Defining optimal thresholds for digital Brix refractometry to determine IgG concentration in ewe colostrum and lamb serum in Scottish lowland sheep flocks

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A B S T R A C T

This research aimed to define thresholds for ewe colostrum and lamb serum Brix refractometer measurements in lowland Scottish sheep. This would facilitate the use of this convenient, sheep-side test, enabling quick and accurate identification of poor quality colostrum and prevention of failure of transfer of passive immunity (FTPI) in lambs. Secondary aims were to identify risk factors for poor colostrum quality and FTPI in lambs. Serum samples (n = 233) were collected from lambs between 24 and 48hrs after birth, from four lowland Scottish meat sheep farms. Pre-suckle colostrum samples (n = 112) were also collected from ewes on two of these farms. Farmers provided information on litter size, ewe body condition score, ewe breed and dystocia. Duplicate digital Brix refractometer measurements were compared with immunoglobulin G (IgG) radial immunodiffusion (RID) testing for all colostrum and serum samples. Receiver operating characteristic (ROC) curves were used to redefine thresholds for Brix testing in colostrum and serum. Linear regression models were constructed with colostrum and serum IgG concentration as the outcomes of interest. Colostrum and serum IgG concentrations were highly variable. The prevalence of inadequate colostrum quality (using <50 g/L IgG on RID) was 4.5% (95% CI = 1.5 – 10.1) and the prevalence of FTPI (using <15 g/L IgG in serum on RID) was 7.73% (95% CI = 4.64–11.93). A ewe's colostrum IgG concentration was significantly and positively associated with the serum IgG concentration of her lamb(s) (p = 0.02). ROC analysis defined a Brix threshold for adequate colostrum quality of > 22.10% (sensitivity 80% (95%CI=28.4–99.5), specificity 90% (95%CI=82.3–94.8)). ROC analysis defined a Brix threshold for serum of > 8.65% for adequate passive transfer of immunity in Scottish lambs (sensitivity 94% (95%CI=72.7–99.8), specificity 82% (95%CI=76.6–87.2)). To optimise passive transfer of immunity in lambs, we suggest that ewe colostrum Brix measurements be defined as 'poor' (<22%); 'fair' (22–26%) and 'good' (>26%); and lamb serum as 'poor' (<8%); 'fair' (8–9%) and 'good' (>9%). It is recommended that these tests are used as for flock screening, using samples from multiple animals.

1. Introduction

The epitheliochorial nature of the ruminant placenta requires that neonatal lambs ingest sufficient high-quality colostrum, promptly after birth, to confer immunity from the dam to the neonate, through a process known as 'passive transfer' (Agenbag et al., 2021). Lambs which suffer from 'failure of transfer of passive immunity' (FTPI) have higher morbidity and mortality rates than their healthy counterparts (McGuire et al., 1983). There is opportunity within the sheep industry to reduce lamb morbidity and mortality rates through identification of poor quality colostrum and to promote effective colostrum management (Christley et al., 2003; Holmøy et al., 2012).

The 3 pillars of colostrum management are: 1) adequate immunoglobulin G (IgG) concentration in colostrum (quality); 2) adequate

quantity of colostrum (Alves et al., 2015); 3) timing of consumption (Dwyer et al., 2016). Colostrum IgG concentrations range widely between individual animals (Kessler et al., 2019), but the optimum threshold is not known for sheep. Two different values have been proposed 20 g of IgG per L (Kessler et al., 2021) and 50 g/L (Dwyer et al., 2016), the former estimated using volume of colostrum produced by ewes and capacity for consumption by lambs, the latter extrapolated from cattle. The relationship between colostrum and lamb serum IgG concentration has not been defined in UK flocks (Page and Lovatt *per comms*), however, lambs from ewes with low pre-suckle colostrum IgG concentrations have been reported to have lower serum IgG concentrations (McGuire et al., 1983). Lamb serum IgG concentrations are maximal 24–48 h after birth (Hernández-Castellano et al., 2015). No universally accepted critical value of serum IgG concentration exists to characterize the 'failure of transfer of passive immunity' (FTPI) in lambs, however, the threshold value of 15 g/L of serum has been widely adopted (Alves et al., 2015; Hunter et al., 1977; Turquino et al., 2011), with the assumption that passive transfer of immunity is not successful below this value. A value of 10 g/L is widely used in cattle (Tyler et al., 1996; Weaver et al., 2000).

The Brix refractometer is a dual-purpose device that can be used 'lamb-side' to measure both colostrum quality and serum IgG concentrations (Santiago et al., 2020). It measures the total solids in a liquid, which approximates IgG concentration in serum and colostrum, through the refraction of light. Brix refractometry has been shown to be highly correlated to direct measures of IgG (ELISA) in sheep colostrum samples (Kessler et al., 2021). An extensive body of literature exists that has defined Brix refractometer thresholds in cattle colostrum and serum (Deelen et al., 2014; Cuttance et al., 2017; Denholm et al., 2022), but little has been published in sheep (Torres-Rovira et al., 2017).

The aim of this research was to define thresholds for dam colostrum and lamb serum Brix measurements in Scottish lowland sheep. Secondary aims were to assess litter size, ewe body condition, breed and exposure to dystocia as risk factors for inadequate colostrum quality and FTPI in the lambs studied.

2. Materials and methods

2.1. Farm recruitment

Four sheep meat farms (Table 1) were recruited to the study in March and April 2022. Three farms were commercial farms with farmer-reported pre-existing neonatal lamb disease and/or mortality, and the fourth was a university-owned farm with a research sheep flock. All samples were collected with appropriate ethical and legal approval (Home Office PPL number PF10145DF and University of Glasgow ethics committee approval reference EA11/22).

2.2. Sample collection and storage

Serum samples were collected from 24 to 48-hour old lambs by jugular venepuncture. On the commercial farms, convenience sampling was employed; whereas on the university farm, samples were collected as part of a larger research study. Blood samples were transported on ice to a laboratory where they were centrifuged (3000 rpm for 15 min). Serum was harvested into sterile plastic tubes and frozen at − 20 °C.

Ewe pre-suckle colostrum samples were collected immediately post-lambing, from a convenient sample of ewes on two farms (Table 1). Teats were cleaned, and samples were collected into sterile universal containers by 2–3 expressions of colostrum directed at a 45° angle into the container. Colostrum samples were stored on ice within 1 h of collection, were transported to the laboratory and frozen at − 20 °C. Litter size, ewe body condition score, ewe breed and dystocia were recorded for each enrolled ewe.

2.3. Radial immunodiffusion measurements

All samples were defrosted and vortexed; colostrum samples were diluted 1:3 with phosphate-buffered saline (PBS) before testing. Where test samples exceeded the range of the assay, they were further diluted with PBS and retested: 31% of colostrum samples were diluted 1:8 (35/112), and 84% of serum samples 1:1 or 1:2 (195/233). IgG was measured in colostrum and serum using radial immunodiffusion (RID) plates (Triple J Farms, Washington USA, Lot 3284A21) following manufacturer instructions. Briefly, 5 μL of each of 3 standard sera were inoculated into the RID plates alongside 5 μL of each colostrum or serum sample in duplicate. The plates were incubated in a moist chamber at 24 °C for 24 h and diffusion zones measured using digital vernier callipers (IP54 Water resistant Louisware electronic, accuracy 0.01 mm, range 0–150 mm). The zonal diffusion measurements for the standard sera were squared and plotted against the IgG concentrations, and the IgG concentrations of the samples calculated. The intra-assay coefficient of variation (CV) for colostrum averaged 3.91% and serum 6.48%.

2.4. Brix refractometer measurements

On the same day as the RID plates were inoculated, the IgG concentration of the test sample was indirectly estimated using a digital Brix refractometer (Brix 0–85%, Model HI96801, Software 2.00, HANNA Instruments, Woonsocket, USA). The refractometer was calibrated with distilled water, before testing, after every 50 samples and if the ambient laboratory temperature changed by more than 5 °C. Briefly, 2–3 drops of colostrum or serum at room temperature (24 °C) were placed on the test well, and the readings were recorded. All samples were tested in duplicate, and a mean measurement for each sample was calculated. To remove any fatty residues, the refractometer well was cleaned between samples. The mean CV for Brix refractometry measurements of colostrum was 2.56% and serum was 2.45%.

2.5. Statistical analysis

All statistical analyses were conducted using Stata (StataCorp LLC

Table 1
Details of the lowland Scottish flocks from which ewe colostrum and lamb serum samples were collected.

Farm	Type	Samples collected	Number of ewes	Scanning percentage	Ewe breed (s)	Sire breed (s)	Pre-lambing nutrition	Feed space
1	Commercial	Ewe pre-suckle colostrum Lamb serum	350	178	Blue faced Leicester, Beltex, Mule, Scottish blackface, Swaledale	BFL, Beltex, Texel	Energy block, 20% crude protein soya roll, grass or hay	> 45 cm
2	Commercial	Lamb serum	400	155	North country hill cheviots	North country hill cheviots, Texels	Haylage, mineral blocks, 18% crude protein ewe rolls	50 cm
3	Commercial	Lamb serum	400	164	Mule	Texel	300 g ewe feed 18%	< 45 cm fully stocked
4	Research	Ewe pre-suckle colostrum Lamb serum	149	111	Easycare	Easycare pure bred	TMR with silage plus 18% crude protein sheep blend, with soya and minerals	50 cm

version 15). Normality of the test measurements was assessed by plotting frequency histograms and performing Shapiro-Wilk tests, and descriptive statistics were calculated.

Receiver operating characteristic (ROC) curves were constructed to determine the optimal threshold for accurately predicting inadequate colostrum quality and FTPI using Brix based on the corresponding RID test results. Colostrum was defined as inadequate quality when RID IgG concentrations were < 50 g/L and < 60 g/L, and lambs were defined as having FTPI when RID IgG concentrations were < 15 g/L and < 10 g/L. Using ROC analysis, the area under the curve (AUC) was determined, indicating the ability of a test to discriminate between inadequate and adequate quality colostrum and between lambs with and without FTPI. The Youden's J statistic was calculated to predict the optimal threshold for each test, based on the sum of sensitivity (Se) and specificity (Sp) being maximised, giving equal weight to false positive and false negative results.

The Se, Sp, likelihood ratio for positive results (LR+) [sensitivity / (1- specificity)], likelihood ratio for negative results (LR-) [(1- sensitivity) / specificity] and accuracy [(true positive + true negative)/total number of samples] of the Brix measurement was calculated using thresholds for inadequate colostrum quality and FTPI.

Linear regression models were constructed using log-transformed colostrum IgG concentration (since colostrum IgG concentrations were not normally distributed) or serum IgG as the outcomes of interest. Univariable models were initially constructed for each risk factor (Table 2). Breed was dichotomised into 'Easycare', and all other breeds and ewe body condition scores (BCS) were categorised into BCS ≤ 2.75; BCS 3–3.25 and BCS ≥ 3.5 BCS. Risk factors with univariable significance of $p \leq 0.2$ were included in further modelling. All biologically plausible interaction terms were explored (including interactions between ewe breed and body condition score and litter size and dystocia), and confounding variables were included if model coefficients varied by > 20%. In all models, farm was included as a fixed effect. Risk factors were excluded from multivariable modelling using a backward, stepwise elimination process, and the likelihood ratio test was used to compare the models ($p < 0.05$). For the subset of matched colostrum and serum samples (where the sampled colostrum was fed to a specific lamb which was also sampled), linear regression was used to determine associations between colostrum IgG and serum IgG concentration (and, therefore, FTPI). Model construction was as described for the colostrum risk factor linear regression models.

Postestimation and model diagnostics were performed using the 'predict' function in Stata for all multilevel logistic regression modelling. Residuals were found to lie within 2 standard deviations of the mean in all cases.

3. Results

3.1. Descriptive results

Table 3 shows the summary statistics for the IgG concentrations and Brix values for colostrum and serum across all four farms. Both colostrum and serum IgG concentrations varied markedly across the samples assayed. The prevalence of inadequate colostrum quality among the ewes tested was 4.5% (5/112, 95%CI=1.5–10.1) using colostrum IgG concentration < 50 g/L and 11.6% (13/112, 95%CI=7.8–16.9) using colostrum IgG concentration < 60 g/L. The prevalence of FTPI in the lambs tested was 4.7% (11/233, 95%CI=2.4–8.3) when using a threshold of 10 g/L IgG and 7.7% (18/233, 95%CI=4.6–11.9) using a threshold of 15 g/L serum IgG.

Fig. 1 shows the relationship between RID and Brix for colostrum and serum. There was high correlation between colostrum RID and Brix (r = 0.74, p < 0.0001) and between serum RID and Brix (r = 0.69, p < 0.0001).

3.2. ROC analysis results

Figs. 2 and 3, show the ROC curves used to determine optimal thresholds for colostrum and serum respectively. Optimal sensitivity and specificity of the test was achieved when the area under the curve (AUC) was greatest and the values of Se, Sp and accuracy were optimised (Table 4). The uppermost left hand corner of the ROC curves maximises test sensitivity and specificity (Nahm, 2022). ROC analysis defined a Brix threshold of < 22.10% and < 25.50% for inadequate colostrum quality when IgG thresholds of < 50 g/L IgG or < 60 g/L IgG were used, respectively. ROC analysis defined a Brix threshold of < 8.30% and < 8.65% for FTPI in Scottish lambs when serum IgG thresholds of < 10 g/L and < 15 g/L were used, respectively. These Brix refractometry thresholds all performed well compared with the RID IgG thresholds, with high Se, Sp and accuracy, high LR+ and low LR-.

3.3. Risk factor analysis

From the two farms on which colostrum samples were collected, ewe colostrum IgG concentrations were positively associated with serum IgG concentrations in the lambs born (n = 141) to those ewes (n = 112) (r = 0.9, SE= 0.04, 95% CI=0.01–0.17, p = 0.02). Farm did not have a significant effect on pre-suckle colostrum IgG concentrations, but litter size was significantly associated with log colostrum IgG concentration (p < 0.01, Table 5). Ewes that had twins produced colostrum with a higher IgG concentration compared with ewes that had a single lamb. No ewe-associated categorical predictor variable or combination of variables were significantly associated with colostrum IgG concentrations.

4. Discussion

This study has established the thresholds for Brix refractometer readings for ewe colostrum and lamb serum for Scottish sheep flocks and demonstrated a positive association between colostrum IgG

Table 2
Categorical predictor variables for poor colostrum quality from ewes from 2 Scottish lowland farms.

Variable	Category	n (%)
Breed	Beltex	3 (2.68)
	Blue faced Leicester	11 (9.82)
	Easycare	88 (78.57)
	Mule	5 (4.46)
	Missing	5 (4.46)
BCS	2	17 (15.18)
	2.5	16 (14.29)
	2.75	1 (0.89)
	3	54 (48.21)
	3.5	7 (6.25)
	3.75	1 (0.89)
	Missing	16 (14.29)
Dystocia	Yes	23 (20.54)
	No	81 (72.32)
	Missing	8 (4.46)
Litter size	Single	43 (38.39)
	Twin	54 (48.25)
	Triplet	7 (6.25)
	Missing	8 (4.46)

Table 3
Descriptive statistics for 24–48 h old lamb serum (n = 233) and pre-suckle colostrum samples (n = 112) collected from Scottish lowland farms in March and April 2022.

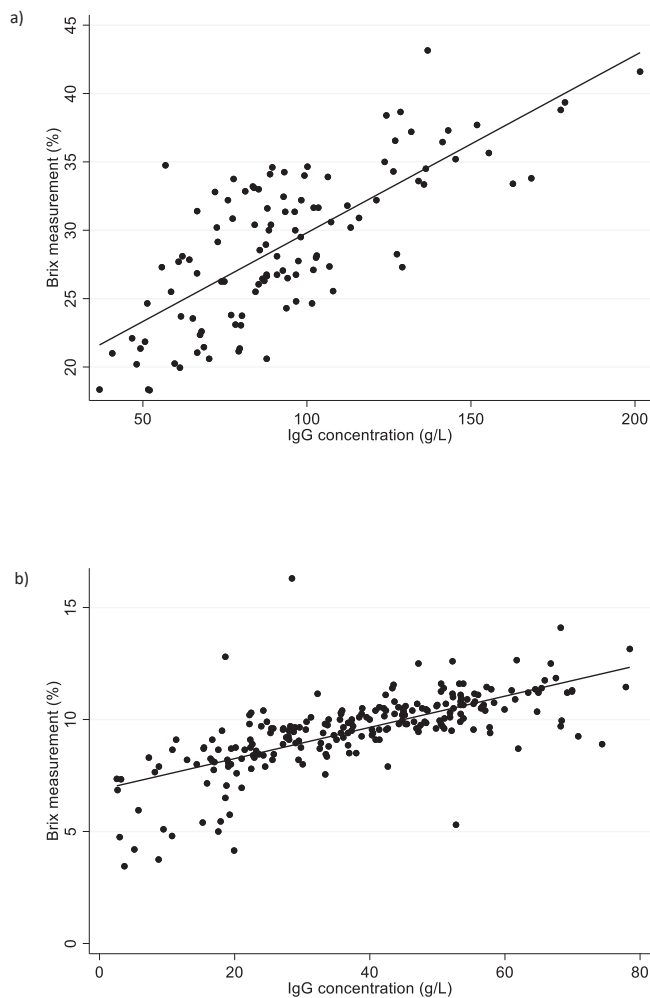| Variable	n	Mean	SD	Minimum	Maximum
Serum IgG (g/L)	233	38.04	16.94	2.56	78.48
Serum Brix (%)	233	9.51	1.72	3.45	16.30
Colostrum IgG (g/L)	112	94.04	31.60	36.78	201.55
Colostrum Brix (%)	112	29.04	5.53	18.30	43.15

a)

b)

Fig. 1. Scatter graphs to show the relationship between digital Brix refractometry measurements (%) and IgG concentration (g/L) (a) in pre-suckle colostrum from 112 ewes from 2 Scottish lowland farms and (b) in 24–48 h old lamb serum from 233 lambs from 4 Scottish lowland farms.

concentration and lamb serum IgG concentration (p = 0.02). These results could be used by Scottish sheep farmers and veterinarians to improve colostrum management within sheep flocks and improve lamb survival.

Brix measurements for colostrum (r = 0.69, p < 0.0001) and serum (r = 0.74, p < 0.0001) were well correlated with RID measures. This result is similar to previous reports of ovine colostrum (where IgG was quantified using ELISA) (Kessler et al., 2021) and goat kid serum (Batmaz et al., 2019). This indicates that Brix can be used as a reliable sheep-side method for the estimation of ewe colostrum and lamb serum IgG concentrations on farm.

ROC analysis defined a Brix threshold of 22.1% for ewe colostrum, when a colostrum IgG threshold of 50 g/L was used. This Brix threshold is in the range of that previously suggested, 26.5% (95% CI: 0.8335–0.9577), by Kessler et al. (2021). ROC analysis also indicated that the sensitivity of Brix measurement as an indicator of poor colostrum quality in our study was moderate (Table 4). It is recommended, therefore, that Brix readings, as an estimate of colostrum IgG concentrations, are used as a screening test rather than an individual animal diagnostic tool. As a screening test, it is desirable to maximise the specificity of the test, and at a Brix threshold of 22.1%, this test has a specificity of 89.7% (95%CI=82.3–94.8), which means that there will be minimal false positive results.

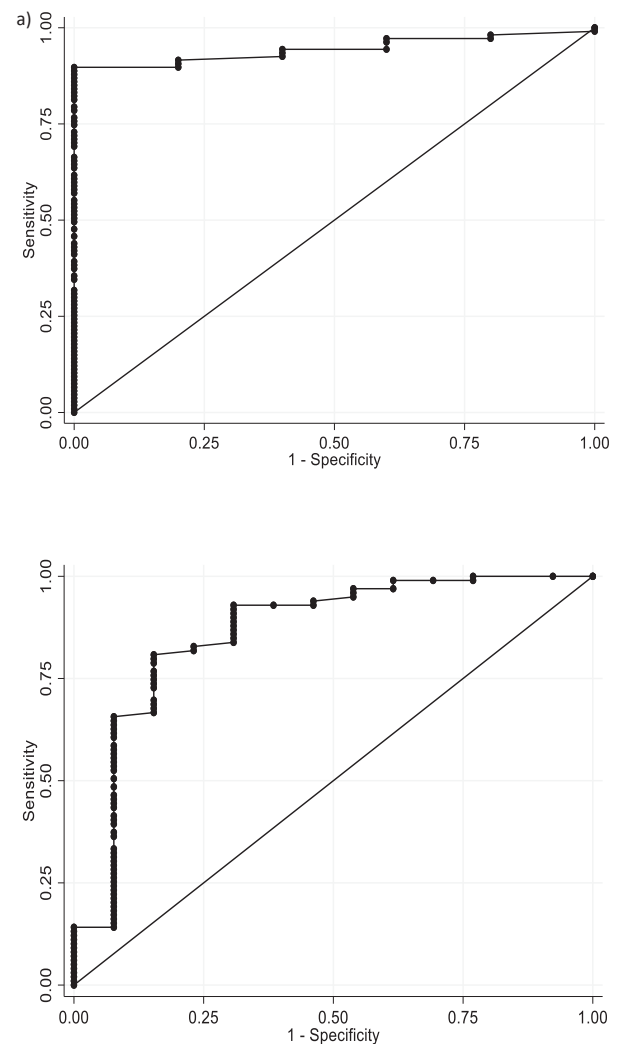While adequate colosturm IgG concentrations are well defined in

a)

b)

Fig. 2. Receiver operating characteristic curves used to determine optimal thresholds for diagnosing inadequate pre-suckle colostrum IgG concentration (n = 112) from 2 lowland Scottish farms (defined as concentration of IgG in colostrum <50 g/L and <60 g/L in graphs (a) and (b) respectively). Area under the curve was 0.94 for colostrum IgG < 50 g/L and 0.86 for colostrum IgG < 60 g/L. Footnote: The uppermost left hand corner of the curve denotes maximal sensitivity and specificity of the test (Nahm, 2022).

cattle (Morrill et al., 2012) two different thresholds have been suggested for ewe colostrum IgG concentrations to facilitate transfer of passive immunity to lambs: 20 g/L (Kessler et al., 2021) and 50 g/L (Dwyer et al., 2016). In the current work, all colostrum samples had IgG concentrations higher than the lower estimate of 20 g/L, and 95.5% of samples had IgG concentrations which exceeded the higher estimate of 50 g/L. These results would suggest that the prevalence of inadequate concentrations of colostrum IgG in our samples was much lower than the previous estimates of UK prevalence of around 20% (Dwyer et al., 2016; Page et al., 2022). This may be explained by a selection bias, as the enrolled farms may have been more proactive in their management of pregnant ewes than the UK average.

Determining which IgG concentration threshold to use for colostrum quality is not simple, as there is little data to support either 20 g/L (Kessler et al., 2021) or 50 g/L (Dwyer et al., 2016). However, the threshold used is important, as demonstrated by the positive association between lamb serum IgG and colostrum IgG in the current study. This association had previously been suggested by McGuire et al. (1983), who reported that lambs from ewes with low pre-suckle colostrum IgG concentrations had lower serum IgG concentrations; but the association
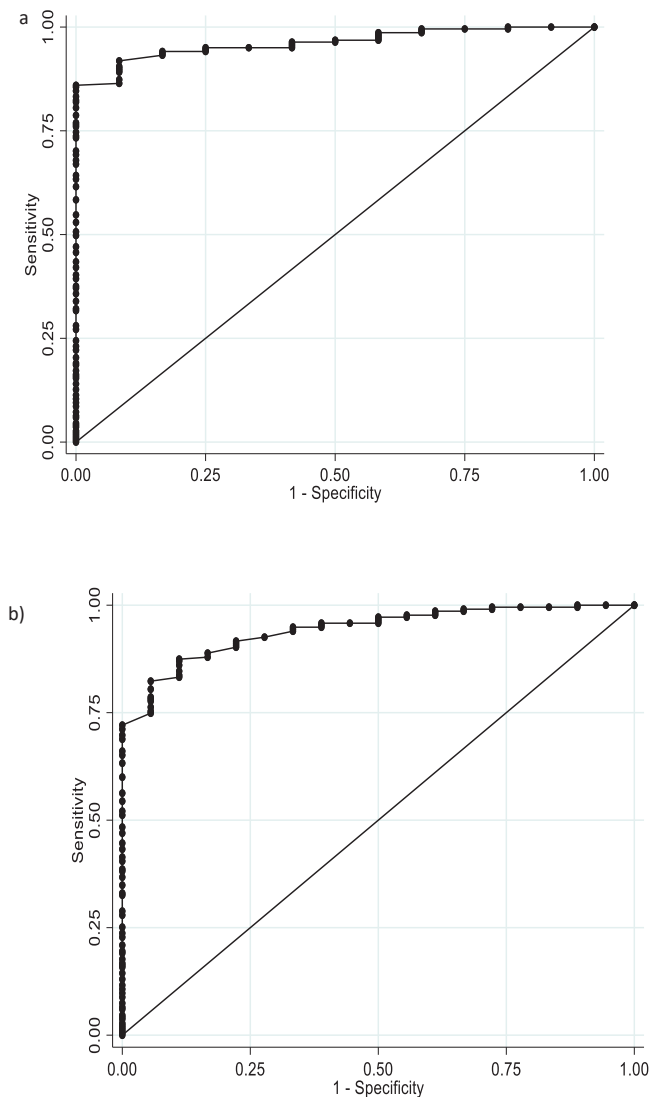
Fig. 3. Receiver operating characteristic curves used to determine optimal thresholds for diagnosing failure to transfer passive immunity in 24–48 h old lambs (n = 233) from 4 lowland Scottish farms (defined as concentration of IgG in serum <10 g/L and <15 g/L in graphs (a) and (b) respectively). Area under the curve was 0.96 for serum IgG < 10 g/L and 0.94 for serum IgG ≤ 15 g/L. Footnote: The uppermost left hand corner of the curve denotes maximal sensitivity and specificity of the test (Nahm, 2022).

had not been clearly demonstrated until now. Despite this positive association, colostrum IgG concentration is only one of the factors in colostrum management that can affect the prevalence of FTPI. Other factors include volume and timing of colostrum ingestion (Alves et al.,

2015; Dwyer et al., 2016) and these two factors should be considered when determining thresholds for adequate colostrum IgG to prevent FTPI in lambs. Measurement of the volume of colostrum ingested by neonatal lambs was beyond the scope of this work, and the timing of the first colostrum feeding was not recorded for all samples and so was not included in the analysis.

Previous work has shown that ewe colostrum production from under- and well-fed ewes averaged 495 ml and 2340 ml per lamb, respectively, in the first 18 h postpartum and increased with litter size (Mellor, 1990). It has been suggested that lambs ingest between 180 and 210 ml/kg bodyweight (BW) of colostrum within the first 18 h of life to prevent FTPI and hypothermia (Mellor, 1990; Banchero et al., 2015), although, Mellor (1990) also reported that lambs fed ad libitum colostrum may consume up to 270 ml/kg BW in the first 18 h of life. In addition, recommendations concerning lamb IgG intake suggest that lambs need to ingest 30 g of IgG in their first 24 h of life (Alves et al., 2015) or between 4 and 8 g IgG/kg BW within the first 24 h (Hernández-Castellano et al., 2015) to prevent FTPI. Consequently, for ewes producing high volumes of colostrum per lamb with good lamb colostrum intake, a colostrum IgG concentration of 20 g/L could be considered adequate. However, where colostrum availability and/or lamb intake may be limited, a threshold of 50 g/L should be used to define colostrum quality.

It has been demonstrated in cattle that more voluminous first-milking colostrum is lower in IgG due to dilution (Pritchett et al., 1991; Chuck et al., 2017; Denholm et al., 2018), but the same has not yet been shown in meat sheep. In fact, in adult sheep both colostrum quality (Amanlou et al., 2011) and volume (Mellor, 1990) have been shown to reduce with reduced nutrition. This is also the case for ewe lambs, although excess feed in has also been demonstrated to have detrimental effects on both colostrum quantity and IgG content within the first three hours post-partum in Rambouillet ewe lambs in the United States of America (Meyer et al., 2011; Swanson et al., 2008). Therefore colostrum volume cannot be used as an indicator of quality.

Using a Brix threshold of 8.65% (equivalent to 15 g/L IgG), as defined by ROC analysis; the LR+ and LR- of the serum Brix test in the current work was good (Table 4). The FTPI (<15 g/L RID) prevalence observed was 7.3%, which was lower than international prevalence estimates of approximately 40% (Alves et al., 2015). The low prevalence of FTPI in lambs in the current study may again reflect the selection bias

Table 5

Final multivariable linear regression model predicting log transformed pre-suckle colostrum IgG concentration (g/L) (n = 112) for ewes from 2 Scottish lowland farms.

Risk factor	Category	Coefficient	SE	95% CI	P-value
Farm	1	ref	ref	Ref	ref
	4	-0.02	0.04	-0.09–0.05	0.54
Litter size	Single lambs	ref	ref	Ref	ref
	Twin lambs	0.11	0.03	0.06–0.16	< 0.01
	Three or more lambs	0.10	0.06	-0.01–0.21	0.08

Table 4

Test results for Brix measurements used to predict inadequate quality of pre-suckle ewe colostrum (defined as colostrum concentration of <50 and <60 g/L) and failure to transfer passive immunity in 24–48 h old lambs (defined as a serum concentration of <10 g/L IgG or <15 g/L determined by RID at 24 h). Brix (%) thresholds were based on optimised values from receiver operating characteristic curve analysis of study data.

Sample	RID threshold (g/L)	Brix threshold (%)	Se (%)	95% CI Se (%)	Sp (%)	95% CI Sp (%)	LR+	LR-	Accuracy (%)
Colostrum	50	22.10	4/5 (80)	28.4–99.5	96/107 (89.7)	82.3–94.8	7.78	0.223	100/112 (89.3)
	60	25.50	10/13 (76.9)	46.2–95.0	81/99 (81.8)	72.8–88.9	4.23	0.282	91/112 (81.3)
Serum	10	8.30	11/12 (91.7)	61.5–99.8	191/221 (86.4)	81.2–90.7	6.75	0.0964	202/233 (86.7)
	15	8.65	17/18 (94.4)	72.7–99.8	177/215 (82.3)	76.6–87.2	5.34	0.0674	194/233 (83.3)

95% CI Se – 95% confidence interval for the sensitivity

95% CI Sp – 95% confidence interval for the specificity

LR+ – Likelihood ratio for detecting poor colostrum or poor passive transfer of immunity

LR- – Likelihood ratio for detecting good colostrum or good passive transfer of immunity

noted above, as the enrolled farms may also have been more proactive in their management of neonatal lambs than the international average.

For its practical use in cattle, Brix estimates of serum IgG used to identify FTPI have been defined as poor, fair, good and excellent (Lombard et al., 2020). Based on the results of the current study, which had moderate Brix sensitivity and high variability within the colostrum and serum IgG concentrations, it is recommended that a similar approach is applied to sheep. As such, we would recommend that ewe colostrum Brix measurements are classified as 'poor' (<22%); 'fair' (22–26%) and 'good' (>26%); and lamb serum Brix measurements are classified as 'poor' (<8%); 'fair' (8–9%) and 'good' (>9%). It is important to stress that given the limitations of Brix refractometry, there is a need for multiple samples (both colostrum and serum) to be collected from any flock at each timepoint throughout the lambing period to draw conclusions on the prevalence of low colostrum IgG concentration and FTPI.

In the current study, ewe colostrum IgG concentrations and lamb serum IgG concentrations compared favourably with those reported in other work (Table 3). The mean colostrum Brix measurements in the current study were 29.04%, similar to that reported for Welsh ewes (28.5%; range=15.4–40%) (Page et al., 2022). Studies by many authors (Gilbert et al., 1988; Swanson et al., 2008; Hernández-Castellano et al., 2015), found mean IgG concentration between 65 and 100 g/L in first milking colostrum (mostly in sheep meat breeds), which corroborates with the mean IgG concentration of 94.0 g/L (range 36.8 – 201.6) found in this study. By contrast, IgG concentration of colostrum collected within 12 h of parturition from Awassi fat-tailed ewes in Turkey, with no monitoring of prior suckling, was reported to average 60.9 $\pm$ 21.4 mg/ml (Higaki et al., 2013). However, this may reflect differences in the time of colostrum sample collection. If lambs suckle colostrum prior to sampling and testing, IgG concentration will decrease. In bovines, this decrease in IgG is due to colostrum sampled after suckling being more akin to second and third milking colostrum and bovine colostrum IgG concentration declines with time (Moore et al., 2005; Morin et al., 2010; Denholm et al., 2018). Ewe colostrum would be expected to behave in a similar manner. Nevertheless, a Brazilian study reported low average colostrum IgG concentrations of 37.15 $\pm$ 3.82 mg/ml from Santa Ines haired meat ewes, when colostrum was collected shortly after parturition and suckling by lambs was not permitted (Alves et al., 2015). In terms of lamb serum, IgG concentration of 38.04 $\pm$ 16.94 g/L at 24–48 h after birth in the current study was higher than reported by Hernández-Castellano et al. (2015) in Gran Canaria (7.04 $\pm$ 2.23 g/L and 8.46 $\pm$ 2.12 g/L at days 1 and 2 postpartum respectively). Whereas another study found lamb serum IgG, measured by ELISA, to be 26.7 $\pm$ 4.4 g/L in lambs 36 h after birth (Alves et al., 2015). These variations between colostrum IgG concentrations and serum IgG concentrations reported in the current study and those reported elsewhere could result from variations in ewe nutrition, flock management and breed. It would be anticipated that the Welsh ewes that had similar colostrum Brix readings would be managed in a similar manner to Scottish ewes, more so than those in Gran Canaria, Turkey or Brazil. These differences in management reflect significant differences in climate, pasture type and output expectations globally.

In the current study, ewes which gave birth to twin lambs were more likely to have higher IgG concentrations in their colostrum than ewes with single lambs. This result is similar to earlier work by (Gilbert et al., 1988; Higaki et al., 2013; Page et al., 2022). The higher IgG concentrations in twin bearing ewes in the current study could be related to husbandry as nutrition of ewes with singleton pregnancies is normally reduced compared with those carrying multiple foetuses, and nutrition is known to affect colostrum quality (Banchero et al., 2015). However, in the study by Page et al. (2022) there was a trend towards supplementary feed increasing the likelihood of adequate colostrum in ewes that gave birth to multiple lambs, but not those that gave birth to single lambs.

Conversely, ewe BCS was not found to be associated with colostrum IgG concentration, contrary to reports by Campion et al. (2019) and a

non-statistically significant trend in Page et al. (2022). This may be because in the current work BCS values at lambing were used rather than changes in BCS between 6–8 weeks prepartum and 24 h postpartum. In the current study, there was also no association between breed and colostrum IgG concentration, however the vast majority of the ewes enrolled were Easycare (78.57%) and all were meat breeds. This differs from the findings of Kessler et al. (2019), who reported colostrum IgG concentration varied significantly between breeds, with dairy breeds having lower IgG concentrations than meat breeds.

5. Conclusion

Defining thresholds for the Brix refractometer should improve the utility of this convenient, sheep-side test by farmers (colostrum samples) and veterinarians (colostrum and serum samples) to quickly and accurately identify inadequate colostrum quality and the possibility of FTPI in lambs. Furthermore, demonstrating an association between colostrum IgG and lamb serum IgG levels improves confidence in colostrum quality being a worthwhile assessment and this link has not previously been demonstrated in UK sheep. Brix refractometry can be used to provide an estimation of colostrum and serum IgG. Three categories for demonstrating colostrum quality and FTPI are suggested as defined by Brix in ewe colostrum: 'poor' (<22%); 'fair' (22–26%) and 'good' (>26%); and in lamb serum: 'poor' (<8%); 'fair' (8–9%) and 'good' (>9%). The thresholds defined from this work for ewe colostrum and lamb serum should improve the accuracy of using a Brix refractometer to identify inadequate colostrum quality and the possibility of FTPI in lambs, which should, in turn, decrease morbidity and mortality in neonatal lambs and positively contribute to farm profitability and sustainability.

Declaration of Competing Interest

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prevetmed.2023.105988.

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