THE NEWBORN LAMB AS A NEW MODEL FOR STUDYING GASTROESOPHAGEAL REFLUX

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Short title: Gastroesophageal reflux in newborn lambs

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ABSTRACT

We aimed to determine whether the newborn lamb at term is a valid model for studying gastroesophageal reflux. Seven bottle-fed lambs, aged 2-3 days, underwent an esophageal Multichannel Intraluminal Impedance-pH monitoring (MII-pH). A total of 196 reflux episodes were recorded, including 73% alkaline and 27% weakly acid. No acid refluxes were observed. Median bolus clearance time was 4s [range: 3-5.5], and proximal reflux extent 35% (26). This first report of MII-pH in a newborn mammal paves the way for future studies with physiological and clinical relevance to human neonates.

Gastroesophageal reflux (GER) is a physiologic process, which is virtually universal in newborns. It is considered pathologic (GER disease, GERD) when it causes excessive symptoms or complications such as esophagitis, apneas or apparent life-threatening events [1]. While transient lower esophageal sphincter relaxation is the predominant mechanism of GER in preterm and term infants, characterization of neonatal esophageal motor function remains largely incomplete [2].

To our knowledge, there is no animal model of neonatal GER. Meanwhile, ovine models have been used for studying numerous physiological processes and pathological conditions in the neonatal period for more than one century [3-5], including for reflux laryngitis [6]. Previous *in vivo* studies have demonstrated similarities between the ovine and human esophagus with respect to thickness and histological structure [7]. In addition, during the first two weeks of life, the preruminant lamb is essentially monogastric, the milk being digested in the abomasum, while the reticulum and the rumen are bypassed [9-11]. The aim of the present study was to test the hypothesis that the newborn lamb is a relevant model for studying neonatal GER using esophageal Multichannel Intraluminal Impedance-pH monitoring (MII-pH).

SUBJECTS AND METHODS

Study Protocol

Seven full-term lambs aged from 2 to 3 days and weighing 3.3 Kg (0.7) were involved in the study. The study was approved by the ethics committee for animal care and experimentation of the Université de Sherbrooke. The non-sedated lambs underwent a 24 h MII-pH monitoring while freely moving in a Plexiglas chamber and able to bottle-feed with ewe milk *ad libitum*. Positioning of the catheter 3 cm above the cardio-esophageal junction was confirmed by X-ray and verified at necropsy. MII-pH recordings were analyzed with the MMS Software and visually checked. Definitions of refluxes and their characteristics have been described previously [12]. Tracings were analyzed during

preprandial (30 mn before feeding), feeding and postprandial (30 mn after feeding) periods. Further

details on the methodology are available in the online-only supplemental data file.

Statistical Analysis

Descriptive statistical analyses were performed using Prism software version 5.04 (GraphPad

Software, San Diego, CA). Data are presented as mean (SD) or median [Q_{25} ; Q_{75}].

RESULTS

A mean of 7 (2) milk feedings was noted during the 24h-recordings. An example of a

recording is given in Figure 1, and results for the 7 lambs are shown in table 1. Overall, esophageal

pH was > 4 and > 7 during 100% and 91% (12) of recording time respectively. Most refluxes [55%

(30)] were purely liquid.

While the mean number of refluxes was identical during pre - and postprandial periods [0.5

(0.6) and 0.5 (0.5) respectively], weakly acid refluxes were predominantly observed in postprandial

periods and alkaline refluxes in fasting periods. Only 10 refluxes (8 gaseous and 2 mixed) were

observed in perprandial periods. Further results are available in the online-only supplemental data

file.

DISCUSSION

Results from the present study lead us to propose the newborn lamb as the first animal

model to study neonatal GER. Indeed, we have showed that: a) 24h-MII-pH recordings, the

recognized gold standard to study GER and GERD, can be easily performed in freely moving, non-

sedated newborn lambs; b) apart from the absence of acid refluxes, the many refluxes recorded

present a morphology and a migration up to the proximal esophagus bearing striking similarities with refluxes in human infants.

While the number of reflux events (1.2/hour) was slightly lower than in healthy human preterm (2-3/hour) [13] and full-term infants (2.6/hour) [14], the present study highlights many similarities of our preruminant, ovine model with the healthy human infant. Indeed, the morphology of the impedance bolus is identical to that recorded in humans, the number of liquid and gas refluxes is close to that reported in the human infant [15] and the proximal liquid reflux extent is within the values published previously [15]. As in healthy human preterms, the bolus clearance after reflux appears very fast and more efficient than in term human newborn (4 s *vs* 13 s) [14]. Our data confirm the maturity of esophageal primary and secondary peristalsis in newborn lambs.

The most significant difference between newborn lambs and the human newborn is the absence of acid refluxes in lambs. The higher esophageal pH in lambs is probably related to the low amount of hydrochloric acid secreted by the abomasum at birth; this amount dramatically increases in the first weeks of life due to the increased number of parietal cells [16]. Of note, because of their frequent feedings, healthy human newborns have also prolonged periods with buffered gastric contents, leading to a higher proportion of weakly acid refluxes (73%) or even alkaline than acid refluxes (27%) [17].

An extensive review of the literature showed that there are only a few animal models for studying GER (rats, dogs, cats, pigs) [8, 18]. Most of these models were specifically designed to characterize GER in non-physiological conditions and during short-term measurements (mostly < 1 hour) [8, 19]. In addition, there are no animal models of GER in newborns. The temperament and size of the lamb make it especially suitable for research involving chronic catheterization and repeated studies in ambulatory animals. In addition, several manometric and electromyographic investigations of the digestive tract of the sheep have been reported [3, 20] and could readily be added to MII-pH recording.

Thus, the newborn lamb appears as a useful model for investigating GER physiology and GERD pathogenesis in various conditions. The latter include the relationships between GER and apneas-bradycardias, the consequences of hypoxia or nasal respiratory support on GER or the use of new medications against GER in full-term and preterm lambs.

ACKNOWLEDGMENTS

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TABLE

TABLE 1. MII-pH variables in 7 healthy, full-term lambs

Recording duration, hh:min	23:52 (00:58)
Mean lower esophageal pH	5.7 (0.6)
Reflux index, % of recording duration	0
Impedance-detected refluxes	
Total Reflux (bolus) number, n	196
Mean number of refluxes, n	28 (24)
Refluxes per hour, n	1.2 (1)
Acid refluxes, %	0 (0)
Weakly acid refluxes, %	27 (35)
Alkaline refluxes, %	73 (13)
Bolus Exposure Index, % of recording duration	0.1 (0.1)
Median Bolus Clearance Time, s	4 [3 ; 5.5]
Proximal reflux extent	
Total (% at 13 cm Z1)	35 (26)
Weakly acid refluxes (% at 13 cm Z1)	11 (20)
Alkaline refluxes (% at 13 cm Z1)	40 (26)

Values are mean (SD), Z1: Impedance channel 1.

FIGURE LEGENDS

FIGURE 1. MII-pH recording sample of a newborn lamb. The black arrow indicates a gastroesophageal reflux. Z1 to 6: Impedance channels 1 to 6, pH: esophageal pH channel.

FIGURE 1.

Z1   Reflux   Reflux

Z2

Z3

Z4

Z5

Z6

pH

1 second