stabilized after d 21 (Fig. 4). Apparent OV-IgG in lambs that received control injections on d 1 and d 15 or on d 28 and d 42 of age changed over time, but after d 7 of the sampling period they remained less ( $P < 0.005$ ) than the values in ovalbumin-treated lambs (Fig. 4).

During the period after weaning, injection type (control, 0.93 odu, vs. ovalbumin, 1.38 odu;  $P < 0.0001$ ), but not initial injection schedule (d 1 and d 15, 1.12 odu, vs. d 28 and d 42 of age, 1.19 odu;  $P = 0.59$ ), affected OV-IgG (pooled SE = 0.013 odu), and the injection type  $\times$  injection schedule interaction was not significant ( $P = 0.08$ ). Antiovalbumin IgG changed ( $P < 0.0001$ ) with time after injection (Fig. 5), and the injection type  $\times$  time interaction was significant ( $P < 0.0001$ ). The injection schedule  $\times$  time and injection



**Figure 4.** Experiment 3: Lambs ( $n = 20$ /group) were from ewes that had not been inoculated with ovalbumin. The following treatments were assigned to lambs soon after birth: 1) control injection (open squares, solid line; 1 mL of adjuvant + 1 mL of sterile isotonic saline) on d 1 of age + control injection on d 15 of age; 2) ovalbumin injection (open circles, solid line; 12 mg of ovalbumin + 1 mL of aluminum hydroxide gel adjuvant + 1 mL of sterile isotonic saline) on d 1 + ovalbumin injection on d 15; 3) control injection (closed squares, dotted line) on d 28 + control injection on d 42; and 4) ovalbumin injection (closed circles, dotted line) on d 28 + ovalbumin injection on d 42. All injections were subcutaneous. Jugular blood samples were collected before the injections and at weekly intervals for 5 wk for antiovalbumin IgG (OV-IgG) quantification. Serum from each sample was diluted 1:100 in PBS; 100  $\mu$ L of diluted sample was assayed; and data were expressed as optical density units (odu). Injection type (control vs. ovalbumin;  $P < 0.0001$ ), but not injection schedule (d 1 and d 15 vs. d 28 and d 42 of age;  $P = 0.59$ ), affected OV-IgG. The injection type  $\times$  injection schedule interaction was not significant ( $P = 0.84$ ). Time after injection affected ( $P < 0.0001$ ) OV-IgG, and the injection type  $\times$  time and injection schedule  $\times$  time interactions were significant ( $P < 0.0001$ ). The injection type  $\times$  injection schedule  $\times$  time interaction was not significant ( $P = 0.34$ ). Values are least-squares means, with a pooled SE of 0.01 odu.

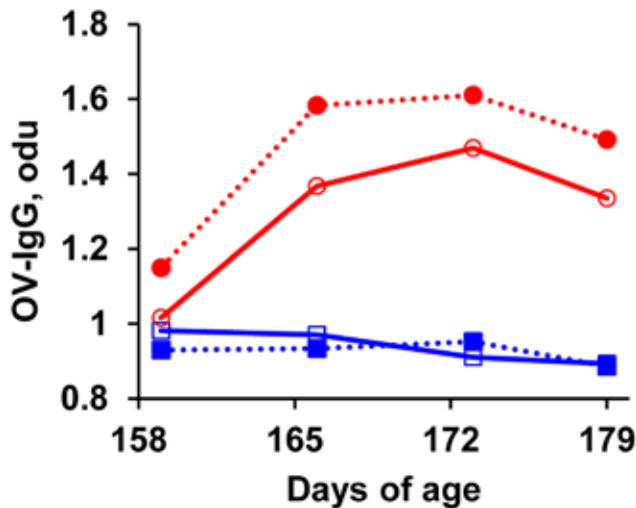
type  $\times$  injection schedule  $\times$  time interactions were not significant ( $P = 0.86$  and  $0.68$ , respectively).

Antiovalbumin IgG in lambs that had received ovalbumin injections early in life (i.e., d 1 and d 15 or d 28 and d 42 of age) increased ( $P \leq 0.003$ ) after the d-159 ovalbumin injection (Fig. 5). In lambs that received ovalbumin injections on d 28 and d 42 of life, average OV-IgG in blood samples collected just before the d-159 booster was greater ( $P < 0.006$ ) than that in lambs in the other groups (Fig. 5). Apparent OV-IgG in lambs that received control injections remained less than 1.0 odu throughout the sampling period (Fig. 5).

## DISCUSSION

In this study, inoculating ewes with ovalbumin during the last approximately 6 wk of pregnancy increased OV-IgG in blood serum and in colostrum. Maternal OV-IgG was apparently transferred to lambs because OV-IgG was greater in lambs born to ovalbu-





**Figure 5.** Experiment 3: Lambs ( $n = 20/\text{group}$ ), which were from ewes that were naïve to ovalbumin, had received one of the following treatments: 1) control injection (open squares, solid line; 1 mL of adjuvant + 1 mL of sterile isotonic saline) on d 1 of age + control injection on d 15 of age; 2) ovalbumin injection (open circles, solid line; 12 mg of ovalbumin + 1 mL of aluminum hydroxide gel adjuvant + 1 mL of sterile isotonic saline) on d 1 + ovalbumin injection on d 15; 3) control injection (closed squares, dotted line) on d 28 + control injection on d 42; and 4) ovalbumin injection (closed circles, dotted line) on d 28 + ovalbumin injection on d 42. Also, at an average age of 159 d, which was soon after weaning, lambs received either a control or an ovalbumin injection that was consistent with their original injection type. All injections were subcutaneous. Jugular blood samples were collected immediately before the d-159 injections and at weekly intervals for the next 4 wk to quantify antiovalbumin IgG (OV-IgG). Serum from each sample was diluted 1:100 in PBS; 100  $\mu\text{L}$  of diluted sample was assayed; and data were expressed as optical density units (odu). Injection type (control vs. ovalbumin;  $P < 0.0001$ ), but not initial injection schedule (d 1 and d 15 vs. d 28 and d 42 of age;  $P = 0.59$ ), affected OV-IgG. The injection type  $\times$  injection schedule interaction was not significant ( $P = 0.08$ ). Time after injection ( $P < 0.0001$ ) affected OV-IgG, and the injection type  $\times$  time interaction was significant ( $P < 0.0001$ ). Injection schedule  $\times$  time and injection type  $\times$  injection schedule  $\times$  time were not significant ( $P = 0.86$  and  $0.68$ , respectively). Values are least-squares means, with a pooled SE of 0.013 odu.

min-treated ewes than in lambs born to control ewes. These effects of ovalbumin inoculation during late pregnancy and the relationship between colostrum IgG and passive immunity in lambs are consistent with published data (Hunter et al., 1977; Sawyer et al., 1977). Moreover, these responses underlie the recommendations to vaccinate ewes against common pathogens, such as *Clostridium perfringens* and *C. tetani*, during late pregnancy and to ensure that lambs receive adequate colostrum soon after birth (ASIA, 2015).

The changes over time in OV-IgG in lambs were evaluated in Exp. 1 and 2. In Exp. 1, OV-IgG in lambs born to ovalbumin-treated ewes was increased on d 1, compared with that in lambs born to control ewes, and OV-IgG remained increased and fairly stable throughout the 6-wk sampling period. However, in Exp. 2, OV-IgG in control lambs, also born to ovalbumin-treated ewes, decreased, compared with d-1 values, during the first 3 wk of life and then remained de-

creased throughout the sampling period. The changes over time in Exp. 2 are consistent with the expected decay of passive immunity during the first few weeks of life and with changes in concentrations of antibodies to  $\epsilon$ -toxin of *C. perfringens* type D in lambs (Watson, 1992; Tizard, 1996; de la Rosa et al., 1997). By contrast, the lack of substantial changes over time in Exp. 1 is not consistent with previous data, and we have no plausible explanation for this apparent maintenance of passive immunity to ovalbumin.

Experiments 2 and 3 were conducted to address questions surrounding the response of young lambs to immunization. In Exp. 2, ovalbumin inoculation of lambs on d 1 of life reduced OV-IgG, compared with control treatment; lambs in this experiment were from ewes that had been inoculated against ovalbumin. We had anticipated a reduction in OV-IgG after ovalbumin inoculation because IgG of maternal origin can neutralize vaccine antigens, and this can reduce antibody concentrations (Tizard, 1996; Chappuis, 1998; Roitt et al., 1998; Premenko-Lanier et al., 2006; Demirjian and Levy, 2009).

In Exp. 3, ovalbumin inoculation of lambs, which were from ewes not inoculated against ovalbumin, on d 1 and d 15 or on d 28 and d 42 of life increased OV-IgG, as did ovalbumin inoculation of lambs on d 15 in Exp. 2. The injection schedule (i.e., d 1 and d 15 vs. d 28 and d 42) had little overall effect on OV-IgG during the immediate 35-d sampling period. Based on previous publications, we had expected ovalbumin inoculation to induce some production of OV-IgG. Even though newborns may not have a fully competent immune system, they can produce antibodies (Tizard, 1996; Chappuis, 1998). The lingering questions about sheep, and other animals, focus on whether neonates can produce protective amounts of antibodies and whether inoculating neonates against common pathogens reduces passive immunity enough to make them more susceptible to the pathogens. The scientific literature does not provide clear answers to these questions, but there is clear evidence that inoculating young lambs (i.e., d 1 and d 21 or d 42 of age) against *C. perfringens* did not induce a significant immune response or improve survival rates and feedlot performance (Hoeffler and Hallford, 1985; de la Rosa et al., 1997).

In Exp. 3, ovalbumin inoculation on approximately d 159 of age increased OV-IgG in lambs that had been inoculated with ovalbumin on d 1 and d 15 or on d 28 and d 42 of life, but the experimental design did not allow us to determine whether the d-159 injection acted as a booster or as a primary inoculation. Nevertheless, the response to the d-159 inoculation was consistent with the recommendation to inoculate lambs at the time of weaning (ASIA, 2015).

## Implications

Maternal antibodies that were produced in response to inoculations during late pregnancy were transferred to lambs via colostrum, and this validated a critical element of the experimental model. Inoculating young lambs may reduce circulating maternally derived antibodies, but it can induce an immune response in the lambs. However, the design of this study did not permit us to determine whether a reduction in maternally derived OV-IgG would make lambs more susceptible to common pathogens or whether the immune response was adequate to protect young lambs from common pathogens. Overall, the results of this study support the recommendations to vaccinate ewes against common pathogens during late pregnancy and to ensure that lambs receive adequate colostrum soon after birth. The results of this study do not support the notion of inoculating newborn lambs, which may or may not be able to produce an adequate immune response, instead of inoculating late pregnant ewes and gaining the colostrum-mediated advantages of passive immunity.

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