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Improved vaccination protocol to enhance immunity in lambs of Kuwait farms

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Abstract

The susceptibility of lambs to infective diseases should be minimized by improving the immunity of lambs through effective vaccination procedures to abate lamb mortality in sheep farms. In this regard, field experiments were carried out with 180 pregnant ewes of Naeemi sheep breed at the PAAFR (Public Authority for Agriculture and Fisheries Research Station, Kuwait). The results revealed that the concentration of serum Ig's in blood samples collected from ewes vaccinated during pregnancy with *Pasteurella*, *Clostridia*, FMD (Foot and Mouth Disease) and PPR (*Peste des Petits* Ruminants) vaccines was significantly elevated as vaccination against these four diseases agents would have ultimately resulted in circulating antibodies protecting the animals against natural exposure. The concentration of colostral Ig's produced by vaccinated ewes during the pregnancy period was significantly ($P<0.05$) higher than the normal colostrum secreted from unvaccinated ewes during pregnancy, ensuring the positive role of the colostrum in transferring passive immunity. The results clearly illustrated no cases of mortality and abortion, as vaccination has played a role in maintaining the health of ewes by overcoming the targeted pathogens and preventing any abnormalities in the pregnant ewes. The present study confirmed the improved immune status of young lambs born to ewes vaccinated twice during pregnancy, reflecting improved health status. It is recommended to maintain 21 days' intervals in vaccinating ewes with different vaccines for specific diseases.

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Introduction

The high rate of mortality of newborn lambs is a severe concern in the sheep industry of Kuwait, accounting for 25 to 35 percent, especially in the first three weeks of birth, causing significant economic losses for the sheep producers (Unpublished data, Public Authority of Agricultural Affairs and Fish Resources (PAAFR). Kuwait accounts for 588,618 heads of sheep, and 11-12 percent of the red meat need of the country is met by the sheep industry (1). Efforts to minimize lamb mortality could enhance the productivity of red meat by the Kuwaiti Sheep industry. Mortality of lambs could be due to a low level of immunity in the initial days of birth (2), along with infectious diseases like diarrhea (3) and pneumonia (4). Newborn lambs attain immunity by sufficient colostrum intake after birth. Lambs are borne lacking immunity, and the only source of Igs is to consume enough colostrum for dams (5,6). Colostrum is the earliest nutritious milk produced by the mammary glands during late pregnancy, secreted for few hours. The newborns should suckle a sufficient quantity of colostrum to enrich them with immunity and activate their immune system (7). Colostrum contains several antibodies that protect the newborn from infectious diseases. Lambs lacking these antibodies get infected even to minor diseases, eventually leading to death. Colostrum must be consumed within the first 12 to 18 hours of production as it will be ceased within 24 hours of birth; therefore, to get enough antibodies that enhance immunity, newborn lambs should consume sufficient colostrum within 24 hours of birth (8). The neonatal receptor FcRn is

responsible for absorption of colostrum in the intestinal cells through the endocytosis process by transport vacuoles (9), which is active in the initial 6 hours of birth and starts to decline in the next 6 hours and ends by 24 hours of birth (10). Therefore, lambs should consume a sufficient quantity of highly nutritious colostrum in the first six hours of birth as the ability of the intestine to absorb Igs decreases significantly after this period. Lamb serum shows a sudden upsurge in Igs concentration after colostrum consumption, enriching the immune system to Figureht against infectious pathogens (11). To be immune from infections, a healthy lamb suckles 100 to 200 ml of colostrum in a single feeding, about 600 ml in three feedings in the first 12 hours of birth. Colostrum should be collected and fed to the lambs through stomach tubes (12). Lambs that fail to take a sufficient amount of colostrum during the first three days of life are susceptible to infectious diseases leading to mortality.

Vaccination is an essential practice in sheep to overcome infections. Generally, sheep get vaccinated against *Clostridial* diseases such as enterotoxemia and tetanus, common among sheep. Enzootic pneumonia causes significant loss among housed sheep. Foot rot is another disease-causing profound effect and is difficult to control because of the extreme antigenic diversity of the causal agent. *Peste de petits* (PPR), or sheep plague, the highly contagious disease that can infect up to 90 percent of the herd, is another concern. In this background, a research gap is realized in the existing protocol in immunization of lambs, and an innovative vaccination protocol was proposed to enhance lamb immunity. Based on previous studies, KISR opted for a proposed vaccination protocol to be vaccinated twice during the delivery period with *Clostridia* sp., *Pasteurella* sp., FMD and PPR vaccines solely combin*ed Clostridia* sp., *Pasteurella* sp., and PPR vaccines. The present study aims to investigate the effect of the proposed vaccination protocol on the immunity of lambs by increased colostrum production, thereby reduced mortality.

Materials and methods

Field experiments were carried out with 180 pregnant ewes of Naeemi sheep breed at the PAAFR (Public Authority for Agriculture and Fisheries Research Station) Research Station, Kuwait. The selected 180 ewes were divided into six groups, each constituting 30 ewes. The ewes were identified and monitored for their performance with the aid of ear tags. Since the age of ewes could affect the mortality rate of newborn lambs, the ewes were selected carefully around 1-2 years of age with a live weight of 38±2.89 kg (13).

The housing of experimental animals

The experimental ewes were separated from the herd in separate enclosures sanitized to eliminate any possible infection from the previous sheep flocks. The sheds were cleaned thoroughly, bedding sand was changed, and water racks were cleaned.

Feeding and nutrition

The ewes and rams of the study were fed with the same ration of concentrates and roughage at 70:30, while the C: R used for lambs was 80:20. The feed ration of C: R (80:20) for lambs was reported to be the best during the pre-weaning, suckling, and post-weaning periods. The ration used for ewes with a C:R (70:30) was the best feeding regime to complete the production cycle in Kuwait's feedlot system irrespective of the season (14).

Care of lambs after lambing

Newborn lambs were maintained in a clean, dry room with a temperature of 40-60°C, and lambs were housed in individual pens (enclosure) with their dams after lambing. In addition, ewes were checked for mastitis, and excess wool was removed from around the udder. Dams usually nurse their lambs just after birth to ensure the receipt of a sufficient amount of colostrum.

Breeding of ewes

A straight breeding program of mating Naeemi rams with Naeemi ewes was done. The reproduction and mating procedures involved synchronizing the estrus induction of ewes earlier introduced at KISR for the Naeemi breed and were used in the present field experiment (15). Controlled breeding technology was used in the adult ewes to synchronize estrus by inserting the vaginal sponge chronogest for both ewes to improve conception rates. One ram was allowed to mate a flock of six to eight ewes by natural mating, and successful jumping and mating were marked.

Diagnosis of pregnancy

Diagnosis of pregnancy was carried out by ultrasound scanner in 42-50 days. Post successful mating of ewes that found not pregnant was rebred.

Lambing of ewes

The parturition process causes energy loss, and the amount of nutrients needed by the pregnant ewes depends mainly on the age, weight of the pregnant females, and the number of fetuses inside their uterus. Newborn lambs have very little fat in their bodies, making them highly susceptible to hypothermia. They were kept inside the enclosure during lambing, especially in the winter season.

Vaccination protocol

Ewes were vaccinated on the side of the neck, using a syringe with 18-gauge needles under the skin after cleaning with alcohol. The injection area was cleaned with alcohol, and the vaccines were administered. Ewes were vaccinated twice, first at the commencement of pregnancy, and the second booster dose was given four weeks before lambing as follows: T_1 - Ewes were vaccinated with *Pasteurella* vaccine (CZCZ Veterinaria S. A, Spain). T_2 - Ewes were vaccinated with *Clostridial* vaccine, which consists of seven species of

antigen, including *Cl. septicum; Cl. Perfringesns type A, C, and D; Cl. Sordellii and Cl. Novy, type B toxoids* (CZCZ Veterinaria S.A, Spain). T_3 - Ewes were vaccinated with foot and mouth disease (FMD) vaccine (CZCZ Veterinaria S.A, Spain). T_4 - Ewes were vaccinated with *the Peste des petits* ruminants (PPR) vaccine (CZCZ Veterinaria S.A, Spain). T_5 - Ewes were vaccinated with *Clostridial sp., Pasteurella sp*., and PPR vaccines. T_6 - Unvaccinated ewes during the pregnancy period.

Birth weight of lambs

Birth weight was recorded for all newborn lambs immediately after lambing and was expressed in kg (16).

Weaning weight of lambs

The weight of lambs taken when lambs reached weaning age at 60 days. Early weaning takes place when lambs are 60 days old. Lambs should reach about 20 kg at this time, while late weaning could occur when lambs are 120 days old. The weaning weight was taken before feeding in the morning, and body weight was expressed in kg (17).

Mortality rate of lambs

The number of sheep that lost their lives within 21 hours of birth. The following equation could calculate the mortality rate of lambs: [MR=100 - (Survival Rate)] (16).

Body condition score of ewes

BCS indicates the fatness and muscles that cover the sheep. It symbolizes the nutritional status of the animals and is rated from one to five, where one is considered emaciated (skinny), and five is considered very fat. The BCS value was mainly determined manually by feeling the degree of fat and muscles deposition over the vertebrae central spinal column and loin vertebrae. Ewes with BCS value 3.0-3.5 ensures successful feeding to newborn lambs and increases the possibility of their survival (17).

Chemical and biochemical analysis

Blood samples were collected before the morning feeding from the jugular vein in 10 ml vacutainer tubes (18). Blood samples were incubated at room temperature for 2 hrs and then centrifuged at 2,000 xg for 10 min at 4°C (Universal 32R, Hettich-Zentrifugen Tuttlingen, Germany) to separate, and the serum samples were stored at -20°C until analysis.

Collection of colostrum samples

Teats were grasped between the thumb and forefinger and were gently squeezed to force the milk downward within the teat canal to collect the colostrum samples. Colostrum was collected in clean plastic screw-top containers. Colostrum samples were stored in a freezer at -20°C until analysis by ELISA Kits.

Enzyme linked immunosorbent assay

The ELISA is a test that detects and measures antibodies in the blood. The test determines the antibodies related to certain infectious conditions. The concentration of Ig's in the colostrum and serum samples was determined ELISA kits (SunLong, China), with purified sheep Ig's, as standard references. Results were expressed as milligrams (mg) of Ig's per milliliter (ml) of serum or colostrum. Samples were individually analyzed in triplicate (19).

Serum neutralization test

The analysis was carried out according to the Office of International Epizootics (OIE), also known as Organization of Animal Health, the guidelines used in PAAFR laboratories. Antibody titters were expressed as the reciprocal of the final serum dilution (log 10) that neutralized 100$TCID_{50}$ of FMD viruses in 50 percent of the wells. The $TCID_{50}$ is the tissue culture infectious dose that will infect 50 percent if the cell monolayers are challenged with the defined inoculum of the virus. Tenfold serial dilutions of the virus were made in Hank's maintenance medium. The virus serum neutralization (SNSN) results were calculated by the method of 50 percent end virus neutralization dose according to formula and expressed as log SNSN index (SNI) (20).

Statistical analysis

All data descriptions and analyses were performed using the Statistical Analysis System (SAS software). Measures of central tendency (means and medians) and dispersion (standard deviation and means) among the five different vaccination groups concerning the concentration of Ig's in the serum and colostrum were computed using SAS. Other variables, including mortality rate of lambs (%), birth weight, weaning weight, and abortion rate of ewes (%), were also computed using SAS. Significant differences among the different groups were compared using the analysis of variance (ANOVA) statistical technique. Moreover, post hock tests to perform pair-wise comparisons between groups using the Duncan Multiple Range Test (DMRT) (21).

Results

Birth weight of lambs

The birth weight of lambs was enumerated in Table 1. The birth weight of lambs in ewes vaccinated with *Pasteurella* vaccine, *Clostridial* vaccine, which consists of seven species of antigen, FMD vaccine, PPR vaccine, and combined vaccine of *Clostridial sp., Pasteurella sp*., and PPR vaccines was 4.356 kg, 4.283 kg, 4.406 kg, 4.256 kg and 4.267 kg respectively. In contrast, the number of lambs born to ewes unvaccinated during the pregnancy was 4.22 kg. In the present experiment, the birthweight was without significant difference (P>0.05) in all the experimented treatments with more than 4 kg.

Weaning weight of lambs

The weaning weight of lambs (kg) of the experimental lambs are listed in Table 2. The weaning weight of lambs in ewes vaccinated with *Pasteurella* vaccine, *Clostridial* vaccine, which consists of seven species of antigen, FMD

vaccine, PPR vaccine, and combined vaccine of *Clostridial sp.*, *Pasteurella sp*., and PPR vaccines were 26.0, 25.83, 25.56, 25.83 and 25.44 kg respectively. In contrast, in control, the lambs born to ewes unvaccinated during pregnancy period was 25.0kg. There were no significant (P>0.05) differences among the treatments in the weaning weight of born lambs. The weaning weight was taken before feeding in the morning; lambs were weighed to record the growth average of lambs.

Table 1: Birth weight (Mean±SD) of newborn lambs

Treatments	Birth weight (kg)	Weaning Weight (kg)	Mean±SD (BCS)
T1	4.356±0.201	26.0±1.75	3.0
T2	4.283±0.204	25.83±1.89	3.5
T3	4.406±0.234	25.56±1.85	3.5
T4	4.256±0.162	25.83±1.098	3.5
T5	4.267±0.263	25.44±1.76	3.5
T6	4.22±0.861	25.0±1.01	3.0

T1: Ewes vaccinated with *Pasteurella* vaccine, T2: Ewes vaccinated with *Clostridial* vaccine, which consists of seven species of antigen, T3: Ewes vaccinated with FMD vaccine, Treatment 4: Ewes vaccinated with PPR vaccine, Treatment 5: Ewes vaccinated with *Clostridial sp.*, *Pasteurella sp*., and PPR vaccines Treatment 6: Ewes unvaccinated during pregnancy period.

Weaning weight of lambs

The weaning weight of lambs (kg) of the experimental lambs are listed in Table 2. The weaning weight of lambs in ewes vaccinated with *Pasteurella* vaccine, *Clostridial* vaccine, which consists of seven species of antigen, FMD vaccine, PPR vaccine, and combined vaccine of *Clostridial sp.*, *Pasteurella sp*., and PPR vaccines were 26.0, 25.83, 25.56, 25.83 and 25.44 kg respectively. In contrast, in control, the lambs born to ewes unvaccinated during pregnancy period was 25.0kg. There were no significant (P>0.05) differences among the treatments in the weaning weight of born lambs. The weaning weight was taken before feeding in the morning; lambs were weighed to record the growth average of lambs.

Body condition score of ewes

Ewes from the field experiment were chosen with BCS value 3.0 to 3.5 for lambs to get good nutrition from their dams during pregnancy and after lambing. This BCS was chosen because it gives a good indication of ewes' status, and the ewe would likely successfully feed their lambs, which would increase the possibility for the newborn lambs to survive, as shown in Table 1. The chosen BCS value for ewes is appropriate to meet the objective of the experiment.

Lambing rate of ewes

Lambing rate of ewes in treatments 1 to 5 was 100%, symbolizing each ewe managed to have one lamb, which is a typical average.

Management

Prevention of mis-mothering by moving lambed ewes and their lambs to individual pens improves the survival rate of lambs. Lambs born in winter are more likely to survive as the weather in Kuwait is very hot and dry. The factors mentioned above were maintained in the field experiment that could be the reason for the successful lambing rate of ewes.

Abortion rate of ewes

The abortion rate of ewes in the experimented treatments 1 to 5 was zero, which could be interpreted as there was no infection of ewes during the pregnancy period.

Comparison of *Pasteurella* vaccine during ewe pregnancy

The mean titter for the immune response of ewes from treatment 1 (*Pasteurella* vaccine), 5 (proposed combined vaccine), and 6 (Control), before and after immunization with *Pasteurella* vaccine is listed in table 1. Animals vaccinated with the proposed combined vaccine reported around two times the titter, 60.12±0.24; after vaccination as compared to before vaccination, 36.56±0.12 and dropped slightly four weeks after lambing, 55.78±0.94. This shows a positive response in elevated production of quality colostrum, which potentially overcomes disease infection. The animals vaccinated with *Pasteurella* vaccine alone in T1 had also reported twice the titter, 52.96±0.1 compared with before vaccinated, 25.28±0.56 and unvaccinated ewes, 25.00±0.33, implying good response to exposure and potential for producing good quality colostrum. The mean titters after lambing dropped, 48.06±0.38, and the comparison between before vaccination and after lambing showed a statistically significant difference between the levels of Ig's although the level of circulating antibodies dropped significantly at lambing, it started to elevate again after 3-4 weeks (after lambing). The comparison of the immune response in treatments 1 and 5 displayed a similar pattern in the level of responses, and there was no significant difference between the two treatments (Table 2). The decline of antibodies titter in serum of ewes at lambing could be due to the transfer of immunoglobulin to the colostrum three weeks before lambing.

The antibody titter of lambs' serum for both lambs born to vaccinated ewes during pregnancy in T1 with *Pasteurella* vaccine alone, T5 with combined proposed vaccine and the unvaccinated (T6) ewes were enumerated in Table 2. The results revealed that the concentration of lambs' Ig's born to unvaccinated ewes was significantly (P<0.05) lower than the serum Ig's of lambs born to vaccinated ewes. The concentration of Ig's reached 19.2±0.153 mg/ml for lambs born to unvaccinated ewes. The antibody titter of lamb's serum born to vaccinated ewes was significantly (P<0.05) higher than the antibody titter of lamb's serum born to unvaccinated and reaches 39.29±0.23 mg/ml and 42.17±0.03 mg/ml, in T1 and T5 respectively, which implies that both

vaccinations approach elucidated almost similar levels of response, with the combined vaccine responding higher.

Comparison of *Clostridia* vaccine during ewe pregnancy

Ewes in treatments 2 and 5 were vaccinated with *Clostridia* vaccine, twice during pregnancy as sole *Clostridia* vaccine and combined vaccine. Figure 1 shows the concentration of serum Ig's of ewes in T2 for vaccinated and unvaccinated animals (T6) during the enrolment period. The X-axis shows the weeks from enrolment, zero indicates the parturition date, and 2-6 weeks indicates the post-parturition period. The ordinate shows the levels of the antibodies in milligrams (mg) per millimetre (ml). The results revealed that the levels of Ig's in vaccinated ewes during the pregnancy period increased significantly ($P<0.05$)), and then declined at lambing (week zero), and increased again at 2-6 weeks after lambing. The antibody titter was increased significantly ($P<0.05$) up to 35.1 mg/ml from 23.1 mg/ml (after the first vaccination) and reached the highest titter of 50.1 mg/ml after the second vaccination. At lambing, the concentration of Ig's decreased and reached 32.1 mg/ml (week zero) for vaccinated ewes and 15.3 mg/ml for unvaccinated ewes (T6). The concentration of Ig's was measured for six weeks after parturition. The Ig's levels decreased at week zero but gradually increased post-parturition again until 46.2 mg/ml at week six after parturition (Figure 1).

Table 2: The immune response of ewes and lambs with *Pasteurella* vaccine

Treatments		IgG mg/ml (Mean±SDSD)
T1	Ewes before vaccination	25.28±0.56
	Ewes after vaccination during pregnancy	52.96±0.13
	Ewes 4 weeks after lambing	48.06±0.38
	Lambs born to vaccinated ewes (3 days of age)	39.29±0.23
T5	Ewes before vaccination	36.56±0.12
	Ewes after vaccination during pregnancy	60.12±0.24
	Ewes 4 weeks after lambing	55.78±0.94
	Lambs born to vaccinated ewes (3 days of age)	42.17±0.03
T6	Unvaccinated Ewes	25.00±0.33
	Lambs born to unvaccinated ewes (3 days of age)	19.2±0.153

T1: Ewes vaccinated with *Pasteurella* vaccine, T5: Ewes vaccinated with *Clostridial sp.*, *Pasteurella sp*., and PPR vaccines, T6: Ewes unvaccinated during pregnancy period.

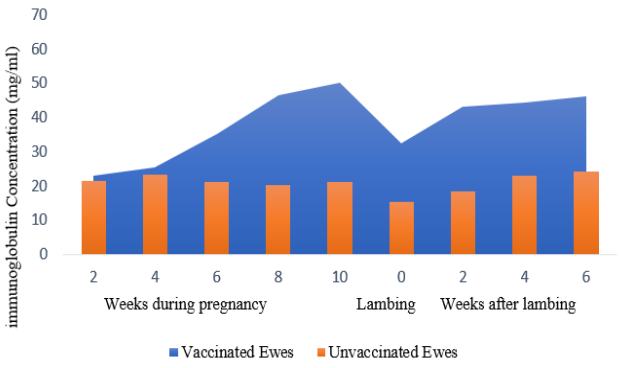

Figure 1: Comparison of concentration of Serum Ig's of Ewes after *Clostridia* Vaccination (T2) and Unvaccinated Ewes (T6).

Figure 2 portrayed the comparative antibody titters for ewes during pregnancy and after lambing in T2 and T6. Vaccinating ewes during the pregnancy period was found to increase the levels of Ig's significantly ($P<0.05$), but the levels of antibodies were declined at lambing (week zero). The antibody levels increased after the first vaccination and reached 36.4 mg/ml, while 55.2 mg/ml after the second. At lambing, the levels of Ig's decreased and reached 38.3 mg/ml for vaccinated ewes and 15.3 mg/ml for unvaccinated ewes. The levels of Ig's were measured for six weeks after parturition. Figure 2 showed that the Ig's level was increased again after lambing and reach 54.8 mg/ml for vaccinated ewes and 20.4 mg/ml for unvaccinated ewes. It was also observed from the present experiments that none of the ewes in treatments 2 and 5 demonstrated clinical signs of the disease.

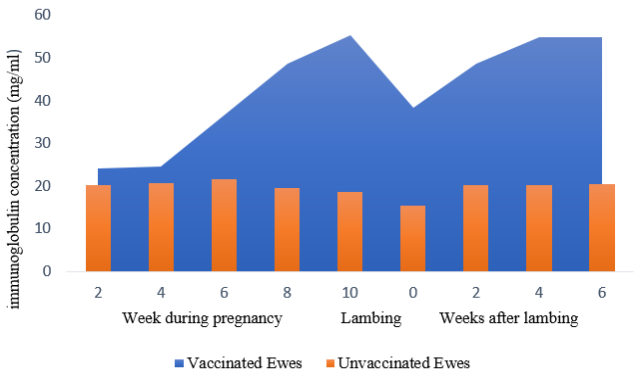

Figure 2: Comparison of serum Ig's concentration of ewes after *Clostridia* vaccination (T2) and unvaccinated ewes (T6).

Comparison of foot and mouth disease (FMD) vaccine during ewe pregnancy

The immune response of unvaccinated (T6) and vaccinated ewes with FMD vaccine (T3) during pregnancy and after lambing are shown in Figure 3. The results revealed that the maternal antibody level with serum neutralizing indices ranged from 0.2 to 0.4 before the vaccination of ewes. As shown in Figure 14, immunity of ewes was elevated significantly after the first vaccination (at week -16 before lambing), the levels of neutralizing antibody response of ewes were 1.8 (at week -14 before lambing), and continued to increase until it reached 3.8 (at week ten before lambing). Ewes were vaccinated again at week six before lambing. After the second vaccination, serum neutralization value was elevated until it reached five (at week six before lambing). At lambing, the levels of Ig's were declined significantly (P<0.05) until it reached two, the serum neutralizing values increased again and reached 3.9, 4.6, and 4 at weeks 30, 60, and 90 post-lambing, respectively. Only one ewe from a vaccinated group showed moderate lesions on the tongue, and no secondary lesions were seen in this group. On the other hand, unvaccinated ewes during pregnancy showed primary and secondary lesions. Thus, it is evident from the study that vaccinating ewes twice helps lower the incidence of severe symptoms of the disease, whereas unvaccinated ewes in the control group showed moderate symptoms, which influenced poor Serum Neutralization Index values ranging from 0.2 to 0.4 at the time of testing.

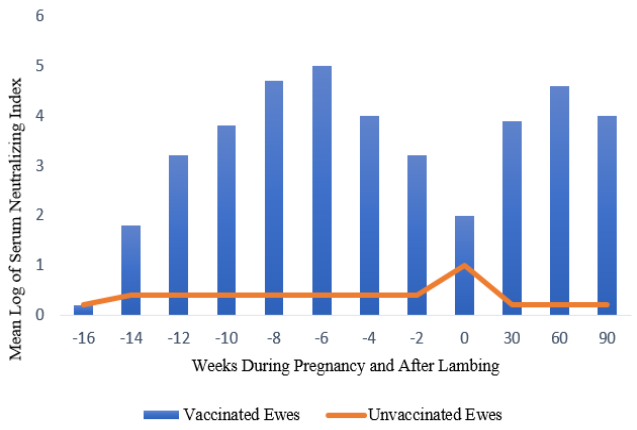

Figure 3: Comparative Immune Response of FMD vaccinated ewes (T3) and unvaccinated (T6) ewes during pregnancy and after lambing.

The Ig's levels/index of lambs born to unvaccinated (T6) and vaccinated ewes (T3) are shown in Figurer 4. The serum neutralizing index values for newborn lambs immediately after lambing were 0.2 for lambs born to unvaccinated ewes and 1.8 for vaccinated ewes. Vaccinating ewes during pregnancy effectively enhanced the immunity of new borne lambs after lambing and suckling colostrum from their dams. Blood samples were collected from young lambs until day 80 of age.

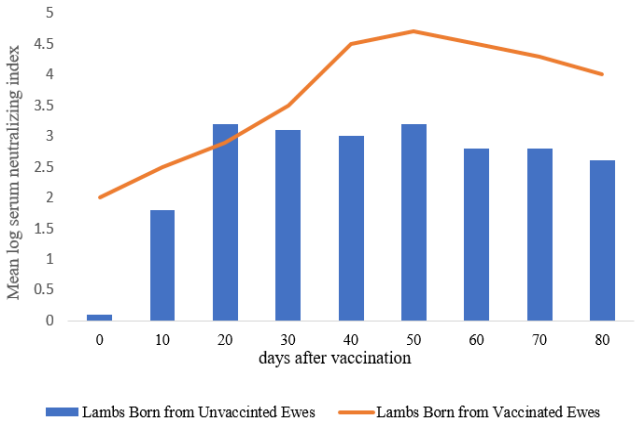

Figure 4. Comparative Immune Response of lambs borne FMD vaccinated ewes (T3) and unvaccinated (T6) ewes.

Comparison of PPR vaccine during ewe pregnancy

The immune response of vaccinated ewes with PPR vaccine (T4), combined KISR vaccine (T5), and unvaccinated (T6) ewes during pregnancy and after lambing are shown in table 3. The results indicated no Ig's detected in blood samples collected from unvaccinated ewes (T6) and ewes before vaccination in both treatments, T4 and T5. After the first vaccination, the antibody titter level in ewes' serum was significantly increased (P<0.05) and reached 39.32±0.61 mg/ml and 39.39±0.6 mg/ml, respectively. Antibody titres, continued to increase after the 2nd vaccination, the increase of Ig's level was significantly (P<0.05) higher than the Ig's level of ewes' serum after the 1st vaccination, and reached 52.91±0.22 mg/ml and 52.54±0.99 mg/ml, respectively. At lambing, there was a significant (P<.05) decline in the mean value of the concentration of Ig's in vaccinated ewes after lambing and reached 14.32±0.06 mg/ml and 13.63±0.09 mg/ml, respectively. The concentration of Ig's starts to increase again after lambing and reached 45.45±0.23 mg/ml and 44.70±0.01 mg/ml, respectively. It was concluded from this experiment that the levels of PPR-specific serum antibody titter in ewes after vaccination could help in increasing their passive immunity transferred to newborn lambs and hopefully protect against the development of the disease.

The concentration of PPR-specific serum antibodies of lambs born to unvaccinated and vaccinated ewes in both treatments was enumerated in Table 4. The results revealed no Ig's detected in blood samples collected from lambs born to vaccinated ewes. Antibody titter for blood samples collected from lambs borne to vaccinated ewes was significantly (P<0.05) higher than the antibody concentration in lamb's serum born to unvaccinated ewes. The level of PPR-specific serum antibody reaches 36.79±0.35 mg/ml and 44.43±0.44 mg/ml in both groups

after the vaccination, respectively. It was concluded from this experiment that the levels of PPR-specific serum antibody titter in lambs born to vaccinated ewes during pregnancy with PPR could help in improving their immune status after suckling hyperimmune colostrum secreted by their dams. Vaccinating ewes during the pregnancy helps increase the passive immunity transferred to the newborn and hopefully protect against the development of the disease.

Table 3: Immune response of ewes and lambs before and after vaccination with ppr vaccine and after lambing

Treatments		IgG mg/ml (Mean±SD)
T4	Ewes before vaccination	0
	Ewes after 1st vaccination	39.32±0.61
	Ewes after 2nd vaccination	52.91±0.22
	Ewes at lambing	14.32±0.06
	Ewes after lambing	45.45±0.23
	Lambs born to vaccinated ewes	36.79±0.35
T5	Ewes before vaccination	0
	Ewes after 1st vaccination	39.39±0.60
	Ewes after 2nd vaccination	52.54±0.99
	Ewes at lambing	13.63±0.09
	Ewes after lambing	44.70±0.01
	Lambs born to vaccinated ewes	44.43±0.44
T6	Ewes	0
	Lambs	0

T4: Ewes and lambs were vaccinated with PPR vaccine, T5: Ewes and lambs were vaccinated with *Clostridial sp., Pasteurella sp*., and PPR vaccines. T6: Ewes and lambs were unvaccinated during the pregnancy period.

Ig's concentration of colostrum

The concentrations of Ig's in colostrum samples treatment was highest in ewes vaccinated with *Pasteurella* vaccine, 65.96±0.711 mg/ml, followed by ewes vaccinated with *Cloristidial* vaccine, 64.49±0.065 mg/ml, Combined vaccines of *Clostridial* sp*., Pasteurella sp*., and PPR (63.88±0.087 mg/ml), Peste des petits ruminants vaccine (PPR), 63.22±0.047 mg/ml, and Foot and Mouth Disease (FMD) vaccine, 63.16±0.078 mg/ml, while the control groups without vaccination recorded poor Igs concentration in colostrum, 44.3±4.33 mg/ml. Vaccinating ewes during the pregnancy period with any of the vaccines appeared to increase significantly ($P<0.05$) the levels of Ig's in the colostrum, as compared to the Ig's levels of colostrum samples collected from unvaccinated ewes during the pregnancy period, 44.21±4.33 mg/ml. These results portrayed that enhancing the immunity of ewes by vaccinating them twice during the pregnancy period would help elevate the levels of Ig's in their blood and consequently in the colostrum, which could have a positive effect on increasing the resistance of newborn lambs to infection with this pathogen (Table 4).

Table 4: Concentrations of Ig's in colostrum's samples

Treatments	IgG mg/ml (Mean±SD)
T1	65.96±0.711
T2	64.49±0.065
T3	63.16±0.078
T4	63.22±0.047
T5	63.88±0.087
T6	44.3±4.33

T1: Ewes vaccinated with *Pasteurella* vaccine, T2: Ewes vaccinated with Clostridial vaccine, which consists of seven species of antigen, T3: Ewes vaccinated with FMD vaccine, T4: Ewes vaccinated with PPR vaccine, T5: Ewes vaccinated with *Clostridial sp., Pasteurella sp*., and PPR vaccines. T6: Ewes unvaccinated during pregnancy period.

Discussion

The diverse variables measured in lambs displayed a positive response. Birth weight was recorded as it is one of the essential indicators affecting the survival of newborn lambs. It could indicate the mother's quality and amount of nutrition to their lambs during pregnancy and after lambing. Lambs borne with birth weight less than 3.0 kg were considered weak, and they are difficult to survive, while lambs with a birth weight of 3.5 kg give a good indication that lambs had an adequate nutritional level from their dams and likely to survive, and lambs with birth weight more than 3.5 kg, considered strong lambs with high chances of survival. Recording birth weight is vital as it correlates with animal survival, growth, and performance (22). The awareness of animal weight is a prerequisite for determining the dosage amount of medicine and the amount of feed to the animal.

Furthermore, animal weight is highly prone to individual variation according to the amount of feed and water present in the gut. To minimize variation by measuring weight at a constant time of day and best done in the morning before feeding. The results showed that the amount and quality of feed given to the lambs were sufficient (18).

The Body Condition Score (BCS) is yet another criterion chosen as it gives a good indication of ewes' status, and the ewe would likely successfully feed their lambs, which would increase the possibility for the newborn lambs to survive. BCS value equal to or higher than 4, ewes could have a significant risk of dystocia (that is, the lamb getting stuck during birth). Thus the chosen ewes with BCS = 3.0-3.5 would have a low possibility to have dystocia. BCS values = 2.0-2.5 are called skinny sheep. This score indicated that ewes could have a slight problem feeding their lambs. These skinny sheep suffer from underfeeding or are infected with a worm disease. The BCS could show clearly the condition of muscling and fat development. The BCS value was determined by manually feeling the fat and muscle deposition degree over the vertebrae, central spinal column,

and loin vertebrae. The chosen BCS value for ewes is appropriate to meet the objective of the experiment.

The lambing rate of ewes in treatments 1 to 5 was 100%, symbolizing that each ewe had one lamb, a typical average. The positive lambing rate is due to vaccination during the pregnancy period that impacted the immune status of ewes, strong enough to face any infection of common diseases found in the surrounding environment. Improving the immune status of ewes during pregnancy could help them decrease the percentages of abortion and have a high lambing rate. In general, the lambing rate in sheep farms is affected by Hygenic facilities; Sanitary, hygienic, and well-managed facilities could have a high lambing rate and reduced abortion rate. In addition, ewes fed with a balanced diet, with selenium (Se) and vitamin E before pregnancy increases lambing rate.

Prevention of mis-mothering by moving lambed ewes and their lambs to individual pens soon after lambing improves the survival rate of lambs. The abortion rate of ewes in the experimented treatments 1 to 5 was zero. The reason is that there was no infection of ewes during the pregnancy period-with usual pathogens causing abortion such as; *Campylobacter fetus subsp., fetus fibrosis*, *Campylobacter jejuni, Chlamydophila abortus* (enzootic abortion of ewes), *Lister monocytogenes (listeriosis*), *Salmonella abortus- ovis Brucella melitensis, Toxoplasma gondii*, Bluetongue and border disease (23). The vaccination protocols helped the ewes ensure adequate antibodies to pathogens of concern and thereby avoided abortions.

Comparison of *Pasteurella* vaccine during ewe pregnancy

Ewes vaccinated with the proposed combined vaccine as well as *Pasteurella* vaccine alone has recorded double the titter after vaccination, which slightly dropped four weeks after lambing, which positively contributed to elevating production of quality colostrum, thereby overcome disease infection. The levels of Ig's of circulating antibodies dropped significantly at lambing and started to elevate again after 3-4 weeks after lambing. The immune response of lambs born to vaccinated ewes at three days of age was elevated as the lambs received colostrum from the pregnant vaccinated ewes, which implies that the Ig's produced in ewes due to vaccination was transferred efficiently from dams to their lambs. The elevation in the Ig's in the secreted colostrum was reflected in the Ig level in lambs' serum, which was adequate to protect them, as it helped protect newborn lambs from infection (24). The current results portrayed that the secreted antibodies were protective to newborn lambs as none of the lambs exhibited any symptoms of pneumonia infection, which means the isotype of antibodies secreted was specified to the *Pasteurella* sp., which is the right isotype for *Pasteurella* sp. found. Studies also suggest that the cellular immune mechanisms could play a chief role in protecting animals against *P. haemolytica* infection (24). More investigations are required to understand the specific role of neutrophil cytotoxin produced by *P. haemolytica* (25).

Comparison of *Clostridia* vaccine during ewe pregnancy

The levels of Ig's in ewes vaccinated with proposed combined vaccine as well as *Clostridia* vaccine during pregnancy period increased significantly and then declined at lambing (week zero), and increased again at 2-6 weeks after lambing. The antibody titter was increased significantly after the first vaccination and reached the highest titter after the second vaccination, and at lambing, the concentration of Ig's decreased and increased again after three weeks. Moreover, ewes vaccinated with the proposed combined vaccine and *Clostridia* vaccine have not demonstrated any clinical sign of the disease. In addition, even the unvaccinated ewes did not show any subclinical symptoms. A speculative explanation could be that ewes in the control group develop immunity in their system due to previously subclinical infection, which could help develop enough immunity against *Clostridia sp.* (26).

Comparison of FMD vaccine during ewe pregnancy

Immunity of ewes was elevated significantly after the first vaccination (at week -16 before lambing), the levels of neutralizing antibody response of ewes were 1.8 (at week -14 before lambing), and continue to increase until it reaches 3.8 (at week ten before lambing). Ewes were vaccinated again at week six before lambing. After the second vaccination, serum neutralization value was elevated until it reached five (at week six before lambing). Thus, it is evident from the study that vaccinating ewes twice helps lower the incidence of severe symptoms of the disease, whereas unvaccinated ewes in the control group showed moderate symptoms, which influenced poor Serum Neutralization Index values ranging from 0.2 to 0.4 at the time of testing.
The Ig's levels/index of lambs born to unvaccinated (T6) and vaccinated ewes (T3). The serum neutralizing index values for newborn lambs immediately after lambing were 0.2 for lambs born to unvaccinated ewes and 1.8 for vaccinated ewes. Vaccinating ewes during pregnancy was very effective in enhancing the immunity of newborn lambs after lambing and suckling colostrum from their dams. Blood samples were collected from young lambs until day 80 of age. The results indicated that the level of Ig's was high until 80 days of age for lambs born to vaccinated ewes. Thus it is a very effective technique to protect newborn lambs. The experimental results agree with several previous studies (27) that passive immunity from dams persists in lambs up to 2 to 6 months of age. The present experiment showed that the elevation of Ig's in lamb's serum was high until 80 days of age (2.5 months of age).

Comparison of PPR vaccine during ewe pregnancy

There were no symptoms of Ig's detected in blood samples collected from lambs' born to PPR vaccinated ewes. Antibody titter for blood samples in vaccinated ewes was significantly higher than unvaccinated ewes. It was concluded that the PPR-specific serum antibody titter in ewes after vaccination could help in increasing their passive

immunity transferred to newborn lambs and hopefully protect against the development of the disease. The secreted antibodies could help improve the immunity of newborn lambs, which helps in reducing their mortality rate (28).

Ig's Concentration of Colostrum

Having high levels of Ig's in colostrum would help in improving the passive transfer of antibodies of lambs deeding on colostrum. As a result, lambs could have enough resistance to infection, more specifically in the first three weeks of their lives, until their immune system becomes fully activated; It is essential to enhance the colostrum quality of colostrum in terms of antibody levels. To increase passive transfer and hopefully the survival rate of new lamb, as colostrum is the only source of nutrition and antibodies (29).

According to the observations from the current study, it can be suggested that lambs could be vaccinated from the age of three months onward. The results from the present study showed that high maternal Ig's in lambs born to vaccinated ewes could affect the immune response in these lambs after vaccination. The study also demonstrated that vaccinated ewes have a significant rise in antibodies titter, which helped in the secretion of hyperimmune colostrum that could help in improving the transfer of passive immunity to young lambs. It was also previously reported that the dose size used in the vaccination could also affect the immune response of ewes (30). From the present study, it can be speculated that colostral antibodies could interfere with the activation of immune response in newborn lambs, but to a certain extent, yet not block it completely. In addition, this interference could be only limited to the response to primary vaccination. At 21 days of first vaccination, vaccinating the lambs resulted in a typical immune response (data not shown in the report). These findings suggest that vaccinating ewes twice during pregnancy could effectively improve the transfer of passive immunity to young lambs, and vaccinating young lambs twice could be at 21 days intervals (31). In the present field experiment, which was carried on a single farm, the team used Naeemi sheep. Naeemi sheep are well adapted to the Kuwaiti environment. Thus, ewes usually respond to the different vaccinations used in the present study. Several factors affect the response to vaccination against the targeted potential diseases. Stress is one of the crucial factors that could affect the efficiency of immune response by the animals after vaccination; some imported animals were found not responsive in the same way to vaccination as others. This could be that these animals suffer from adaptability stress (data not published) that suppresses their immune system. Furthermore, vaccination of ewes during the pregnancy helped produce high-quality colostrum, consequently reducing the mortality rate of newborn lambs by transferring protective antibodies. Therefore, in such a scenario, it is essential to vaccinate young lamb at age four to six months to ensure the production of antibodies and protection of offspring (32).

Conclusion

Vaccinating ewes during pregnancy with the vaccines such as *Pasteurella*, *Clostridia*, FMD, and PPR, was very effective in protecting newborn lambs from the infection of different diseases. In the present study, none of the lambs exhibited any symptoms of these diseases. There was no mortality between lambs borne to vaccinated ewes, and there were no symptoms of infection by any pathogen between newborn lambs, which symbolizes that the isotype of antibodies secreted as a response to this vaccination is the right isotypes for these diseases. Thus lambs were protected from infection. Vaccinating ewes during pregnancy aided the abundant production of an adequate number of antibodies to protect ewes during pregnancy and lambs after lambing. The elevation of serum Ig's in lambs after suckling confirmed that the antibody gained by lambs is from the colostrum, which proves that Ig's were transferred efficiently from dams to their lambs. Vaccinating the ewes twice during pregnancy is very effective in improving the immune status of young lambs, and vaccinating ewes with different vaccines could be recommended at 21 days intervals.

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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بروتوكول التحصين المحسن لتعزيز المناعة في الحملان في مزارع الكويت

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الخلاصة

يجب التقليل من تعرض الحملان الصغيرة للأمراض المعدية وذلك عن طريق تحسين مناعة هذه الحملان من خلال إجراءات التطعيم الفعالة للحد من نفوقها في مزارع الأغنام. وفي هذا الصدد، أجريت تجارب حقلية على ١٨٠ نعجة حامل من سلالة النعيمي التابعة للهيئة العامة لشئون الزراعة والثروة السمكية. أظهرت النتائج أن تراكيز الأجسام المضادة في عينات الدم التي تم جمعها من النعاج والتي تم تحصينها أثناء الحمل بلقاحات الباستيوريلا والكلوستريديا والحمى القلاعية ومرض طاعون المجترات الصغيرة قد أدى إلى ارتفاعها بشكل ملحوظ. كما أوضحت النتائج أن تراكيز الأجسام المضادة في الحليب الأول الذي تنتجه النعاج المحصنة خلال فترة الحمل قد ارتفع بشكل ملحوظ (أ<٠,٠٥) من تراكيز الأجسام المضادة في الحليب الأول الذي يفرز من النعاج الغير محصنة، مما يثبت الدور الإيجابي للحليب الأول في نقل المناعة السلبية. ولقد أظهرت النتائج أيضا وبوضوح عدم وجود أي حالة وفاة أو إجهاض بين الأغنام حديثة الولادة، حيث لعب التطعيم دوراً فعالا في الحفاظ على صحة النعاج من خلال التغلب على مسببات الأمراض المستهدفة ومنع حدوث أي تشوهات في النعاج الحامل. أكدت الدراسة الحالية تحسن الحالة المناعية للحملان الصغيرة التي ولدت لنعاج تم تطعيمها مرتين أثناء الحمل، مما يعكس تحسن الحالة الصحية. كما توصي الدراسة بالاحتفاظ بفترات ٢١ يومًا لتحصين النعاج باللقاحات المختلفة.