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Invited Review: The preterm pig as a model in pediatric gastroenterology

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Abstract

At birth, the newborn mammal undergoes a transition from a sterile uterine environment with a constant nutrient supply, to a microbe-rich environment with intermittent oral intake of complex milk nutrients via the gastrointestinal tract (GIT). These functional challenges partly explain the relatively high morbidity and mortality of neonates. Preterm birth interrupts prenatal organ maturation, including that of the GIT, and increases disease risk. Exemplary is necrotizing enterocolitis (NEC), which is associated closely with GIT immaturity, enteral feeding, and bacterial colonization. Infants with NEC may require resection of the necrotic parts of the intestine, leading to short bowel syndrome (SBS), characterized by reduced digestive capacity, fluid loss, and dependency on parenteral nutrition. This review presents the preterm pig as a translational model in pediatric gastroenterology that has provided new insights into important pediatric diseases such as NEC and SBS. We describe protocols for delivery, care, and handling of preterm pigs, and show how the immature GIT responds to delivery method and different nutritional and therapeutic interventions. The preterm pig may also provide a sensitive model for postnatal adaptation of weak term piglets showing high mortality. Attributes of the preterm pig model include close similarities with preterm infants in body size, organ development, and many clinical features, thereby providing a translational advantage relative to rodent models of GIT immaturity. On the other hand, the need for a sow surgical facility, a piglet intensive care unit, and clinically trained personnel may limit widespread use of preterm pigs. Studies on organ adaptation in preterm pigs help to identify the physiological basis of neonatal survival for hypersensitive newborns and aid in defining the optimal diet and rearing conditions during the critical neonatal period.

Keywords

colitis; formula; immunity; intestine; microbiota; milk; newborn

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INTRODUCTION

Birth is a major transition in the life of mammals that involves abrupt changes in the physiologic and metabolic functions of many essential organs, including the lungs, the liver, cardiovascular system, kidneys, and the gastrointestinal tract (**GIT**) that are required for extrauterine survival. Prenatal trajectories of development vary among organ systems and differ widely among mammalian species (Sangild, 2006). In humans, most tissues and organs reach a relatively advanced stage of maturity by late gestation, and newborn infants are generally well prepared for extrauterine life despite that tissues such as muscle and the central nervous system remain relatively immature even at normal term (~40 wk gestation). This pattern contrasts with that for pigs, which are born with muscular and nervous systems that are relatively mature while the GIT is relatively immature, making newborn pigs more dependent on maternal milk for survival and growth than human neonates. Awareness of these species- and organ-specific developmental differences is very important when translating patterns of ontogenetic development from one species to another.

Human infants born at <37 wk postconception are defined as preterm. These newborns represent 10% of all live births worldwide (Simmons et al., 2010) and suffer greater morbidity and mortality (Oestergaard et al., 2011). Preterm pigs delivered at <95% gestation (<110 d of 116 d gestation) suffer from many of the same organ immaturities as preterm infants (Sangild et al., 2002a), including an increased sensitivity to necrotizing enterocolitis (**NEC**). Necrotizing enterocolitis is a gut inflammatory condition that develops in 5 to 10% of all hospitalized preterm infants (Lin et al., 2008) and, in both species, NEC development is diet- and colonization-dependent (Sangild et al., 2006). Infants suffering from severe NEC may be subject to intestinal resection and subsequent development of short bowel syndrome (**SBS**). During the past 10 yr, our groups have studied the etiology and pathogenesis of NEC using preterm pigs, with our results published in >50 scientific papers. A goal for this review is to critically evaluate the validity of the preterm pig as a translational model for NEC and SBS. We describe some of the model considerations that are important, but not described in detail in the original research papers. For additional insights into the biological basis of NEC in pigs, we refer the reader to our original papers and reviews (Sangild, 2006; Siggers et al., 2011a; Buddington and Sangild, 2011; Cilieborg et al., 2012; Buddington et al., 2012). Our focus in this contribution is on the unique advantages and limitations of preterm pigs, including biological aspects, technical considerations, and economic and ethical constraints. Studies in preterm pigs may also provide valuable information for understanding the physiological basis of neonatal adaptation in newborn term pigs.

Preterm Pigs, Necrotizing Enterocolitis, and Conventional Pig Production

Perinatal mortality in modern pig production herds is relatively high (15 to 25%) compared with values for other production animal species (Rootwelt et al., 2013). Newborn term pigs are susceptible to external stressors such as adverse nutritional conditions and microbiological challenges (Jacobi and Odle, 2012) and intestinal infections and digestive complications are major causes of neonatal pig morbidity (Pedersen et al., 2011). While limitations induced by intensive production systems may contribute to the perinatal morbidity, it is evident that pigs are born at a stage of organ maturation that still requires appropriate maternal care. The 10% of farmed pigs that are born a few days before normal term show markedly increased mortality (Vanderhaeghe et al., 2011). Pigs delivered even earlier (e.g., 5 to 10 d before term, 92 to 96% gestation), due to maternal infection or induced premature labor, have very low viability under normal rearing conditions (Silver et al., 1983; Pejsak et al., 1997). Hence, the preterm pig is a highly sensitive model to investigate factors that affect survival in near-term pigs born under farm conditions.

The neonatal pig digestive disease, necrotizing enteritis (Schäfer et al., 2012), shares many similarities with NEC, but the two diseases are not identical. The β toxin of *Clostridium perfringens* type C can be identified as a causative agent for necrotizing enteritis in pigs born from sows that are not vaccinated against this toxin (Songer and Uzal, 2005). Clostridia overgrowth from several serotypes is often, but not always, associated with necrotizing enteritis in preterm pigs (see also, later section on gut microbiota). Intestinal immaturity, coupled with general bacterial colonization and excessive feeding, are the key factors that precipitate clinical NEC. A dysregulated intestinal immune system may be a common base for the two disease entities, but the roles of the gut microbiota and diet (colostrum, milk, formula) differ between the immature and the fully mature intestine.

Strong genetic selection pressure for lean tissue growth and large litter size in pig production herds could be linked with immature organ maturation at birth. Sows with large litters have shorter gestation lengths and give birth to less mature piglets (Vanderhaeghe et al., 2011). Likewise, genetic selection for muscle growth in conjunction with prolonged postnatal maturation of some tissues (e.g., muscle versus adipose tissue) could potentially result in the GIT and other organs being less functionally mature at critical life stages, such as birth and weaning. This speculation is consistent with the increased sensitivity of modern production herds to diseases such as necrotizing enteritis relative to outbred pig strains or minipigs.

Perinatal organ maturation is in part mediated via adrenal cortisol secretion (Sangild et al., 1994, 1995a). Circulating cortisol levels of pigs are positively correlated with neonatal survival (Leenhouwers et al., 2002), adaptation to weaning (Chapple et al., 1989), and increased fat accretion in slaughter pigs (Foury et al., 2007). These findings support the hypothesis that the breeding goals of pig production partly counteract maturation of essential internal organs like the GIT. This remains a hypothesis, but does provide a theoretical framework to understand why problems of perinatal mortality and weaning associated digestive diseases remain significant challenges in modern pig production. It may also explain why we have observed that the sensitivity of preterm pigs to NEC, delivered at the same gestational age, seems to vary widely among different breeding herds and countries. In addition to intestinal immaturity per se, circumvention of the prepartum cortisol surge by preterm, caesarean delivery (Sangild et al., 1995b, 2006) potentially contributes to hypersensitivity to diet- and colonization-dependent intestinal lesions in preterm pigs. Preterm pigs may be a model for term newborn pigs with impaired survival capacity, in part related to lowered cortisol secretion (Leenhouwers et al., 2002). There is a great need to better understand both the genetic and the physiological basis of neonatal survival and adaptation (e.g., sensitivity to NEC and necrotizing enteritis) in preterm and term neonates.

Preterm Versus Term Animal Models

Term newborn pigs have already served as a valuable large animal model that has contributed to our understanding of GIT development, adaptation, and nutrient metabolism. This includes responses to different feeding regimens, parenteral nutrition (**PN**), surgical resection, and to existing and experimental diet ingredients (e.g., Urschel et al., 2007; Elango et al., 2009; Turner et al., 2011; Stoll et al., 2012). Specific questions related to preterm infants have also been addressed using near-term pigs as acute and chronic models (Di Lorenzo and Krantis, 2002; Ewer et al., 2004; Aquilina et al., 2007; Gill et al., 2012). However, term pigs do not adequately model the challenges caused by an immature GIT after preterm birth, and do not simulate accurately the spontaneous constellation of features that are the hallmarks of NEC that develops in preterm infants. Similarly, even though term pups of laboratory rodents (mice and rats) are considered to be immature, they are born with a GIT well prepared for processing dam milk. Fetal and preterm lambs have been used as preterm models, but the digastric adult gut limits the relevance for studying development of

the GIT in monogastric species like pigs and humans. Although nonhuman primates, notably baboons, are valuable model animals, they pose limitations (e.g., specialized facilities, restricted availability, herbivorous adult diet). Key attributes of the preterm pig include similarities with preterm infants with respect to size (e.g., 0.6 to 1.1 kg) and the impaired respiratory, nutritional, immunological, and metabolic responses after preterm birth (Sangild et al., 2002a; Sangild, 2006; Lennon et al., 2011). Preterm pigs are amenable to handling and surgical procedures and have GIT characteristics similar to those of infants (Sangild, 2006).

The interactions among dietary, microbial, and immunological factors that occur after birth influence the health and NEC risk of preterm neonates. Our studies using preterm pigs have demonstrated that the degree of immaturity at birth is a critical and necessary element that makes the pig model of NEC especially relevant to the clinical findings for preterm infants. In our experience, only pigs delivered before 94% gestation (<109 d gestation) show signs of organ immaturity and spontaneously develop NEC-like symptoms (Sangild, 2006). As noted above, this may however vary, depending on the genetic constitution of different sow herds.

The medical and surgical complications caused by infant intestinal resection and SBS differ markedly from those of existing animal models of intestinal and metabolic adaptation to SBS. This led us to develop the preterm pig as a model for intestinal resection. We have learned that the immediate postsurgical adaptations after intestinal resection are reduced in preterm versus term piglets (Sangild et al., 2009). These findings have implications for human preterm infants requiring intestinal resection for treatment of NEC and other disorders. Moreover, the model provides opportunities to evaluate existing and experimental interventions for SBS.

Prematurity in Preterm Pigs and Preterm Infants

Organ maturation is accelerated in the fetal pig during the last 14 d of gestation (Sangild, 2006; Buddington et al., 2012). Corresponding with this, morbidity and mortality of the preterm pig are inversely related with gestational age at delivery. Because organ development is less linear in pigs, relative to humans, prematurity expressed as a percentage of gestation does not correlate directly for preterm pigs and infants. As a litter bearing mammal, the gestation length of a sow is also much more closely regulated and less variable than in humans. Figure 1 illustrates schematically how three key organs, the lungs, the GIT and the brain develop in relation to birth and weaning for humans, pigs, and rats. Lung development correlates closely with the time when mammals first become viable ex utero. The time when the GIT and brain undergo rapid and dramatic functional maturation determine when species acquire the capacity to digest diets other than mother’s milk (earlier for infants) and develop neurological control of locomotion (earlier for pigs). However, even within the same organ (e.g., lungs, gut, brain) different functions may show different developmental trajectories in relation to birth and weaning.

The limited access to tissues from preterm infants at different stages of gestation limits our understanding of organ development in infants. Regardless, some reasonable assumptions have been made about GIT development (e.g., Montgomery et al., 1999), allowing us to make comparisons with pigs. Our initial studies with pigs described the patterns of ontogenetic development of the stomach, pancreas, and small intestine in fetal pigs at different gestational ages (Sangild et al., 1994, 1995a, 1995b; Buddington and Malo, 1996). These were followed by studies using preterm and term pigs born either vaginally or by caesarean section (Sangild et al., 1997). To date, our investigations have been dominated by preterm pigs delivered by elective caesarean section at 104 to 107 d of gestation. This corresponds to 89 to 92% of gestation, with term = 117 d in Danish production herds and 115 d in some US production herds, again suggesting that gestational ages and patterns of

development may differ slightly between breeding herds. Mortality of preterm pigs delivered at 89 to 92% of term is 10 to 20%, with moderate intensity of clinical care (Che et al., 2010). However, survival drops markedly when delivery is at 100 d (85%) of gestation, even with intensive respiratory, hemodynamic, and nutritional interventions. Still, preterm pigs at 92% of gestation show distinct signs of prematurity relative to full-term, caesarean-delivered pigs. Pigs delivered at this stage spontaneously ventilate, but respiratory distress syndrome is common. They also display enteral food intolerance (vomiting of gastric residuals), have poor locomotory skills, impaired temperature regulation, and hemodynamic instability, and immaturity of enteric and systemic immunity (Sangild et al., 1996, 1997, 2002a; Cilieborg et al., 2011a). Other organs, such as the kidneys, pancreas, and brain are also immature. Collectively, these observations led to our estimation that the 90% gestation preterm pig is a good model for the 75% gestation (30 to 32 wk) preterm infant who rarely requires prolonged ventilation support, but is at risk of NEC and other acute and chronic diseases associated with preterm birth (Sangild, 2006). This estimation mainly relates to GIT functions and does not necessarily apply to other organs and tissues (e.g., brain, Fig. 1).

Body growth and GIT maturation tend to be more variable in preterm than in term pigs (Cilieborg et al., 2011a; Sangild et al., 2002a, 2002b). This may reflect that trajectories of development differ among litters in the last weeks before term, that gestation length varies among different litters, and that there are differences in the exact time when embryos implant and establish placental connections. Despite this, we have not detected consistent differences in immediate postnatal survival and the risk of NEC among preterm pigs obtained at 90 to 92% of term from different litter sizes or from sows with different genetics or parity. We have been surprised to see how well intrauterine growth restricted (**IUGR**, <700 g body weight) preterm pigs survive and thrive with only a marginal increase in neonatal mortality and NEC sensitivity compared with normal-weight siblings (Che et al., 2010). We have successfully raised preterm IUGR pigs as small as 300 g at birth for 10 d after birth. Conversely, large preterm newborn pigs (>1300 g; Fig. 2E) do not appear to have a specific survival advantage and suffer from the same developmental immaturities as their normal- and low-birth-weight littermates. In fact, some studies indicate that large-for-gestational-age infants have increased risk for short- and long-term developmental abnormalities (Djelantik et al., 2012).

Delivery and Postnatal Care of Preterm Pigs

Some of the typical protocol time lines and pictures of operative and clinical care procedures we have established for our NEC and SBS preterm pig studies are presented in Fig. 3. The compressed developmental trajectory of pigs during late gestation makes it essential that pregnant sows of known breeding dates are available. Additional desired attributes of the sows include specific pathogen-free, known parity, and consistent genetics. Sows can be transported to the surgical facility in the days before caesarean section or several weeks before. Litter size in pig production has increased markedly in recent decades as a result of the breeding policy. Litter sizes of 15 to 20 pigs are common, and a litter size up to 25 pigs is not unusual.

The mode of delivery (spontaneous vaginal, induced vaginal, elective caesarean section, caesarean section after induction of parturition) has marked effects on the postnatal characteristics of several organs (lungs, liver, GIT, heart), regardless of whether pigs are born preterm or at full-term (Siggers et al., 2008b; Sangild et al., 1995b, 1996b, 1997). The fact that NEC develops in both vaginally and caesarean-delivered preterm pigs and infants indicates that immaturity plays a more significant role than birth mode. Possibly, the maturational effects of vaginal birth (e.g., improved thermoregulation, respiration, and gut

functions) are not sufficient to counteract the adverse effects of feeding and bacterial colonization in triggering NEC (Siggers et al., 2008b).

The caesarean section used to harvest preterm pigs occurs after an overnight fast, with general anesthesia induced using intravenously administered anesthetics, like thiopental sodium or intramuscular injection of zolazepam, tiletamin, xylacin, ketamine, and butorphanol with or without inhalation anesthesia (e.g., isoflurane, with or without endotracheal intubation). As specific anesthesia proto-canesthesiaols may vary among performance sites, the acute treatment of the newborn pigs may also vary. Specifically, following injection with compounds (like xylacin) that compromise circulatory and respiratory function, it may be helpful to reverse the effect in the newborn pigs with antidotes like yohimbin, tolazolin, or atipamezol.

The caesarean section is performed aseptically with the sow in lateral recumbency. After local anesthesia of the abdominal wall (lidocaine alone or in combination with noradrenalin), an abdominal incision is made to expose the uterine horns. Individual preterm pigs are removed through incisions. To maximize blood volume after delivery, cord blood is squeezed into the fetal systemic circulation before clamping and severing the umbilical cord (at this time, cord blood is available for sampling for later analysis of baseline and preterm birth parameters). Fluid is aspirated from the mouth and nose to facilitate respiration. A critical concern is keeping the newborn pigs warm. This can be done by wrapping them in a warmed, dry cloth after delivery and rapidly transferring them to an oxygenated and thermoregulated transport incubator. The delivery of an entire litter requires 30 to 45 min after induction of anesthesia, depending on litter size. After the preterm pigs have been removed, maternal blood is collected aseptically by accessing uterine veins, and the sterile plasma fraction is transfused into the newborn pigs to provide passive immunity. If the surgical site is not immediately adjacent to the rearing facility, it is necessary to have a heated transport chamber (incubator) that includes an oxygen supply. Newborn preterm pigs can be group housed for the first few hours after birth because direct contact between littermates may stimulate initiation of breathing reflexes. The respiratory stimulant doxapram hydrochloride can be provided sublingually or via an umbilical vessel to newborn pigs that do not ventilate adequately.

Enclosed incubators are the preferred choice for rearing preterm pigs, although open cages that have a heat source may be sufficient for preterm pigs of moderate prematurity (>94% gestation). Conventional infant incubators offer tightly controlled temperature, humidity, oxygen concentration, shield from environmental microbes, and have the potential to provide ventilation support such as continuous positive airway pressure systems. On the negative side, infant incubators are expensive, require space, and make surveillance of many pigs from large pig litters (e.g., 20 to 25 pigs) more difficult. Over the years, our groups have developed incubator systems and protocols that include the most critical features and procedures (Fig. 2).

The first 12 h after delivery are critical. As mentioned previously, newborn preterm pigs may require respiratory and circulatory support while they recover from the anesthesia from the caesarean section. In fact, the influence of anesthesia is prolonged in preterms (3 to 5 h), relative to term pigs (1 to 2 h), presumably as a result of a reduced ability to metabolize and excrete the anasthetic drugs. Constant 24-h clinical attention is required for about 5 d after delivery to ensure maximal survival of preterm pigs. The immediate spontaneous postnatal mortality after preterm birth (e.g., before 12–24 h) is attributed to immaturity of various tissues and organs (lungs, liver, brain, kidney, cardiovascular; Fig. 1). This is consistent with the direct relationship among improved respiratory care of infants after preterm birth, increased survival, and lower incidence of chronic diseases. Oxygen saturation among

preterm pigs is generally reduced for about 12 h postpartum (Sangild et al., 2002a; Bjørnvad et al., 2008) before blood gas values, including carbon dioxide, lactate, and pH values, normalize. Lung atelactasis (impaired bronchio alveolar expansion) is a frequent finding among preterm pigs at autopsy, even beyond the first wk of postnatal life. Apparently, even a partially expanded lung volume is sufficient to ensure normal blood gas values, at least short term. We compensate for immature lung function by providing 1 to 2 L/min of 100% oxygen into the incubator environment for the first 6 to 12 h after birth. Thereafter, supplemental oxygen is provided only to pigs showing signs of respiratory distress (e.g., depressed ventilation rate, low partial oxygen pressure pallor). This is accomplished by placing a mask over the snout of the pig to allow direct inhalation of supplemental oxygen. Prolonged provision of pure O_2 is detrimental and decreases rather than increases survival rate, and in infants this may also predispose to later complications such as neurodevelopmental defects (Sorensen and Greisen, 2009). A proportion of preterm pigs suffer from persistent ductus arteriosus (**PDA**), disturbing blood flow and oxygenation for several days after birth. Similar to preterm infants, PDA in preterm pigs is unrelated to the risk of NEC, and resolves itself spontaneously within the first week after birth, as assessed by echocardiography and histology (Norgaard, Sangild and Agerholm, unpublished observations). It is possible to provide ventilation and vascular support to preterm pigs, but these interventions require additional equipment and expertise and impose intense technical demands on research personnel. Constant surveillance of individual preterm pigs can be performed by installation of web-based cameras that allow clinical consultations between on-site primary care takers and external support staff (Fig. 2F).

Preterm pigs have immature thermoregulatory capabilities, especially after caesarean delivery (Siggers et al., 2008b). Low-weight preterm pigs (<700 g) are more sensitive than normal-weight preterm pigs (Bjørnvad et al., 2008; Che et al., 2010). A temporary moderate degree of hypothermia for the first few hours after delivery may be important to activate the sympatico-adrenal response that triggers many organ adaptations. However, our experiences show that preterm pigs that develop severe hypothermia (<35°C internal temperature) have increased mortality during the first postnatal day. Hence, it is critical that the incubators provide a thermal environment that prevents both hypo- and hyperthermia.

Preterm pigs and preterm infants have an immature skin epithelium that is prone to bacterial infections and an increased loss of body fluids by transpiration. A thin film covering the entire skin of preterm pigs can be removed using a damp cloth. Alternatively, the film can be left and will dry and peel off over the first 1 to 2 d after birth (Fig. 2E). Leaving the film may protect the skin of neonates from percutaneous fluid loss and bacterial invasion until barrier functions and immunological responses of the epidermis are acquired (Levy, 2007). Epidermal development has not been investigated in preterm pigs, but nonspecific or Staphylococcus-related skin inflammatory rashes are common, probably modeling the immature innate immunity reported for infants (Levy, 2007). Maintaining humidity at 80 to 100% in the incubators for the first 1 to 2 d reduces percutaneous fluid loss, although this may increase bacterial growth.

There is increasing awareness of the importance of external stimuli (maternal contact, sound, light intensity) for infant development. *Minimal touch* clinical practices are common in neonatology, and are combined with reducing the exposure to ambient noise and light. On the other hand, advocates of *neonatal kangaroo care* argue that maternal care, physical contact, and even gentle massage, could be important for postnatal development of the preterm child (Simmons et al., 2010). We generally practice minimal touch for preterm pigs based more on intuition than on evidence for specific beneficial effects. Most preterm pigs open their eyes within 24 h of delivery, become partly mobile within 1 to 3 d, and can be transferred within the first week of life from the highly controlled environment of the

incubators (Fig. 2D) to larger, open space cages, allowing for explorative behavior. Considerable variation exists among litters delivered at ~90% gestation as to the trajectories of postnatal development of eye lid opening and normal movement (1 d to 1 wk). While such clinical signs of maturity are obvious, we have not found any consistent relation to later NEC development. Much research remains to be done to investigate the more long term effect of piglet prematurity on the GIT as well as other organ systems.

Surgical Preparation

Within 3 to 4 h after delivery, each pig has an orogastric feeding tube placed and one or more catheters inserted through the umbilical stump for vascular access (Fig. 2B). The orogastric feeding tube (5 or 8 French size) is introduced via a small incision made in the cheek, behind the teeth, to prevent the pig from chewing the tube, and passed into the stomach or distal esophagus. The surgically placed feeding tube avoids the possible tissue damage and stress caused by repeated insertion of a feeding tube and the misplacement of the tube into the trachea by inexperienced personnel. The feeding tube is fixed to the skin and the pigs can then be fed with a minimum of stress (Fig. 2E).

The insertion of a sterile catheter via the umbilical stump provides vascular access for blood sampling and provision of maternal serum, parenteral nutrition (PN), supplemental fluids, and therapeutic compounds (Fig. 2B). The umbilical vessel catheters can be inserted into one of the two umbilical arteries (**UAC**) and advanced 17 to 20 cm/kg of birth weight. This distance is sufficient to place the end of the catheter in the dorsal aorta in the thoracic region. This ensures good mixing of infused hyperosmotic nutritional solutions such that the gut, kidney, and hind limbs are only exposed to diluted infused nutritional solutions. Exposure to hyperosmotic nutritional solutions may cause local necrosis in the kidney if the catheter is not placed correctly. Attempts to catheterize the umbilical vein have not been successful, as the catheter does not spontaneously enter into the ductus venosus and further into the vena cava caudalis. Instead, the tip of the catheter may end close to the liver parenchym, causing local necrosis if hyperosmotic nutritional solutions are infused. However, umbilical venous catheters (**UVC**) are commonly used for preterm infants and can, in fact, also be placed in preterm pigs. When a UVC is placed it should be advanced about 5 to 6 cm from the abdominal wall, placing it close, but not too close, to the hepatic-portal junction. Since the internal venous structure normally disintegrates 3 to 5 d postnatally to form the permanent ligamentum teres hepatis, UAC tend to be better than UVC for repeated blood sampling and for longer-term provision of PN. A small-diameter catheter (e.g., 3.5 French) is preferred and to prevent coagulation around the tip of the catheter, which may cause embolization and infarction in downstream organs. We often coinfuse heparin at 1 U $kg^{-1} \cdot h^{-1}$. The catheters are secured in the cord stump by two or more ligatures. Additional sutures or soft cotton strings are used to occlude the other umbilical vessels and avoid bleeding. After the UAC or UVC is inserted, it is critical that it is secured to avoid loss by leg movements or when the cord tissue dries after 3 to 5 d. A common feature is that we secure the remnant cord stump (3 to 4 cm) and catheters to the skin, with catheter access on the dorsal surface of the pigs. This includes the use of cyanoacrylate-based adhesives, adhesive tape, sutures, and surgical staples. If intravascular infusions (PN, fluid restitution, or other) are to be provided after preterm pigs become fully mobile in their cages, the catheters and incubators should be fitted with swivel systems to allow free movement of the pigs without excessive twisting and kinking of the catheters. For longer periods of PN infusion and vascular access, a catheter can be placed into a jugular vein and exteriorized in the neck region, either immediately after birth or later.

The placement and securing of the feeding tube and vascular catheter requires 15 to 20 min per pig. With the use of several work stations and experienced personnel, an entire litter of

20 pigs can be ready for the first feedings within 4 to 5 h of delivery, which is important to prevent hypoglycemia and dehydration. Most preterm pigs remain adequately anesthetized from the maternal anesthesia at caesarean section for the placement of the feeding tube and UAC or UVC. Few require supplemental anesthesia (e.g., intramuscular doses of zolazepam, tiletamin, xylazine, and ketamine or gas anesthesia, such as isoflurane). Analgesia can be provided (e.g., intramuscular doses of butorphanol). However, the possible suppressive effects on respiration and cardiac function (e.g., xylazine) need to be considered and an antidote (e.g., atipamezol) should be immediately available, if needed.

Sterile procedures must be used throughout placement of the feeding tube and umbilical catheter as newborn preterm pigs have very immature epithelial barriers (skin, lungs, GIT) and immune functions. In contrast to many other species (e.g., rodents, humans), the pig placenta does not transfer maternal immunoglobulins to the fetus. As a consequence, newborn pigs are devoid of systemic passive immunity, and acquire passive immunity entirely by postnatal uptake of colostral antibodies. The combination of reduced intestinal immunoglobulin uptake capacity in fetal and preterm pigs (Sangild et al., 1997, 1999) and the immature intestinal responses to colonizing bacteria (Sangild et al., 2002a) predispose newborn preterm pigs to a greater risk for health challenges. Passive transfer of immunity can be partially provided by orogastric feeding of sow colostrum (Sangild et al., 1997, 2002a). When it is desirable to avoid the GIT maturational and protective influences of colostrum, passive immunity can be provided using sterile plasma collected from the sow during the caesarean section (see earlier) that is administered via the UAC during the first 24 h after delivery, either as several boluses or constantly by inclusion in the parenteral fluid (Fig. 2D). An infusion of 10 to 20 mL/kg maternal plasma, with ~30 mg/mL maternal immunoglobulin G, results in circulating levels of 5 to 10 mg IgG/mL. This is considered adequate to provide initial basic passive immunity and infection resistance (Sangild et al., 1999, 2002a). A dose of 20 mL/kg represents a relatively large expansion of the total plasma volume (about 30%), but is comparable to the plasma expansion induced by immunoglobulin uptake from ingestion of sow colostrum by newborn pigs (McCance and Widdowson, 1959). Apart from a few cases of transient piloerection, we have not observed adverse reactions to the plasma transfusion. Although the provision of systemic or enteral immunoglobulins may combat local and systemic infections, this does not prevent NEC, consistent with several studies in preterm infants.

Parenteral Nutrition

Parenteral nutrition allows preterm pigs and infants to have positive nutrient and energy balances and normoglycemic conditions after birth. Our standard preterm pig protocols, which are patterned after those used in neonatal intensive care units, use continuous infusion of PN solutions for 2 to 5 d after delivery (Fig. 2D, 3) before the transition to enteral feeding (Fig. 2E, 3). The PN solution has been formulated to meet the energy and nutrient requirements of preterm pigs and includes (per liter) 3200 to 3400 kJ as amino acids (45 to 54 g), glucose (72 to 116 g), and lipids (21 to 31 g), with added vitamins and minerals. The actual sources of nutrients vary among study sites. The PN solution is typically administered via the umbilical arterial or jugular venous catheter using syringe or infusion pumps at rates from 2 to 12 mL $\cdot$ kg^{-1} $\cdot$ h^{-1} (up to 240 mL $\cdot$ kg^{-1} $\cdot$ d^{-1}). Because of the high osmolarity of the PN solution, supplemental fluid (e.g., lactated Ringers or 0.9% NaCl) may be administered to maintain fluid homeostasis, as is done clinically with preterm infants.

Provision of PN is especially critical for infants born extremely preterm (<28 wk gestation) who have excessive catabolism for the first weeks of life (Ziegler, 2011). Similarly, PN is essential for the smallest preterm newborn pigs (Che et al., 2010). Despite the necessity of PN, long-term PN is associated with generalized GIT atrophy, resulting in diminished structure and functions, liver damage and dysfunction, septicemia, and increased risk of

disease (Niinikoski et al., 2004; Stoll et al., 2010, 2012; Jain et al., 2012). The GIT of preterm pigs develops an increased sensitivity to enteral feeding and risk of NEC after a few days of TPN (Bjornvad et al., 2008; Oste et al., 2005; Van Haver et al., 2008a, 2009; Oste et al., 2010a; Siggers et al., 2011b). The direct effects of longer term PN (1 to 2 wk) on NEC risk in the model are unknown. In the weakest preterm pigs and infants, there is typically a need for long-term PN to ensure adequate nutrient and fluid intake, and to avoid overload of the immature GIT with enteral nutrients, causing food intolerance, maldigestion, and NEC.

Preterm pigs tolerate less PN than term pigs, and easily develop PN-related hyperglycemia, azotemia, and fluid overload (Sangild et al., 2002a; Stoll et al., 2012). Whereas provision of PN formulated with different sources of lipid and carbohydrate for short periods does not alter liver and GIT characteristics (Vegge et al., 2009), longer-term total parenteral nutrition (**TPN**) induces liver steatosis, insulin resistance, and metabolic dysfunctions (Jain et al., 2012). As in preterm infants, long-term exposure to PN lipid emulsions, especially those based on saturated and n-6 vegetable oils, may be detrimental (Duro et al., 2008). This finding is relevant for SBS preterm infants that often rely on TPN for extended periods (months). During TPN, in the absence of any enteral food, administration of the gut tropic hormone, glucagon-like peptide 2 (**GLP-2**), markedly improves gut growth and adaptation in preterm and term pigs, with and without SBS (Burrin et al., 2000; Petersen et al., 2001, 2002; Sangild et al., 2007; Vegge et al., 2013). Somewhat surprisingly, GLP-2 failed to prevent NEC in preterm pigs (Sangild et al., 2006; Benight et al., 2013). Apparently, this promising novel drug candidate only acts under certain physiological conditions, and studies in pigs may help to define therapeutic potential of GLP-2 for infants dependent on PN. Collectively, our findings indicate that the preterm pig is a relevant large animal model to examine how to optimize the composition and use of PN for the preterm infant.

Enteral Nutrition

There is universal agreement that enteral feeding is preferred to reduce the detrimental consequences of PN and to meet the high energy and nutrient requirements of preterm infants (Ziegler, 2011). Our long-term objective has been to define the optimal formula composition and feeding protocol that will support growth and maturation without increasing the risk of NEC. Starting enteral feeding from the first day after birth has, in recent years, become a widely accepted method to enhance structural and functional development of the GIT after preterm birth, and most neonatologists begin the transition 1 to 10 d after birth. However, the protocols for the transition are very diverse and can be controversial. We are using the preterm pig to better define the optimal time (after preterm birth or intestinal resection), amount (minimal or standard amounts), and composition (breast milk, colostrum, or various milk replacers) of enteral nutrition and the relevance to preterm infants at risk of NEC and SBS (Fig. 3).

Preterm pigs delivered at 90 to 92% of gestation require tube feeding because of immature abilities to coordinate suckling, swallowing, and breathing that limit the ability to suckle or to be bottle fed (Rasch et al., 2010). Similarly, preterm infants may require tube feeding depending on the extent of immaturity. The GIT of the preterm and in utero fetal pig responds to enteral feeding (Jiang et al., 2011b; Bjornvad et al., 2005; Sangild et al., 2002a), although some functional markers are less responsive compared with term pigs (e.g., intestinal motility, immunoglobulin absorption, brush border enzymes, nutrient absorption, permeability; Sangild et al., 2002a). Relative to preterm infants, formation of solid curds from milk proteins (especially casein) is more advanced in preterm pigs due to the greater activity of milk clotting enzymes (chymosin). Hence, the common clinical method of monitoring gastric residuals in preterm infants as an indicator of food intolerance is difficult to apply in preterm pigs, as the content may be less fluid.

Typically, preterm infants are initially provided small volumes of enteral food in addition to PN to induce GIT growth and maturation without overloading the immature GIT (Fig. 3). This approach is commonly known as minimal enteral nutrition (**MEN**). We have found low-dose enteral feeding (e.g., 2 to 4 mL/kg every 3 h) should be started the day of delivery for optimal survival and health of preterm pigs, and the optimal feeding advancement rate may be highly diet-dependent (Cilieborg et al., 2011c). Although MEN has been proposed to induce GIT adaptation in SBS preterm infants, the evidence for clinical benefits are considered weak (Bombell and McGuire, 2008).

Most neonatologists gradually increase the volume of enteral feed to what is considered as *full enteral feeding*, which is 150 to 180 mL $\cdot$ kg^{-1} $\cdot$ d $^{-1}$ for preterm infants (Agostoni et al., 2010). Advancing the volume too rapidly has been associated with food intolerance and increased risk of NEC, though this is controversial. Some of our studies have used the clinically relevant protocol of increasing the volume gradually over a 3- to 5-d period to a standard volume of 15 mL/kg every 3 h (120 mL $\cdot$ kg^{-1} $\cdot$ d $^{-1}$). This volume is about 50% of the milk intake ingested by normal-term pigs (200 to 250 mL $\cdot$ kg^{-1} $\cdot$ d^{-1}). Our studies indicate ~90% gestation preterm pigs are intolerant to feeding volumes in excess of 200 mL $\cdot$ kg^{-1} $\cdot$ d^{-1} during the first week of life (Fig. 3), which is consistent with intolerance to normal oral feeding and immature GLP-2 responses (Petersen et al., 2003; Sangild et al., 2007).

Composition of enteral feeds influences the risk of NEC. When mother's milk is not available, natural milk and colostrum products (porcine, bovine, human) are markedly better in protecting against NEC in pigs than any milk formula, regardless of whether this diet is fed immediately after birth or later (Sangild et al., 2006; Cilieborg et al., 2011d; Jensen et al., 2013). The protective benefits of colostrum are enhanced when volumes fed are advanced slowly by gradually replacing PN (Cilieborg et al., 2011b; Bjornvad et al., 2008; Siggers et al., 2011b). The formulas we have used are generally adjusted to match the macronutrient contents in sow's milk (per liter: 4000 to 4500 kJ energy, 55 to 65 g protein, 40 to 50 g carbohydrate, 60 to 70 g lipid) and are typically formulated from products used for hospitalized infants. Our studies have evaluated various formula ingredients, including whey and casein proteins (Thymann et al., 2012), minerals, vitamins, and lipids (Vegge et al., 2009). Although bioactive milk factors, like oligo-saccharides, gangliosides, osteopontin, lactoferrin, and antioxidant vitamins (E and C) are reported to support GIT maturation and protect from bacterial invasion and inflammatory reactions, our studies using formula-fed preterm pigs have provided few consistent maturational responses or NEC protective effects of these factors, relative to intact colostrum or milk.

Industrial processing of milk-based formula products (fractionation, spray-drying, freeze-drying, pasteurization) may reduce milk bioactivity (Chatterton et al., 2013) and could be a factor that partly explains the reduced protective effect of formula on the immature GIT of preterm pigs. Independently of processing, however, high amounts of maltodextrin (from hydrolyzed corn syrup) contribute significantly to NEC in formula-fed preterm pigs. This was first verified by our comparisons of maltodextrin versus lactose as the source of carbohydrate in formula (Thymann et al., 2009; Buddington et al., 2012). Our results indicate that formula with large amounts of maltodextrin induces NEC by adversely altering the responses of the immature enteric immune system to bacterial colonization (Siggers et al., 2011a). This immature response may be associated with excessive fermentation in the colon of carbohydrate that passes undigested through the small intestine. This mechanism includes accumulation of SCFA and lactate in the colon lumen, which may cause osmotic diarrhea and systemic acidosis. The increased risk of NEC caused by formula with maltodextrin occurs even when the transition to full enteral feeding is gradual (Hang et al., 2009; Oste et al., 2010b; Cilieborg et al., 2011c; Siggers et al., 2011b).

Similarity of Necrotizing Enterocolitis in Preterm Pigs and Infants

Most animal models used to study the pathophysiology of human diseases suffer from limitations that compromise relevance to humans. This does not necessarily preclude a given animal model from providing insights into human disease progression and possible interventions. With regards to NEC, it is important to recognize that the disease process involves a range of predisposing factors that, in combination, lead to necrosis of the intestine and colon. While uterine infections and inflammation contribute to preterm birth in humans, these conditions do not show any clear relationship with NEC sensitivity among infants or when preterm pigs are challenged with endotoxin in fetal life (Cilieborg et al., 2011d). A key advantage of the caesarean delivered preterm pig is the ability to study preterm individuals that have not experienced the highly variable maternal and fetal factors that trigger premature parturition and affect postnatal responses.

Human preterm infants with NEC show abdominal distension, food intolerance, regurgitation, and lethargy in the early phases of the disease. Radiographically, >50% of NEC infants show signs of pneumatosis intestinalis, that is, the bacteria-dependent accumulation of gas in the submucosa and serosa. Histologically, NEC causes multiple pathological changes in all parts of the intestinal wall, including necrosis of enterocytes, tissue edema, hemorrhage, leucocyte infiltration, and separation of the submucosal and lamina propria layers. The histopathologic changes in the tissue reflect those of a coagulative and hemorrhagic necrosis and range from erosion of the epithelial layer to transmural necrosis across the entire gut wall. Anatomically, the pathological changes are most prevalent in the distal small intestine and colon of preterm infants, but in rare cases, NEC lesions can be present throughout the GIT from the stomach to the rectum (Blakely et al., 2006; Lin et al., 2008).

The incidence of NEC in preterm pigs fed colostrum (<5%, Sangild et al., 2006; Bjornvad et al., 2008) is much lower than the 30 to 90% when preterm pigs are fed milk replacer (formula), especially with larger feeding volumes (>100 mL $\cdot$ kg^{-1} $\cdot$ d^{-1}) and with maltodextrin as a source of carbohydrate (Bjornvad et al., 2008; Thymann et al., 2009; Møller et al., 2011; Siggers et al., 2011a). Similarly, the incidence of NEC is lower among preterm infants fed human colostrum and milk compared with formula, although NEC rates remain variable. Interestingly, bovine colostrum and human banked milk has similar efficacy in preterm pigs (Jensen et al., 2013). Spontaneous NEC rates are variable among preterm pig studies, even using identical protocols. While this is not ideal for model stability and predicting sample size in NEC studies, it reflects the normal epidemiology of NEC in infants. The greater NEC incidence among preterm pigs relative to preterm infants confers an experimental advantage by reducing the sample size required to test treatment modalities.

The symptoms and histopathology of NEC in preterm pigs (Sangild et al., 2006; Bjornvad et al., 2008; Siggers et al., 2011b) share striking similarities with infant NEC (Fig. 3). Preterm pigs commonly develop pneumatosis intestinalis, a hallmark histopathological sign of NEC that relates to gut microbial colonization and gas production. It has been critical to establish a standardized, blinded score system for evaluating NEC at necropsy, and to potentially correlate with histopathology scores (Siggers et al., 2011b). We evaluated several scoring systems and combinations, and now rely mainly on a simple categorical 1 to 6 system that defines NEC as a preterm pig having a score of 3 in at least one of 5 GIT regions (stomach, proximal, middle and distal small intestine, and colon). The scoring is 1 = absence of lesions; 2 = local hyperemia, inflammation, and edema; 3 = hyperemia, extensive edema, and local hemorrhage; 4 = extensive hemorrhage; 5 = hemorrhage, local necrosis and pneumatosis intestinalis; and 6 = extensive necrosis, hemorrhage and pneumatosis intestinalis. Actual necrosis may not always be present in the severe cases, which may stem from the fact that disease progression can be extremely fast in pigs and, therefore, the

hemorrhage per se is the only visible pathological change. In a minority of affected pigs, the disease is better categorized as spontaneous intestinal perforation (**SIP**). In SIP, isolated bowel perforation is seen together with mucosal ulceration, edema, and leakage of intestinal fluid into the abdomen, but initially without pneumatosis, inflammation, and necrosis (Blakely et al., 2006). It is difficult to differentiate between NEC and SIP in infants, and they require similar medical and surgical interventions (enteral food withdrawal, antibiotics, intestinal resection and/or peritoneal drainage).

Before euthanasia and necropsy, we frequently incorporate a clinical score system similar to the Bell grading system used for preterm infants. Optimally, the proportion of pigs that develop NEC should be relatively consistent within an experiment to allow for necessary power calculations and to evaluate the efficacy of interventions. In our experience, a sample size of 15 pigs for each treatment is enough for statistical analysis of most outcome measures.

The NEC disease progresses to a critical, life-threatening state earlier in preterm pigs after they start enteral feeding (as early as 6 to 8 h), compared with infants (typically 1 to 4 wk after birth). A rapid advancement of enteral feeding volumes with a maltodextrin-based formula (e.g., achieving 15 mL $\cdot$ kg^{-1} $\cdot$ 3h^{-1}, 120 mL $\cdot$ kg^{-1} $\cdot$ d^{-1} within 12 h) predispose to a rapid and severe disease onset. Pigs showing the first signs of NEC after the start of enteral feeding tend to be affected the most. Death can occur within 6 h of observing the first clinical signs of discomfort, such as cyanosis, abdominal distension, lethargy, fatigue, bloody stools, labored breathing, and lowered abdominal oxygenation (Gay et al., 2011). The different characteristics of NEC between preterm pigs and infants may in part be related to the absence of some therapeutic interventions for pigs (e.g., broad spectrum antibiotics, enteral food withdrawal, hemodynamic, vascular and hydration support), and how newborn preterm pigs are immunologically unprotected at birth. Necrotizing enterocolitis develops later and less frequently in preterm pigs with the use of extended minimal enteral and parenteral feeding protocols, underlying the importance of such clinical protocols that allow the infant GIT to mature before greater volumes are introduced. Corresponding with this, we consistently experience that if preterm pigs do not develop NEC within the first 48 h of enteral feeding (>100 mL $\cdot$ kg^{-1} $\cdot$ d^{-1}), the risk and severity of NEC symptoms decline over the following 2 to 10 d. In the rodent models of NEC, the combination of formula feeding and artificial hypoxic and/or hypothermic insults in the postnatal period cause intestinal lesions 2 to 4 d later in rats (Liu et al., 2009) and 10 to 14 d in mice (Gribar et al., 2009). The spontaneous development of NEC in preterm pigs after the start of enteral feeding and without artificial insults is an obvious advantage that allows for translation to the spontaneous NEC that develops in preterm infants.

Pigs appear to have a greater incidence of hemorrhagic lesions in the stomach compared with infants. This may reflect a species-specific difference in structural GIT development that influences the NEC risk. The low acid secretory capacity of the preterm pig stomach relative to infants (Sangild et al., 1995b) predisposes to greater bacterial densities in this region (Cilieborg et al., 2011c), and this may increase bacteria dependent inflammatory lesions in the gastric epithelium.

It is uncertain whether the intestinal cellular mechanisms leading to mucosal inflammation and dysfunction in preterm pigs are shared with preterm infants. Studies in infants and rodents (Liu et al., 2009; Gribar et al., 2009) demonstrate that overexpression of Toll-like receptors (**TLR**) is involved in NEC pathogenesis, especially TLR-4, which recognizes gram-negative bacteria. However, we have not been able to demonstrate that increased TLR-4 expression explains the greater sensitivity to gram-negative intestinal inflammation of preterm versus term pigs (Bering et al., 2011). A series of studies on the intestinal

proteome in pigs with and without NEC (Jiang et al., 2008, 2011a, 2011b, 2012a) verified that bacterial colonization is crucial in the early onset of NEC, acting via proteins that are central for mucosal structure, nutrient metabobolism, antioxidants, stress, and immunological responses. The mechanisms identified as underlying the NEC disease process depend on gestational age at birth (Jiang et al., 2013b) and share some similarity with the pathways reported for rodent models and human infants (Jiang et al., 2013a).

Gut Bacterial Colonization, Immunity, and Necrotizing Enterocolitis

Microbial colonization of the epithelial surfaces of the GIT, skin, and lungs contribute to neonatal immune development and adaptation, and play a role in the immune dysregulation associated with NEC. Preterm infants and pigs have a slower and different pattern of GIT colonization, relative to term neonates (Magne et al., 2005; Cilieborg et al., 2011a). Feeding formula with maltodextrin to preterm pigs reared in sterile isolators, or fetal pigs in utero, does not induce NEC (Bjørnvad et al., 2005; Sangild et al., 2006) or compromise gut structure and functions (Siggers et al., 2007). While these findings confirm the central role of the microbiota in the development of NEC (Azcarate-Peril et al., 2011), it is also clear that the immature immunological responses of the preterm newborn contribute to the disease process.

We have not been able to associate NEC development in preterm pigs to any specific predisposing gut microbiota component (Cilieborg et al., 2012). The general overgrowth of pathogens, including Clostridia (Sangild et al., 2006; Bjornvad et al., 2008; Cilieborg et al., 2011a) appears to occur as a result of the NEC lesions, and is not directly causing NEC. Changes in the metabolic characteristics of the resident bacteria may be as important as the species composition and densities of bacteria. Specifically, metabolites, including short-chain fatty acids produced by the gut bacteria, can provide benefits or be damaging with effects depending on both the gestational age at birth, postnatal age, and diet composition (Buddington and Sangild, 2011).

The GIT of formula-fed, preterm, caesarean-derived pigs is rapidly colonized by a diversity of bacteria at densities of 10^8 to 10^{10} bacteria per gram colon contents (Siggers et al., 2008b; Cilieborg et al., 2011a, 2011c). Adhesion of bacteria to the intestinal epithelium is more pronounced in preterm, relative to term pigs (Cilieborg et al., 2011a) and this may initiate detrimental immune responses (Jiang et al., 2013b). An altered colonization pattern may also explain the NEC-inducing effect of a few days of TPN (Bjornvad et al., 2008; Van Haver et al., 2009; Siggers et al., 2011b). Conversely, colostrum may reduce bacterial adherence and stimulate intestinal mucous production (van Haver et al., 2009; Puiman et al., 2011). Admistration of probiotic strains of bacteria may support initial colonization in preterm newborns, but there are concerns about the optimal time, dosage, and strains of probiotics (Agostoni et al., 2010; Mihatsch et al., 2012). Our studies using preterm pigs support a cautious approach towards the use of probiotics as two similarly designed studies resulted in positive (Siggers et al., 2008a) or negative (Cilieborg et al., 2011b) GIT responses. Further, natural colonization of the preterm GIT with maternal microbiota via vaginal birth did not improve NEC resistance in preterm pigs (Siggers et al., 2008b).

Broad spectrum, prophylactic antibiotics are routinely administered to newborn preterm infants and term pigs for prevention of infections and sepsis (Lin et al., 2008; Simmons et al., 2010; Ohlsson and Shah, 2009; Nguyen et al., 2012). Similarly, administering antibiotics to preterm pigs reduces infections caused by microbial contamination via catheters, surgical procedures, and other challenges. However, broad spectrum antibiotic treatment markedly lowers NEC risk in preterm pigs and interferes with endpoints dependent on microbial colonization and natural development of immunity (Cilieborg et al., 2011a; Jiang et al., 2012a). For this reason, antibiotics are not normally administered to preterm pigs being used

to study NEC. On the other hand, the immunological immaturity and increased sensitivity of the preterm pig to pathogens provides a translational model to define the optimal class, time, dosage, and administration mode (enteral and/or parenteral) of antibiotics to prevent bacteria-dependent inflammatory reactions in newborn infants and pigs and to evaluate the consequences on development of immunity and the assemblages of GIT bacteria.

Intestinal Resection and Short Bowel Syndrome

Short bowel syndrome is defined as a condition with marked malabsorption as a result of anatomical or functional loss of a significant length of the small intestine. More severe cases of SBS are referred to as “intestinal failure” that result in an inability to maintain fluid, nutrient, and energy balances (O’Keefe et al., 2006; Aunsholt et al., 2012). Extensive intestinal resection for NEC is a cause of SBS among preterm infants. Preterm pigs to be used for intestinal resection and SBS studies (Sangild et al., 2009; Vegge et al., 2013) have umbilical vessel catheters and feeding tubes placed in the same manner as described for the NEC model. Typically, SBS pigs are fed enteral diets for 1 to 2 d before the resection to adapt the pigs and the GIT to luminal nutrition (Fig. 3). Pigs fed diets other than sow or cow colostrum receive transfusions of maternal plasma for passive immunity. Enteral feeding is stopped 6 to 9 h before surgery to empty the stomach and small intestine of food, with PN provided continuously thereafter to sustain metabolic stability, normoglycemia, and blood volume and pressure during the perioperative period.

After anesthesia is induced (as described above), a 5-cm ventral midline incision is made to expose the bowel. The distal small intestine is separated from the mesentery using an ultrasonic scalpel (Fig. 3), beginning from the ileocolonic junction and proceeding proximally. When an estimated 50% of the small intestine has been separated (total intestinal length is estimated as 300 cm × body weight$^{0.65}$), the distal end is closed before the segment is excised. A stoma is created by exteriorizing 1 cm of the distal end of the remnant intestine through an incision in the dorsolateral part of the abdomen. The exteriorized portion is everted to expose the mucosa and secured to the skin with 2 to 3 sutures. A sterile silastic tube approximating the lumen diameter is inserted 5 cm into the intestine through the stoma and fixed to the skin with sutures. This approach enhances drainage of fluid from the stoma. Pigs remain on PN, increasing from an initial 4 up to 8 mL $\cdot$ kg^{-1} $\cdot$ h^{-1} over the first 48 h after surgery. Supplemental fluids in excess of PN are required post surgery to maintain body fluid homeostasis because of excessive fluid loss via the stoma and the loss of hindgut functions (Fig. 3). This is provided by coinfusion of isotonic saline or lactated Ringer’s solution with the PN at a rate of 2 mL $\cdot$ kg^{-1} $\cdot$ h^{-1} beginning about 24 h after surgery. Fluid stability is considered critical for resected pigs, and especially for preterm pigs that are highly sensitive to dehydration. Hydration status must be closely monitored to avoid both dehydration and overhydration. The pigs receive broad spectrum antibiotics for 3 d after resection (combination of gentamycin, pentrexyl, and enrofloxacin).

Some of the challenges associated with constructing and maintaining a stoma can be partly overcome by anastomosing the remnant small intestine to the hindgut (Nagy et al., 2004; Turner et al., 2011; Barnes et al., 2012). This approach is relevant to infants who retain a functional colon after resection. We consider a surgical model with the remnant intestine fitted with a jejonostomy to be more translational for the majority of preterm SBS infants who have a stoma during the immediate postresection period. It is only after the remnant small intestine has healed that the stoma in such infants is removed and the bowel is made continuous. The preparation of a stoma in preterm pigs allows for the collection of intestinal content in stoma bags fitted onto the skin of the piglet (Fig. 3) for nutrient balance studies (Vegge et al., 2013) and for evaluating interventions to improve intestinal adaptation during SBS.

We have not attempted to resect the intestine from preterm pigs with severe NEC because of the difficulties associated with stabilizing pigs with NEC long enough to allow surgical intervention. Nevertheless, the clinical complications after 50% distal intestine resection in healthy preterm pigs are very similar to SBS complications for infants that require intestinal resection for other reasons (e.g., segmental absence of the intestinal musculature, gastroschisis, intestinal atresia, midgut volvulus). We observed that the postsurgical adaptational responses to enteral food intake are reduced in preterm versus term SBS pigs, and the clinical complications, such as hemodynamic instability, hypothermia, intestinal dysmotility, dehydration, respiratory distress and peritonitis, are more severe in preterm versus term pigs (Sangild et al., 2009; Aunsholt et al., 2013). These findings coincide with a lack of postsurgical increases in protein synthesis, villous height, crypt depth, enterocyte proliferation, and digestive enzyme activities when compared with the corresponding intestinal segment in unresected control pigs (Vegge et al., 2013). The lack of adaptation contrasts with the structural and functional adaptation of the remnant intestine in newborn term pigs (Turner et al., 2011; Barnes et al., 2012), older suckling pigs (Nagy et al., 2004), and adult rats (Józsa et al., 2009). This has led us to conclude that there is a need to develop interventions that are specifically tailored to support intestinal adaptation in preterm newborns. The preterm pig model of SBS may be well suited for accomplishing this goal.

Ethical Considerations of Preterm Pig Studies

Animal disease models are often associated with a degree of suffering for the animals involved. Is it justifiable to expose newborn pigs to maladaptation syndromes by premature delivery and suboptimal rearing conditions? When exactly would a pig need to be euthanized? In our studies on preterm pigs, we aim to limit the spontaneous, immaturity-related neonatal mortality to levels occurring in normal sow herds (10 to 20%). With our present level of intensive care, spontaneous mortality of preterm pigs delivered at ~90% gestation is within this range or lower, even for large litters of >20 pigs. Adherence to international guidelines for the use and care of animals is particularly important in studies of severe diseases like NEC and SBS, where an element of stress and pain caused by disease progression is difficult to avoid. Death is not an endpoint in our studies of pigs (in contrast to many previous rodent NEC protocols), and careful observation of clinical disease progression by trained personnel is required to evaluate if and when individual pigs that become moribund need to be euthanized. Still, death may occur before euthanasia in some individuals due to the rapid progression of NEC and SBS complications. We recognize the need for a close dialog among care takers, researchers, and the public to balance effective disease modeling with humane endpoints in accordance with ethically acceptable standards. The fact that preterm pigs serve as translational models for thousands of immature preterm infants and millions of weak newborn production pigs helps to justify the continuous refinement of this relatively expensive, laborious, and technically demanding model.

CONCLUSION AND PERSPECTIVES

During the last 10 yr the preterm pig has become established as an important preclinical model for preterm infants, particularly in studies of NEC and SBS. New diet-dependent preventive and curative therapies have been discovered, and manipulations of the gut microbiota have led to new knowledge about neonatal GIT development. The preterm pig is a highly translational large animal model for improving parenteral and enteral feeding regimens for preterm infants, with some findings relevant also for the clinical care of term newborn infants and pigs. As such, the preterm pig model represents an exciting collaborative research platform for basic biology, pediatric medicine, and agricultural and veterinary science. These research fields differ markedly in language and target populations, but translational neonatal research brings them together in the common goal to improve survival and health of the newborn.

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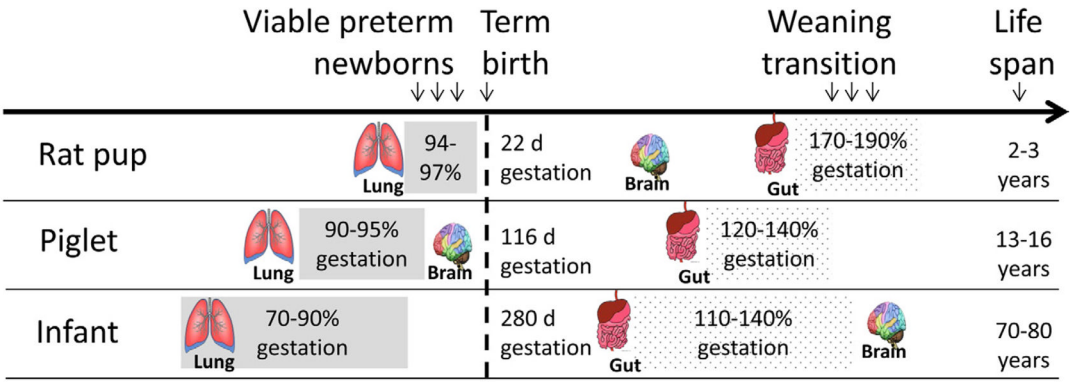

Figure 1.

Maturation (as percentage of gestation) of lungs, gut, and brain relative to term birth, weaning, and total life span for rats, pigs, and humans. Symbols for lungs, gut, and brain indicate the time period when ontogenetic maturation is sufficient to secure general viability of preterm newborns (dark grey boxes), that is, independent breathing (determined by lung alveolar respiration), digestion of nonmilk food (determined by gut functions), and locomotion (in part determined by brain motoric control). See online version for figure in color.

Figure 2.
Piglet neonatal intensive care unit. (A–C) Incubator types, room design, and clinical care procedures have been adjusted over the years to improve the care and neonatal adaptation of 20 to 25 preterm piglets per litter. After (B) placement of an orogastric feeding tube and a vascular catheter into a jugular vein or umbilical artery, (C, D) the piglets are placed in heated, ventilated, humidified and oxygenated incubators. (D) Syringe or infusion pumps deliver intravenous or oral nutrition or other therapies. (E) Growth-restricted or normal-weight preterm pigs may also be fed manually by providing intermittent boluses, both before and after they become mobile at 1–5 d after birth. (F) Continuous clinical surveillance is supported by web based cameras. See online version for figure in color.

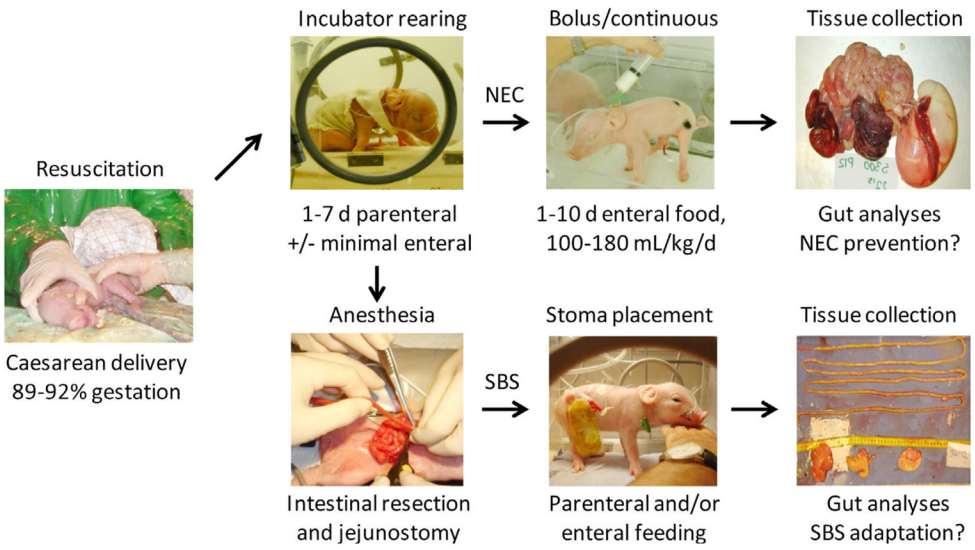

Figure 3.

Flow of events in short-term protocols using preterm pigs delivered by caesarean section at ~90% gestation to investigate factors that prevent necrotizing enterocolitis (NEC, upper panels) or stimulate intestinal adaptation following intestinal resection and short bowel syndrome (SBS, lower panels). See online version for figure in color.