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Initial Digestive Potential of Alimentary System in Newborns

By Penzhoyan G.A., Model G.Y. & Korotko G.F.
Kuban State Medical University

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Systems of different hydrolases during antenatal life are asynchronous.

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Initial Digestive Potential of Alimentary System in Newborns

Penzhoyan G.A. [α], Model G.Y. [σ] & Korotko G.F. [ρ]

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Identification of the enzymes in three mentioned bio liquids of the newborn is advisable for the reasonable estimation of the digestive potential and precise prognosis of lacto trophy.

Resume: Initial enzyme digestive potential is presented by fatally organized alimentary system hydrolase activities in a newborn. Both lacto trophy efficacy and breast milk hydrolases depend on it.

Quantitative indicator of the potential was measured by analyzing lipase, α- amylase, alkaline phosphatase, pepsinogens (I, II) in umbilical cord blood serum, amniotic fluid, aspirate gastric content in a newborn at the end of the delivery, in venous blood of 36 new mothers with full-term pregnancy, in 40 new mothers with incomplete gestation as well as in their newborns.

During antenatal life systems of different hydrolases were formed asynchronously. In the case of immature gestation, the concentration of hydrolases and zymogens in the examined bio liquids was reduced except lipase, which was active. Hydrolases of gastric contents are most informative towards the digestive potential and its low variability, while hydrolases in the newborn umbilical cord blood serum and in the amniotic fluids are less informative. Poly-enzyme analysis of three bio-liquids of a newborn proved to be reasonable for the conclusion concerning the morph functional maturity of the digestive system of a newborn.

Initial digestive potential makes it possible to expect lacto trophy efficacy and individualize the program for both breast and mixed feeding.

Author α σ ρ: SBEI "Regional Clinic N2", Ministry of the Health Care of the Krasnodar Territory, Krasnodar, Krasnykh Partizan Str., 6, Building 2. e-mail: korotko@rambler.ru

Keywords: newborn, hydrolases, amniotic fluids, gastric content, blood serum, digestive potential.

I. Introduction

Breastfeeding proved to be the "gold standard" for a newborn thanks to its unique nutritional, immune, regulatory, electrolyte, vitamin characteristics, as well as to the microbiota of the breast milk and its numerous substitutes. Milk nutrients are ingested by a newborn, but first they need to be hydrolyzed in the digestive system, this process is performed by hydrolyzing enzymes of the digestive glands and small intestine of a newborn according to the self-digestion pattern, and by first milk and mature milk enzymes according to autolytic digestion. Autolysis of lipids and proteins (casein) of first milk and milk is induced and realized in the gastric and small intestine cavities by the hydrolases of the newborn's digestive glands that developed during his antenatal period. Their hydrolases make up the initial digestive potential of the alimentary tract of the newborn. The digestive potential has not yet been investigated in perinatology, neonatology, or pediatrics either in terms of theory or in applied medicine. The notion has recently been put forward by the authors. But it should be taken into consideration that the reducing of this morpho-functional potential may threaten the development of a newborn.

II. Materials and Methods

Among seventy-six examined new mothers 36 had full-term (37-41 weeks) and 40 premature (27-36 weeks) pregnancies. Forty-seven children were born during vaginal birth and 29 by cesarean section. The investigation began after the written consent was signed by the parents under the current Federal "Law on Health Protection of Citizens" and the decision of the Ethics Committee. In newborns, anthropometric data, Apgar score and some anthropometric parameters and obstetric history were assessed under the Order of the Ministry of Health of the Russian Federation "On approval of the Order of Medical Care in the Profile «Neonatology»". The above-mentioned parameters were significantly lower in premature newborns than in mature newborn infants (see Table 1).

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Table 1: Indicators of new mothers with mature newborns (36-numerator) and premature newborns (49-denominator)

Variables	Average	Median value	Minimum	Maximum	Lower quartile	Upper quartile	Shift direction, statistical significance
Mother's age (years)	27,67 30,75	28,0 31,5	14,0 19,0	41,0 44,0	24,5 27,5	30,5 35,0	$p < 0,5$
Gestional age (weeks)	38,03 32,00	38,0 33,0	38,0 27,0	39,0 35,0	38,0 30,0	38,0 34,0	↓ $p < 0,001$
Mass (g)	3546,4 1765,2	3595,0 1715,0	2460,0 670,0	4800,0 3130,0	3170,0 1335,0	3835,0 2150,0	↓ $p <$ 0,001
Height (cm)	53,47 41,27	54,0 42,0	46,0 33,0	59,0 48,0	52,0 37,0	55,5 46,0	↓ $p <$ 0,001
Head circumference (cm)	34,14 28,58	34,0 30,0	31,0 17,0	37,0 35,0	33,0 26,0	35,5 31,0	↓ $p < 0,001$
Breast circumference (cm)	33,31 26,28	33,0 27,0	27,0 16,0	37,0 33,0	32,0 24,0	35,0 29,0	↓ $p < 0,001$
Apgar 1 (scores)	7,9 5,5	8,0 6,0	7,0 1,0	8,0 7,0	8,0 5,0	8,0 6,0	↓ $p <$ 0,001
Apgar 5 (scores)	8,7 6,0	9,0 6,0	8,0 1,0	9,0 8,0	8,0 6,0	9,0 7,0	↓ $p <$ 0,001
Latency period (hours)	3,4 2,05	2,0 0,0	0,0 0,0	21,0 41,0	0,0 0,0	5,0 0,1	↓ $p < 0,001$

New mothers' amniotic fluids were obtained in sterile syringes and then centrifuged (10 min., 3000 revolutions). The newborns' blood was obtained from their umbilical cords; the mothers' blood was obtained from the ulnar vein.

In newborns fasting gastric content was aspirated, then it was homogenized and centrifuged (10 min., 3000 revolutions). In amniotic fluid and gastric aspirate supernatants, umbilical cord blood serum of the newborn and mother's blood serum lipase, α-amylase, alkaline phosphatase were determined by colorimetric methods with standard reagent kits for in vitro diagnostics (Roche) on a modular platform for biochemical and immunochemical analysis Cobas-8000 (module C 702). α-1- antitrypsin (reagent F1-Antitrypsin) was determined on a biochemical analyzer Architect C 8000 (Abbott) by the turbidimetry method. Pepsinogens I and II were determined by chemiluminescent immunoassay analysis on microparticles by Abbott reagents using immunological analyzer Architecr plus: 2000.

Statistical data processing was implemented within the Statistica 6 package by nonparametric statistics methods since the above-mentioned parameters had a large spread, and their empirical values did not correspond to the standard distribution law. Correlation analysis of enzyme parameters was carried out.

III. Results and Discussion

The aim of the research is the quantitative characteristics of the initial digestive potential of the alimentary tract in newborns and methods for its determination that includes the determination of digestive glands hydrolyzes in the newborn's blood serum, gastric aspirate, and amniotic fluids.

The amount of digestive glands hydrolases in human blood serum depends on the number and activity of glands producers granulocytes of the correlative enzymes [9]. In the blood serum of new mothers, the amount of hydrolases is higher (Table 2) than that in the blood serum of the newborns (Table 3).

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Table 2: Blood Serum Hydrolases in New Mothers

Enzymes	Average	Median Value	Minimum	Maximum	Lower Quartile	Upper Quartile	Shift direction Statistical significance
Lipase (U/l)	28,80 30,30	27,29 30,30	7,10 8,40	63,50 51,90	22,35 23,35	31,10 35,20	p > 0,10
Amylase (U/l)	52,97 52,28	52,97 52,28	4,00 3,00	82,00 78,00	48,50 41,00	61,50 65,50	p > 0,10
ALP (U/l)	182,5 120,8	181,0 118,5	94,0 56,0	441,0 235,0	137,0 87,0	185,5 136,0	↓p < 0,001
Pepsinogen I (ng/ml)	50,74 56,23	49,62 55,30	8,40 4,50	106,00 215,90	36,65 38,35	58,80 66,35	p > 0,10
Pepsinogen II (ng/ml)	8,88 7,79	8,74 7,55	2,70 1,70	33,10 17,50	4,90 5,65	10,70 8,75	p > 0,10
α-1-antitripsin (g/l)	0,30 0,47	0,30 0,30	0,30 0,30	0,30 2,38	0,30 0,30	0,30 0,38	p < 0,025

Note: ALP - alkaline phosphatase. (numerator – mature newborns, denominator - premature newborns)

It proves the incomplete development of the digestive glands enzymatic potential. Hydrolases differ in the initial level of their content in the blood serum that indicates that morphofunctional maturation of the enzyme systems of the fetus and the newborn is asynchronous. Producers of pepsinogen - the stomach glands (especially pepsinogen I) and producers of α-amylase - salivary and pancreas glands are most retarded. The antitrypsin activity of umbilical cord blood serum in the newborns was 4.5 times as high as that of the mother's.

Table 3: Hydrolases of the Umbilical Cord Blood Serum in Newborns

Enzymes	Average	Median Value	Minimum	Maximum	Lower Quartile	Upper Quartile	Shift direction Statistical significance
Lipase (U/l)	10,72 10,49	10,12 10,00	5,70 5,40	21,30 18,80	8,30 8,30	12,10 12,60	p > 0,10
Amylase (U/l)	9,00 4,72	8,50 4,00	1,00 0,00	52,00 16,00	5,00 2,00	9,00 6,00	↓p < 0,01
ALP (U/l)	157,5 166,5	157,5 171,0	97,0 11,0	243,0 274,0	119,0 134,0	181,5 200,5	p > 0, 10
Pepsinogen I (ng/ml)	10,92 4,82	8,45 4,20	3,40 1,10	55,10 12,20	6,75 2,60	10,92 6,10	↓p < 0,001
Pepsinogen II (ng/ml)	5,55 3,44	3,80 1,85	1,80 0,40	36,70 30,00	2,40 0,90	5,55 4,65	↓p < 0,001
α-1-antitripsin (g/l)	1,33 1,30	1,33 1,29	0,84 0,48	2,95 2,66	1,22 0,97	1,40 1,62	p > 0,10

Note: ALP - alkaline phosphatase. (numerator – mature newborns, denominator - premature newborns)

The new mothers that gave birth to both premature and mature newborns did not differ in the content of blood serum enzymes, except for alkaline phosphatase.

The concentration of amylase and pepsinogen (I, II) in the umbilical cord blood serum of the premature newborns was lower than that in the umbilical cord blood serum of mature newborns (Table 3). Reduced amyl lytic activity of the glands secrets in premature newborns can cause maldigestion in the case of mixed and artificial feeding of infants as most infant formula milk contains α-amylase- hydrolyzed polysaccharides. It is not contained in breast milk. Incomplete gestation reduces premature peptic potential of the fund-antro-duodenal producers of pepsinogens. It may affect the hydrolysis and protein metabolism in premature newborns, the formation of regulatory peptides (mainly breast milk casein) [5–13], and the process of proteolysis in the lacto trophy. This statement results from the postulate of the interaction of breast milk

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proteases and digestive glands excretions in the gastrointestinal tract [6, 7, 10], that have recently been confirmed by peptidomics and mass spectral chromatography. According to this fact the excretions proteinases (gastric aspirate) increase the hydrolytic effect of breast milk proteinases of lactating women by 1.5 -2.5 times [10]. The milk proteinases (like other hydrolases) have specific self-regulating dynamics during lactation [1, 14].

Premature birth did not affect the lipase content in the umbilical blood serum in the newborns. It speaks for the formation of the low initial level of lipase production by the digestive glands of the fetus during earlier gestational periods than other considered enzymes.

The reduced content of three hydrolases (α-amylase, pepsinogen I and II) in the umbilical cord blood serum of the newborn proved immature initial digestive potential in preterm pregnancy. No significant data concerning other hydrolases were found. It is explained by the fact that enzymatic homeostasis in the blood is provided not only by transporting the corresponding enzymes and zymogens using increment and resorption but removing the same enzymes from the bloodstream by different mechanisms. It has been the subject of quite a number of experimental and clinical research (Review: [5, 16]). Therefore, the relatively constant content of hydrolases at one or another level is the result of the balance of the given complex multidirectional regulated processes. In newborns we observed three low hydrolases content in the blood and their severe vibrations, that prove low enzyme potential, its variability, and, consequently, limited diagnostic information value.

The Digestive Glands Hydrolases in Amniotic Fluids

The volume and composition of amniotic fluids have been studied under normal and pathological conditions by lots of researchers at different times. The presence of enzymes in the amniotic fluids, including digestive gland hydrolases, has been established. However, the informational hydrolases criteria concerning the enzyme potential of the glands have not been studied in full, especially the mechanisms of origin of this group of enzymes in the amniotic fluids [15]. In different periods of gestation, hydrolases in the amniotic fluid are of different origin, but at the end of the gestation they come mainly from the digestive glands of the fetus. We cannot deny participation of hydrolases of amniotic fluid and placenta in the genesis [17], as well as the transport of hydrolases from the blood of a pregnant woman [15, 17]. They seem to be additional sources of enzymes in the amniotic fluids. These problems have recently been under our consideration [14].

In the amniotic fluids of new mothers with full-term gestation, the composition of hydrolases (see Table 4) differs from that in the blood serum of newborns (Table 3) and their new mothers (Table 2). The concentration of α- amylase, α-1-antitrypsin, pepsinogen I and especially pepsinogen II in the amniotic fluids is much higher than that in the blood serum of new mothers. What concerns alkaline phosphatase the differences are insignificant. The lipase composition in the amniotic fluid is five times as low as in the umbilical cord blood serum and even 15 times as low as the average blood serum index of the new mother.

Table 4: Hydrolases of delivery waters

Enzymes	Average	Median value	Minimum	Maximum	Lower quartile	Upper quartile	Shift direction, Statistical significance
Lipase (U/l)	2,00 4,64	1,90 4,05	0,90 0,90	4,90 33,40	1,45 2,10	2,35 4,64	↑p< 0,01
Amylase (U/l)	182,73 67,40	146,50 65,70	37,00 16,00	537,00 162,00	92,00 47,00	229,00 84,50	↓p< 0,01
ALP (U/l)	182,94 38,74	132,50 23,50	18,00 0,00	999,00 396,00	74,00 14,00	167,50 44,00	↓p< 0,01
Pepsinog en I (ng/ml)	33,95 19,28	29,85 17,90	10,40 7,00	106,00 63,10	24,85 13,40	38,00 19,90	↓p< 0,01
Pepsinog en II (ng/ml)	545,79 254,47	493,05 124,40	100,00 14,10	1635,90 1631,60	320,80 57,15	733,70 254,47	↓p< 0,01
α-1-antitrypsi n (g/l)	4,43 1,21	0,30 0,30	0,30 0,30	30,00 30,00	0,30 0,30	0,30 0,39	↑p< 0,005

Note: ALP – alkaline phosphatase (numerator – full-term newborns, denominator – premature newborns)

We cannot but mention the moderate statistically significant correlation between the hydrolase content in amniotic fluids and blood serum of umbilical cord: for α-amylase r=0,63; for pepsinogen I r=0,68; for pepsinogen II r=0,50; for alkaline phosphatase r=0,52. Hence, the hydrolases of amniotic fluids are informative

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concerning the individual morphofunctional immaturity of digestive glands in both fetuses and newborns.

In incomplete pregnancy, the amniotic fluids contain all types of hydrolases except lipase in a less concentration than in full-term pregnancy. The decreased content of hydrolases is statistically highly significant ($p<0, 01$).

High concentration of pepsinogen II in amniotic fluids that differ greatly from pepsinogen I prove the differences in development mechanisms of hydrolases of digestive glands in the systemic bloodstream of newborns, their mothers, and amniotic fluids. This phenomenon can be explained by the early development of enteric enzyme producers in the fetus [3, 18-20]. Pepsinogen II is mostly synthesized by pyloric and duodenal glands. That is why the concentration of this isoproenzyme in amniotic fluids in incomplete pregnancy as well. So, the regurgitated stomach content is transported to the amniotic fluids as the result of the duodenal, gastric and oral reflux which is common for both fetuses and newborns, while in their stomach content we found out higher concentration of pepsinogen II in comparison with pepsinogen I. Evidences of these differences are given in Table 5.

The high hydrolytic activity of amniotic fluids, the high volume of their transfer into the digestive tract of the fetus by swallowing, breathing and inhaling makes it possible to conclude that hydrolases of amniotic fluids take part in the hydrolysis of nutrients of the gastrointestinal tract which provides the amniotic trophism with its specific autolytic and self-digestion. It is necessary for the nutrition of the digestive tract mucous coat structures.

Hydrolases of Aspirated Stomach Content of Newborns

Fasting stomach content of a newborn is a mixture of gastric glands secretions, duodenal contents (pancreas secretion, duodenal secretions, and bile secretions), swallowed oral liquid (secretions of salivary glands and crevicular fluids) and amniotic fluids. Due to the absence of recurring activities of the digestive system in neonates [1], the volume and composition of the aspirated stomach content are relatively stable and demonstrate the total secretory activity of the above mentioned digestive glands, including their enzyme production.

Table 5: Hydrolases of stomach content in newborns

Enzymes	Average	Median value	Minimum	Maximum	Lower quartile	Upper quartile	Shift direction, statistical significance
Lipase (U/l)	43,68 40,47	10,65 20,50	0,30 0,10	270,00 244,10	2,5 4,45	48,75 41,94	p> 0,10
Amylase (U/l)	278,03 92,03	204,00 92,03	12,00 3,00	1289,00 237,00	140,00 44,00	340,00 103,00	↓p< 0,001
ALP (U/l)	423,23 55,31	70,00 46,00	21,00 3,00	4988,00 397,00	32,00 19,50	389,00 55,31	↓p< 0,001
Pepsino gen I (ng/ml)	133,74 42,70	90,85 42,70	4,10 0,00	907,50 150,10	44,60 19,80	133,75 58,15	↓p< 0,001
Pepsino gen II (ng/ml)	1125,03 573,64	1108,96 388,15	100,60 0,00	3087,80 2648,70	679,25 148,55	1761,10 861,00	↓p< 0,001
α-1-antitryps in (g/l)	0,32 0,31	0,30 0,30	0,30 0,30	0,45 0,48	0,30 0,30	0,32 0,31	p> 0,10

Note: ALP – alkaline phosphatase (numerator – full-term newborns, denominator – premature newborns)

Judging by the data given in Table 5, polysecretion aspirated from the stomach possessed the high concentration of α-amylase, lipase and pepsinogens, especially pepsinogen II; all 76 samples of stomach content demonstrated the higher level of pepsinogen II than pepsinogen I. The same result was received after the analysis of amniotic fluids. The level of similar hydrolases both in the gastric aspirate and amniotic fluids had moderate statistically significant correlation coefficients: for amylase $r=0,57$; for pepsinogen II $r=0,60$. We registered a strong correlation among five enzymes of amniotic fluids and gastric aspirate: the index of canonical correlation was $R_{pcc}=0,82$ (that characterizes the stage and interaction force between two variable lists). The results of these findings prove the above- formulated discovery of one more physiological mechanism, namely the development of digestive glands hydrolases of high concentration in amniotic fluids: duodenogastrooral regurgitation (reflux) into the amnion.

High enzyme activity of gastrointestinal contents provided by the fetal enzymes of both digestive glands

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and enterocytes performs the cavitary, parietal, and intracellular digestion of the fetus, including its amniotic trophism. In neonatal and subsequent stages of the child's development the hydrolases of his digestive tract that made up his initial digestive potential provide (together with the breast milk hydrolases) the lacto trophy with its peculiar proper and autolytic types (including the induced subtype) of digestion.

Saliva proteases increase the activity of casein by pepsins and trypsin in vitro. Similar interaction of proteinases in lacto trophy takes place in the stomach and small intestine under appropriate conditions (pH of the medium) [1]. In several recent works devoted to enzyme peptidomics, the summing up of the proteolysis produced by secretory proteases in the baby's stomach and similar proteases of mother's milk incubated in the stomach (2 h) by nano-chromatographic identification of peptides formed mainly during hydrolysis of β casein was established. At the same time, the effects of plasmin did not change, or they reduced by 1.3 times. Cathepsin D actions increased by 2.3 times, of pepsin by times, of elastase by 1.6 times, of chymotrypsin by 2.5 times, and those of prolineendopeptidases by 1.5 times. Hence, milk autoproteolysis was increased twice as much by secretory proteases in the stomach of the infant by the proteases [16]. The authors verified the relevance of intragastric proteolysis in the formation of regulatory peptides, most of which have acknowledged effects.

Pediatricians take an interest in the lipolytic activity of milk and its lipids, which play energetic, plastic, nutritional and protective role in the lacto trophy of the child. The lipolysis technology is multistage: it is performed by lipases of saliva and gastric secretion in the stomach cavity, then by lipases of milk and pancreatic secretion in the small intestine with the participation of bile salts inducers (promoting milk lipase) and colipase (promoting the effect of pancreatic lipase) [15]. Triglycerides are released from milk fat globules in the stomach by hydrophobic lipases of saliva and gastric secretion, that act as inducers of lingual and gastric lipases of the infant as well. The material of the globules membranes is recognized as a valuable product for the infant and has recently been added to milk mixtures. By the way, during the period of lactation, the lipolytic activity of milk is reducing more slowly than the content of other hydrolases in milk [15].

In human breast milk, there is no substrate for α-amylase, but its activity is high in the gastric aspirate. It is significant for the polysaccharide hydrolysis in complementary foods in the case of mixed and artificial feeding of infants. Hydrolysis of the principal carbohydrate of lactose milk is carried out by milk lactases and the small intestinal mucosa. Lots of researchers have lately focused their attention on these enzymes. Lactase is one of the disaccharides of enteric membrane digestion it was not included in the secretory potential and was not found in the gastric aspirate. The results shown in Table 5 indicate a significant decrease in hydrolases content (except lipase and antitrypsin) in the gastric aspirate of premature newborns if compared to full-term newborns. These data are extremely informative about the secretory digestive potential of newborns.

IV. Summary

The technology of lacto trophy makes it possible to conclude that the secretory hydrolases of the digestive glands, which form the digestive potential of the newborn, are of fundamental importance for its implementation. In this regard, its quantitative characteristic should be taken into account not only in incomplete gestation periods, but also in normal ones.

It is all the more important because hydrolase levels proved to be higher than the average in gastric aspirate, amniotic fluid, and umbilical cord blood serum in the group of premature infants, who had mainly a reduced digestive potential, while in infants of the group with standard gestational age enzymatic indicators of three bio liquids were reduced in comparison with average values. This phenomenon took place at the gestational borderline. Such results make it possible to acknowledge the digestive potential of newborns during childbirth the diagnostic test in the trophological prognosis of the development of newborns. Due to its digital variability and quantitative insufficiency, the material obtained does not allow determining the reference enzyme parameters of the standard initial digestive potential. That is why further fact-finding inquiry is necessary. At the current state of knowledge only a sharp decrease in the quantitative initial digestive potential of hydrolases in amniotic fluids and in umbilical cord blood serum can serve a reliable prognostic sign of trophological dysfunction in a newborn.

V. Conclusions

1. Hydrolases of the secrets of the digestive glands and small intestine of newborns make up the prenatally formed initial digestive potential of their digestive system.
2. The digestive potential characterized by the enzymes of the cord blood serum of the infant is significantly lower than that of the mother's venous blood serum and proves the incompleteness of the digestive potential in the antenatal period.
3. Low enzymatic activity of this potential requires proper and autolytic digestion of breast milk hydrolases to participate in lacto trophy.
4. Morphofunctional maturation of producers of different digestive system hydrolases of the fetus and the child are asynchronous: the digestive system of the small intestine matures earlier than the others, next come to the lipase producing

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glands followed by fetal zymogenic proteases and α-amylase.

5. The adequate initial digestive potential of the digestive system of newborns is marked by the content of hydrolases in gastric aspirate; the content of hydrolases in amniotic fluid and umbilical cord blood serum are less informative.
6. It is recommended to characterize the initial digestive potential of newborns by the parallel with the results of determination of several hydrolases and zymogens mentioned above that were obtained from gastric aspirate at the end of the delivery as well as in the umbilical cord blood serum and amniotic fluids.
7. In immature gestation, the digestive potential of the digestive system turns out to be reduced differently in different hydrolase systems, but not in the lipase system.
8. The determination of the initial digestive potential of the digestive system of newborns is promising for justifying the management of their natural, mixed, and artificial feeding.

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