Variation in colostral immunoglobulin G concentration in fat tailed sheep and evaluation of methods for estimation of colostral immunoglobulin content

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Abstract

In ruminants, colostrum is a vital source of immunoglobulins that provide passive immunity for their offspring during the neonatal period. It is suggested that colostral immunoglobulin G (IgG) concentration varies between and within breeds and could also be affected by maternal factors. The aim of this study was to investigate possible effects of litter type and ewe parturition number on colostral IgG concentration in two Iranian fat-tailed breeds of sheep (Shaul and Lori Bakhtyari) as well as usefulness of different methods for estimation of IgG concentrations in colostrum. The colostral IgG concentrations were measured in 38 Shaul and 59 Lori Bakhtyari ewes by single radial immunodiffusion, zinc sulphate turbidity and Biuret methods. Measurement of IgG by single radial immunodiffusion revealed that Lori Bakhtyari ewes had significantly ($P < 0.05$) lower colostral IgG levels (48.82 ± 2.10 mg/ml) than Shaul ewes (62.86 ± 2.48). With regard to the effect of litter type and parturition number, a significant ($P < 0.05$) difference in IgG concentration of colostrum was only observed between the first (65.17 ± 5.74 mg/ml) and third parturition (41.10 ± 4.60 mg/ml) of Lori Bakhtyari ewes. The colostral IgG concentration was not associated with ewe serum IgG concentration ($P > 0.05$). The mortality rate was higher in lambs born to ewes with lower IgG in their colostrum. Single radial immunodiffusion did not correlate either with zinc sulphate turbidity method (r = -0.253, $P > 0.05$) or with Biuret method (r = -0.005, $P > 0.05$). We can conclude that concentration of colostral IgG could be influenced by breed but not by litter type and parturition number.

Colostrum, parturition, litter, radial immunodiffusion, zinc sulphate turbidity, Biuret method

Adequate transfer of colostral immunoglobulin (Ig) to newborn lambs is essential as lambs with insufficient amount of antibody are at higher risk of contracting diseases and thus higher mortality rate (Sawyer et al. 1977; Ahmad et al. 2000). The quantity of colostral immunoglobulin transfer could be influenced at different levels including production, ingestion and absorption of IgG. Low production of colostral IgG is one of the major problems culminating in failure of passive immunity transfer (Paulík et al. 1984; Tizard 2004). Influenced by maternal genetics, colostral IgG concentration varies widely between and within breeds (Halliday 1978; Gilbert et al. 1988; Tyler et al. 1999; Gulliksen et al. 2008; Zhang et al. 2009). Concentration of colostral Ig could also be modulated by other factors including parturition number and litter type (Gilbert et al. 1988; Higaki et al. 2012).

Single radial immunodiffusion (SRID) has been long applied as an accurate method to measure IgG concentration of colostrum and serum but it is time-consuming and expensive. Other methods have also been considered to estimate serum IgG concentration such as precipitation of globulins by zinc sulphate or assessment of total protein by Biuret method

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(Tizard 2004; Sedlinská et al. 2005; Massimini et al. 2006); however, the usefulness of these test, in comparison to SRID, for prediction of IgG concentration in colostrum remains to be determined.

The present study was conducted to determine colostral IgG concentration in Shaul and Lori Bakhtiari breeds, which are two native Iranian fat-tailed sheep (Tavakkolian 2000), and to evaluate the impact of maternal colostral IgG concentration on lamb mortalities. We also investigated the possible effects of litter type and ewe parturition number on colostral IgG concentration as well as correlation of ewe serum IgG concentration with colostral IgG concentration. Finally, the efficacy of zinc sulphate turbidity assay (ZST) and Biuret methods in measurement of colostral IgG concentration in comparison to SRID was assessed.

Materials and Methods

Animals

A total of 38 Shaul and 59 Lori Bakhtyari ewes were randomly selected for the study. Shaul and Lori Bakhtyari ewes were housed at animal research farms of the Faculty of Veterinary Medicine, University of Tehran and Research Centre of Animal Husbandry of Shahrekord (Chaharmahal and Bakhtyari Province, Iran), respectively. The ewes had no apparent signs of illness and were clinically healthy. The body condition of ewes was almost similar across different groups and the average body condition score was similar. The animals were managed in a semi open shed confinement system. Their nutrition consisted of sufficient amounts of alfalfa hay, wheat bran, barley grain, corn silage, and wheat and barley stover. The Lori Bakhtyari ewes also had occasional access to local pasture in summer. The animals were divided into different groups according to their parturition number and litter type (Table 1). It should be noted that total number of Shaul ewes in litter type groups is 29 due to missing information of 9 ewes. During the first month after parturition, mortalities in the offspring of Shaul and Lori Bakhtyari ewes were recorded.

Table 1. The colostral IgG concentration (Mean ± SEM) in Shaul and Lori Bakhtyari sheep

	Colostral IgG concentration (mg/ml)					
	Shaul breed		Lori Bakhtiyari breed		Total	
	n	mean ± SEM	N	mean ± SEM	n	mean ± SEM
Parturition number						
1	5	68.39 ± 4.85	7	65.17 ± 5.74*	12	66.51 ± 3.78
2	8	58.36 ± 7.87	12	52.62 ± 4.32	20	54.92 ± 4.00
3	9	62.19 ± 3.89	16	41.10 ± 4.60*	25	48.69 ± 3.81
≤ 4	16	63.75 ± 3.72	24	47.30 ± 2.62	40	53.88 ± 2.49
Litter type†						
Single	14	66.55 ± 3.32	42	51.48 ± 2.21	56	55.25 ± 2.03
Twin	11	62.18 ± 5.44	17	42.26 ± 4.83	28	50.08 ± 4.02
Triplet	4	62.5 ± 12.5	0	0	4	62.5 ± 12.5

*Values differ significantly ($P < 0.05$)

†The inconsistency in the total number of Shaul ewes is due to missing information of 9 ewes.

Colostrum whey and serum preparation

Immediately after parturition, 10 ml of colostrum was obtained from each ewe. For colostrum whey preparation, 1.5 ml of colostrum samples was centrifuged for 5 min at 800 g. Then the upper fat layer was removed and 100 µl of rennin solution (1%) was added to the rest of sample. Skimmed colostrum was kept at 37 °C until the clot separated from whey. Finally the clots were precipitated by centrifugation at 700 g for 5 min. The supernatant was harvested and stored at –20 °C until analysis.

Three ml of blood was prepared by jugular venipuncture just after parturition. After blood coagulation at room temperature, serum was separated by centrifugation at 700 g for 10 min and stored at –20 °C until analysis.

Immunoglobulin and total protein measurement

Total immunoglobulin measurement was performed by a qualitative spectrophotometric zinc sulphate turbidity assay (Pfeiffer et al. 1977). To determine IgG concentration, SRID was carried out in 1% agarose in phosphate

buffer saline, pH 7.2, containing 0.5% chicken anti-sheep IgG (Nikbakht et al. 2008) according to the method of Mancini et al. (1965). Considering the particular characteristic of chicken immunoglobulin, 2% polyethylene glycol 6000 was added to agar solution in order to clarify the precipitation zone. The total protein concentration was measured by Biuret method kit according to manufacturer's instructions (Baharafshan, Iran).

Statistical analysis

A two-way analysis of variance was used to determine the effect of parturition number (1, 2, 3, ≥ 4) and litter type (singles or twins) on colostral IgG concentration between the Shaul and Lori Bakhtyari breeds. Bonferroni *post hoc* test was performed for multiple comparisons between groups. The correlation between SRID and either ZST or Biuret methods was analyzed by Pearson correlation test. The association between colostral IgG concentration and ewe serum IgG concentration was also examined by Pearson correlation test. The level of significance was set at $P < 0.05$ and Statistical Product and Service Solutions (SPSS) software (version 15) was used to perform all statistical calculations.

Results

Colostral IgG concentration measured by single radial immunodiffusion varied from 25 to 100 mg/ml and from 7.35 to 90.65 mg/ml in Shaul and Lori Bakhtyari ewes, respectively. Shaul ewes had a higher average of colostral IgG concentration with 62.86 ± 2.48 mg/ml than Lori Bakhtyari ewes with 48.82 ± 2.10 mg/ml ($P < 0.05$). There was no significant ($P = 0.81$) difference between parturition number of Shaul ewes and IgG content of colostrum, but a significant ($P < 0.05$) difference was observed for Lori Bakhtyari breed (Table 1). Comparison of colostral IgG concentration between Shaul and Lori Bakhtyari sheep revealed no significant ($P = 0.30$) difference regarding to ewe parturition number and litter type. During the first month after parturition, the offspring of two Shaul and 12 Lori Bakhtyari ewes were found dead. Data about *post mortem* signs, cause and exact time of death were not available. The mean IgG concentration in colostrum of Lori Bakhtyari ewes with dead lambs was 41.93 ± 6.07 mg/ml, which was significantly ($P < 0.05$) lower than the average for ewes with no lamb mortality (50.58 ± 2.17 mg/ml); this was not significant in Shaul breed. No association between ewe's serum at the time of parturition and colostral IgG concentration was found ($P = 0.82$). The mean value obtained by ZST and Biuret methods was 125.78 ± 8.09 (ranging from 36.69 to 215.39) and 115.89 ± 1.77 (ranging from 84.1 to 158.1), respectively. No correlation was observed between results obtained by SRID and either ZST (r = -0.253, $P = 0.177$) or Biuret methods (r = -0.005, $P = 0.97$).

Discussion

In our experiment, it was hypothesized that colostral IgG concentration in fat-tailed sheep could be affected by breed, litter type and ewe parturition number. Colostral IgG varied widely among different individuals in both Shaul and Lori Bakhtyari ewes. We found that breed had a significant effect on colostral IgG concentration. In Shaul ewes the IgG concentration in colostrum was similar to those found in other breeds such as Rambouillet (64 mg/ml), Targhee (67 mg/ml), Columbia (64 mg/ml), Finn crossbreed (69 mg/ml), Lacaune (67 mg/ml) and Ostfriesisches Milchschaf (64 mg/ml) (Gilbert et al. (1988) and Waelchli et al. 1994). The mean colostral IgG concentration found in Lori Bakhtyari breed was similar to those reported for Blackface (45 mg/ml) and Suffolk s(54 mg/ml) (Dwyer and Morgan 2006). This inter-breed variation in colostral IgG concentration could be associated with polymorphism in the neonatal Fc receptor gene, which plays a role in transferring of IgG from serum to colostrum (Tizard 2004; Zhang et al. 2009). We found that breed and parturition number had no interactive effect on colostral IgG concentration; this is in agreement with Tyler et al. (1999).

Similarly to data reported by Villette and Levieux (1981), parturition number had no effect on the colostral IgG concentration in Shaul sheep. In contrast, Gilbert et al. (1988) and Higaki et al. (2012) found significant difference between primiparous and multiparous

ewes. In our study, this was only observed for Lori Bakhtyari sheep between their first and third parturition. Higaki et al. (2012) suggested that higher colostral IgG concentration in primiparous ewes could be due to production of a lower colostrum volume. Taken together, after the first parturition in multiparus ewes, colostral IgG seems to be not affected by the number of parturitions.

There was no association between the litter type and colostral IgG concentration in both Shaul and Lori Bakhtyari ewes, which agrees with findings of Dwyer and Morgan (2006). However, Gilbert et al. (1988) and Higaki et al. (2012) observed that ewes which delivered more lambs had much higher colostral IgG concentration. We could not find a clear explanation for this inconsistency.

In accordance with data reported by Sawyer et al. (1977), serum IgG concentration of ewes at the day of lambing could not be appropriate criteria for the prediction of the IgG level in colostrum.

It was found that SRID did not correlate with either ZST or Biuret methods. The latter methods showed higher values for IgG than those from SRID. Sedlinská et al. (2005) also observed that ZST values were approximately 2 × higher than those obtained by SRID, although they found high correlation between SRID and ZST. Since ZST and Biuret test are not specific to detect IgG, the inability of these methods to appropriately predict the IgG amount could be attributable to the high concentration of immunoglobulins and proteins in colostrum. Based on our results, ZST and Biuret tests may not be recommended for prediction of IgG concentration in sheep colostrum.

In conclusion, considering the genetic effects on colostral IgG concentration, it would be worthwhile to improve colostrum quality by selection of breeds with higher colostral IgG concentration to ensure adequate transfer of IgG to offspring. The evidence of this improvement was found by Gilbert et al. (1988) in colostral IgG concentrations of Polypay ewes, which were developed by the intermating of Dorset and Targhee and Finn and Rambouillet breeds.

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