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The preterm piglet – a model in the study of oesophageal development in preterm neonates

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Abstract

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Aim—Preterm infants have difficulty attaining independent oral feeding. This can ensue from inadequate sucking, swallowing, and/or respiration. In impeding bolus transport, immature oesophageal motility may also be a cause. As studies on the development of oesophageal motility are invasive in preterm infants, the preterm piglet was investigated as a potential research model.

Methods—Oesophageal motility (EM) of term (n=6) and preterm (n=15) piglets were monitored by manometry for 10 min immediately following bottle feeding on days 1-2 and 3-4 of life.

Results—Piglets' oral feeding performance and EM were similar to those of their human counterparts. Term piglets readily completed their feeding whereas their preterm counterparts did not. They also presented with greater peristaltic activity and propagating velocity. Peristaltic activity remained unchanged over time in preterm piglets, but an increase in synchronous and decrease in incomplete motor activity were noted. Preterm piglets that developed symptoms analogous to necrotizing enterocolitis (NEC) demonstrated uncharacteristic oesophageal activity.

Conclusion—Immature EM may cause oral feeding difficulties. NEC-like symptoms may adversely affect EM. The piglet is a valid research model for studying human infant oral feeding and oesophageal development.

Keywords

oral feeding; prematurity; oesophageal motility

INTRODUCTION

Among the numerous medical issues facing preterm infants, gastrointestinal (GI) maturation, such as tolerance of enteral nutrition and oral feeding, is a sustained concern for caregivers. Immature sucking, swallowing, respiration, or incoordination of these three

functions put infants at risk for oxygen desaturation, apnea, bradycardia, aspiration, as well as inability to complete oral feedings (1,2,3,4). Clinical studies have gained a better understanding of the development of sucking and respiration than that of swallowing. This is likely due to the availability of less invasive technologies than those used for the monitoring of the different phases of swallowing (5,6,7). Swallowing is composed of an oral, pharyngeal, and oesophageal phase, each involving the participation of different anatomical structures (8,9). In brief, the bolus is formed in the oral cavity during the oral phase, transported to the upper oesophageal sphincter during the pharyngeal phase, and down to the stomach during the oesophageal phase. Oesophageal motility (EM) patterns are divided into peristaltic and non-peristaltic contractility (10,11). Peristalsis can be propagating in an ante- or retro-grade direction whereas non-peristalsis describes synchronous contractions or incomplete peristalsis. The use of video fluoroscopy in human neonates has shown that poor bolus formation, improper laryngeal closure, and poor clearance of pharyngeal residue increase risks of penetration/aspiration that can lead to adverse events such as those mentioned above (8,9). Studies on preterm infants born between 25 to 35 weeks gestation and monitored between 33 and 38 weeks postmenstrual age (PMA) showed significantly less propagating peristaltic than non-peristaltic motor patterns (11). The possibility that abnormal swallowing or dysphagia may be a resultant of inappropriate oesophageal motility is supported by the study of Jadcherla et al (12). These investigators observed that dysphagic preterm infants, as diagnosed by an abnormal videofluoroscopic modified barium study, presented with dysfunctional pharyngo-oesophageal motility characterized by infrequent swallows, failure of complete peristaltic propagation, prolonged distal oesophageal waveforms, higher upper oesophageal sphincter (UES) tone and prolonged UES relaxation. Infants with gastroschisis presented with some of the same dysfunctions (13). These recent studies support the notion that immature oesophageal function may be involved in the oral feeding issues of preterm infants. In studying postnatal oesophageal maturation of healthy preterm neonates (27.5 ± 0.6 weeks gestation, GA) between 33.8 and 39.2 weeks PMA, Gupta et al suggested that the differences observed in the maturation processes implicated in the proximal striated and distal smooth regions may reflect the maturation of central and peripheral neuromotor mechanisms (14). Insofar as these studies were conducted by delivering varied volumes of liquid or air in the pharynx, it remains to be clarified whether similar oesophageal activities would be observed during actual oral feeding sessions when frequent swallows are implicated.

Thus, our interest was to verify whether oral feeding difficulties is associated with immature primary oesophageal motor function, namely activity ensuing directly from a swallowing event. Due to the invasiveness of using endoscopic procedures during oral feeding in preterm infants, we wanted to first test this hypothesis in the preterm piglet model. This was based on the similarities in GI developmental profiles between piglet and human infant and the availability of a preterm piglet model (15-18). We hypothesized 1. that oesophageal maturation would be delayed in preterm vs term piglets as indicated by a reduced proportion of peristaltic motility and 2. that the preterm piglet is a valid research model for preterm infants in the study of upper GI maturation.

STUDY DESIGN AND METHODS

Animals

Piglets of either gender born from timed-pregnant, artificially inseminated sows resulting from crossings between Danish Landrace, Danish Yorkshire and Danish Duroc were obtained from the same supplier. Preterm piglets (n=15) were delivered by caesarean section at a postconceptional days of 105 days (91% of full gestation) as described previously (18). Term piglets (n=6) were delivered vaginally at 115 postconceptional days (full gestation).

All procedures were approved by the National Committee on Animal Experimentation (Denmark).

Standard Care—Preterm piglets, immediately after delivery, were weighed and placed in heated (30–37 °C) incubators (Air-Shields, Hatboro, PA) at 80–100% moisture and oxygen supplementation (0.5–2 l/min) the first 8–10 hours of life. An umbilical arterial catheter (infant feeding tube 4F; Portex, Kent, UK) was placed for subsequent total parenteral nutrition (TPN) and secured by ligating the umbilical cord to the skin. An orogastric tube (6F, Portex, Kent, UK) for enteral feeding was passed through the cheek and secured to the nape of the neck to prevent damage from chewing. For the first 2 postnatal days, preterm piglets received TPN (Nutriflex Lipid plus; Braun, Melsungen, Germany) with or without minimal enteral nutrition. When given, this was delivered at 108 kcal/kg/day (144 ml/kg birth weight /day) and constituted 21% (22.7 kcal/kg/day or 30.2 ml/kg/day) of the energy content of standard TPN. Given enterally every 3 hours, it consisted of cow's colostrum or a mix of three commercial human infant formulas (80g/l Pepdite 2-0, 70 g/l Maxipro and 75 ml/l Liquigen-MCT; SHS International, Liverpool, UK). Colostrum from cow rather than sow was used because of its similar preventive GI effects on intestinal mucosal weight, villus morphology, and brush border enzyme activities and greater availability (19). To achieve the same energy content as TPN, colostrum was diluted by 2/3 with water. On postnatal day 3–4, piglets were weaned from TPN and enterally fed with cow's colostrum or formula (118 kcal/kg/day or 120 ml/kg/day) every 3 hours. With the exception of the differing diets, all the animals received the same postnatal intensive care.

Term piglets, taken from their sow within 12 hours of birth, were bottle fed every 3 hours with the same cow's colostrum as that offered to their preterm counterparts (118 kcal/kg/day or 120 ml/kg/day).

Study Design

Prior to monitoring an oral feeding session, the oro-gastric feeding tube was removed. Bottle feeding was offered for no more than 15 min and stopped in the presence of sustained adverse events (see below). EM activity was monitored immediately after oral feeding for 10 minutes. Any milk not taken by mouth was gavaged following the monitored session after the oro-gastric tube was re-inserted. All animals were first monitored on postnatal day 1–2. Healthy preterm and term piglets were monitored again 48 hrs later on postnatal day 3–4. Preterm piglets showing any clinical sign of necrotizing enterocolitis (NEC)-like symptoms (18), e.g., abdominal distension, bloody diarrhea, lethargy, were monitored that same day. This occurred within 24 h from start of enteral feeds. The integrity of the GI tract was assessed at time of sacrifice following their second motility session from stomach to colon. Severity of NEC-like symptoms was scored according to the scale developed in our laboratory (18).

Methods

Bottle feeds used a Pritchard Flutter Valve bottle nipple (NASCO, Ft Atkinson, WI). Immediately after the feeding, a multichannel oesophageal catheter was inserted with the ports positioned as described below. All the piglets were held down gently prone or on the right side by the same investigator (SR) who ensured that the catheter remained in place. By placing lightly an index finger over the piglets' hyoid, it was possible to identify the elevation of the hyoid, commonly used as a marker for the onset of swallowing (3). The timing of that event along with behavioral states and movements were continuously noted on the recording chart.

EM was monitored by manometry using a 4-port multichannel oesophageal catheter (33-35 cm, OD 2-2.4 mm). Data were recorded onto a 4-channel manometric set-up and transferred to a BIOPAC system for later analyses or directly onto the BIOPAC system (BIOPAC AcqKnowledge® Version 3.9.0, BIOPAC Systems, Inc., Goleta, CA). Prior to each recording session, the system was calibrated at 0 and 75 cm water pressure. To ensure correct placement of the catheter above the LOS, the length from the snout to the sphincter of each piglet was measured prior to the first recording session using a Veterinary Foley catheter (6F; SurgiVet, Smiths Medical, Inc., Waukesha, WI). The catheter was inserted till its tip was in the stomach (resistance against further insertion), the balloon was inflated with 1.5 cc of air and the catheter pulled back until resistance from the balloon against the LOS was felt. Based on the distance between snout and sphincter as noted from the marks on the Foley catheter, the multichannel oesophageal catheter was inserted such that the ports were positioned at approximately 1, 3, 5, and 9 cm above the LOS.

Outcome Measures

Tracings were analyzed for 5 different wave patterns, propagating and retrograde peristaltic waves, synchronous and incomplete non-peristaltic waves as described by Omari et al (11) and double synchronous propagating waves (DSP) as described below. Only pressure fluctuations occurring in the absence of overt movements were included in the analyses. The following criteria were used for characterizing the 5 types of oesophageal pressure waves. 1) An antegrade propagating peristaltic wave (Prop) required the presence of a peak pressure on all channels with an overall propagating pattern occurring in an aboral direction, i.e., upper oesophageal sphincter to stomach. The propagation velocity was computed by the distance between the most proximal and distal ports divided by the time interval between the peak pressures at both ports (cm/s). 2) A retrograde propagating peristaltic wave (Retro) similarly defined, but with a propagating pattern occurring in an orad direction, i.e., from stomach to the upper oesophageal sphincter. 3) A synchronous non-peristaltic wave (Synch) was identified when the peak pressure on all channels occurred simultaneously. 4) A wave that did not meet any of the 3 above criteria was classified as incomplete (Inc), i.e., non peristaltic and/or not present on all channels. 5) A double synchronous-propagating wave (DSP) described the presence of a Synch and Prop wave occurring close to the same time, i.e., the Synch wave present on all channels occurring just prior to the onset of the peak pressure of a Prop wave beginning in the most oral channel (9 cm above the LOS) followed immediately by the Prop wave itself (representative patterns shown in Fig. 1). Within each category of piglets, the frequency (#/min) of the total EM activities (combined Prop, Retro, Synch, Inc), and individual patterns were computed. Within the Prop pattern, the frequency of DSP was calculated. The percent distribution of each pattern (Prop, Retro, Synch, Inc, DSP) over total EM activity within healthy preterm (no NEC-like symptoms) and term piglets was computed to determine whether particular motility patterns significantly increased or decreased over time.

Data Analyses

Data are presented as mean $\pm$ SEM. Frequencies of total EM activities of any types, Prop, Retro, Synch, Inc, DSP activity (#/min) and Prop velocity (cm/s) were first calculated for each recording. The overall means $\pm$ SEM of these outcomes within each group at each time point were then computed. Independent t-test was used to compare term vs healthy preterm and healthy vs NEC preterm piglets. A 2-way repeated measures ANOVA (SPSS v. 16, Chicago IL) was used to compare outcomes over time and between groups. Upon significance in group, time, and/or group*time effect, post-hoc paired or independent t-tests were used accordingly. Statistical significance was established at p-values $\leq$ 0.05.

RESULTS

A significant difference in birth weight was found between preterm and term animals ($1264\text{g} \pm 86\text{g}$ vs. $1573\text{g} \pm 69\text{g}$, respectively, $p = 0.046$). Of the 15 preterm piglets, 6 developed NEC-like symptoms within 24 hours from the start of enteral feeding. No difference was noted in birthweight between preterm piglets that did and did not develop NEC ($1151 \pm 154\text{g}$ vs. $1229 \pm 91\text{g}$, respectively). All preterm animals had difficulty initiating sucking and none finished their prescribed feeding volume. Milk leakage, coughing, choking, gagging and regurgitation along with limited endurance were commonly observed during bottle feeding. This contrasted with their term counterparts that readily took to bottle feeds and rapidly completed their feeding with no sign of difficulty.

The integrity of the GI tract from stomach to colon was assessed following the second EM monitoring for all piglets as we have demonstrated in an earlier article that preterm piglets fed enterally can develop NEC (18). Severity of NEC-like symptoms was scored according to the 6-level scale developed in this earlier study: no sign of gastroenterocolitis (grade 1), mild acute focal gastroenterocolitis (grade 2), moderate acute locally extensive gastroenterocolitis (grade 3), severe acute locally extensive hemorrhagic gastroenterocolitis (grade 4), severe peracute locally extensive hemorrhagic and necrotic gastroenterocolitis (grade 5), extensive peracute extensive hemorrhagic and necrotic gastroenterocolitis (grade 6). NEC severity grade in term animals was at 1.0, healthy preterm piglets ranged from 1.0 to 1.2, and their NEC counterparts ranged between grades 4 to 6.

Term vs. healthy preterm piglets

Term piglets demonstrated a significantly greater #EM/min ($p=0.001$, Fig 2) and #Prop/min ($p<0.001$, Fig. 3a) than their healthy preterm counterparts with no significant change over time and group*time. Retrograde activity (#Retro/min) was similar between the 2 groups (Fig. 3b). Frequency in Synch activity (#Synch/min, Fig. 3c) was similar between groups, but with a significant time ($p=0.029$) and group*time effect ($p=0.001$). Post-hoc analyses showed that term piglets exhibited a significant decrease in #Synch/min over time ($p=0.008$) with a greater Synch activity on postnatal day 2 than their healthy preterm counterparts ($p=0.025$). Frequency in Inc activity (#Inc/min, Fig. 3d) was greater in term vs healthy preterm piglets (group effect, $p=0.006$) with no significance effect over time and group*time. Velocity of Prop waves showed a significant group effect with term and healthy preterm piglets averaging over time 3.61 ± 0.33 and 1.66 ± 0.24 cm/s, respectively ($p=0.003$). Similarly, # DSP/min between the 2 groups, averaged 0.79 ± 0.15 vs 0.33 ± 0.04 , respectively ($p=0.010$). No differences over time and group*time effect in the latter 2 measures were observed.

Percent distribution of Prop, Retro, and Inc over time in term piglets did not change and a trend in a decline of % Synch ($p=0.058$) was noted. Preterm piglets did not change over time in % Prop and Retro, but there were a significant increase in % Synch and decrease in % Inc ($p\leq 0.047$). Percent DSP on days 1-2 vs. 3-4 were 49% vs. 93% in preterm piglets and 82% vs. 70% in term counterparts, respectively. The change over time was only significant for the preterm group ($p=0.038$).

Healthy vs NEC preterm piglets

Frequency of EM activity was greater in NEC vs healthy counterparts ($p=0.043$) with a significant time effect ($p=0.005$). Post-hoc analysis showed that over time, the NEC group showed a significant decline ($p=0.036$). No differences were observed in #Prop/min, #Retro/min, and #Synch/min. Significant group ($p=0.009$) and time ($p=0.038$) effects in #Inc/min were observed with healthy preterm piglets demonstrating a lower incidence of Inc activity

than their NEC counterparts. Post-hoc analysis showed a decline over time in healthy piglets ($p=0.027$). No significant difference in velocity of Prop wave was noted between the 2 preterm groups of piglets.

It is of note to mention that although we did not measure the amplitudes of the individual motor patterns studied, we observed that, within an animal and during individual monitored post-feeding sessions, the amplitudes of the Prop, Sync, and Inc motor patterns varied widely on all channels (~ 2 to 30 cm H₂O). It is conceivable that these variations resulted from the manner in which the piglet was held by our investigator. Studies designed to specifically focus on motor pattern amplitudes are needed to investigate the amplitude of oesophageal contractions in the developing conscious preterm piglet.

DISCUSSION

This study aimed to understand the degree to which oesophageal motility in preterm infants can interfere with their transition from tube to oral feeding. As such studies are difficult to conduct in the clinical arena, we took advantage of the availability of the preterm piglet as a research model to characterize the developmental EM profile following oral feeds in an animal model that shares many GI similarities with the human infant.

Preterm piglets, like their human counterparts, had difficulty sucking, swallowing, and exhibited similar signs of poor oral feeding skills, e.g., milk leakage, regurgitation, and limited endurance. None were able to ingest their prescribed volume by mouth. This contrasts with the performance of term piglets that, similarly to term infants, readily took the volume offered with minimal milk leakage or sign of difficulty.

Oesophageal activity is dependent on a number of functional constituents maturing at different times, i.e., anatomical, neuro-chemical and -mechanical features, along with peripheral and central neural innervations (15,20-24). As it relates to primary peristalsis, some understanding has been gained regarding the neuromuscular mechanisms involved in the integrated activity between the proximal striated and distal smooth muscle portions of the esophagus (25). However, due to the complexity of this system, no studies have yet investigated the temporal maturation sequences in which EM occurs.

In an attempt to do so, we used our monitored outcomes as indices of the level of upper GI sensitivity in response to bolus transport/clearance in preterm and term piglets during their first 4 days of life. Comparison between groups provided insight into the postconceptional maturation as there was a difference in in-utero maturation of 10 days between the 2 groups (105 vs 115 days, respectively).

Additionally, comparison between preterm animals that did and did not develop NEC offered preliminary observations on the effect of NEC-like condition on upper GI maturation. It is of interest to note that, like their human counterparts, preterm piglets can develop NEC-like symptoms, whether they received formula or colostrum, albeit with a lower incidence when mother's milk is offered (18).

To put these maturational changes within and between groups in better perspective, we propose a 3-phase model for the EM maturational process in response to oral feeding (Fig. 5). Phase 1, the early stage, pertains to the period of neuromuscular immaturity represented by the predominance of Inc pattern. During phase 2, an intermediary stage, Synch activity predominates as peripheral neuromuscular maturation advances. Phase 3 corresponds to the time when central neural regulation mature allowing for improved sequential control of peripheral neuromusculature as reflected by the increasing presence of propagating peristalsis, be them ante- or retro-grading. Using this theoretical framework, healthy preterm

piglets are progressing from a phase 1 to 2 of maturation, as over time, they demonstrated a decrease in percent occurrence and frequency of Inc activity concurrent with increase in the percent occurrence and frequency of Synch activity. A number of clinical studies lend support to the above model. Omari et al showed that preterm infants, born between 25 and 35 weeks GA and monitored once between 33 and 38 weeks PMA, exhibited 26.6% peristaltic vs. 73.4% non-peristaltic motor patterns. Among the non-peristaltic patterns, 31.3% were Sync and 34.6% Inc (11). These authors concluded that these non peristaltic EM may contribute to the poor clearance in refluxed material as their infants were tube fed. According to our model, these infants appear to be progressing from a phase 1 to 2 of maturation. Jadcherla et al (26) observed that preterm infants (29.9 ± 2.5 weeks GA) monitored twice, around 33 and 36 weeks PMA, and receiving mid-oesophageal liquid infusions demonstrated significantly greater complete peristalsis at the later age (80% vs 90%). Their subjects in contrast to those of the previous study would appear to be progressing from a phase 2 to 3 of our model. The observations that infants with dysphagia or gastrochisis present with dysfunctional pharyngoesophageal motility similar to those observed in preterm neonates, e.g., failure of complete peristaltic propagation, prolonged distal oesophageal waveforms, higher upper oesophageal sphincter (UES) tone and prolonged UES relaxation suggest that inappropriate maturation of oesophageal motility can lead to oral feeding difficulties (12,13).

Term piglets showed a progression from a phase 2 to 3 of maturation as reflected by a decline in Synch activity along with increased Prop activities and velocity. This parallels the observations of Staiano et al (22). In a study on preterm and term neonates (32.9 ± 2.6 and 38.9 ± 1.6 weeks GA, respectively), these authors using high-resolution manometry observed that complete propagating peristalsis was present in significantly smaller number in preterm than term infants (26% vs 55%, respectively). An increase in propagating motor pattern would be expected to increase oral feeding performance; such an observation was made in the present study with the term piglets. In an earlier study comparing oral feeding performance in infants born prematurely vs. term, we observed similarly that preterm neonates performed at a lesser level than their term counterparts (3).

The greater Inc activity in term vs. healthy preterm animals appears to contradict our model. However, term piglets, at both times, ingested larger volumes than their preterm counterparts as they completed their feedings. It is conceivable that they may not have had the full maturity to handle the rate at which they ingested their feedings despite their success in completing them with no adverse effect. This is a possible drawback that term infants may also encounter when flow rate is too rapid. Such apparent discrepancy is reminiscent of our earlier study where we observed that infants born between 26/27 and 28/29 weeks GA attain independent oral feeding performance at the same time, despite significant difference in the maturation of specific oral feeding skills (27).

The absence of marked changes in frequency of Retro activity in all groups at both times suggests that central maturation for the generation of retrograde activity occurs at a later time than that of antegrade activity (phase 3) in the piglet.

The velocities of Prop waves noted in our preterm and term piglets are within the ranges observed in infants and adults, respectively, 1.2 ± 0.2 cm/s and 4.4 ± 1.8 cm/s (10,28). The presence of DSP is novel. To our knowledge, the occurrence of a simultaneous Synch and Prop wave in the esophagus has not been described in the literature. The initial synchronous-propagating signal, spreading rapidly down the esophagus, may be a 'forewarning' of the presence of a bolus to downstream structure(s) such as the LOS. The greater occurrence of DSP in term piglets lends support to the notion that they are at a more advanced phase 3 of maturation than their preterm counterparts. Additionally, the significant increase in percent

DSP over time in the preterm piglets supports their advance towards a phase 2 to 3 of development. It is evident that additional studies are necessary to confirm the existence and identify the physiological function of DSP waves in the pig.

Healthy and NEC preterm piglets showed similar EM profiles, but for a greater total EM and Inc activity in the NEC group. It is likely that the greater total oesophageal activity in the NEC piglets resulted from their greater Inc activity due to an adverse NEC-like oesophageal development. One case report has described the involvement of the distal esophagus in an infant who developed massive NEC (29). Unfortunately, we did not investigate oesophageal integrity in our piglets as it is not routinely conducted in infants who succumbed from NEC. The significant increase in Inc activity in the NEC piglets may be due to a gradual degeneration of neuromuscular mechanisms innervating the esophagus. As healthy piglets demonstrated a lower incidence of Inc than their NEC counterparts, it is conceivable that early determination of Inc activity in preterm piglet or infants, while still receiving TPN, may predict the development of NEC at a later time. Studies are needed to verify such possibility. The decline in Inc at the later time point is likely due to the fact that the NEC piglets were failing.

In summary, we confirmed our hypotheses. First, EM maturation, at similar postnatal days, is delayed in preterm vs. term piglets. This is illustrated by an overall lower EM activity accompanied by a greater occurrence of non-peristaltic than peristaltic patterns in preterm than term piglets. These preliminary observations raise the queries about 1. the possibility that immature EM function may explain some of the oral feeding issues encountered by preterm infants; 2. the concept that intrinsic/peripheral maturation occurs before external/central maturation, and 3. the notion that NEC can affect the upper GI as it does the rest of the GI tract. Additional studies will be necessary to confirm our present observations. Second, the piglet is a good research model for the study of upper GI development and oral feeding performance of human infant as the preterm and term piglet and their human counterparts share similar developmental characteristics in oral feeding performance and oesophageal activities.

Finally, following a premature birth, it is recommended that whenever oral feeding difficulties arise, not only should the oral and pharyngeal phase of swallowing be evaluated, but its oesophageal phase as well.

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Figure 1.

Sample tracings of Prop, Retro, Synch, and Inc waves. Prop tracing shown with DSP: a peak pressure in the most oral channel initiates the onset of the Prop wave as well as a simultaneous DSP (dashed line) on all channels; Retro tracing: a peristaltic wave is seen retrograding along all channels. Note the respiratory movements recorded at approximately 50 breaths/min; Synch tracing: a synchronous wave present on all channels; Inc tracing: an incomplete wave with peak pressure not seen on all channels. Note that Inc waves may consist of peak pressures present in nonconsecutive channels.

Figure 2. Frequency of total oesophageal activities (#EM/min) in preterm and term piglets. Significance between same symbols: (a) $p=0.001$; (b) $p=0.043$; (c) $p=0.036$.

Figure 3.

Frequency of Prop, Retro, Synch, and Inc waves (#/min) in preterm and term piglets (same legend as in Fig. 2). Significance between same symbols: (a) $p<0.001$; (b) $p=0.008$; (c) $p=0.025$; (d) $p=0.006$; (e) $p=0.009$; (f) $p=0.027$.

Figure 4.

Maturation model of oesophageal motility. Phase 1: predominance of Inc pattern when peripheral and central constituents are immature. Phase 2: intermediary stage, predominance of Synch activity when peripheral maturation advances. Phase 3: maturation of central regulation with predominance of peristalsis, ante- or retro-grade in direction.