Diagnostic accuracy of a digital Brix refractometer for assessing colostrum quality and failure of passive immune transfer in neonatal lambs

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Introduction

- Failure of transfer of passive immunity (FTPI)
  - Major risk factor for neonatal diseases and mortality (Sawyer et al, 1977, Mc Guire 1983)
  - Influence longevity, future milk production, weight gain in cattle (Raboisson et al, 2016)
  - Still remain a major issue in many flocks, despite widely spread recommendations on colostrum management
- Need to develop simple and cost-effective diagnostic tools
  - To evidence FTPI at the lamb and flock level
  - To investigate colostrum quality as a risk factor
  - To select / discard colostrum for a colostrum bank

Introduction

- Assessment of FTPI in neonatal ruminants
  - Diagnosed when serum IgG concentration
    - < 10.0 g/L (Mc Guire, 1983)
    - < 15.0 g/L (Hunter et al., 1977; Flaiban et al., 2009; Silva et al., 2009; Turquino et al., 2011, Alves et al 2015)
  - Historical gold standard for serum IgG concentration : radial immunodiffusion (RID)
  - Numerous indirect methods already evaluated in calves…
    - Serum Total Protein (STP) (direct or indirect)
      - Brix refractometer
    - GGT
  - … but much less in lambs

Introduction

- Colostrum IgG concentration
  - Cows
    - Increasingly popular in the past decade
    - Poor quality colostrum classically defined when IgG concentration < 50 g/L
    - Indirect methods (i.e. %Brix) largely used (Bielmann et al., 2010; Buczinski and Vandeweerd, 2016; Gross et al., 2017)
  - Ewes
    - High variability of colostrum IgG concentration also (Gilbert 1988, Maden et al 2003, Loste et al 2008, Nunes et al 2006, Hashemi et al 2008, Kessler et al 2019)
    - Use of Brix refractometer : scarce studies (Kessler et al 2021)

Study objectives

- Assessing the diagnostic performances of a digital Brix refractometer for the assessment of
  - colostrum quality in ewes
  - failure of transfer of passive immunity (FTPI) in neonatal lambs

Material and Methods

- Colostrum samples
  - 233 meat ewes (Noir du Velay and Blanche Massif Central breeds)
  - Manual milking
    - immediately after lambing n = 153
    - 6 hours after lambing : n = 38
    - 12 hours after lambing : n= 42
  - Frozen immediately at -20°C until analysis

- Plasma samples from neonatal lambs
  - 223 2 to 4 day-old, reared under their dam
  - Jugular venipuncuture in 2 vaccum tubes with lithium heparinate anticoagulant
  - Centrifugated and frozen at -20° within 30 minutes until analysis

Material and Methods

- Determination of colostrum and plasma IgG concentration
  - RID with a calibration set on each plate (Sheep IgG Ring Test, IDBiotech, France)
- Determination of colostrum fat concentration
  - Gerber method

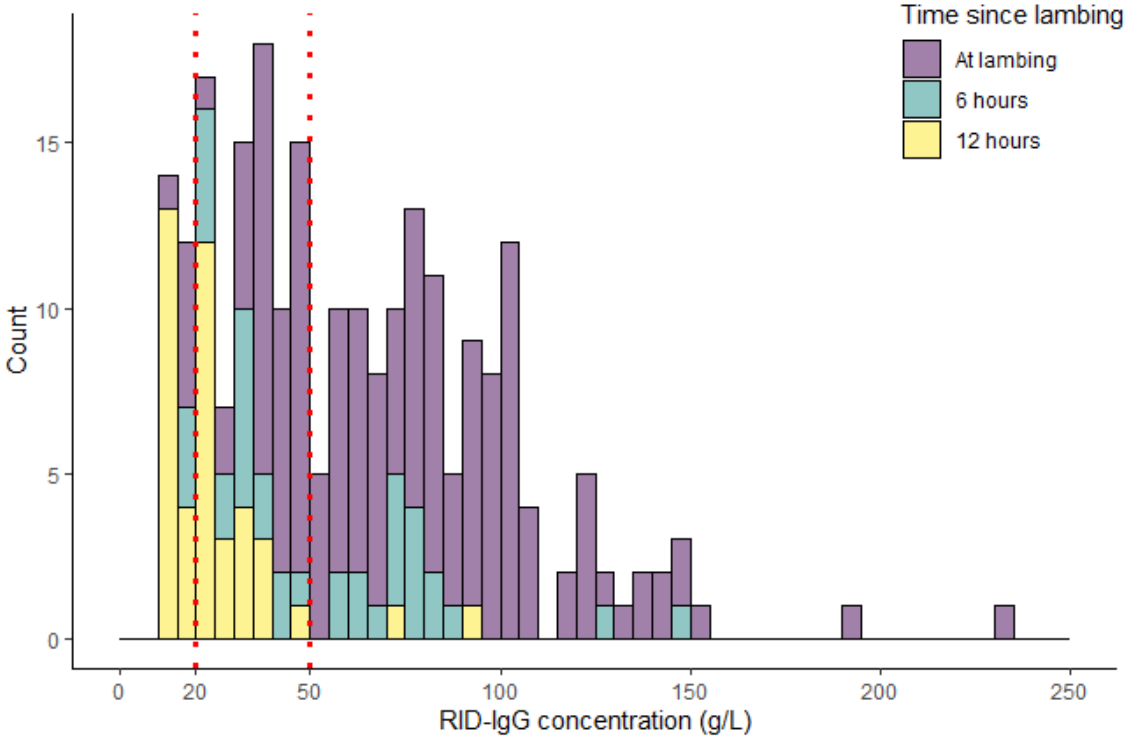
- Digital Brix refractometer
  - Hanna HI 96801 (accuracy: ±0.2% Brix)
  - Samples allowed to thaw at room temperature and homogenized
  - Triplicate measures

Material and Methods

- Statistical analysis
  - Pearson r correlation between %Brix and RID-IgG
  - Optimal cutoff point : ROC curves (pROC package R software, Robin et al, 2011)
    - Youden's J statistic : Se + Sp -1
    - Distance to the left-hand corner of ROC space (d^2)
  - FTPI: RID-IgG < 10 g/L or <15 g/L
  - Colostrum quality: RID-IgG < 50 g/L

Results

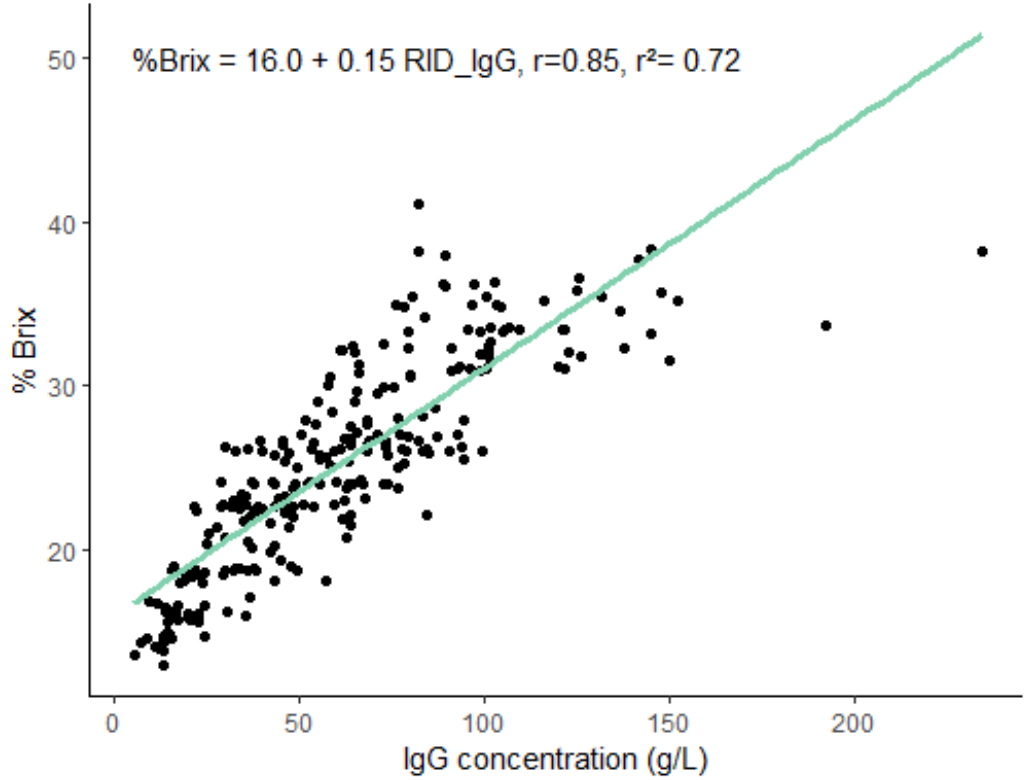
- Colostrum IgG concentration



Mean	62.0 g/L
Q1	32.9 g/L
Q3	84.7 g/L
< 50 g/L	108 (46.3%)

Results

- Colostrum : correlation between RID-IgG and %Brix



Inlfuence of IgG concentration

Each increase of 10 g/L of IGG

↓

Increase in %Brix by 1.5 ± 0.05 ($p<10^{-6}$)

Influence of fat content

%Brix = 13.5 + 0.14 RID_IgG + 0.03 FAT

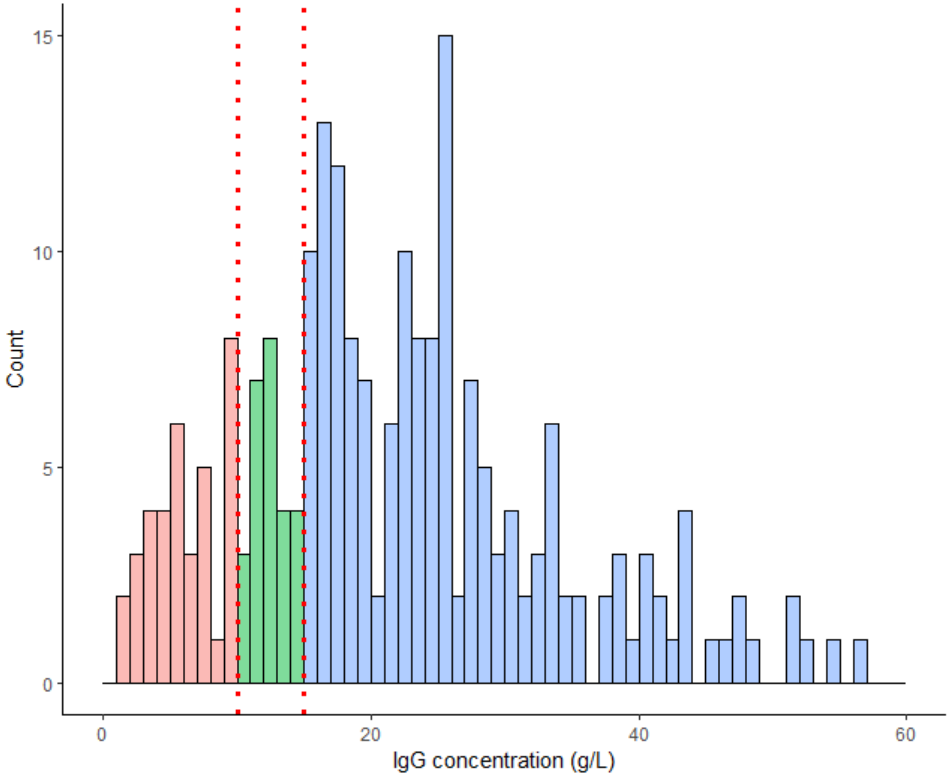
Each increase of 10 g/L of fat

↓

Increase in %Brix by 0.3 ± 0.05 ($p<10^{-6}$)

Results

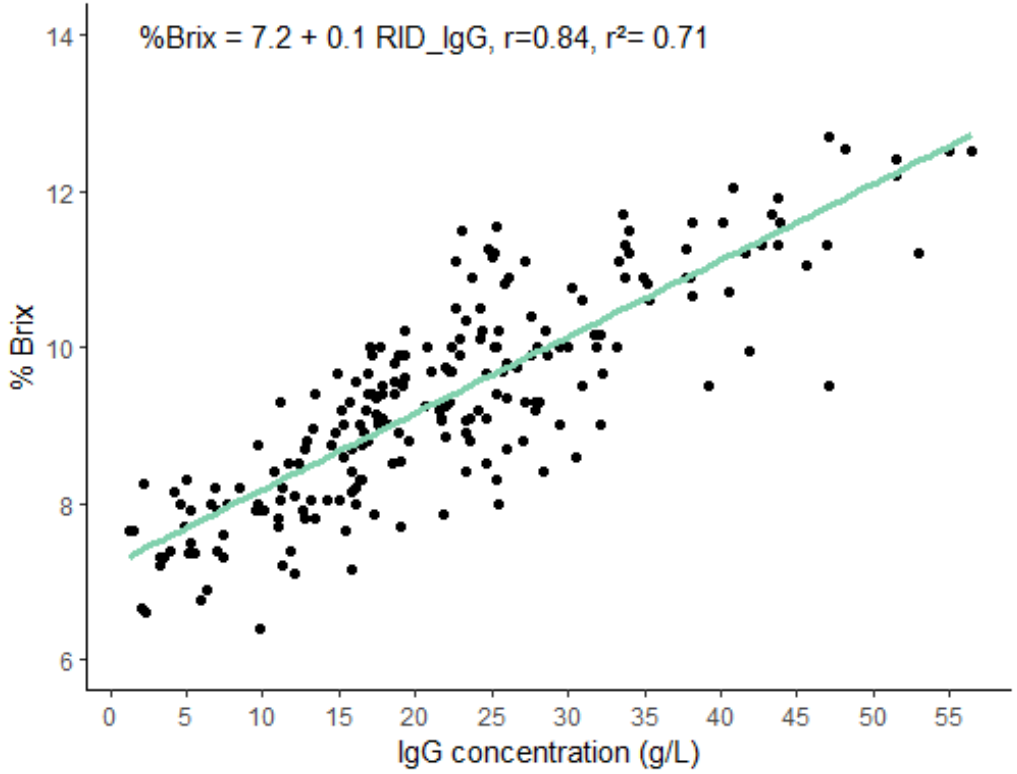
- Plasma IgG concentration



Mean	21.6 g/L
Median	19.5 g/L
Q1	13.4 g/L
Q3	27.6 g/L
< 10 g/L	36 (16.1 %)
< 15 g/L	113 (27.8%)

Results

- Plasma : correlation between RID-IgG and %Brix



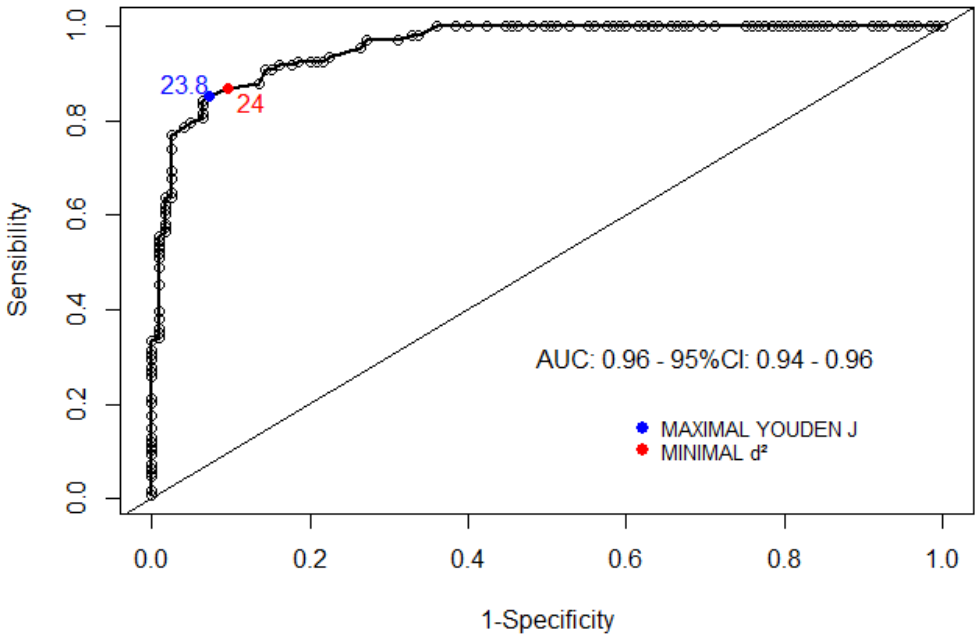
Each increase of 5 g/L of IgG

↓

Increase in %Brix by 0.5 ± 0.02 ($p<10^{-16}$)

Results

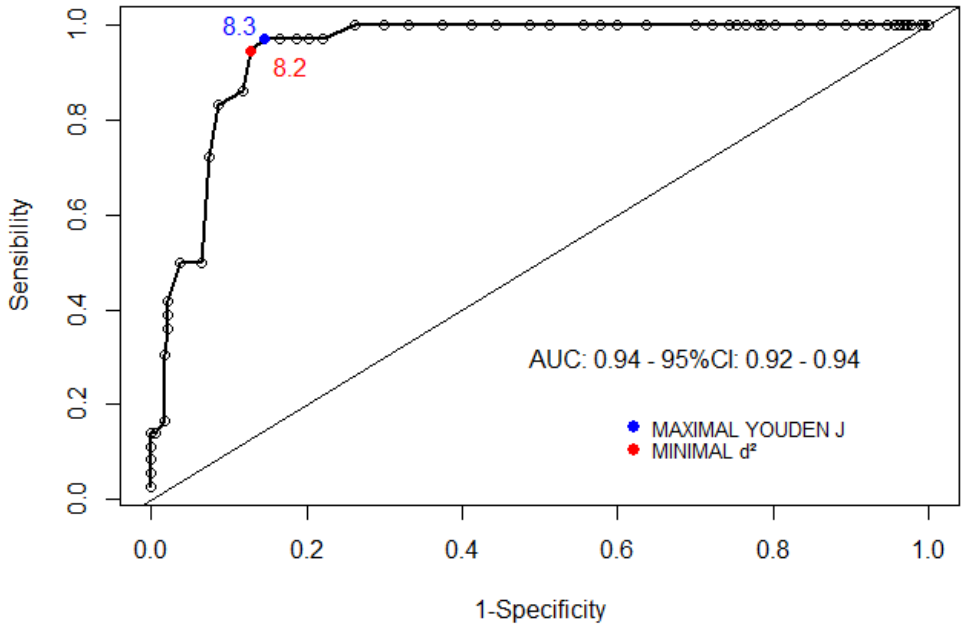
- ROC curves for detecting colostrum with IgG < 50 g/L



Cutoff	Sensitivity (%)	Specificity (%)	Youden J	d²
23.8	85.2 [77.1 – 91.3]	92.8 [86.8 – 96.6]	0.78	0.15
24.0	87.0 [79.2 – 92.8]	90.4 [83.8 – 94.9]	0.77	0.16

Results

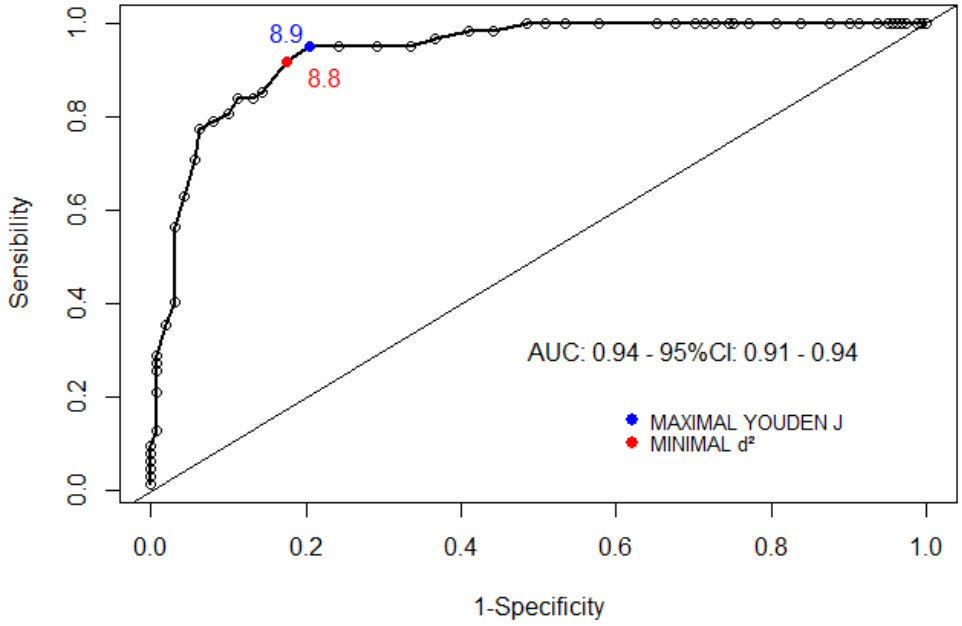
- ROC curves for plasma, FTPI = RID-IgG < 10 g/L



Cutoff	Sensitivity (%)	Specificity (%)	Youden J	d^2
8.3	97.2 [85.5– 99.9]	85.6 [79.7 – 90.3]	0.83	0.15
8.2	94.4 [81.3 – 99.3]	87.2 [81.5– 91.6]	0.81	0.14

Results

- ROC curves for plasma, FTPI = RID-IgG < 15 g/L



Cutoff	Sensitivity (%)	Specificity (%)	Youden J	d^2
8.8	91.9 [82.2 – 97.3]	82.6 [75.8 – 88.1]	0.74	0.19
8.9	95.1 [86.5 – 99.0]	79.5 [72.4 – 85.4]	0.75	0.21

Discussion and conclusion

- Ewe colostrum
  - % Brix thresholds slightly higher (24 % Brix) than reported in cattle (21-23 %Brix)
    - May be due to a higher fat content (mean = 90.0 ± 34.7 g/L)
  - Our results differ from the only published study on sheep
    - Kessler et al 2021 : lower IgG concentration, higher % Brix thresholds
    - Kessler et al 2021 : ELISA IgG : poor agreement with RID (Gelsinger et al 2015; Dunn et al 2018)
  - **High accuracy for detecting poor / good quality colostrum**
    - High interest for selecting colostrum that should / should not be kept for a colostrum bank
- Lamb plasma
  - % Brix thresholds ≃ those reported in calve (8.1- 8.4 % at IgG <10 g/L) (Lombard et al, 2020)
  - **High accuracy for detecting lambs with FTPI**
    - High interest at the flock level to detect TPI issues

Thank you for your attention.

Any question ?

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