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EFFECTS OF IMMUNIZING PREGNANT EWES AND DOES ON THE HUMORAL IMMUNE RESPONSE OF SECRETED COLOSTRUM

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ABSTRACT

To produce hyperimmune colostrum of ewes and does by vaccinating them during their pregnancy and to examine the performance of their lambs/kids receiving the colostrum from the dams. Animals were divided as those under control (T-1) without vaccination and those under 3 treatments of vaccinated flocks (T2-T4). The vaccines used in this study were; *Clostridia sp* (CZ Veterinaria S.A Spain), *Pasteurella sp*. (CZ Veterinaria S.A Spain), and *Sheeppox* (Biosciences, Jordan). Study variables were concentration of immunoglobulins (Igs) in both serum and colostrum of ewes and does as well as quantifying the IgG classes in the serum of offspring of both species. Body condition scores (BCS) and live weight changes of both species were also examined. Effects of vaccination of pregnant ewes and does were clearly indicated by doubling of serum Igs in vaccinated dams compared to control. Likewise, the colostrum of the dams of both species considered as hyperimmune contained (20.89±1.22 mg/ml) in T-1 (control without vaccination), and with vaccination T-2 (*Clostridia sp.,* 43.45±0.06 mg/ml), T-3 (*Pasteurella sp.*, 46.22±0.150 mg/ml) and T-4 (*Sheep pox* 47.13±0.11mg/ml); the corresponding values for does were T-1 19.25±0.22 mg/ml, T-2 39.25±0.5 mg/ml, T-3 40.5±1.3 mg/ml, and T-4 45.2± 2.3 mg/ml, respectively. Hyperimmune colostrum intake by the offspring had a significant positive impacts on the concentration of total of immunoglobulins-gamma (IgG) and Ig classes (IgG, IgM, IgA) in the blood sera. Most important positive effects were in increasing IgG from control (22.0±2.0 mg/ml) to T-2 (33.0±0.32 mg/ml), T-3 (35.0±1.20 mg/ml), and T-4 (32.0±0.87 mg/ml), respectively in lambs; the corresponding values for kids for T-1, T-2, T-3, were T-1 (19.12±0.91 mg/ml), T-2 (36.0±0.39 mg/ml), T-3 (33.0±0.56) and T-4 (35.0±0.21 mg/ml), respectively. Our studies concentrated initially in challenging the ewes/does with any individual vaccines of *Clostridia sp*., *Pasteurella sp.,* and *Sheep pox*. Morbidity and mortality rates during the early 7 days of age of offspring were almost none in vaccinated flocks compared to those without vaccination. The benefits of pregnant small ruminant vaccination were clearly demonstrated in our studies. However, the effects of combined vaccine use for pregnant dams are yet to be investigated (underway) on offspring until weaning.

Key words: Hyperimmune colostrum, immunoglobulin, vaccination, mortality, lambs, kids.

INTRODUCTION

Colostrum is secreted by the mammary gland, and contains immunoglobulins, enzymes, hormones, nutrients, in addition to unidentified growth factors. It is the only source of Igs for newborn lambs/kids survivals. Lambs/kids during their first 18-36 hours of age require about 180-290 ml of colostrum/kg of live weight (Nowak and Poindron (2006). Igs cannot pass through the placental barrier to fetus due to their large size, therefore, feeding colostrum to newborn lamb/kids is vital for neonatal's immunity Ocak' *et al.* (2005). After suckling, the concentration of Igs of serum of offspring increased rapidly during the first few hours with peak level at about 24 hours after birth Cortese (2009); Castro *et al.* (2011). Igs are known to be synthesized by plasma cells of the mammary gland epithelium during the last 5 weeks of pregnancy. The concentration of Igs in colostrum will be elevated due to the pinocytosis process, and then Igs will be moved from the blood to the colostrum Guedes *et al.* (2010). The main reason of this action is the elevation of estrogen level during gestation, which will cause specific receptors in the mammary gland epithelium to bind to immunoglobulin-G and take it by the cell through transcapillary change Guedes *et al.* (2010). During this process IgG will be moved through the lumen of the mammary gland to colostrum. Small ruminants in hot arid zone, especially Kuwait, are raised in the intensive system (zero-grazing) due to desertification. The mortality rate of lambs/kids are high and could be as high as 30-40% when exposed to arid zone in confined housing. In such management, dams are not challenged by the prevalent diseases, therefore, their colostrum was found to be low in immune bodies Burezq *et al.* (2016). The present study addresses this issue of immunizing dams and producing hyperimmune colostrum for their offspring.

MATERIALS AND METHODS

Animals. A total of 24 first parity ewes and does were randomly distributed to four flocks as a control (T1) without vaccination during pregnancy, and treatment flocks having flocks of ewes and does were vaccinated; (T2), (T3), and (T4) were vaccinated against *Clostridia sp., Pasteuralla sp.,* and *sheep pox*, respectively, twice at the beginning of their pregnancy and 4 weeks before lambing/kidding. Both flocks, Naeemi fat-tailed (Awassi) ewes and the Shami does, were of 1-2 years old. One mature ram and one buck were used for mating the dams. All animals were housed at Kuwait Institute for Scientific Research (KISR's) Research and Innovation Center (KRIC).

Housing in Insensitive System: A total of 50 animals (24 ewes, 24 does, a ram, and a buck), were housed in a closed steel shed of size 60 m X 20 m the space allocated for each animal 3 m^2 with access to outside fence of the total 900 m^2. Six ewes/does were grouped in fenced pens having feeding managers and water troughs.

Feeding and Management: The feed consisted of concentrates, roughages, vitamins, and minerals as shown in Table 1. Rams were provided with 1.0 kg/head/day mixed feed. Two weeks before joining ewes and does, the amount of feed was increased to 1.25 kg. Pregnant ewes and does were provided with 70% concentrate and 30% roughages (Table 1). Young lambs after weaning were provided with 80% concentrate and 20% roughages. Dry ewes were offered 0.8 kg/head/day of the same feed given to the rams. This amount was gradually increased to 1.25 kg/head/day during the last month of pregnancy, according to the National Research Council (NRC, 2007) recommendations, and KISR's feedlot feeding and nutritional studies (Razzaque, 1995).

Table 1. Ration Ingredients (%) of DM / Chemical Composition (%) of Dry Matter (kg/100 kg DM).

Composition	DM %	CP %	EE %	Ash %	ADF %	NDF %
Corn	10.0	1.401	0.189	0.5398	0.501	2.0
Barely	40.5	5.674	0.756	2.186	2.029	8.0
Wheat Bran	10.0	1.401	0.189	0.5398	0.501	2.0
Soya Bean	6.5	0.911	0.123	0.351	0.326	1.3
Premix	1.0	-	-	-	-	-
Limestone	1.0	-	-	-	-	-
Salts	1.0	-	-	-	-	-
Alfalfa Hay	15.0	2.102	0.284	0.809	0.752	3.0
Straw	15.0	2.102	0.284	0.809	0.752	3.0
Total	**100%**	**14.01**	**1.89**	**5.398**	**5.01**	**20.0**

DM: Dry Matter; **CP:** Crude Protein; **EE:** Ether Extract; **ADF:** Acid Detergent Fiber; **NDF:** Neutral Detergent Fiber.

Mating and Pregnancy Diagnosis: A straight breeding program of mating Naeemi ewes X Naeemi rams, and Shami does X Shami bucks was used. The reproduction and mating procedures involved synchronizing the estrus induction of ewes/does earlier introduced at KISR Razzaque *et al*. (1995). The diagnosis of pregnancy was carried out by ultrasound scanner (Rheintechnic, Germany), during the 42-50 day of pregnancy.

Vaccination: Pregnant ewes/does were divided randomly to four treatment groups. These groups were without vaccination control group (T1) and vaccinated groups (T2, T3 and T4). Blood samples were collected from dams before vaccination and 24-48 hrs after lambing/kidding. Serum samples were collected and stored at -20ºC for immunological analysis. Treatment of groups of pregnant ewes/does, were vaccinated against *Clostridia sp., Pasteurella sp.,* and *Sheep pox*. For T2, two ml of an inactivated *Clostridia* vaccine containing *Cl. septicum, Cl. perfringens* Types A, C and D, *Cl. sordellii, Cl. novyi* Type B toxoids, together with whole culture of *Cl. chauvoei,* formaldehyde inactivated and adsorbed onto Aluminium hydroxide gel (1.4 mg Al/ml) was used. For T3, two ml of inactivated cultures of *Pasteurella multocida sp.,* and *Pasteurella haemolytica sp.,* adsorbed onto Aluminium Hydroxide Gel was used. Each dose of 1.5 ml of *sheep pox*/*goat pox vaccine* contains a minimum of $10^{2.5}$ $TCID_{50}$ of freeze dried live attenuated virus from the reference strain KSGP O-240. Animals were given initial vaccination 3-4 weeks before starting the experiment, and then pregnant ewes and does were vaccinated twice during pregnancy period, at the beginning of confirmed pregnancy and 4 weeks before lambing or kidding Somasundaram (2011).

Serum and Colostrum Collection: Blood samples were collected (a) before lambing/kidding and (b) after lambing. Serum separation blood samples, was carried out according to technique of Stevanovic' *et al.* (2015). Colostrum samples were collected from dams after parturition at 0 hrs, 12 hrs, and 24 hrs. Samples were centrifuged at 100,000 x g, and then stored at −20°C, for ELISA test for immunoglobulin assessment.

Live Weight. Live weight of ewes, does and BCS, confirmation of pregnancy, total protein contents of colostrum of samples collected from ewes/does, mortality rate, abortions and colostrum quality were also measured.

ANALYTICAL PROCEDURE:

Total Protein Analysis (TP) of Colostrum: Colostrum samples were analyzed using Kjeldahl method (AOAC, 2012) for total protein content.

Immunoglobulin Analysis of Serum: ELISA kits were used to measure total Igs in serum samples. In addition to measuring the concentration of different Ig classes (IgM, IgG and IgA) as per manufacturer instructions (Alfa Diagnostic International, USA). Optical densities were read at 450 nm, using an ELISA reader (Epoch Biotek).

RESULTS

Total Protein (TP) of Colostrum of Ewes and Does: Total protein (TP) content of ewes' colostrum of T1 was 5.32 %, while TP content of ewes' colostrum from vaccinated flocks (T2-T4) was increased significantly (*P*<0.01) to 10.75-10.88%. TP content of does' colostrum of control flock was 4.50 %. Vaccinated flocks (T2-T4) resulted in significant increase of TP (*P*<0.01) to 9.63-9.81%. Both species consistently had higher TP contents showing better quality colostrum immunologically for the newborn offspring (Fig. 1).

Immunoglobulin's of Serum of Ewes during Gestation: The concentration of total Igs in ewe's serum of (T1) flock was 20.89±1.22 mg/ml (Table 2). This concentration was increased significantly (*P*<0.01) after the 1st vaccination with *Clostridia sp*, *Pasteurella sp.*, and *Sheep pox*. Concentration of total Igs was increased by 68-88 %, as compared to the control flock. Igs continued to increase significantly (*P*<0.01) after the second immunization by 19-24 % (Table 2) (Gilbert *et al*. 2014; Hashemi *et al*. 2008), which proves the importance of immunizing dams during the pregnancy period. Although the concentration of Igs were significantly (*P*<0.05) increased as compared to the control group, there were no significant difference between the treatment groups (T2, T3, and T4) as shown by the *t*-test.

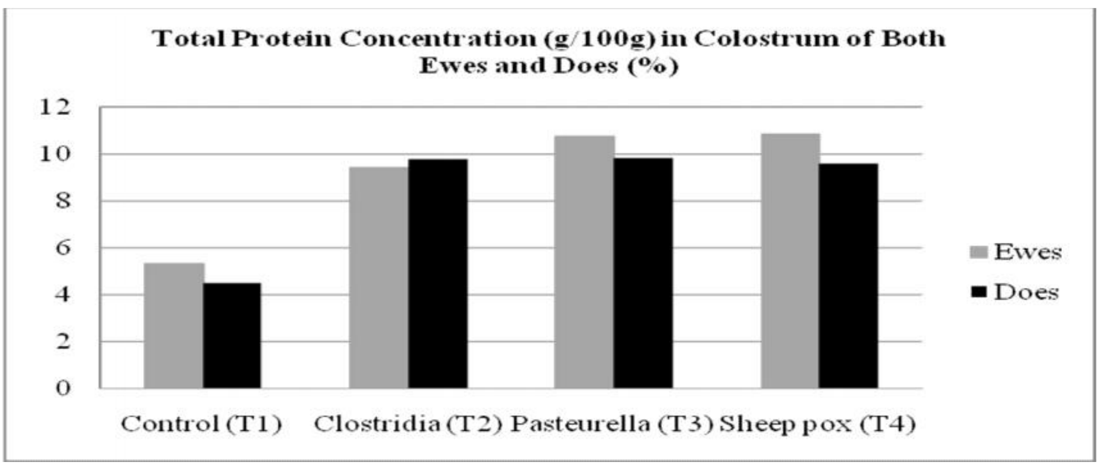

Figure 1. Total Protein Concentration (g/100g) in Colostrum of Both Ewes and Does (%)

Table 2. Concentrations of IgG in Serum of Ewes and Newborn Lambs (Mean ± SD).

Treatment	IgG mg/ml					
	Pregnant Ewes		Newborn Lambs	Pregnant Does		Kids
Control –T1	20.89 ± 1.22		22.0 ± 2.0	19.25±0.22		19.12±0.91
	1st *	2nd **		1st *	2nd **	
Vaccinated *Clostridia*-T2	35.1 ± 0.23	43.45 ± 0.22	33.0 ± 0.32	36.0 ± 0.39	39.5 ± 0.5	36.0 ± 0.39
Vaccinated *Pasteurella*-T3	37.2 ± 0.13	46.22 ± 0.14	35.0 ± 1.20	33.0 ± 0.56	40.5± 1.3	33.0 ± 0.56
Vaccinated *Sheep pox*-T4	39.3 ± 0.33	47.13 ± 1.13	32.0 ± 0.87	35.0 ± 0.21	45.2 ± 2.3	35.0 ± 0.21

*The first vaccination was at the beginning of the pregnancy period. **The second vaccination was 4-6 weeks before lambing (P<0.01). All animals were vaccinated against Clostridia and Pasteurella two months before starting the experiment as per Public Authority for Agriculture Affairs and Fish Resources (PAAFR) vaccination protocol.

IgG in Serum of Lambs Fed with Colostrum: Lambs were fed with 180-290 ml of colostrum/kg of live weight. The concentration of IgG of lambs' serum after feeding these animals with colostrum collected from T1 flock was (22.0±2.0 mg/ml). The concentration of IgG was significantly increased (*P*<0.01) by 66%, after feeding newborn lambs with colostrum collected from vaccinated ewes/does (Table 2), showing a significant positive impact of high quality colostrum feeding offspring at 24 hours after birth.

Immunoglobulin Classes (IgG, IgM, IgA) of Ewes' Colostrum: IgG concentration of colostrum of T1 flock was (54.3±4.3 mg/ml), as shown in Figure 2a. The concentration of IgG increased significantly (*P*<0.01) in the ewes, colostrum after vaccinating pregnant dams twice during the pregnancy period with *Clostridia sp., Pasteurella sp., and Sheep pox*. Results showed significant increase of IgG concentration by 20-23 % in the colostrum samples collected from vaccinated flocks, as compared to the control flock. The concentration of Igs in the colostrum started to decrease after 48 h until it reached the control flock level (Sanglid, 2003) (Fig. 2a).

Ewe's colostrum IgM of T1 flock was 5.3±0.32 mg/ml (Fig. 2a). It was observed that there was a non-significant increase in the concentration of IgM of colostrum after immunizing dams during pregnancy period with (5.515±1.3 mg/ml), (6.68±0.22 mg/ml), and (6.597±0.10 mg/ml), respectively. Ewe's colostrum IgA of T1 flock was (8.145±1.44 mg/ml). Results showed a non-significant elevation in the concentration of IgA after immunizing dams during pregnancy.

Immunoglobulin (Igs) of Serum of Does during Gestation: Concentration of total Igs in does, serum of T flock was (19.25±0.22 mg/ml) (Table 2). Igs concentration started to increase significantly (*P*<0.01) after the 1st vaccination with *Clostridia*, *Pasteurella*, and *Goat pox*. It was observed that the concentration of total Igs was increased by 57-67 %, as compared to the control flock. Furthermore, the concentration of Igs continued to increase significantly (*P*<0.01) after the second vaccination by 29-40 % as compared to the first vaccination (Table 2). There were no significant differences in Igs concentrations between the 3 treatment flocks (T2, T3 and T4) as shown by the *t*-test.

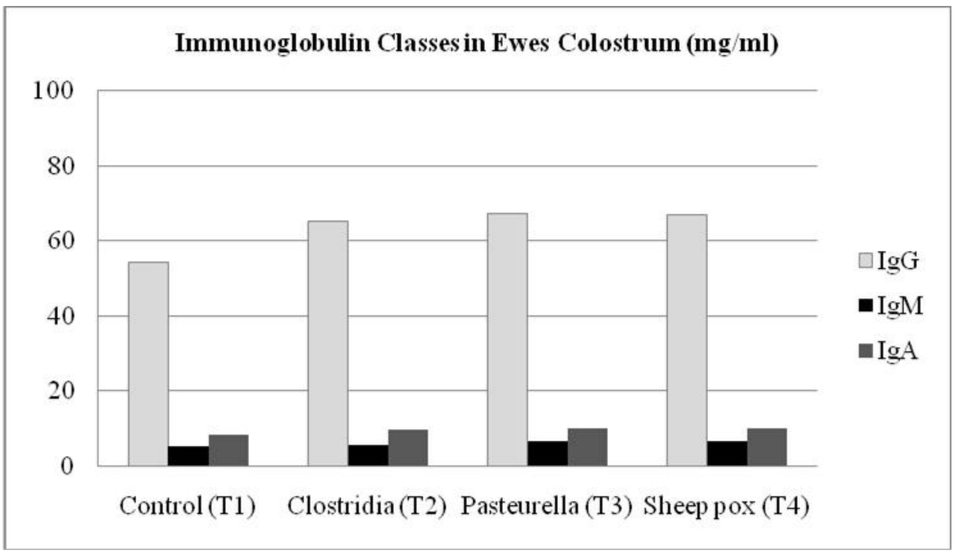

Figure 2a. Concentrations of Total Ig Classes in Ewes' Colostrum

IgG in Kids Serum Fed with Colostrum: The concentration of IgG in kids' serum after feeding them with colostrum of T1 flock was (19.12±0.91mg/ml). IgG increased significantly (*P*<0.01) by 73-89% after feeding kids' with hyperimmune colostrum of vaccinated flocks (Table 2).

Immunoglobulin Classes (IgG, IgM, and IgA) of Does' Colostrum: The concentration of IgG of colostrum collected from T1 flock was (50.2 ± 2.33 mg/ml) (Fig. 2b). Total IgG of serum and colostrum of does increased significantly (*P*<0.05) after vaccinating does twice during their pregnancy with *Clostridia sp.*, *Pasteurella sp.*, and *Goat pox*. Results showed a significant increase of IgG concentration by 48% in the colostrum collected from vaccinated flocks, as compared to the control flock. The concentration of Igs in the colostrum started to decrease after 48 h and reached the control group level (Fig. 2b) (Sanglid, 2003).

Does colostrum IgM of T1 flock was (3.52±0.12 mg/ml) (Fig. 2b). It was observed that there was a non-significant increase in the concentration of IgM of colostrum after immunizing dams twice during gestation period, and reaches (4.13±0.04 mg/ml), (4.05±0.33 mg/ml), and (4.13±0.15 mg/ml), respectively, as compared to the control Flock (Fig. 2b). The concentration of IgA of does' colostrum of T1 flock was (7.53±1.87 mg/ml) (Fig. 2b). Results showed a non-significant elevation of the IgA of colostrum collected from immunized flocks during pregnancy period (Fig. 2b).

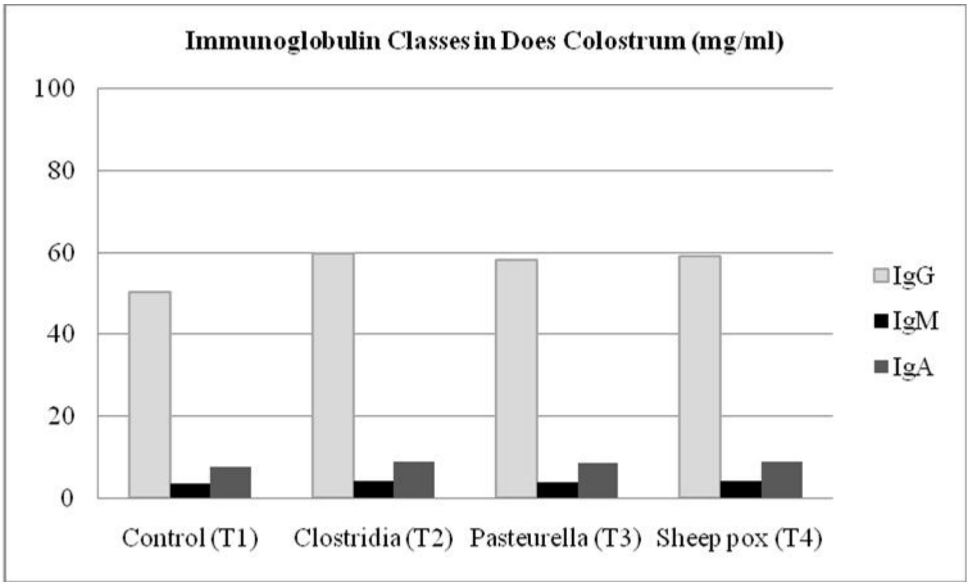

Figure 2b. Concentration of Immunoglobulin in Does Colostrum

Live Weight Changes and Body Conditions: After mating, the mean live weights of ewes of T-2 (vaccinated with *Clostridia sp*.), T-3 (vaccinated with *Pasteurella sp*.), and T-4 (vaccinated with *sheep pox* or *goat pox*) were similar, with some individual variations (data not shown). However, the live weight of ewes steadily increased as expected till the end of the fifth month of pregnancy. The mean live weight of does before mating, ranged between 37.6 and 39.3 Kg with large individual variations (data not shown). During late gestation period, pregnant does gained live weight as expected almost in a similar pattern as that of ewes. The mean BCS for ewes ranged from 2.7 to 2.9 (1-5 scale) over the entire 1st to 5th month of pregnancy period without showing any clear trends of changes, indicating that the animals maintained a steady body condition during the pregnancy period and thus had a good nutritional status. Furthermore, the BCSs of does consistently showed a lower scores starting from the 1st to 5th month of pregnancy. The BCS ranged from 2.3 to 3.0, and the does also maintained the BCS steadily during the study (Corner-Thomas, 2015). Mortality rates in newborn animals reached 28.0-33.3 % in young lambs and kids in T1 flock, respectively (Fig. 5).

Mortality Rate of Lambs: The mortality percentages of newborn lambs in the control flocks reached 28.0%, which was found from birth (day one) to weaning at 10 wks (Burezq *et al*. 2016). In contrast, there was no mortality between lambs receiving hyperimmune colostrum (T2, T3, and T4) in the first seven days of age. Vaccination of pregnant ewes resulted in improving immunity of lambs. Therefore, morbidity and mortality of young animals from birth to weaning was reduced to zero.

Mortality Rate of Kids: The mortality percentages of newborn kids in the control flocks reached 33.3% from birth (day one) to weaning at 10 wks (Burezq *et al.* 2016), but no mortality was found in kids receiving hyperimmune colostrum (T2, T3, and T4) in the first seven days of age. Vaccination of pregnant does resulted in improving the immunity of kids; therefore, morbidity and mortality of young animals from birth to weaning will be reduced.

DISCUSSION

Immunoglobulin's (Igs) in Serum of Ewes/Does during Gestation. A significant elevation of Igs in dam's serum was observed during pregnancy period and before lambing/kidding (Figs. 3 and 4), reaching about 43.45 to 47.13 % in ewes and 39.5 to 45.2 % in does over the control (T1 flock). Igs started to drop sharply ante-partum until it reached control level at parturition as shown in figures 3 and 4, and then started to rise again during the next four weeks after parturition. These findings agree with previously published results of Saucedo *et al*. (2011) and Vatankhah (2013). The sharp loss of immunity after lambing/kidding is due to hormonal changes, which are usually related to lambing and lactation; these changes are the main source of the supply of protein and energy, which are important to maintain immunity. The energy and protein requirement of the lambing ewes increases 2 to 3 fold, respectively, in the 4 weeks period between lambing and milk production (www.wormboss.com.au/news/articles/nonchemical-management/why-are-lambing-ewes-susceptible-to-worm-infection.php).

Immunoglobulins in Colostrum of Ewes and Does: Vaccination of dams during their pregnancy period in housed flocks was very effective in boosting Ig levels significantly (*P*<0.5) in their blood and producing of hyperimmune colostrum (Nikbakht *et al*., 2010; Rudovsky *et al*., 2008; Weaver *et al*. 2000). The mechanism of boosting serum Igs and production of hyperimmune colostrum by vaccinating pregnant dams is an interesting topic of study, especially the biochemical process. However, it was found that Igs were synthesized in mammary gland epithelium during the later 5 wks of pregnancy due to hormonal influence, mainly by estrogen (Cortese, 2009; Castro *et al*. 2011).

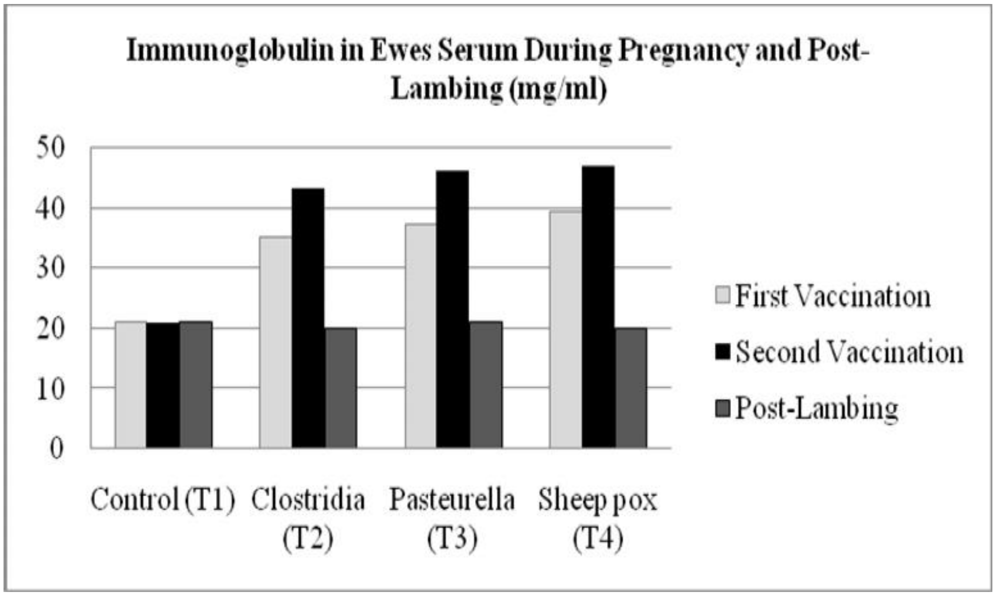

Figure 3. Concentrations of Total Immunoglobulins in Serum of Ewes

Immunity of Lambs and Kids: Feeding of hyperimmune colostrum to 24 h old lamb/ kids was found to have great significance for their survivals. Three different classes of Igs including IgG, IgM, and IgA are usually synthesized during the last few weeks of pregnancy by the plasma cells located in the submucosa of the mammary gland epithelium (Yilmaz and Kasikci, 2013). These antibodies are transferred into the colostrum by selective receptor-mediated intracellular route (Kacskovics, 2004). Fc is the specific receptor for transferring IgG antibody from the serum to mammary gland, and it is regulated by hormonal changes during the pregnancy period (Yilmaz *et al.* 2011; Tabatabaei *et al.* 2013). Among three classes of Igs (IgG, IgM and IgA), only IgG was found to play main passive immunity functions in small ruminants (sheep, goats) (Table 2). These factors are of interest to study in intensively raised small ruminants.

There are many functions of the Fc receptor, such as IgG metabolism and prevention of the degradation of IgG in maternal circulation. It also plays an important role in determining the concentration of IgG in the colostrum (Brujeni *et al.* 2010; Kacskovics, 2004; Baintner, 2007; Lerias *et al*. 2014). The expression of FcRn receptor increased in dry ewes in late gestation period, and decreased during colostrogenesis (i.e., transfer of Igs from the dam blood circulation into the mammary secretions, which occurs in the last two weeks before parturition) and lactation period (Hine *et al.* 2010; Hernandez-Castellano *et al.* 2014).

Anti-mortem and Post-mortem Symptoms of Sick Lambs and Kids. The young lambs showed sever diarrhea and difficulty in breathing. Dehydration started from day 0 and continued to 10 days maximum. usually most lambs died within the first week of life, similar symptoms were observed in kids. Both species of young animals showed watery to bloody diarrhea coughing and were unable to stand alone. No interventions such as fluid therapy were provided to the animals of control and treatment flocks. The post mortem examination showed hemorrhage in the intestine and congestion of lungs and entire the respiratory tract. Diarrheas appeared to be associated with septicemia and in some cases death especially within 24 hrs of the lambs/ kids were born.

Our findings should be treated with caution because of the small size of lambs/kids and their different time of birth. In addition, further studies were not carried out to diagnose the causative pathogens. In Kuwait, intensive production and management of dairy cattle were observed to face serious losses due to high rate of morbidity and mortality of diary calves, especially during the first seven days of their lives (Razzaque *et al.* 2009a, Razzaque *et al*. 2009b) due to the following causative antigens found in calves including *E. Coli, Pasteurella sp*., and *Salmonella sp.* (Razzaque *et al*. 2009C).

Annual and biannual vaccination protocols for dry ewes, were carried out 4 weeks before starting the experiment, would have protected animals in all flocks against *Clostridia sp. Pasteurella* sp., and *Sheep pox* or *goat pox*. Vaccination of pregnant ewes/ does boosted the production of hyperimmune colostrum for both lambs/ kids especially for the first seven days of their live. In the present study, we managed to decrease the mortality rate significantly in newborn lamb/kids by vaccinating the pregnant dams with single pathogen (i.e., *Clostridia sp.,* alone, *Pasteurella sp.,* alone, and *sheep pox* or *goat pox* alone). Gilbert *et al*. (2014) and Hashemi *et al*. (2008) indicated positive effects of immunizing dams during the gestation period, yet the mortality rate of treated flocks was about 8-10% after the first week of life, which was mainly due to the single vaccination in the present study. Possibly, use of combined vaccines against multi-pathogens would help in controlling the infection caused by *Clostridia sp.,* and *Pasteurella sp.,* and *sheep pox* or *goat pox* in newborn lambs/kids for longer period of time.

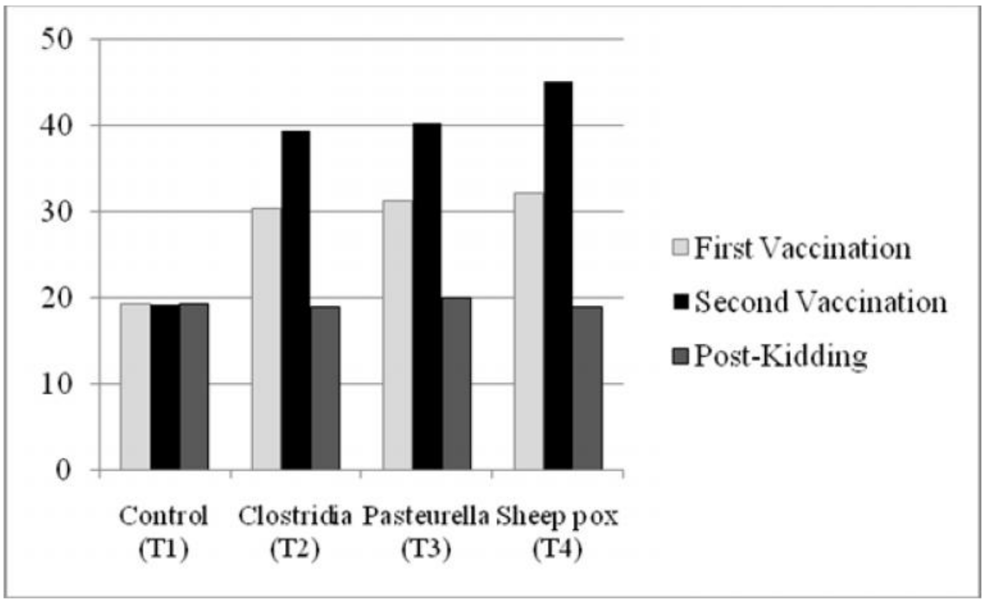

Figure 4. The Concentration of IgG in Does Serum

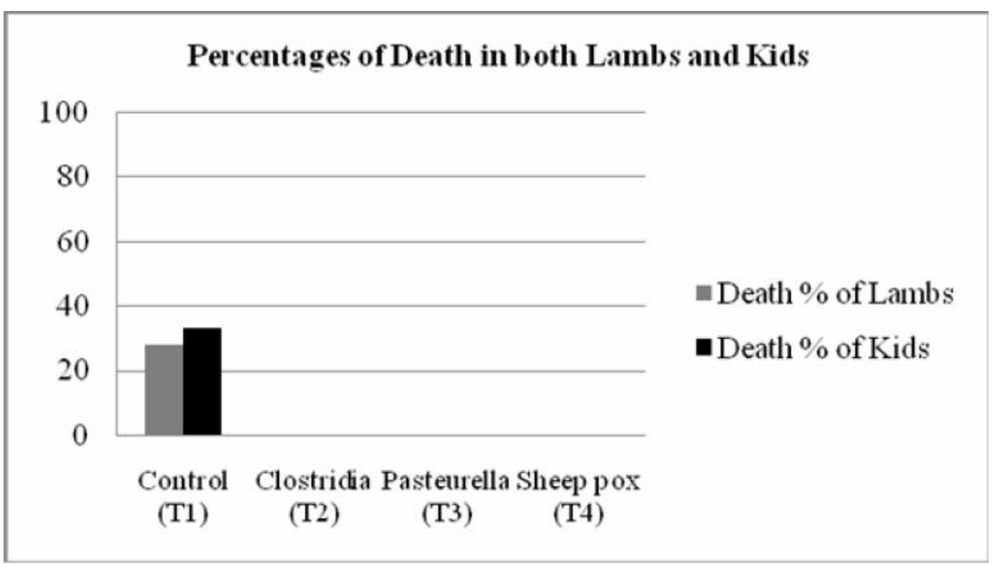

Figure 5. The Percentages of Lambs/ Kids Mortality Fed on Hyperimmune Colostrum

Conclusion: Vaccination of pregnant ewes/does was effective in producing hyperimmune colostrum for lambs/kids. Newborn lambs/kids receiving hyperimmune colostrum raised their immune status by double. Findings showed benefits of vaccinating pregnant ewes/does. The results of vaccinating pregnant dams twice during their pregnancy using a single pathogen vaccine showed significant positive impacts on the quality of colostrum and improved survival of lambs/kids during their first seven days. The mechanism of action for raising the quality of colostrum by single species vaccination of pregnant animals is not known. Therefore, further studies are needed for using the vaccination protocols, i.e., single species vaccine.

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