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Research Note: Sheep Antiserum as an Antibody Supplement in Newborn Lambs

J.L. Pommer[1]

[1] *Direct correspondence to: J. L. Pommer, MS, Ovis, LLC, Canton, SD 57013, (605) 321-7627, Email: jpommer@ovissheep.com*

One of the greatest management challenges facing lamb producers today is keeping newborn lambs alive and healthy. More than 20 percent of lambs do not reach weaning, with 80 percent of those losses occurring in the first 3 days of life (Held, nonreference summary of professional observation). Starvation, hypothermia and scours account for most of those death losses. It has been estimated that 45 percent of all lambs that die during the first few days of life can be contributed to inadequate colostrum intake (McGuire et al., 1983). This can be contributed to ewe's colostrum being poor quantity or quality; bad udders (mastitis, hardbag); dysfunctional teats; multiple births (triplets, quads); neglect from the ewe; orphaned or weak lambs; and diseases, such as ovine progressive pneumonia (OPPV) and Johnes.

The lack of or reduced colostrum intake leaves the newborn lamb without adequate antibody protection to certain infectious diseases causing the newborn lamb to become sick and possibly die (Sawyer et al., 1977). The most common infectious agents causing death in lambs are *Clostridium perfringens* (type C & D), *Salmonella* spp., *Escherichia coli* (*E.coli*), and Mannheima hemolytica ((Rook et al, 1990). Navel ill, septicemias, *E.coli* enteritis and peracute pneumonia are more common in 2- to 3-day old lambs that lack passive immunity. *E.coli* endotoxemia tends to show up at 7 to 10 days of age. A study at the U.S. Sheep Experiment Station showed that 46 percent of lamb mortality was caused by scours and 8 percent by pneumonia, both of which are likely related to the lack of immune protection in the newborn lambs (Gates et al., 2000).

An important management tool for protection to infectious diseases is to ensure that newborn lambs receive adequate antibody intake within the first hours of life (Vihan, 1988). Colostrum provides energy, protein, minerals, vitamins, water and, most importantly, antibody protection against the infectious diseases mentioned above. Lambs are born antibody deficient and have comprised immune systems until they ingest colostrums. Adequate antibody intake is important for all lambs to ensure good health, survivability, and performance (Rook et al, 1990).

The purpose of this research project is to determine if a sterile, irradiated, hyper-immune serum product derived from healthy, hyper-immunized sheep can be used as an antibody supplement in newborn lambs.

Sterile, Irradiated-Antiserum Product

Two healthy Cheviot male (wether) adult sheep were vaccinated using commercial vaccines multiple times (4x at 21-day intervals) against specific *E.coli* endotoxin and *Cl. perfringens* enterotoxins, that are produced by major bacteria that cause illness in newborn lambs. The sheep were monitored for antibodies titers to *E.coli* endotoxin by a qualified, enzyme-linked immunoassay (ELISA, see below). Once adequate titers were achieved (1:32,000), 500 ml of whole blood was collected 21 days following the final vaccination. The serum, which contains the protective antibodies, was harvested by centrifugation (3,000 rpms), pooled, sterile-filtered (.45ul), bottled (100 ml), and sterile-irradiated to ensure product quality. The antiserum product was frozen (-20°C) until ready for use. The product was tested on an *E.coli* endotoxin-ELISA and had a sheep antibody (IgG) titer of 1:32,000.

Ewe's Colostrum

Colostrum was collected from three Cheviot ewes from the 2008 lambing season. The collected colostrum was pooled, mixed and aliquoted into 4 oz feedings and was frozen (-20°C) until

ready for use. The colostrum product was tested on an *E.coli* endotoxin-ELISA and had a sheep antibody (IgG) titer of 1:6,400.

Lamb-Milk Replacer

Lamb-milk replacer (Merrick, Inc, Middleton, Wisc.) was purchased and mixed according to label instructions. The milk was also tested on an *E.coli* endotoxin-ELISA and had a bovine (IgG) titer of 1:800.

Lamb Care and Use

Twelve (12) newborn Cheviot lambs were collected at birth from ewes that had multiple births (triplets, twins), ewes with bad udders, or older ewes. Lambs were not allowed to nurse the ewe. Lambs were given an ID tags, and navels were disinfected with 7-percent iodine. Blood was drawn from lambs prior to receiving their first designated feeding, approximately 30 minutes following birth.

Blood samples (3 ml) were collected from the jugular vein using 18-ga x ½-inch needle and 3cc syringe. Collected blood was transferred to 5 ml vacutainer blood tubes, which were labeled with the lamb's ID and collection date. Collected blood tubes were refrigerated (4°C) overnight, then centrifuged (15 min at 3,000 rpm) for serum collection. Serum was transferred to a small tube, labeled, dated and frozen (-20°C).

The newborn lambs were assigned into four (4) groups with three (n=3) lambs per group. Group A received one, 4 oz feeding of pooled colostrum. Group B received orally one feeding of 20 cc of antiserum in 4 oz of milk replacer. Group C received 10 cc dose subcutaneous (two, a 5 cc dose per upper shoulder) and oral feeding of 4 oz of milk replacer. Group D received oral feeding of 4 oz of milk replacer only. Following the first feedings, all lambs received lamb milk-replacer (Merrick) according to label instructions.

All lambs were evaluated at the time of feedings for appetite, activity, and illness. Appetite and activity were scored 0 to 5 with 0 = none, 2.5 = moderate, and 5 = strong. Possible illness was determined by lack of appetite and activity and by body temperature (102.4°F = normal). Lambs were evaluated for diseases of the respiratory, digestive, muscular and skeletal, and central nervous systems. Illness was scored 0 to 5 with 0 = severe, 2.5 = moderate, 5 = none. All evaluation scores, temperatures and treatments were recorded.

Lambs that appeared to be ill (lack of activity and appetite with temperature) were treated with supportive medicine (antibiotics and anti-inflammatories). Lambs that died during the study were submitted to South Dakota State University Animal Diagnostic Research and Diagnostic Laboratory, Brookings, S.D. for diagnostic evaluation.

Lambs also had blood drawn at 24 hrs, 1 week, 2 weeks and 4 weeks of age after receiving their first designated feeding. Blood samples (3 ml) were collected, processed and frozen as stated above. All collected serum samples were evaluated for endotoxin antibody titer levels using a qualified endotoxin ELISA assay that measured sheep IgG.

Lambs had access to fresh water and lamb creep starter (18-percent protein). All lambs had tails docked 1 week after birth, and male lambs were castrated after 4 weeks of age. All lambs were off study at 4 weeks of age (28 days) and were weaned after 5 weeks of age. All lambs were vaccinated at 28 days for clostridial diseases (type C and D).

The Institutional Animal Care and Use Committee (IACUC) at Ovis LLC approved the protocol for this experiment, according to guidelines provided by the *Guide For the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (1998)

Anti-Endotoxin Enzyme-Linked Immunosorbent Assay (ELISA)

An ELISA assay described by American Research Products was used to test collected lamb serums for the presence of specific antibodies to lipopolysaccharide (LPS) from *E.coli* J5 (Sigma-Aldrich, St. Louis, Mo.). Briefly, ninety-six well MaxiSorp ELISA plates (Nunc) were used to coat LPS to the plates. A commercial sheep serum (American Research Products, Belmont, Mass.) was used as the endotoxin positive control, with a titer of 1:4,000. Newborn-lamb serum (Ovis LLC, Canton, S.D.) was collected after birth to be used as a negative control (<1:100). Test serums and controls were diluted in phosphate-buffered saline, pH 7.4, Tween 20 (nonionic detergent) (PBST) (Sigma Aldrich, St. Louis, Mo.) starting at 1:100 dilution and serial diluted on the plates to have 100 uL per well. Test-and controls-serum samples were replicated twice on each plate. The plates were incubated at 37°C for one hour in a humidified incubator and then washed three times with PBST wash buffer. Rabbit anti-sheep IgG (H and L) alkaline phosphate conjugate (Kirkegaard and Perry Laboratories, Gaithersburg, Md.) was added at a concentration of 1:2000 by diluting in PBST and adding 100 uL per well. The plates were incubated for 30 minutes as stated above. The plates were washed three times with PBST and color was developed by adding 100uL per well of TMB substrate solution (Kirkegaard and Perry Laboratories, Gaithersburg, Md.). The plates were read 20 minutes after substrate addition at an optical density (OD) of 630nm with a microplate reader (ELx800 Automated Microplate Reader, DiaLab, Austria). The data reduction was performed using Gen 5 software with the microplate reader.

Group A: Ewe's Colostrum

Three lambs (A-500, A-575, and A-576) that received 4 oz of ewe's colostrum orally had strong appetites (score = 5) and activity (score = 5) with no illnesses (score = 5) seen during the 28-day trial (Table 1). All three lambs had negative (<1:100) endotoxin titers at birth (Table 2). Endotoxin titers (933.3) were detected at 24 hrs after birth with a slight decrease (466.7) at 1 week of life. Endotoxin titers did decrease slightly (333.3) at 2 weeks of life but increased (933.3) at 4 weeks of life.

Group B: Antiserum Product Oral

Three lambs (B-494, B-495, and B-513) that received 20cc of antiserum product orally in 4 oz of lamb milk replacer had strong appetites (score = 5) and activity (score = 5) with no illnesses (score = 5) seen during the 28-day trial (Table 1). All three lambs had negative (<1:100) endotoxin titers at birth (Table 2). Endotoxin titers (666.7) were detected at 24 hrs after birth with an increase (800) at 1 week of life. Endotoxin titers did decrease (233.3) at 2 weeks of life but increased (466.7) at 4 weeks of life.

Table 1. Individual Lamb Health Scores

Group & Lamb #	Activity*	Appetite*	Illness**	Comment:
Group A: Ewe's Colostrum - 4 oz orally				
500	5	5	5	healthy
575	5	5	5	healthy
576	5	5	5	healthy
Group B: Antiserum orally - 20 ml in 4 oz Lamb-milk replacer				
494	5	5	5	healthy
495	5	5	5	healthy
513	5	5	5	healthy
Group C: Antiserum sq - 10 ml				
493	5	5	5	healthy
519	5	5	5	healthy
580	NA	NA	NA	removed from study
Group D: Lamb-milk Replacer - 4 oz orally				
487	5	5	5	healthy
526	1	2	0	died; septicemia
570	2	3	2	joint ill; treated with antibotics

* score 0-5 (0 = none, 2.5 = moderate, 5 = strong)
** score 0-5 (0 = severe, 2.5 moderate, 5 = none)
sq = subcutaneous NA (not available)

Table 2. Individual Lamb Endotoxin Titers

Group & Lamb #	Birth	24 hrs	1 week	2 weeks	4 weeks	Comment:
Group A: Ewe's Colostrum – 4 oz Oral						
500	<100	800	800	200	800	healthy
575	<100	1600	400	400	400	healthy
576	<100	400	200	400	1600	healthy
Group B: Antiserum orally - 20 ml in 4 oz Lamb-milk replacer						
494	<100	800	400	400	800	healthy
495	<100	800	1600	200	400	healthy
513	<100	400	400	100	200	healthy
Group C: Antiserum sq - 10 mls						
493	<100	3200	400	200	400	healthy
519	<100	3200	1600	800	800	healthy
580	<100	NA	NA	NA	NA	removed from study
Group D: Lamb-milk Replacer - 4 oz orally						
487	<100	<100	200	200	400	healthy
526	<100	<100	100	NA	NA	died, septicemia
570	<100	<100	100	400	400	joint ill, treated

sq = subcutaneous NA = not available < = less than

Group C: Antiserum Product Subcutaneous

Two lambs (C-493 and C-519) that received 10cc of antiserum product subcutaneous had strong appetites (score = 5) and activity (score = 5) with no illnesses (score = 5) seen during the 28-day trial (Table 1). Both lambs had negative (<1:100) endotoxin titers at birth (Table 2). Endotoxin titers (3200) were detected at 24 hrs after birth with a decrease (1000) at 1 week of life. Endotoxin titers did decrease slightly (500) at 2 weeks of life but increased (600) at 4 weeks of life. One lamb in this group (C-580) escaped the lambing pen and was reunited with its mother and therefore removed from the study.

Group D: Milk Replacer

Two of the three lambs (D-526, and D-570), that received 4 oz of lamb-milk replacer, had health-related issues. Lamb D-487 remained healthy throughout the

study (Table 1). One lamb (D-526) died at 9 days of age due to a bacterial septicemia (*Klebsiella* sp). Lamb D-570 developed a joint-ill condition starting at 2 days of age, which continued throughout the 28-day study. The lamb was given antibiotics to treat the condition. Lamb D-487 had a strong appetite (score = 5) and activity (score = 5) with no illnesses (score = 5) seen during the 28-day trial. All three lambs had negative (<1:100) endotoxin titers at birth (Table 2.). Endotoxin titers remained negative (<1:100) at 24 hrs after birth with a slight increase (133.3) at 1 week of life. Endotoxin titers did increase slightly (300) at 2 weeks and (400) at 4 weeks of life.

All the lambs in Groups A, B and C remained healthy throughout the 28-day trial compared to Group D. When evaluating endotoxin titers, both Groups A and B had comparable endotoxin titers throughout the 28-day study. Group C had much higher titers at 24 hrs when compared to Group B, which may have to do with gut absorption vs subcutaneous receipt of the antiserum product. The lambs in Group D had negative endotoxin titers (< 1:100) at 24 hrs, which probably contributed to the two lambs health problems.

Lamb D-526 was noticed ill at 2 days of age with lack of appetite and activity. The lamb's temperature was normal (102.4°F). The lamb was treated with antibiotic (penicillin) with a noticed improvement but became ill several days later. The lamb was thin and had a temperature (103.1°F), and central nervous symptoms (occasional seizure). The lamb died overnight. The carcass was submitted to SDSU ADRDL for diagnostic findings. The diagnostic report indicated a severe septicemia (*Klebsiella* sp.), probably due to the lamb being colostrum-deprived.

Lamb D-570 was noticed ill at 3 days of age with lack of appetite and activity. The lamb's temperature was slightly elevated (103°F) and it had difficulty walking and inflamed front joints. The lamb was treated with penicillin and banamine with no improvement. The attending veterinary prescribed tetracycline and continued banamine treatment. The lamb improved in appetite but continued to have a stiff gate throughout the 28-day study.

Even though lamb D-487 had a negative endotoxin titer at 24 hrs, the lamb remained healthy throughout the 28-day trial.

This study demonstrated that newborn lambs that received antibody supplement orally and subcutaneously remained just as healthy during the 28-day study, as newborn lambs that received 4 ounces of pooled ewe's colostrum. The use of the antibody supplement by subcutaneous delivery gave a much higher level of antibody response and should have further research completed to determine its use in lamb-production operations. Control lambs that did not receive any antibody supplement or colostrum, were not as healthy and came down with illnesses associated with the lack of passive immunity.

This sterile-irradiated-antibody supplement could be used as a management tool for newborn lambs less than 24 hours of age, when adequate ewe's colostrum is not available or for newborn lambs that have difficulty nursing. Further research in lamb-production operations should be conducted to confirm efficacy.

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