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Research Article

Interrelationships of Serum and Colostral IgG (Passive Immunity) with Total Protein Concentrations and Health Status in Lambs [1]

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Abstract

This study was designed to determine relationship between sheep serum before lambing, colostrum and 1-day-old lamb serum total protein (TP) and immunoglobulin-G (IgG) concentration and their effect on neonatal diseases and also the linear relationship between serum TP and IgG concentration (STPC and SIgGC, respectively) in different days of the neonatal period thereby determining the feasibility of TP concentration in the prediction of colostrum quality and passive immunity and to define a cut-off point for STPC and SIgGCat 24 h after birth (STPC-24 and SIgGC-24, respectively) associated with increased risk of illness or death in lambs. For this purpose, blood was obtained from the lambs and ewes at day 1 (n=325), at day 0 (before colostrum intake) and on different days in neonatal periods (n=50) and blood (10-15 days before lambing) and colostrum were obtained from the respective ewes related to the lambs. Mean serum TP and IgG concentrations on days 1, 2, 4, 7, 14 and 28 were significantly higher than values on day 0 (before colostrum intake) from the lambs remained healthy in neonatal period. The STPC-24 was significantly lower in diseased and dead lambs when compared to healthy lambs in the neonatal period (P<0.001 and P<0.001 respectively). The STPC-24 in lambs that died or became ill was 62% to 67% in SIgGC-24, respectively. Mean colostral TPC was significantly (P<0.05) higher in dams (n=254) of healthy lambs when compared to those of sick lambs in neonatal period. There was a significant correlation between the dams' STPC and both the SIgGC in dams and SIgGC-24 in their lambs (R=0.454, R=0.342, respectively). The study revealed that STPC-24 >55 g/L and SIgGC-24>600 mg/dL is probably consistent with adequate level of passive transfer. It was also noted that in addition to determining colostrum quality, STPC plays an essential role in the prediction and prevention of neonatal diseases in lambs. In conclusion, immediate and inexpensive determination of TP concentrations is beneficial in making timely management and treatment decisions related to failure of passive transfer (FPT).

Keywords: *Neonatal lamb, Colostrum, Total protein, IgG, Passive transfer failure*

Kuzularda Serum ve Kolostral IgG (Pasif İmmünite) Konsantrasyonlarının Total Protein ve Sağlık İle İlişkisi

Öz

Bu çalışma kuzulamadan önce koyun serum, kolostrum ve 1. günde kuzularının total protein (TP) ve immunoglobulin-G (IgG) seviyeleri arasındaki ilişkinin ve bu parametlerin neonatal hastalıklar üzerindeki etkisinin ve ayrıca neonatal periyodun farklı günlerinde serum TP ve IgG konsantrasyonları (STPK ve SIgGK) arasındaki linear ilişkinin belirlenmesi, böylece TP konsantrasyonunun pasif immunite ve kolostrumun kalitesinin belirlenmesinde kullanılabilirliğinin belirlenmesi ve doğumdan sonra 24. saat STPK ve SIgGK pasif immunite ve kuzularda hastalık ve ölüm riskini artıran eşik değerinin (STPK-24 ve SIgGK-24, sırasıyla) belirlenmesi için dizayn edildi. Bu amaçla tüm kuzulardan 1. gün (n=325), kolostrum almadan önce (0. saat/gün) ve sonraki günlerde (n=50) ve annelerinden kuzulamadan 10-15 gün önce kan ve ayrıca kolostrum örnekleri alındı. Neonatal periyotta sağlıklı olduğu belirlenen kuzulardan yaşamın 1, 2, 4, 7, 14 ve 28. günlerinde ortalama serum TP ve IgG seviyelerinin 0. güne göre (kolostrum almadan önce) önemli seviyede yüksek olduğu belirlendi. Neonatal dönemde hastalanan ve ölen kuzuların STPC-24'ları sağlıklı olanlara göre önemli seviyede düşük olduğu saptandı (sırasıyla P<0.001 ve P<0.001). Neonatal sağlıklı kuzuların annelerinin (n=254) ortalama kolostral TPK hastalanan kuzularınkine göre önemli seviyede (P<0.05) yüksek bulundu. Annelerin STPK'nun hem anne SIgGK'u hem de kuzularının SIgGK-24 ile arasında önemli bir korelasyon olduğu belirlendi (sırasıyla R=0.454 ve R=0.342). Bu çalışma STPK-24'nun >55 g/L ve SIgGK-24'nun >600 mg/dLolması yeterli konsantrasyonda pasif transferini işaret etmektedir. Bu çalışma ile ayrıca KTPK'larının kolostrumun kalitesinin belirlenmesinin yanında, neonatal hastalıkların belirlenmesi ve önlenmesinde önemli bir rolü olduğu da tespit edildi. Sonuç olarak total protein konsantrasyonlarının hızlı ve ucuz bir şekilde belirlenmesi pasif transfer yetmezliği ile ilgili sevk-idare ve tedavi kararlarının zamanında alınması açısından faydalıdır.

Anahtar sözcükler: *Neonatal kuzu, Kolostrum, Total protein, IgG, Pasif transfer yetmezliği*

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INTRODUCTION

Neonatal morbidity and mortality are important causes of economic loss for sheep farms [1-4], thus making this period the most critical [5-9]. The syndesmochorial placenta in ruminants does not allow the transfer of maternal antibodies, also known as immunoglobulins (Ig), to the fetus during pregnancy. Thus, lambs are born hypogammaglobulinemic. Therefore, neonatal lambs depend on ingestion and absorption of maternal antibodies in colostrum to provide humoral immunity during the neonatal period. This process is termed passive transfer and is determined by measuring serum IgG concentrations. To ensure adequate passive transfer of immunity, lambs should receive a sufficient volume and quality of colostrum within the first 12 h of life [5,8-11]. Inability of neonatal lambs to obtain and absorb sufficient amount of colostral IgG is a secondary immunodeficiency disorder termed as Failure of Passive Transfer (FPT). FPT results in hypogammaglobulinemia which in turn predisposes neonates to develop diseases and death [1,2,5,8-16]. Therefore, passive immunity plays a critical determining role in the short-term health and survival for lambs until their own immune system begins functioning fully. Numerous studies in the past three decades correlated neonatal diseases with inadequate serum IgG, in other words, FPT in animals and thus suggested the importance of IgG in the prevention of infections and enhancing growth performance in neonates [1,2,5,8,9,14,16-18].

Currently, the incidence of FPT in lambs ranges from 3.4% to 20%, with mortality rates fluctuating between 45% and 50% in the first 2 weeks of life, particularly the first week [3,5,11-13].

Major economic losses may occur in the farms experiencing FPT frequently. Thus, FPT is a major economic concern for producers. Therefore, it is prerequisite for sheep producers to prevent lamb sickness and losses by monitoring immune status of lambs [3,8,9].

Several methods are currently being used to detect FPT in newborn ruminants. SRID [19] and ELISA [20] are the most accurate tests for direct measurement of serum IgG concentration (SIgGC). However, these tests require significant diffusion time and are expensive [20]. Therefore, for the detection of FPT in individual herds, screening with indirect methods and confirmatory diagnosis with SRID or ELISA may be more appropriate [20]. Other tests, including the determination of serum total protein concentrations (STPC) [5,12,15], serum GGT activity [5,21,22] and serum γ-globulin concentrations [5,14], zinc sulfate turbidity (ZST) test [12,15,23] and the serum glutaraldehyde coagulation test [3], provide an approximate assessment of SIgGC based on the concentration of total globulins or other proteins associated with IgG during passive transfer in lambs [8,9,22]. The ability to obtain fast and accurate test results on the farm is imperative in making timely management and treatment decisions. Furthermore, the accurate and rapid availability of test results is important in terms of clinical practice, for example in the evaluation of prognosis and determination of alternative treatments of neonatal diseases [5,24].

Different studies were carried out in calves to quantify increased risk of death associated with low SIgGC. However, there is a scarcity of data concerning this association in lambs [5,20]. In neonatal calves, an increased risk of death and illness was associated with SIgGC below 1,000 mg/dL as determined by single radial immunodiffusion (SRID) [5,8,9]. However, a dividing line between hypogammaglobulinemia and normal SIgGC in neonatal lambs has not yet universally been accepted. Studies investigating association between risk of developing death and SIgGC [1,5,13,22]. Nevertheless, there is a lack of universally accepted threshold for the SIgGC below which FPT develops in lambs [5,22]. Furthermore, studies investigating relationship between passive immunity and lamb death beyond neonatal period where ZST test, an indirect method, was used and extended the results to cover neonatal period [14,15]. Studies evaluated passive immunity using ZST test revealed that ZST test units below 10 [12] and/or 20 [15] had increased of contracting disease in neonatal period. Therefore, the association between neonatal lamb death and SIgGC is not yet fully understood [25]. Furthermore, association between passive immunity and diseases encountered beyond neonatal period has not been fully elucidated in lambs. For this reason, the direct role of SIgGC for the prevention of diseases and data regarding the SIgGC or FPT threshold values that increases risk of sickness and death in the lambs is not yet clear.

Different methods are presently available for detecting STPC in newborn ruminants. These tests are practical, quick and inexpensive, as well as suitable for the field use. However, the accuracy of STPC to calculate IgG concentrations has only been evaluated in healthy lambs [5]. Furthermore, data regarding the serum TP threshold values associated with lamb health during the neonatal and subsequent periods and the accuracy of these threshold values as well as the availability of using TP concentration to assess the quality of sheep colostrum are either insufficient or lacking entirely.

The objectives of this study as follows; 1- to determine the relationship between the level of TP and IgG in the ewe's serum, colostrum and that in the one day-old lamb; 2- to determine whether survival or illness of the newborn lamb is correlated with concentration of passively acquired TP byone day-old lamb, thereby defining a cut-off point for STPC and SIgGC associated with increased risk of illness or death in lambs; 3- to identify a relationship between serum or colostral IgG and TP concentrations, thereby determining the accuracy of TP concentrations in the prediction of passive immunity (IgG) and colostrum quality; 4- to determine a relationship between serum IgG and TP concentrations in healthy lambs during the neonatal period, thereby demonstrating the feasibility of using STPC to identify the status of passive immunity in this period.

MATERIAL and METHODS

Animals: The study was conducted after obtaining ethical

approval from the Kafkas University Institutional Ethical Committee for Animal Care and Use (KAÜ-HADYEK, 2008-23). Details of animals, farm selection, farm management practices, clinical examination and blood and colostrums sampling method were given elsewhere [26]. Briefly, 301 ewes and their 347 lambs on two neighboring and similar management practices farms were included in the study.

IgG and Total Protein Assays: Serum IgG concentrations were measured using a commercial ELISA kit (Bio-X Competitive ELISA kit for Ovine blood serum IgG Assay-BIO K 350, Bio-X Diagnostics, Belgium). Colostrum IgG concentration was also tested with the same kit using bovine colostrum calibrator (Bio-X Elisa Kit for Bovine Immunoglobulin Assays-BIO K 165). Serum and colostral total protein (TP) concentrations were measured by using spectrophotometry with a commercial kit (TML, Total Protein, Code; A1279, Tani Medical, Turkey).

Statistical Analysis: The lambs were categorized based on the clinical examination as healthy or sick. In addition, sick lambs were also categorized as dead and recovered. The results of clinical examination were categorized in terms of life period as neonatal period and the period from 5 to 12 weeks of life (postneonatal period) to compare morbidity, mortality and their relations with serum IgG and TP concentrations in lambs. Animals whose serum or colostrum TP or IgG concentrations could not be measured for any reason excluded (n=22, only serum TP were not measured) from the analyses. Data was collected and entered into a database (Microsoft access).

Independent samples T test was used to compare serum or colostral IgG concentrations in different period of life. Time-dependent differences were localized by use of the Tukey HSD test. The relationship between serum IgG and TP concentrations were explored by Pearson correlation and simple/multiple regression analysis (SPSS).

The accuracy of STPC for estimating IgGC was established by using standard linear regression analysis previously described in detail [5,22,26]. Calculations were performed by use of SPSS. Origin 6 program was used to obtain scatter diagrams illustrations. For all analyses, values of $P<0.05$ were considered significant. Morbidity and mortality risk according to different SIgGC-24 and STPC-24 levels were calculated according to X^2 for trend (X^2, Odds ratios).

RESULTS

The morbidity and mortality rates in neonatal and post-neonatal periods, disease reasons were given elsewere (26). The majority of neonatal deaths occurred (84.6%, 11/13) in the first week of life.

The mean± SD (min-max) serum IgG (n=347) and TP (n=325) concentrations at the 24th h after birth were, 2198±1162 mg/dL (19-5302) mg/dL and 73±13 (21-117) g/L, respectively and were significantly (R^2=0.671, P=0.000) correlated. Serum TP and IgG concentrations were significantly ($P<0.001$) higher on days 1, 2, 4, 7, 14 and 28 of the neonatal periods compared to day 0 (before colostrum intake) in healthy lambs *(Table 1, Table 2)*.

Simple and multiple regression models were calculated between variables which had linear correlations *(Table 3)*. Multiple regression models were developed to predict SIgGC based on lamb's age and STPC. STPC was linearly and significantly ($P<0.001$) correlated with SIgGC on different days during the neonatal period *(Table 3)*.

The most accurate result for predicting SIgGC was on day 1 (R^2= 0.562) compared to other days. Additionally, in healthy neonatal lambs, multiple regression model established between variables with data obtained on days 1, 2, 4, 7, 14 and 28 before and after taking colostrum was determined as the most accurate model for calculation of SIgGC from STPC (R^2= 0.701) *(Table 3)*.

The SIgGC and STPC at 24 h after birth was lower in clinically ill, recovered and dead lambs compared to healthy lambs in the first week of life and the neonatal period *(Table 4)*.

Table 1. *Serum TP (g/L) and IgG (mg/dL) concentrations [mean±SD, (Min-Max)] in neonatal healthy lambs*

Parameter	Day						
	0	1 (±1th)	2	4	7	14	28
TP (n=41)	40±6^{a} (21-47)	73±11^{b} (52-107)	73±13^{b} (47-117)	71±9^{b} (49-86)	70±10^{b} (46-88)	63±7^{c} (48-78)	59±7^{c} (46-71)
IgG (n=50)	26±16^{a} (8-62)	2666±1316^{b} (781-5302)	2743±1359^{b} (805-5308)	2295±1110^{b} (709-5029)	1714±816^{c} (493-3518)	1013±401^{d} (295-1893)	935±357^{d} (301-1707)

Different letters refer to significant differences between the values in the same rows (P<0.05). Tukey HSD test was used to detect differences

Table 2. *The correlation between mean serum TP and IgG concentrations in neonatal healthy lambs (n=41)*

Parameter	Day						
	0	1	2	4	7	14	28
Pearson Correlation	0.157	0.749**	0.735**	0.680**	0.620**	0.729**	0.634**
Sig. (2-tailed)	0.327	0.000	0.000	0.000	0.000	0.000	0.000

*Statistically significant (**P<0.01 and *P<0.01). The relationship was determined by Pearson correlation test*

Table 3. *Simple and multiple regression analyses between IgG and TP of the clinical status of the lambs*

Parameter		Days	n	Formulas	R^2	P
Regression analysis in neonatal healthy lambs on different days	Simple Regression Analysis	0	41	IgG= 8.043+(0.449 x TP)	0.025	0.327
		1	41	IgG= (92.16 x TP)-3960.9	0.562	<0.0001
		2	41	IgG= (77.42 x TP)-2826.3	0.540	<0.0001
		4	41	IgG= (84.36 x TP)-3562.2	0.462	<0.0001
		7	41	IgG= (50.46 x TP)-1723.2	0.385	<0.0001
		14	41	IgG= (37.75 x TP)-1294.7	0.532	<0.0001
		28	41	IgG= (33.75 x TP)-1032.5	0.402	<0.0001
	Multiple Regression Analysis	0,1,2,4,7,14,28	287	IgG= [(73.4 x TP) - (29.74 x day)] - 2755.6	0.701	<0.0001
		1,2,4,7,14,28	246	IgG= [(72.4 x TP) - (31.75 x day)] - 2657.5	0.617	<0.0001
		1,2,4,7,14	205	IgG= [(72.1 x TP) - (79.2 x day)] - 2419.9	0.610	<0.0001
		1,2,4,7	164	IgG= [(76.7 x TP) - (125.2 x day)] - 2608.1	0.540	<0.0001
		1,2,4	123	IgG= [(83.8 x TP) - (64.4 x day)] - 3239.3	0.531	<0.0001
		1,2	82	IgG= [(83.4 x TP) + (67.1 x day)] - 3398.1	0.547	<0.0001
Simple regression analysis according to the results of clinical examination	Neonatal period	1	325[a]	IgG= (57.84 x TP) - 2047.9	0.451	<0.0001
		1	268[b]	IgG= (57.25 x TP) - 2000.5	0.327	<0.0001
		1	57[c]	IgG= (58.12 x TP) - 2049.4	0.673	<0.0001
		1	44[d]	IgG= (58.29 x TP) - 2074.8	0.567	<0.0001
		1	13[e]	IgG= (47.24 x TP) - 1649.2	0.616	<0.0001
	Postneonatal period	1	312[a]	IgG= (57.52 x TP) - 1988.6	0.381	<0.0001
		1	208[b]	IgG= (60.79 x TP) - 2181.9	0.383	<0.0001
		1	104[c]	IgG= (48 x TP) - 1429.2	0.322	<0.0001
		1	89[d]	IgG= (43.17 x TP) - 1156.5	0.289	<0.0001
		1	15[e]	IgG= (67.15 x TP) - 2423.8	0.483	0.0004

[a] *General (without categories based on clinical examination),* [b] *healthy,* [c] *ill,* [d] *recovered,* [e] *died*

A significant (P<0.0001) linear relationship was noted between SIgGC-24 and STPC-24 in healty, diseased, recovered and dead lambs (R^2=0.327, R^2=0.673, R^2=0.567 and R^2=0.616, respectively, *Table 3)* in neonatal period. STPC and SIgGC measured at 24 h after birth were associated with developing diseases in the first week of life; but, this was not evident during the last 3 weeks of neonatal life *(Table 4)*.

A significant linear relationship was found between SIgGC-24 and STPC-24 in health, ill, dead and recovered lambs (R^2=0.383, P< .0001; R^2=0.322, P< .0001; R^2=0.483, P< .004 and R^2=0.289, P< .0001, respectively, *Table 3)* in post-neonata period..

SIgGC-24 and/or STPC-24 markedly differed between sick, dead and recovered lambs in the neonatal and postneonatal periods of life. This difference was most obvious between the first week of life and the last three weeks of the neonatal period. Additionally, SIgGC-24 or STPC-24 was lower in clinically ill and dead lambs in the neonatal period compared to the same groups in the postneonatal period *(Table 4)*.

The levels of SIgGC-24 were allocated into various categories *(Table 5)*. As SIgGC-24 concentrations increased morbidity and mortality rate decreased in neonatal period. The critical threshold of SIgGC-24 for increased risk of mortality and morbidity in the neonatal period was <200 mg/dL (OR=Undefined x^2=293, P=0.0000) and ≤600 mg/dL (OR=107.7 x^2=76.5, P=0.0000), respectively. The morbidity risk of lambs with SIgGC-24 <800 mg/dLwas approximately 4.4 times higher in postneonatal period when compared with lambs having SIgGC-24 above 800 ng/mL (OR=4.37 x^2=6.5, P=0.024). However, no specific SIgGC-24 was determined for mortality in this period *(Table 5)*.

The STPC-24 was also categorised *(Table 6)*. As STPC-24 concentrations increased the morbidity and mortality rate decreased during the neonatal period. The critical threshold of STPC-24 for mortality and morbidity in the neonatal period was ≤45 g/L (OR=51.56 x^2=103.8, P=0.000) and ≤55 mg/dL (OR=256.6 x^2=173.2, P=0.000), respectively. The morbidity risk of lambs with STPC-24 <70 g/L was 2.5 times higher in postneonatal period when compared with lambs having STPC-24 above 70 g/L (OR=2.54 x^2=14.5, P=0.00001). However, no specific TP level that increased the risk of death in this period was identified *(Table 6)*.

Colostral IgG concentrations (CIgGC) (n=169) ranged from 1337 to 12877 mg/dL (mean±SD, 6078 ± 2526 mg/dL) and colostral TP concentrations (CTPC) (n=254) were between 14 and 98 g/dL (42±18 g/dL). There were significant correlations (R^2=0.683, P=0.000) between these parameters *(Table 7)*. Furthermore, a significant linear relationship (R^2=0.460, P<0.0001) was determined between CTPC and CIgGC (Regression model; CIgGC=1941.6+91.35*CTPC, *Fig. 2)*. However, there was no significant correlation between STPC orSIgGCin lambs and CTPC or CIgGC *(Table 8)*.

CTPC and CIgGC were significantly (P<0.05) higher in dams

Table 4. *Effect of STPC(g/L) and SIgGC(mg/dl) determined at 24 h of birth on health status of lambs in neonatal and postneonatal periods[a]*

Periods		Health Status			
		Healthy	Ill	Outcome	
				Dead	Recovered
1st week	IgG	2319±1097 (271-5302)	475±508[b***] (19-1601)	54±32[b***] (19-113)	861±419[b***, e***] (271-1601)
	n	324	23	11	12
	TP	74±11 (44-117)	46±11[b***] (21-74)	38±8[b***] (21-46)	52±10[b***, e**] (35-74)
	n	302	23	11	12
2nd to 4th week	IgG	2337±1087 (527-5302)	2179±1174[c***] (271-4837)	1768±687[c***] (1283-2254)	2203±1198[c***] (271-4837)
	n	287	37	2	35
	TP	75±11 (44-117)	72±14[c***] (46-99)	62±4[c**] (60-65)	72±14[c***] (46-99)
	n	268	34	2	32
Whole Neonatal	IgG	2337±1087 (527-5302)	1526±1279[b***, d*] (19-4837)	318±674[b***, d***] (19-2254)	1860±1205[b**, e***] (271-4837)
	n	287	60	13	47
	TP	75±11 (44-117)	61±18[b***, d***] (21-99)	42±11[b***, d***] (21-65)	66±16 [b***, e***] (35-99)
	n	268	57	13	44
Postneonatal	IgG	2409±1113 (371-5302)	1982±1067[b**] (271-5017)	2311±1307 (571-4837)	1925±1018[b***] (271-5017)
	n	225	109	16	93
	TP	76±11 (46-117)	70±12[b***] (35-99)	72±14 (53-99)	70±12[b***] (35-92)
	n	208	104	15	89

*[a] Data are presented as the mean±SD. Numbers in parentheses represent the range of values in that group. Independent samples T-test was used to detect differences in the STPC-24 or SIgGC-24 among healthy, sick, dead and recovered lambs; [b]Significantly different from healthy lambs (b***= P<.0001, b**= P<0.01, b*=P<0.05); [c] Significantly different in the same group between the first week and the last three weeks in the neonatal period within the same parameters (i.e. comparison of STPC-24 in sick lambs between the two different periods, c***= P<0.001, c**= P<0.01, c*= P<0.05); [d] Significantly different in the same group between in neonatal and postneonatal periods within the same parameters (d***= P<.0001, d**= P<.001, d*= P<0.05); [e]Significantly different from deceased lambs (e***=P<0.001, e**=P<0.01, e*=P<0.05)*

of healthy neonates compared to those of sick and recovered lambs (P<0.05) but these values did not significantly differ in neonatal death (P=0.054 and P=0.125, respectively). Similarly, CTPC and CIgGC were not associated with diseases or death in the postneonatal period *(Table 7)*.

Maternal STPC and SIgGC did not significantly differ between healthy and sick and recovered lambs in the neonatal as well as postneonatal period *(Table 7)*.

The mean concentration of maternal STPC and SIgGC(n=41) 10-15 days prior to lambing was 62.4±6.9 g/L (range 52.9-78.3) and 5.463±1.870 mg/dL (range 1.846-11.766), respectively, and there were significant (R=0.454) correlations between these parameters. A significant positive correlation (P=0.01, R=0.388) existed between maternal SIgGC and at of lamb born to them. A significant (R=0.314, P=0.045) positive correlation existed between the SIgGC of dams and STPC of their lambs. Additionally, the correlations between maternal STPC and SIgGC of their lambs was positive and significant (R=0.342, P=0.029) *(Table 8)*.

DISCUSSION

Veterinarians often use tests for passive transfer to guide their decisions in diagnostic, treatment and protection in neonatal diseases. Determination of passive transfer status (PTS) results in two important consequences; either sufficiency which suggests better health and flock management system or insufficiency which requires diagnosis and treatment of such individuals and taking maximum preventive measures [5,8,9]. PTS determination of newborns is also valuable in routine herd health programs, observation during disease investigations and in the assessment of individual neonates with questionable colostral intake [8,9,24]. Passive transfer of immunity prevents disease and enhances growth performance not only in the short term, such as in neonatal period, but also in the long term, such as the post-weaning period in lambs [5,8,9,18,27]. Inadequate PTS may not be effect health provided that sanitation is excellent, but adequate PTS may be sufficient even if poor sanitation and extreme infectious pressure exist [8,9,27]. FPT in sick neonatal ruminants suggests a poor prognosis [5,8,9,24]. Therefore, quick and accurate tests for determining FPT applied on farm are ofparamount importance.

No currently available assay procedure is entirely satisfactory [5,8,9,20,27]. Being capable of directly measuring IgG concentration, ELISA has become available for use in calves

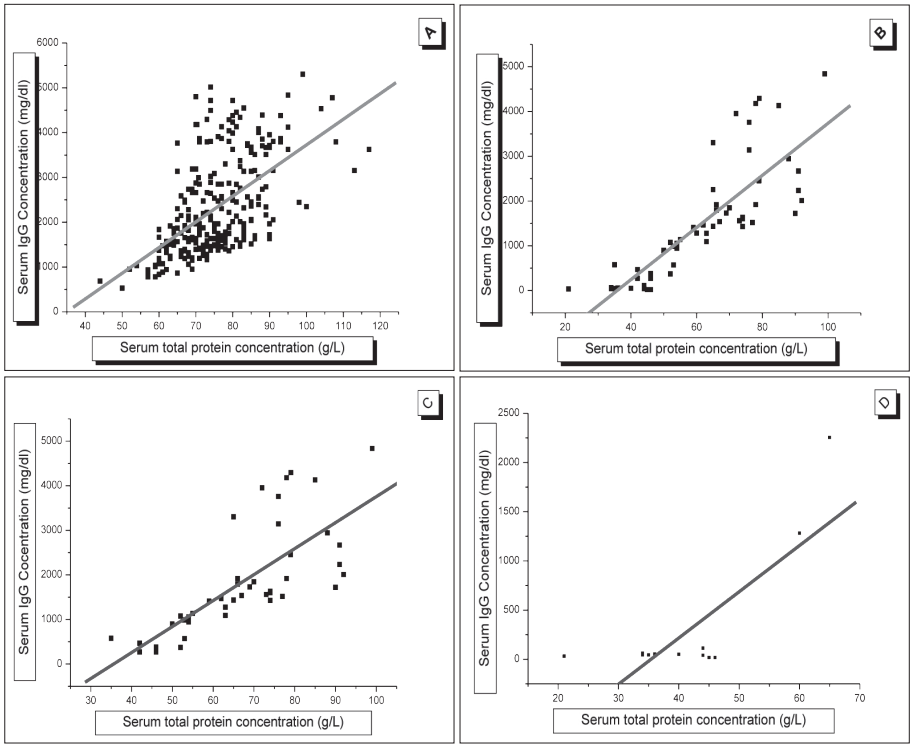

Fig 1. Scatter diagrams illustrating the associations between SIgGC-24 and STPC-24, observed in 268 healthy (A), 57 sick (B), 44 recovered (C) and 13 diseased (D) lambs in the neonatal period. Each data point represents a value for 1 lamb, and each regression line represents the best fit for the data

Table 5. *Lamb morbidity and mortality associated with various categories of SIgGC at 24th h after the birth*

IgG (mg/dL)	Neonatal Period				Postneonatal Period			
	Morbidity		Mortality		Morbidity		Mortality	
Categories	n^1/n^2	%	n^1/n^2	%	n^1/n^2	%	n^1/n^2	%
1-200	11/11	100	11/11	100	0/0	0	0/0	0
201-500	6/6	100	0/6	0	4/6	66.7	0	0
501-600	2/3	66.7	0/3	0	2/3	66.7	1/3	33.3
601-800	0/3	0	0	0	2/3	66.7	0	0
801-1000	3/14	21.4	0	0	6/14	42.9	2/14	14.3
1001-1500	11/67	16.4	1/67	1.5	29/66	43.9	3/64	4.5
1501-2000	11/87	12.6	0	0	25/87	28.7	2/87	2.3
2001-2500	5/42	11.9	1/42	2.4	15/41	36.6	2/41	4.9
>2501	11/114	9.6	0	0	26/114	22.8	3/108	5.3

n^1= number of sick or dead lambs, n^2= total number of lambs in this category

and there is only one study evaluating passive immunity in healthy neonatal lambs [5,25] and there is no such study conducted in diseased lambs. In the current study we used ELISA procedure designed to directly determine serum or colostral IgG concentrations in dams and their lambs. ELISA seems to have some advantages in terms of cost, time, and capacity for measuring large number of samples at once, better diagnostic performance over SRID, a gold standard test [20].

The optimum period to determine passive transfer is 24 h of life because neonatal ruminants are capable of absorbing many proteins, including macromolecular substances due to nonselective absorption by intestines within the first 24 h [8-10]. In this study mean SIgGC-24 determined before colostrums intake was in agreement with previous reports [5,21] and increased significantly after colostrum ingestion. The IgG concentration of day 1 was similar to that reported previously by researchers [5,22,27] but lower than that of others [16,21,28]. This variation might be related to the number of subjects investigated as previous studies used small number of animals, controlled colostrum intake and farm management system (vaccination, good hygiene and feeding practices etc.) [13,28].

Our study found that STPC (40±6 g/L) prior to colostrum intake (hour 0), similar to levels expected in severe passive immune deficiency, were lower than the levels (58-69 g/L) reported by Oztabak and Ozpinar [29], but similar to data from Pauli [30], 40.7 g/L. To the best of our knowledge, there are no other studies in which STPC is determined prior to colostrum intake. The mean STPC on different days of the first week of life (70-73 g/L) in our study was similar to figures reported by Brujeni et al.[16], but comparatively higher than

Table 6. *Lamb morbidity and mortality rates associated with various categories of STPC at 24 h after the birth*

TP (g/L)	Neonatal Period				Postneonatal Period			
	Morbidity		Mortality		Morbidity		Mortality	
Categories	n^1/n^2	%	n^1/n^2	%	n^1/n^2	%.	n^1/n^2	%
1-40	7/7	100	6/7	85.7	1/1	100	0/1	0
41-45	6/7	85.7	4/7	57.1	3/3	100	0/3	0
46-50	5/6	83.3	1/6	16.6	2/5	40	0/5	0
51-55	7/9	77.8	0/9	0	5/9	55.6	2/9	22.2
56-60	2/18	11.1	1/18	5.5	8/17	47.1	2/17	11.8
61-65	6/30	20	1/30	3.3	14/29	48.3	2/29	6.9
66-70	5/52	9.6	0/52	0	21/52	40.4	2/52	3.8
71-75	5/59	8.5	0/59	0	17/59	28,8	2/59	3.4
76-80	7/55	12.7	0/55	0	12/55	21,8	1/55	1.8
81-117	7/82	8.5	0/82	0	21/82	25.6	4/82	4.9

n^1= number of sick or dead lambs in this category, n^2= total number of lambs in this category

Table 7. *Relationship between maternal serum or colostral TP and/or IgG concentrations [mean±SD, (Min-Max)] and lamb health status*

Period	Sample		Clinical Examination			
			Healthy	Ill	Outcome After the Illness	
					Died	Survived
Neonatal	Serum	TP (g/L)	62±7 (53-78)	62±6 (56-73)	-	62±6 (56-73)
		IgG (mg/dL)	5578±2060 (1846-11766)	5054.8±890 (3192-5938)	-	5054.8±890 (3192-5938)
		N	32	9	0	9
	Colostrum	TP (g/dL)	43±18 (15-98)	35± 14[a] (14-82)	41±18 (25-82)	27±33[a] (14-55)
		N	219	35	8	27
		IgG (mg/dL)	6327±2392 (1337-12594)	5123.5±282[a] (1800-12877)	4702±2930 (1887-11127)	5269.4±2829[a] (1800-12877)
		N	134	35	9	26

[a] Significantly lower than healthy lambs (P<0.05)

Table 8. *Correlations between maternal serum or colostral TP and IgG concentration and serum TP and IgG concentration at 24 h after birth in lambs*

Correlations		TP[l]	IgG[l]	TP[c]	IgG[c]	IgG[d]	TP[d]
TP[c]	Pearson Correlation	-0.018	0.018	1.000	.683**	-0.113	-0.160
	Sig. (2-tailed)	0.777	0.781		0.000	0.599	0.457
	N	238	254	254	154	24	24
IgG[c]	Pearson Correlation	0.073	-0.009	0.683**	1.000	0.041	-0.241
	Sig. (2-tailed)	0.353	0.911	0.000		0.881	0.368
	N	162	169	154	169	16	16
IgG[d]	Pearson Correlation	0.314*	0.388*	-0.113	0.041	1.000	0.454**
	Sig. (2-tailed)	0.045	0.012	0.599	0.881		0.003
	N	41	41	24	16	41	41
TP[d]	Pearson Correlation	0.230	0.342*	-0.160	-0.241	0.454**	1.000
	Sig. (2-tailed)	0.148	0.029	0.457	0.368	0.003	
	N	41	41	24	16	41	41

[l] Lamb, [c] Colostrum, [d] Dam

that reported by Loste et al.[28], Massimini et al.[5], Pauli [30] and lower than that of Bekele et al.[2] and Oztabak and Ozpinar [29]. In our study, the mean STPC on day 14 (63 g/L) was lower than that reported by Brujeni et al.[16], Oztabak and Ozpinar [29], but comparatively higher than that of Loste et al.[28]. The mean STPC at day 28 (59 g/L), was lower than that reported by Brujeni et al.[16], but comparatively higher than that reported by Loste et al.[28]. Differences in the course

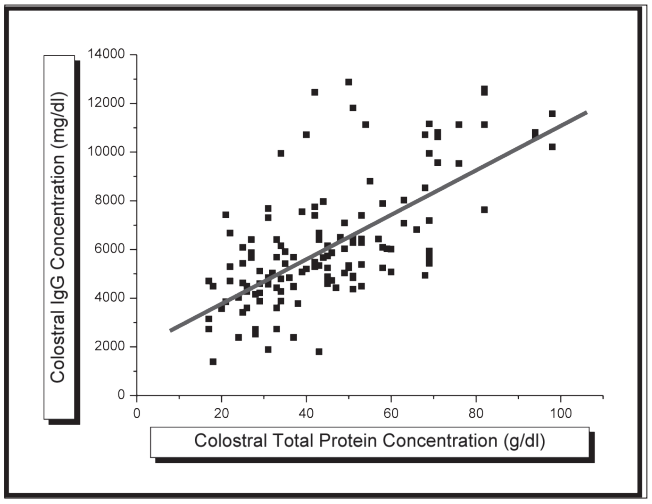

Fig 2. Scatter plot of colostral IgG and TP concentrations observed in 154 sheep. In the graph, the solid line represents the best fit for the data, as determined by means of simple linear regression

of STPC during the neonatal period might be explained based on methods used for measuring STPC such as refractometry [5,23,27], the biuret method [15,27], serum protein electrophoresis [16], spectrophotometric analysis also used in our study adapted to the biuret method [29] and automated biochemical analyzer [28] and management practices such as colostrum quality, the amount of colostrum taken, feeding program implemented. In our study STPC and SIgGC peaked within 24 h after colostrum intake in healthy lambs, generally remained stable during the first week and then declined significantly on day 14 and 28 of the neonatal period. Other studies have reported similar findings [16,28,29]. However, some studies have shown that STPC and SIgGC on day 30 were slightly higher than that of day 15 and have claimed that this could be associated with the balance between resecretion mechanisms and new IgG production or early activation of the immune system in lambs [5,16]. In addition, production of indigenous IgG following antigenic stimulation of the lambs' immune system may cause the slight increase in IgG concentration around day 30 [16]. However, STPC and SIgGC on day 28 were lower than those of day 14 in this study.

In the present study, neonatal losses were mainly encountered in the first week of life (84.6%) as reported earlier [3,11,13-15]. Accordingly, SIgGC were significantly lower in both ill and dead lambs in the first week of life when compared with other periods (last three weeks of neonatal life and postneonatal period) and thus making passive immunity a key factor in the first week of neonatal life. On the other hand, a study by Bekele et al.[2], reported that passive immunity had no effect on neonatal lamb mortality, but the threshold value of serum Ig set above 2300 mg/dL for adequate passive immune status was quite high compared to other studies [5,29]. Universally accepted optimal threshold value of IgG by the veterinary community, below which FPT occurs in lambs, does not exist. Information regarding the risk of illness or death associated with varying categories of SIgGC is limited for lambs [5,22]. Studies, using various threshold values of different indicators, have revealed that 24 to 36 hour-old lambs had an increased risk of death when SIgGC were below 800 mg/dL [11], 1.500 mg/dL [8], 600 mg/dL(IgG_1) [13] and 500 mg/dL(γ-globulin) [14]. The lamb mortality rates of aforementioned studies were 46% (27/59), 18% (2/11), 45% (9/20) and %60 (3/5), respectively. In our study, the mortality rate in lambs having SIgGC-24 below 500 mg/dL, 600 mg/dL, 800 mg/dL and 1500 mg/dL was 64.7% (11/17), 52.4% (11/20), 47.8%(11/23) and 11.5% (12/104), respectively. This finding is in accordance with many studies but slightly differs from some [10,13]. However, use of different strata of SIgGC may be misleading as all lambs having SIgGC-24 concentrations below 200 mg/dL died in our study. Therefore SIgGC-24 below 200 mg/dL may be considered as a significant threshold value for lamb mortality. A study in which the same categorization criteria as ours was used disclosed that the mortality rate in 36-hour-old lambs with serum IgG_1 concentrations below 1.000 mg/dL was 3 to 4 times greater when compared with higher concentrations [1]. However, the mortality rates greatly differed between our study and that of Gilbert et al.[1]. The reasons for this difference may be the cut off value used by Gilbert et al.[1] as the value was much greater and thus variation in mortality might have been wider and also the method used for measurement of IgG concentration was SRID. Studies disclosed that the threshold value of IgG below which passive immunity develops is <1000 mg/dL when SRID used while it was <500 mg/dL when ELISA used and it is known that SRID is prone to overestimation and ELISA is considered more specific [20,26]. It may be concluded that SIgGC-24 >200 mg/dL for 1-day old lambs may be a reasonable goal for producers to decrease the risk of death associated with FPT as all lambs below this figure died in the present study.

The STPC-24 was significantly lower in lambs that died compared to lambs which were healthy or recovered during the first week and neonatal period. This was accordance with previous studies [14,15]. Some studies considered that lambs with STPC-24 of less than 50 g/L [15] or 58 g/L [17] to be hypogammaglobulinemic and claimed that the risk of death in those lambs was high. Our study indicated that STPC-24 of 45 g/L or less could be a threshold that increases the risk of mortality in the neonatal period as 10 of the 13 lambs died in the neonatal period had STPC-24 ≤45 g/L. A close and linear relationship was found between SIgGC and STPC on day 1 in lambs died in the neonatal period, and 62% of the variation in SIgGC could be explained in association with STPC *(Fig. 1 D)*. Furthermore, all lambs with STPC-24 ≤45 and died had SIgGC-24 values of less than 200 mg/dL *(Table 2)*. This level was established as the SIgGC threshold that increases the risk of mortality in the neonatal period in the present study. Therefore, STPC-24 of 45 g/L or less and SIgGC-24≤600 mg/dL could be used as a threshold for passive immunity that indicates a high risk of death in the neonatal period.

In contrast to neonatal calves [31], the relationship between healthy neonatal lamb and SIgGC has not yet been studied in detail and no widely accepted threshold value of SIgGC associated with risk of developing illness is available. The present study revealed that the risk of morbidity was much greater in lambs having SIgGC-24 below 600 mg/dL than those having SIgGC-24 above 600 mg/dL. This figure may be a candidate for threshold concentration for disease development in neonatal lambs. There islimited number of studies where passive transfer deficiency is indicated by cut off values obtained using indirect methods in lambs. Zinc sulfate turbidity (ZST) test was utilized and the results were designated by several researchers as <12 [12] and <20 units [15] in 1- to 2-day-old lambs. The model predicting STPC based on ZST units for 1-day-old lambs revealed that STPC of 5.2 and 5.4 g/dL were equivalent to 10 and 12 ZST units, respectively [18]. This was the only study exist in the literature relating STPC with neonatal diseases in lambs and no STPC threshold was used for morbidity risk. However, this issue has extensively been researched in calves [5,8,9,32]. In our study, the sick lambs had significantly lower STPC-24 than healthy lambs. The morbidity rates in lambs with STPC-24 ≤55 g/L was 3.9 to 11.8 times higher than those with STPC-24>55g/L and close and linear correlations noted between SIgGC-24 and STPC-24 in sick neonatal lambs. Additionally, SIgGC-24 of 23 lambs out of 29 lambs with STPC-24 ≤55 g/L was below the IgG cut-off value (≤600 mg/dL), raising risk of morbidity. These data may indicate that STPC-24 ≤55 g/L could be cut off value predicting illness in advance.

Our study revealed that STPC-24 and SIgGC-24 were not associated on mortality rate of lambs in the neonatal period. However, other studies conducted in calves and lambs [8,9,14,15,31] showed that TP and IgG concentrations or passive immunity were associated with deaths in post-neonatal period and can be used to predict outcomes. In our study, STPC-24 and SIgGC-24 ranges had no significant prevention effects on death of lambs in terms of post-neonatal period. This may be attributed to inappropriate management regimens (poor sanitation, overcrowded housing, absence of vaccination), environmental conditions (temperature, season) as reportedcalves up to 12 weeks [15,31,33].

In the present study, the mean CIgGC was close to that of Maden et al.[21], comparatively higher than that of Zarilli et al.[34] and lower than other studies [1,10,28]. Dams'CIgGC was 2-3 times higher than SIgGC of respective lambs at 24-72 h of birth and did not correlate which each other as reported previously [1]. Lambs born to ewes with low CIgGC and CTPC were more likely to be exposed to disease in our study as reported by Khan et al.[15], but opposite results were also disclosed [13]. No correlation was detected between CIgGC or CTPC and lamb serum TP or IgG concentrations. High CIgGC could not always be protective in lambs due to delayed lactogenesis, malnutrition or under nutrition during pregnancy, poor colostral management (delay in colostrum intake, inadequate amount of colostrum, colostrum quality etc.), infections, prematurity, mismothering, dams' health, low birth weight and weakness of neonates, cold exposure, crowded housing [1,13,28,32]. These factors may have played a role in the present study. Quigley et al.[32] reported a significant linear correlation between colostral protein and IgG (R^2=0.510) in cows. Similarly, a significant correlation (R^2=0.683) and a linear relationship (R^2=0.460) was found between colostral TP and IgG concentrations in our study indicating that TP levels could be beneficial in evaluating colostrum quality.

A positive correlation was found between STPC prior to lambing in ewes and SIgGC-24 in lambs. Similarly, a positive correlation was found between SIgGC in ewes and STPC-24 or SIgGC-24 in their lambs. In addition, positive correlations were found between the individual values of IgG and TP in ewes before lambing. These results were in agreement with that of Andres et al.[17]. Therefore, measuring serum IgG or TP in ewes before lambing would be a valuable indicator of the risk of lamb diseases or passive immunity. Andres et al.[17] suggested that low immunity in lambs may be caused by a lower level of gamma globulin (<1.3 g/dL) and TP (<5.9 g/dL) in the dams. In the present study, mortality rates in the lambs of dams with STPC ≥59 g/L were low. However, dam's serum IgG or TP concentration seemed to have no effect on lamb's health [28] that maternal STPC measured 10-15 days before birth in the first two months had no effect on lamb mortality as reported in the present study.

Passive immunity and growth performance are critical for lambs in the neonatal period [18]. Determining passive immune status accurately and in a timely fashion is important for taking preventive measures [5,22]. In the present study, the accuracy of STPC, a test that can be adapted to the field, for determining passive immunity was demonstrated. The linear models that were used to determine SIgG-24 from STPC-24 in lambs that were healthy (R^2=0.33 to 0.70), diseased (R^2=0.67) or died (R^2=0.62) in the neonatal period were sufficient. However, the calculation of IgG concentrations based on TP concentrations was not clearly specific according to the linear regression formulas. Our study provides useful information for practicing veterinarians in terms of validating an alternative way that is measurement of STPC for FPT in individual lambs and colostrum quality could contribute to the development of passive transfer monitoring programs. Measurement of STPC for FPT in individual lambs and colostrum quality, compared to other tests such as IgG measurement (SRID or ELISA), does not require expensive instrumentation, provides an immediate result, and is also adaptable to field use. These advantages should be beneficial for timely management and therapeutic decisions [5,27]. Furthermore, in our model SIgGC could be calculated from STPC with an accuracy of 70% at any day of neonatal period of lambs.

Overall the present study disclosed that the first week of neonatal life was critical for lamb's health and passive transfer of immunity was of paramount importance for maintenance of health. The study also revealed critical cut off point of SIgGC and STPC at 24 h after birth for increased risk of disease and death in the both periods. STPC using a

<55 g/L and SIgG-24 ≤600 mg/dL threshold value resulted in correct classification of the highest percentage of lambs with regard to their passive transfer status. It was also noted that CIgGC and CTPC have essential role in prevention of diseases in lambs. Furthermore, there was a significant linear relationship between CIgGC and CTPC. Therefore, serum TP concentration was an important consideration in determining passive transfer of immunity and colostrum quality in sheep farms.

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