How does the foetal gastrointestinal tract develop in preparation for enteral nutrition after birth?

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Abstract

At birth, the gastrointestinal tract (GIT) must be able to cope with the shift from parenteral nutrition before birth (via the placenta) to enteral nutrition after birth (oral colostrum/milk intake). In preparation for this event, the GIT grows and matures very rapidly in the weeks before birth. A series of studies in foetal pigs and sheep have shown that both hormonal and luminal factors influence this rapid phase of GIT development in farm animals. Among the potential hormonal regulators of development, cortisol plays a pivotal role. Thus, the normal developmental increases in stomach acid and gastrin secretion, and in certain enzyme activities (chymosin, pepsin, amylase, lactase, aminopeptidases), are stimulated by circulating cortisol. Cortisol also affects the intestinal absorption of immunoglobulins at birth but has limited effects on the GIT in the postnatal period. Ingestion of amniotic fluid by the foetus and of colostrum by the neonate also modulates GIT growth and enzyme activities. These effects may be mediated via luminal actions of growth factors, hormones and nutrients present in the fluids. However, luminal influences on the developing GIT are less pronounced in the foetus than in the neonate. In conclusion, both circulating and luminal factors affect prenatal GIT development to ensure that the foetal GIT is sufficiently mature to support the dramatic changes in nutrition that occur at birth. © 2000 Elsevier Science B.V. All rights reserved.

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1. The prenatal stomach

In the weeks before birth, the growth rates of the porcine stomach and ovine abomasum are similar to that of the whole body (Sangild et al., 1992,1995c) (Fig. 1A) but gastric function (acid and enzyme secretion) changes significantly during this period (Fig. 1B and C).

In the postnatal period, gastric acid is important for the activation and activity of gastric proteolytic enzymes and for prevention of bacterial growth in the upper gastrointestinal tract (GIT). Although gastric acid secretion has been detected in foetal pigs (Sangild et al., 1994b) and sheep (Shulkes et al., 1985,1987), secretagogue-stimulated acid secretion does not mature until some weeks after birth (Cran-
develops either shortly before or just after birth (Guilloteau et al., 1983; Sangild et al., 1991, 1995c) (Fig. 1C). The protease zymogens are produced mainly by the chief cells of the fundic mucosa and their activation and activity in the gastric lumen are stimulated by an acidic environment (pH 2–4). A shift in the nature of protease zymogens occurs during development in a number of species. For example, in the pig and sheep, during early life, the milk-clotting components (chymosins) are the major protease zymogens. With increasing age, they are gradually replaced by components exhibiting more general proteolytic activity (pepsins) (Foltmann, 1992) (Fig. 1C).

2. The prenatal small intestine

In the weeks before parturition, the pig intestine grows more rapidly than the body as a whole and its relative weight increases 70–80% over the last 3 weeks of gestation (Fig. 2A). A similar situation is noted for foetal lambs (Trahair et al., 1986a). In foetal sheep which have been growth retarded experimentally, GIT development is very severely affected. Hence, abnormal GIT growth appears early, before the onset of growth deficiencies of any other foetal organs, and persists at least until late term (Avila et al., 1989; Trahair et al., 1997). In the foetal lamb, the density of villi (villi number per mm² of mucosal surface) declines from 115 days of gestation to parturition, and continues to decline after birth. Therefore, the absolute number of villi is fixed at some point during development or the rate of production of new villi is outstripped by the rate of increase in intestinal diameter (and length). Crypt density increases towards term and after birth. The crypt villus ratio thereby steadily increases. Since cell proliferation also increases towards term (Trahair et al., 1986c), the migration rate rapidly accelerates from the long renewal time in the foetus (up to 20 days) (Trahair et al., 1986d) to the short time of the adult (2–3 days) (Trahair and Robinson, 1986a).

Hydrolysis of substrates in the foetal intestinal lumen is minimal (Britton and Koldovsky, 1989) and most of the digestive processes are intracellular, not luminal, in immature (vacuolated) enterocytes (Din-
gestation in both these species, lactase specific activity decreases in lambs (Sangild et al., 1994a) and increases in piglets (Sangild et al., 1995b) (Fig. 2B) during the last weeks of gestation.

Absorption of nutrients, as measured by the uptake of monosaccharides and amino acids by the intestinal mucosa, is low during the first half of gestation but increases rapidly thereafter (Buddington and Malo, 1996). During the final weeks of gestation, there is a particularly rapid increase in glucose uptake capacity (Sangild et al., 1993; Sangild, unpublished observations) while the ability of the mucosa to take up most amino acids, including lysine (Fig. 2C), remains unchanged. Just after birth, when the newborns begin to suckle, there is a dramatic decrease in the mucosal uptake of both glucose and amino acids which may be associated with colostrum-induced changes in the microvillus membrane (Zhang et al., 1997) (Fig. 2C). Nevertheless, total transport capacities along the entire length of the small intestine remain constant or even increase due to the large increase in intestinal mass (Fig. 2A) (Zhang et al., 1997).

One of the most striking features of the small intestine in newborn farm animals is its ability to take up macromolecules, including immunoglobulins, and to transport them intact across the epithelium into the blood. This ability ceases within the first day or two after birth in both piglets and lambs by a process known as “intestinal closure” (Weström et al., 1984; Sangild et al., 1997a). In the piglet, intestinal macromolecule uptake is present in utero during the last 2 weeks of gestation but it is markedly less in the foetus than in the neonate (Sangild et al., 1999). The prenatal developmental increase in the ability to absorb macromolecules is illustrated in Fig. 2C by data for the capacity to take up bovine serum albumin by non-specific endocytosis.

The intestinal absorption of series of proteins has also been tested in the foetal lamb at 120–125 days of gestation (Sangild and Trahair, unpublished results). Surprisingly, these studies were unable to demonstrate transfer of intact proteins across the intestine, although, by immunohistochemistry, the proteins were shown to be present in the cells lining the small intestine. These observations suggest that the ability to take up and transfer intact proteins from the epithelium into the circulation is a very specific

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**Fig. 2.** Mean (±S.E.) values for some key intestinal functions in foetal and neonatal pigs. (A) Weight of the small intestine relative to body weight, (B) the activity of three brush-border enzymes, lactase-phloridzin hydrolase, maltase-glucoamylase and aminopeptidase N, expressed relative to intestinal weight, (C) absorption of nutrients and macromolecules by the small intestine; the specific mucosal uptake of glucose and lysine as measured in vitro is shown together with the proportion of bovine serum albumin (BSA, protein macromolecule marker) absorbed into the blood stream after an oral dose of BSA + colostrum in vivo (modified from Sangild et al., 1995b,1999).
process that develops close to term in both pigs and sheep. This suggestion is supported by the finding that the capacity for protein absorption is lower in newborn piglets and lambs delivered prematurely than in those born at term (Hough et al., 1990; Sangild et al., 1997a). Hence, the uptake of macromolecules by the newborn farm animal is not merely the result of persisting intra-uterine absorptive processes as suggested previously (Weaver and Walker, 1989) but also reflects a specific maturational process that is timed to maximise uptake just after birth.

3. Regulation of prenatal development by glucocorticoids

Many hormones and growth factors are potential developmental regulators of GIT structure and function. Among these, cortisol from the adrenal cortex has received the most attention in relation to development of the foetal GIT and of other essential organs (e.g., lungs, liver, kidneys). In the pig and sheep, the major developmental increase in adrenocortical function occurs in late gestation, such that glucocorticoid concentrations in foetal blood are highest at term (Liggins, 1976; Hennessy et al., 1982; Silver and Fowden, 1989). Postnatally, glucocorticoid concentrations normally decrease and show only minimal changes around the time of weaning in pigs (Sangild et al., 1991).

In the pig and sheep, the normal development of gastric function seems to be stimulated by the prepartum increases in adrenocortical secretion. In the pig foetus, the major changes in gastric acidity and plasma gastrin concentrations occur concomitantly with the surges in blood glucocorticoid concentrations. In addition, treatment of foetal pigs (Sangild et al., 1992,1994b) with exogenous glucocorticoids induces precocious secretion of acid, gastrin and intrinsic factor. However, elevated glucocorticoids have little effect on gastrin-stimulated acid secretion after birth (Sangild et al., 1992) which suggests that porcine parietal cells may become unresponsive to cortisol once acid secretion has been initiated. In foetal sheep, gastric fluid acidity is not affected by exogenous cortisol infusion or by adrenalectomy (Sangild et al., 1995c). This may reflect the later development of acid secretory function in foetal sheep than in pigs, as abomasal fluid pH normally remains close to neutral until birth in foetal lambs (Sangild et al., 1995c).

In pigs and sheep, exogenous glucocorticoids stimulate the concentrations of chymosin and pepsin A in the stomach (Sangild et al., 1994a,c,1995c) and amylase in the pancreas (Sangild et al., 1994d,1995c) both before and immediately after birth. Adrenalectomy of foetal sheep or treatment of newborn pigs with metyrapone (an inhibitor of adrenal glucocorticoid synthesis) reduces the activity of these hydrolases (Sangild, 1995; Sangild et al., 1994d,1995c). Elevations in glucocorticoid secretion during the first 5 weeks of postnatal life continue to induce small increases in some hydrolase activities (Sangild et al., 1991), but these effects are unlikely to be of physiological importance because blood glucocorticoid concentrations normally decrease during this period.

In studies on foetal lambs, exogenous infusion of cortisol for 7 days from 108 days of gestation was associated with a doubling of the crypt cell proliferation rate and of the enterocyte migration rate along the villus (Trahair et al., 1987b). The proliferation rate was similar to that seen in the late gestation and early neonatal periods, when endogenous cortisol concentrations are high. However, the increase in cell production did not result in enhanced epithelial growth, since there were no significant changes in villus height or crypt depth. Furthermore, in proximal regions, production of new villi declined significantly. As a consequence, there was an increase in total cell number per villus. The villi became wider and accommodated more cells, but were reduced in density per unit area of mucosal surface.

To test whether enhanced growth would eventually occur as a result of increased cell proliferation, the infusion period was extended for a further 7 days (to 14 days) (Trahair et al., 1988). In these sheep foetuses, the proximal villus height, and proximal and distal crypt depth significantly increased by up to 50%. Thus, in the first 7 days of cortisol treatment, proliferation and migration increased precociously. Over the next 7 days of treatment, the increased cell production resulted in hypertrophy of the epithelial surface. Furthermore, in this mid-gestation period, proximal regions of the small intestine responded more quickly and showed more marked changes than
the distal regions. However, increases in cell proliferation and migration did increase in distal regions with significant crypt growth after 14 days of cortisol treatment which suggest that hypertrophy may occur, even distally, after more prolonged treatment.

Abolition of the endogenous cortisol surge near term by bilateral foetal adrenalectomy prevents the increase in villus height that normally occurs along the entire small intestine as parturition approaches (Trahair et al., 1987a). Crypts were also shorter in proximal regions of the foetal small intestine after adrenalectomy. Indeed, very little villus growth occurred during late gestation in the absence of cortisol. The observation that cortisol has little effect on villus growth early in gestation but is essential for the normal ontogenetic increase in villus height indicates that there are critical periods during development when the GIT is glucocorticoid sensitive.

The developmental rise in lactase and aminopeptidase activities in the prenatal pig is, at least in part, regulated by cortisol because infusion of cortisol into immature foetuses stimulates the activity of these enzymes (Sangild et al., 1995b). The lower cortisol concentration observed in piglets after caesarean delivery (as opposed to spontaneous vaginal birth) is also associated with altered GIT enzymic development in the newborn pig (Sangild et al., 1994e, 1996c). Similarly, in the GIT of the sheep foetus, the normal prepartum rise in aminopeptidase N activity and decrease in lactase activity can be stimulated precociously by exogenous cortisol and prevented by foetal adrenalectomy (Sangild et al., 1994a). However, the effects of glucocorticoids on the enzymic development of the small intestine and other regions of the foetal GIT (Sangild, 1995; Sangild et al., 1994c,d,1995b,c) are variable and dependent on the region of the GIT and the specific enzyme. These variable effects may be due to regional differences in the number and activity of the glucocorticoid receptors in the epithelial cells and/or to differences in the molecular mechanisms by which the glucocorticoids act to alter transcription and translation in these cells.

Studies in rats and mice have shown that glucocorticoids stimulate the normal advancement of intestinal closure which occur at 2 to 3 weeks of age in these species (Daniels et al., 1973; Moog, 1979). The mechanism whereby this occurs includes an increased cell replacement in the intestinal mucosa (Trahair, 1989; Henning et al., 1994). Consistent with this, Bate et al. (1991) observed histological changes and a decreased intestinal uptake of bovine immunoglobulin G in newborn piglets from sows with elevated blood cortisol concentrations in late gestation, but in this study it was not known how foetal blood glucocorticoid concentrations had been affected. Several lines of evidence suggest that the cellular mechanisms of closure and the role of cortisol in intestinal closure differ between rodents and the large farm animals. Firstly, the limiting factor for macromolecule uptake in pigs is the transmission of these molecules into the blood (lasting 12–36 h) and not the uptake into the vacuolated cells of the intestinal mucosa (lasting about 3 weeks) (Clarke and Hardy, 1971; Smith and Jarvis, 1978). Secondly, cell migration rate does not normally change during the first days of postnatal life in pigs (Smith and Jarvis, 1978) or after glucocorticoid treatment (James et al., 1987a). Thirdly, neonatal caesarean-delivered piglets and lambs show severely depressed immunoglobulin G uptake into plasma after metyrapone treatment (inhibition of cortisol synthesis) (Patt and Eberhart, 1976; Hough et al., 1990; Sangild et al., 1993). Finally, compromised newborn pigs with elevated cortisol concentrations, such as vaginally-delivered premature pigs or growth-retarded newborn pigs ("runts"), have higher macromolecule uptake than the corresponding caesarean-delivered pigs or normally-sized pigs (Svendsen et al., 1990; Sangild et al., 1997a). Hence, the literature indicates that, in the farm animal species, cortisol stimulates (rather than inhibits) macromolecule transport across the small intestine.

The potential effects of glucocorticoids on prenatal development of the porcine GIT are summarised in Fig. 3.

4. Regulation of prenatal development by luminal fluids

Major developmental changes take place in the gut in foetal sheep following the onset of foetal swallowing (Trahair and Robinson, 1986b) and in neonatal piglets in response to colostrum intake (Widdowson et al., 1976; Zhang et al., 1997). Thus, fluids in the
Fig. 3. Summary of the effects of endocrine factors (e.g., foetal cortisol secretion) and luminal factors (e.g., intake of amniotic fluid by the foetus and of colostrum by the neonate) on GIT development in the perinatal period (+, increase; 0, no change; −, decrease; ?, not investigated). Most effects are similar between pigs and lambs, which are the species for which most experiments have been done. Nevertheless, species differences do exist, and the effects of cortisol, amniotic fluid and colostrum on individual functions may also be highly dependent on foetal and postnatal age. See text for references.

GIT lumen appear to be important in maintaining and stimulating the normal sequence of mucosal differentiation during the perinatal period. Foetal lambs, in which swallowing of amniotic fluid has been prevented by ligating the oesophagus at 90 days of gestation, showed both reduced growth and altered differentiation of the intestine by late gestation (Trahair et al., 1986b; Avila and Harding, 1991; Trahair and Harding, 1992, 1995). Similar effects were observed in foetal lambs or piglets after more short-term oesophageal ligation in late gestation (Trahair and Sangild, 1999). When luminal fluid input was restored after oesophageal ligation, gut development was partly reversed (Trahair and Harding, 1995). Lack of foetal ingestion had the most pronounced effect on intestinal diameter which is a central index of intestinal growth and correlates well with most of the other parameters of gut growth (Trahair and Harding, 1992, 1995; Trahair and Sangild, 1997). While studies on oesophageal ligation in foetal lambs have not been associated with notable changes in body weight, studies in pigs show that foetuses prevented from swallowing amniotic fluid in utero are growth retarded at birth.

While luminal fluid has an important role in the structural development of the small intestine, its effects on the functional development of the GIT is more equivocal (Trahair and Sangild, 1997). In foetal piglets, amniotic fluid-infused animals have significantly higher aminopeptidase activities than foetuses without any luminal input, but this effect is only present in the distal small intestine (Sangild et al., 1997b). Lack of swallowed fluid has no apparent effect on the ability to absorb glucose, amino acids and dipeptides, and it actually increases the activity of lactase in the small intestine (Sangild et al., 1997b). Because enterocyte turnover is slower in the foetal intestine than in the postnatal intestine, the modulating effects of luminal factors on foetal GIT development may also take a relatively long time to
become expressed. Only long term changes in the amount or quality of swallowed amniotic fluid may have effects on intestinal function. The effects that luminal factors may have on early GIT development are summarised in Fig. 3.

Some evidence from studies on both the pig and sheep suggests that the ingestion of amniotic fluid in the foetus and of colostrum in the neonate may stimulate the acid secretory function in the stomach. Experiments on pig foetuses (87–94% of gestation) and sheep foetuses (82–87% of gestation) showed increased plasma gastrin concentrations after 6–7 days of intra-gastric infusion of either amniotic fluid, milk whey or colostrum whey (Sangild et al., 1996a,b). The gastrin-releasing effects of these three fluids were similar despite the large differences in their protein and growth factor content. However, both the absolute concentrations and the relative increases in plasma gastrin over the infusion period were smaller in the foetal sheep than in the foetal pig. Foetuses which received no fluid at all because of oesophageal ligation showed decreased plasma gastrin concentrations (Sangild et al., 1996a,b). Both the lack of luminal nutrients (proteins, peptides) and gastrin-releasing substances in amniotic fluid may be responsible for the decrease in gastrin secretion in such foetuses. In the pig, the infusions of amniotic fluid, milk whey or colostrum whey also decreased gastric fluid pH at birth (Sangild et al., 1996b) which indicates that luminal fluids may enhance maturation of immature parietal cells. The suggestion that enteral input is necessary for the normal development of acid secretory function in the pig during perinatal period is consistent with previous findings in the foetal rabbit (Mulvihill et al., 1985).

In foetal pigs and sheep, the increases in blood gastrin concentrations observed in response to changes in enteral stimuli (Sangild et al., 1996a,b) were relatively small compared with the response to exogenous cortisol administration (Sangild et al., 1994b) or to the normal rise in gastrin that occurs with advancing gestational age (Shulkes et al., 1985; Sangild et al., 1994b). Glucocorticoids appear more important in regulating gastrin secretion in the pig and sheep during the perinatal period than the presence in the gastric lumen of amniotic fluid before birth or colostrum after birth. The same may be true for various other GI hormones.

In long gestation species such as the pig and sheep, the time of intestinal closure to macromolecule transport may be influenced more by luminal factors than by endocrine factors such as glucocorticoids. Studies in neonatal (Weström et al., 1985) and foetal pigs (Sangild et al., 1999) indicate that colostrum itself plays the most important role in the induction of intestinal closure. When colostrum whey was infused into the foetal pig intestine at 101–107 days of gestation, the uptake of macromolecules declined from 104 days onwards (Sangild et al., 1999). In contrast, foetuses infused with amniotic fluid or milk whey remained fully capable of taking up macromolecules by pinocytosis during the entire period of infusion. In these studies, some foetuses had significantly elevated plasma cortisol (probably in response to foetal surgery or treatment) but this did not shorten the time available for macromolecule absorption in these foetuses.

Studies in both foetuses and neonates have shown that colostrum ingestion is associated not only with rapid growth and enhanced pinocytotic activity of the intestinal mucosa but also with changes in the activity of brush-border enzymes. Colostrum ingestion has been reported to either increase or decrease the specific activity of lactase (expressed per intestinal weight, protein or DNA) while consistently increasing the specific activities of enzymes such as maltase and aminopeptidases (Sangild et al., 1996c; Wang and Xu, 1996; Zhang et al., 1997; Fig. 2B). Exposure of the neonatal intestine to colostrum is associated with profound changes, both in mucosal structure and in the biochemical events which lead to the insertion of functional enzymes into the brush-border membrane (Dudley et al., 1996). The nature of the nutrient components or regulatory factors in colostrum that causes these structural and functional changes remains unknown.

5. Perspectives

Perinatal mortality is very high in farm animals, including pigs and sheep (e.g., 10–20%). A large part of this mortality may arise because the offspring of these species are born at a time in gestation when they are only just able to survive. Thus, the final prepartum maturation of the foetal tissues and or-
gans, including the GIT, plays a critical role in determining neonatal viability. Improving neonatal viability therefore depends on identifying the precise nature of the systemic and luminal factors that influence GIT development during the critical period just before birth. Such information will help to optimise the clinical and nutritional care of newborn farm animals.

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