Amino acid metabolism in intestinal bacteria and its potential implications for mammalian reproduction

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ABSTRACT: Reproduction is vital for producing offspring and preserving genetic resources. However, incidences of many reproductive disorders (e.g. miscarriage, intrauterine growth restriction, premature delivery and lower sperm quality) have either increased dramatically or remained at high rates over the last decades. Mounting evidence shows a strong correlation between enteral protein nutrition and reproduction. Besides serving as major nutrients in the diet, amino acids (AA) are signaling molecules in the regulation of diverse physiological processes, ranging from spermatogenesis to oocyte fertilization and to embryo implantation. Notably, the numbers of bacteria in the intestine exceed the numbers of host cells by 10 times. Microbes in the small-intestinal lumen actively metabolize large amounts of dietary AA and, therefore, affect the entry of AA into the portal circulation for whole-body utilization. Changes in the composition and abundance of AA-metabolizing bacteria in the gut during pregnancy, as well as their translocation to the uterus, may alter uterine function and epigenetic modifications of maternal physiology and metabolism, which are crucial for pregnancy recognition and fetal development. Thus, the presence of the maternal gut microbiota and AA metabolites in the intrauterine environments (e.g. endometrium and placenta) and breast milk is likely a unique signature for the programming of the whole-body microbiome and metabolism in both the fetus and infant. Dietary intervention with functional AA, probiotics and prebiotics to alter the abundance and activity of intestinal bacteria may ameliorate or prevent the development of metabolic syndrome, while improving reproductive performance in both males and females as well as their offspring.

Key words: amino acids / bacteria / intestine / metabolism / reproduction

Introduction

Reproduction is a fundamental and important biological process for producing offspring and preserving genetic resources. Sexual reproduction in mammals (including humans and livestock species) is complex and highly organized. This physiological event involves the generation of mature reproductive cells (sperm and oocyte) and associated fluids/secretions (e.g. seminal plasma and uterine secretions) in the reproductive system of both males and females, the transport of sperm to the female reproductive tract, the fertilization of the oocyte, the development of the zygote, pregnancy recognition, implantation of the embryo, the maintenance of pregnancy, conceptus development, parturition and lactation, as well as growth and development of neonates (Owen and Katz, 2005; Bazer, 2013). To successfully complete these biological processes, many essential nutrients, including water, carbohydrates, amino acids (AA), lipids, minerals, and vitamins, are required for the formation and maturation of reproductive cells and the synthesis of varieties of proteins, hormones, and secretions (Wu et al., 2012a; Lin et al., 2014). Thus, it is essential to elucidate the role of nutrients and other dietary factors in reproduction of both sexes in humans and other animals (Wu et al., 2004; Cross and Mickelson, 2006; Gaskins et al., 2012; Bazer, 2013; Mora-Esteves and Shin, 2013).

As major macronutrients in diets, AA are not only the building blocks of proteins and peptides but also are essential for the synthesis of many bioactive molecules that participate in the regulation of signaling pathways and metabolism in the body (Sato et al., 2006; Wu, 2013; Wu et al., 2014). Compelling evidence indicates that AA play a crucial role in both female and male reproduction (Bazer, 2013; Wu et al. 2013a). Studies with humans and animals over the last decades showed that certain AA are abundant in seminal plasma (e.g. serine, threonine, glycine, glutamate, arginine and tyrosine), uterine secretions (e.g. arginine, leucine, proline and glutamine), allantoic fluid (e.g. glutamine, alanine, serine, glycine, arginine, ornithine and citrulline), and milk (e.g. aspartate, glutamate, glutamine, leucine, proline and taurine), indicating their...
important biological functions in fertility, embryonic and fetal survival, as well as offspring growth and development (Brown-Woodman and White, 1974; Fröhlich et al., 1980; Wu and Knabe, 1994; Davis et al., 1994a; Wu, 2009; Wang et al., 2012). Of particular interest, dietary supplementation with specific AA (e.g. arginine, glutamine, leucine, glycine and methionine) has beneficial effects in improving reproductive performance (e.g. embryonic/fetal survival and growth) in both humans and other animals through the regulation of key signaling and metabolic pathways (Wang et al., 2012; DelCurto et al., 2013; Wu, 2013). However, more work is warranted to uncover the important functions of AA in reproduction and the underlying mechanisms, especially in humans and livestock species (Bazer, 2013; Wu, 2013).

Results of extensive studies over the last decades have revealed that bacteria in the gut play an important role in the metabolism and recycling of nitrogenous compounds, including AA (Fuller and Reeds, 1998; Bergen and Wu, 2009; Dai et al., 2011; Macfarlane and Macfarlane, 2012). The gut bacteria or groups of bacteria (microbiota) either utilize the AA derived from food or the host as building blocks for their synthesis of proteins or further metabolize these nutrients via conversion or fermentation to produce varieties of metabolites (ammonia, hydrogen sulfide (H2S), nitric oxide (NO), polyamines, phenolic and indolic compounds) in both the small and large intestine (Dai et al., 2011; Macfarlane and Macfarlane, 2012). Furthermore, intestinal bacteria can de novo synthesize some of the nutritionally essential amino acids (EAA), which may contribute to the regulation of AA homeostasis in the body (Metges, 2000). These findings indicate the importance of AA metabolism in the gut microbiota on the nutrition and physiology of the host (Blachier et al., 2007; Dai et al., 2011; Collins et al., 2012; Macfarlane and Macfarlane, 2012).

Results of extensive studies indicate that the composition of the gut microbiota in mammals changes dramatically during the course of pregnancy and is in parallel with the alteration of whole-body metabolic status (Collado et al., 2008; Santacruz et al., 2010; Koren et al., 2012). For example, the abundance of potential AA-metabolizing gut bacteria (e.g. the Enterobacteriaceae family of bacteria, Streptococcus genus bacteria) increases during the late stage of gestation (Koren et al., 2012). In addition, these bacteria were found to be abundant in overweight compared with normal-weight pregnant women (Collado et al., 2008; Santacruz et al., 2010). Furthermore, dietary supplementation with arginine, which induces a shift in the Firmicutes:Bacterioidetes ratio to favor Bacteroidetes in the jejunum and ileum (Ren et al., 2014a), enhances embryonic survival in mammals, including mice (Ren et al., 2012) and pigs (Mateo et al., 2007; Li et al., 2014). Although many factors affect the gut microbiota composition during gestation, such as diet and hormones, the above findings suggest an important role for the AA-metabolizing bacteria in female reproduction. The underlying mechanisms are unknown but may include: (i) regulation of the digestion and absorption of protein and AA in the small intestine; (ii) availability of dietary AA for use by reproductive organs; (iii) strategic changes in the composition and abundance of the microbiota during pregnancy; (iv) alterations in uterine capacity and function as well as the body metabolic status; (v) programming of metabolic changes in the gut microbiota of the mother and her offspring; and (vi) changes in the production of reproduction-related AA metabolites (e.g. NO, polyamines and glutathione) directly or indirectly linked to reproductive physiology of the host. Details of these aspects are discussed in the following sections.

**AA metabolism and nitrogen recycling in the gut pertinent to reproduction**

As the major source of nitrogen in the gut, dietary protein is digested and its products (free AA, dipeptides and tripeptides) are absorbed into the portal circulation for the synthesis of proteins, enzymes, hormones and metabolites in the body (Winckler et al., 1999; Bergen and Wu, 2009). However, at the same time, relatively large amounts of proteins and AA undergo extensive metabolic recycling in the intestine by either intestinal epithelial cells or the gut bacteria (Stoll et al., 1998; Wu, 1998). The utilization and metabolism of AA in the intestine includes: (i) catabolism of AA for ATP production in small-intestinal mucus cells (e.g. aspartate, glutamate and glutamine); (ii) synthesis of important molecules for maintaining normal nutrition and physiology of the intestine and whole body (e.g. citrulline, proline, NO, polyamines and neurotransmitters); (iii) synthesis of important proteins and peptides for maintaining normal gut structure and function (e.g. mucins, immunoglobulins, defensins and glutathione); (iv) synthesis of proteins and peptides for the growth and cell–cell interactions of gut bacteria (e.g. peptidoglycan, S layer proteins, chemotactic peptides, acyl homoserine lactones); (v) fermentation of proteins and catabolism of AA for energy provision in bacteria especially in the large intestine [products like short chain fatty acids (SCFA), branched-chain fatty acids (BCFA), phenolic and indolic compounds, and H2S]; and (vi) transformation of AA to produce AA metabolites [e.g. biogenic amines, gamma-aminobutyric acid (GABA), dopamine, serotonin (S-HT), and NO] in bacteria for the adaptation to harsh gut conditions (Nast and LeDuc, 1988; Smith and Macfarlane, 1997; Hernández et al., 2005; Konstantinov et al., 2008; Vermeiren et al., 2009, 2012; Wu, 2009, 2010; Blachier et al., 2010; Dai et al., 2011, 2012a; Cryan and Dinan, 2012; Macfarlane and Macfarlane, 2012; Davila et al., 2013).

The metabolic transformation of AA and their nitrogenous metabolites in mucosal epithelial cells and microbes in the small and large intestines constitutes extensive nitrogen recycling in the gut, which plays an important role in affecting the efficiency of AA utilization in the body (Fuller and Reeds, 1998; Bergen and Wu, 2009; Fuller, 2012). The secretion of nitrogen in the form of digestive enzymes, mucins, immunoglobulins and urea to the lumen of the small intestine can be further degraded, utilized and metabolized by the gut bacteria. The de novo synthesis of AA, especially lysine, by gut bacteria takes place in both the small and large intestine (Metges, 2000; Metges et al., 2006). The major nitrogen source for the bacterial synthesis of lysine is ammonia, which is derived from the bacterial catabolism of AA or urea in the intestine (Metges, 2000). In pigs, the absorption of bacterial lysine occurs mainly in the small intestine (Torrallardona et al., 2003a,b). This is of nutritional advantage, as lysine is one of the EAA for all animals and the provision of an extra amount of lysine by the gut microbiota to the host can reduce dietary requirement of lysine. However, to date, the nutritional importance of the bacterial de novo synthesis of AA to the host is still uncertain due to the lack of quantitative data (Metges et al., 2006; Fuller, 2012).

There is growing interest in the utilization and metabolism of AA by bacteria in the small intestine as well as the significance of these biochemical reactions for host nutrition (Booijink, 2009; Miner-Williams et al., 2009; Dai et al., 2010, 2012a). Transcriptomic analysis of gene expression in bacteria from the ileal effluent samples of humans showed that mRNA levels for the genes related to AA utilization and metabolism
comprise ~20% of the total metabolism-related genes (Booijink, 2009). Using the pig as the animal model, Miner-Williams et al. (2009) reported that bacterial protein was the major component of the digesta protein from the ileum, even when the pigs were fed a highly digestible casein-based diet. Also, bacteria in the pig small intestine utilize large amounts of AA, including EAA, and some of the AA were utilized extensively by catabolic pathways (Dai et al., 2010, 2012a). While small-intestinal enterocytes were able to metabolize branched-chain AA (BCAA) (Val, Leu and Ile) but not other EAA (Chen et al., 2007, 2009), small intestinal microbes exerted extensive metabolism of EAA, including BCAA (Yang et al., 2014). These findings indicate that the small-intestinal bacteria play an important role in regulating the AA pool (total amount) and profile (composition of each AA) during the process of the digestion and absorption of AA by the small intestine. This ‘modification’ not only affects the efficiency of AA supplementation to reproductive organs for protein synthesis and cell growth, but also regulates reproduction-related signaling pathways directly or indirectly through production of their metabolites (Cross and Mickelson, 2006; Bazer, 2013; Wu et al., 2013a). However, further studies are warranted to investigate the detailed mode of action.

Diversity of AA-metabolizing bacteria in the gut

Recent studies with the human colonic bacteria have shown that protein- and AA-fermenting bacteria are abundant and diverse in the colon. The abundance of the AA-fermenting bacteria in the large intestine is very high and their number can reach up to $10^{11}$ per gram dry feces (Smith and Macfarlane, 1998). Using the traditional plate counting technique, the authors have also reported that the dominant bacterial species for the utilization of single AA or pairs of AA are very different. For instance, Clostridium bifermens is the predominant bacteria for the utilization of lysine or proline, and pairs of AA (e.g. phenylalanine/leucine, isoleucine/tryptophan and alanine/glycine), whereas Peptostreptococcus spp. bacteria are predominant for the utilization of glutamate or tryptophan. Many species of bacteria utilize the same AA as substrates for growth (Smith and Macfarlane, 1998). Overall, bacteria belonging to the Clostridium spp. dominate in AA fermentation in the human large intestine, but other bacterial species, such as Fusobacterium spp., Bacteroides spp., Veillonella spp., Megaposphera elsenii and Selenomonas ruminantium, may also be important for AA metabolism in the large intestine (Smith and Macfarlane, 1998; Dai et al., 2011).

There are few studies on AA utilization and metabolism in bacteria from the human small intestine. Metatranscriptomic analysis of the human ileal bacteria reveals that bacteria belonging to the Clostridium clusters, the Bacillus-Lactobacillus-Streptococcus group, and Proteobacteria are primarily responsible for the utilization of AA (Booijink, 2009). Results of studies with AA-metabolizing bacteria in the pig small intestine indicate that bacteria belonging to the Escherichia coli, Klebsiella sp., Streptococcus sp., Megaposphera elsenii and Acidaminococcus fermentans, play an important role in utilizing AA in the pig small intestine (Dai et al., 2010). Further, studies with mucin-degrading bacteria suggest that Akkermansia muciniphila makes a substantial contribution to nitrogen cycling in the small intestine (Derrien et al., 2008). Considering that the small intestine is the major site for nutrient absorption and also has immunological and endocrine functions, it is of utmost importance to identify relationships between variation in the bacterial community and AA metabolism in this organ.

Possible links between AA-metabolizing bacteria and pregnancy

Many biological processes take place during pregnancy, such as the development of an embryo to a blastocyst, peri-implantation signaling between the blastocyst and the uterine epithelium, development and morphological changes of the trophoderm, as well as initiation of implantation and placentation (Bazer et al., 2009). Histotroph, including AA, is essential for maintaining normal pregnancy in mammals (Cross and Mickelson, 2006; Bazer et al., 2012a). The composition and activity of the gut microbiota change dramatically with gestational stages (Koren et al., 2012). This, in turn, has a major impact on the progress of pregnancy, especially during critical windows of embryonic survival, growth and development (Cross and Mickelson, 2006; Wu et al., 2013a).

Fluctuations of the gut microbiota during pregnancy

Recent studies with women indicate that the composition of the gut microbiota shifts with different stages of pregnancy and is affected by the maternal body weight (Koren et al., 2012). For example, during late pregnancy in women (third trimester, 33.7 weeks), the abundance of gut bacteria belonging to Proteobacteria and Actinobacteria, especially the Enterobacteriaceae family and Streptococcus genus, increase substantially (Koren et al., 2012). In contrast, butyrate-producing bacteria (e.g. Faecalibacterium and Eubacterium) dominate in the first trimester (13.8 weeks) and decrease in the third trimester (Koren et al., 2012). At early lactation/post-partum, the content of Streptococcus tends to decrease, but remains relatively high when compared with the first trimester (Koren et al., 2012; Jost et al., 2014). Metabolic analysis suggests that, during third trimester, the gut microbiota may induce greater adiposity and insulin insensitivity in the mother to facilitate the maternal storage of dietary energy as triacylglycerols and increase the availability of nutrients in the maternal plasma for transfer to her fetus (Koren et al., 2012). Similarly, compared with normal-weight pregnant women, the numbers of Bacteroides, Staphylococcus and Enterobacteriaceae are higher in overweight pregnant women during the entire gestation (Collado et al., 2008; Santacruz et al., 2010). The increased numbers of Bacteroides and Staphylococcus are associated with enhanced accretion of white adipose tissue in pregnant women and the fetus. These observations suggest that the abundance of bacteria related to intestinal AA metabolism increases at late gestation and in overweight pregnant women. Microbial metabolites of AA that may regulate maternal and fetal whole-body energy metabolism in a tissue-specific manner are unknown.

It is noteworthy that total fecal SCFA concentrations and especially BCFA concentrations are higher in perinatal women, when compared with non-pregnant subjects (Jost et al., 2014). Some of the SCFA produced by the large-intestinal bacteria are derived from the fermentation of AA, whereas BCFA are produced solely from the fermentation of BCAA by AA-fermenting bacteria in the large intestine (Smith and Macfarlane, 1997). Therefore, this phenomenon is correlated with the observation of increased abundance of gut AA-metabolizing bacteria.
at this stage. The metabolic shift of the gut microbiota beneficially aids in adaptation to diet and ATP production in the colon (Jost et al., 2014). It is still unclear whether the driving force for the fluctuations of the gut microbiota and their activity during pregnancy is the microbiota itself, dietary changes, or altered digestive function of the gut. However, the available evidence suggests that many of the AA metabolites produced by intestinal bacteria may play important roles in perinatal women (Jost et al., 2014).

AA nutrition and metabolism for uterine and placenta function

Abundance of certain AA (e.g. glutamine, ornithine and arginine) increases substantially in uterine secretions as well as fetal allantoic and amniotic fluids during early pregnancy due to increased expression of AA transporters in the conceptus (Bazer et al., 2012b; Wu et al., 2013b). Such biochemical changes not only provide nutrients for the growth and development of the fetus but also activate the signaling pathways that support normal gestation (Bazer et al., 2012a; Wu et al., 2013a, b). Studies of the nutritional status in pregnant animals showed that undernutrition led to the development of intrauterine growth restriction (IUGR) and high mortality rate of neonates (Wu et al., 2004; Walther et al., 2011). Under conditions of nutrient restriction, intestinal nitrogen recycling that involves gut micro-organisms is reduced (Allison and Macfarlane, 1989; Fuller and Reeds, 1998) in association with decreased activities of enzymes required for synthesis of NO and polyamines in placenta and endometrium (Wu, 2010) and decreased concentrations of the arginine-family of AA, BCAA and polyamines in fetal fluids (Wu et al., 1998a, b; Kwon et al., 2004). One possibility is that gut bacteria provide precursors (e.g. S-adenosylmethionine and agmatine) for the synthesis of polyamines in the intestinal mucosa and other tissues (e.g. the reproductive tract) (Wang et al., 2014a). Nonetheless, adequate provision of dietary AA is required to maintain the intestinal microbiota (Dai et al., 2010, 2011) whose nitrogenous products (e.g. arginine, NO and polyamines) may be beneficial for the regulation of cell proliferation in the uterus, placenta and embryo/fetus (Kong et al., 2014; Wang et al., 2014a, b, c). Like underfeeding, overnutrition also has negative effects on pregnancy. For example, a high-protein diet during pregnancy and lactation in mice caused lower birthweight, smaller litter size, and a higher mortality rate in the offspring during the lactation period (Walther et al., 2011). Maternal obesity results in increased risks for obstetric problems, including diabetes mellitus, hypertension, pre-eclampsia, thrombosis events, respiratory complications, IUGR, and delivery complications (e.g. Cesarean section and neonatal deaths) (McKnight et al., 2011).

Basal AA and nitrogen requirements for the growth and survival of intestinal bacteria are relatively stable, but may vary markedly with changes in the host’s physiological conditions (Dai et al., 2010, 2011, 2012a). In case of undernutrition, especially protein restriction, the gut microbiota will compete with the host for AA during AA digestion and absorption processes in the small intestine, thereby further reducing the availability of dietary AA for reproductive organs. For a high-protein diet, excessive AA that exceed the capacity of absorption by the host’s small intestine undergo catabolism by gut bacteria to generate excessive amounts of ammonia and toxic metabolites, such as indolic and phenolic compounds in the intestine (Smith and Macfarlane, 1996, 1997, 1998). As noted previously, the microbial AA metabolites can reach reproductive organs through systemic blood circulation or local diffusion (Nishimura et al., 2013). Changes in concentrations of AA metabolites (such as polyamines, NO, H2S, ammonia and serotonin) in the uterus may modulate cellular signaling pathways [e.g. AKT (protein kinase B), AMPK (5’-adenosine monophosphate-activated protein kinase), MTOR (mechanistic target of rapamycin), and RAS (Renin–angiotensin system) signaling] as well as nutrient transport and metabolism in both the uterus and conceptus, which will affect the migration, implantation and development of cells in the conceptus (Kim et al., 2011; Bazer et al., 2012a, c; Gao et al., 2012a, b; Wang et al., 2014a, b, c). The embryo and fetus are very sensitive to the toxicity of elevated levels of ammonia in maternal and fetal plasma, as well as fetal amniotic and allantoic fluids (Wu et al., 2013b).

Presence of the gut microbiota in the female reproductive tract and the links to reproductive health

A large number of bacterial species that reside in the digestive tract has been reported to be present in the female reproductive tract, including the vagina, the endometrium and the placenta (Gajer et al., 2012). The overall diversity and abundance of these bacteria are reduced during pregnancy, with Lactobacillus spp. and the orders of Lactobacillales, Bacteroidales, and Actinomycetales dominating in different vaginal subsites (Aagaard et al., 2012). Similarly, dynamic changes of the vaginal microbiota occur during the menstrual cycle, with Lactobacillus spp. and Streptococcus spp. being the major bacterial species (Eschenbach et al., 2000; Gajer et al., 2012). Disturbance of the vaginal microbiota reduced the abundance of the hydrogen peroxide-producing Lactobacillus spp. (e.g. Lactobacillus crispatus), and an increased abundance of Gardnerella vaginalis and Prevotella spp. is associated with bacterial vaginosis and pelvic inflammatory disease (Eschenbach et al., 2000; Andrews et al., 2006; Martin et al., 2012). Furthermore, bacterial species, such as Streptococcus, Escherichia coli, Shigella, Proteus, Enterobacter, and Candida species which is present in the digestive tract, have been detected in the vagina and shown to be correlated with gestational age in pregnant women (Dechen et al., 2010; Martin et al., 2012). Considering that most of the above bacterial species can metabolize AA, it is important to further uncover their role in female reproductive physiology and health as well as sperm viability and transport in the female reproductive tract and fertility (Suarez and Pacey, 2006; Barbontetti et al., 2011).

In patients with endometritis, bacteria such as Chlamydia trachomatis and Neisseria gonorrhoeae were frequently isolated (Eckert et al., 2002; Haggerty et al., 2004; Swidsinski et al., 2013). However, in peripartum and post-partum women with endometritis, the bacterial isolates were dominated by E. coli, enterococci, Streptococcus, as well as anaerobic bacteria such as peptococcus and Bacteroides fragilis and Ureaplasma urealyticum (Platt et al., 1979; Cicinelli et al., 2009, 2012). Similar observations were reported for uniparous farm animals. For example, in dairy cows, the post-partum endometritis was associated with increased abundance of total bacteria and especially the prevalence of E. coli, Arcanobacterium pyogenes, Bacteroides spp., Fusobacterium spp., Peptostreptococcus spp., Prevotella spp., Ureaplasma spp., Streptococcus spp., Pseudomonas spp., and Staphylococcus spp. in the uterus (Sheldon et al., 2009; Machado et al., 2012; Santos and Bicalho, 2012; Peng et al., 2013). These kinds of variations were also found in accordance with health status and days post-partum of the animals (Machado et al., 2012; Santos and Bicalho, 2012; Peng et al., 2013).
The placenta harbors a unique microbiome, which resembles the oral microbiota (Aagaard et al., 2014). Based on high throughput sequencing of the bacterial 16S ribosomal DNA and whole-genome shotgun metagenomic studies, Aagaard et al. (2014) reported that the non-pathogenic E. coli was the most abundant bacterium in the placenta of most individuals (Aagaard et al., 2014). Bacteria such as Bacteroides spp., Neisseria lactamica, Staphylococcus epidermidis, and Propionibacterium acne were also predominant in the placental samples (Aagaard et al., 2014). Notably, the oral bacteria such as Prevotella tannerae and non-pathogenic Neisseria spp. were detected (Aagaard et al., 2014). Enrichment of Actinomyctales, Alphaproteobacteria, Streptococcus, Acinetobacter, and Fusobacterium nucleatum in the placenta was associated with antepartum infection and increased risk for preterm birth (Aagaard et al., 2014). Analysis of the metabolic pathways of the placental microbiota showed strong correlation between gestational age at delivery and increased abundance of bacterial pathways involved in glycine/serine/threonine metabolism (Aagaard et al., 2014). Due to low abundance of the placental microbiota and to the sampling method used, further studies are warranted to validate these observations.

Collectively, the above findings suggest that the presence of bacteria in the female reproductive tract that originate from the digestive tract through the blood, oral or feces pathways may play important roles in female reproductive health. The driving force for the changes of the microbiota may be either an adaptation of the bacteria to the changing environment (e.g. AA or glycogen concentrations) of the female reproductive tract or a chronic infection with certain bacteria (Cicinelli et al., 2009; Gajer et al., 2012; Martin et al., 2012). Nitrogen metabolism in the bacteria may increase as AA concentrations in vaginal and uterine secretions increase during pregnancy and post-partum. Changes in the abundance of metabolites of bacterial proteinolyis and AA catabolism may serve as signals to modulate the function of the female reproductive tract, such as hormone secretion, ovulation, and endometrial receptivity (Sheldon et al., 2009; Aagaard et al., 2014). These physiological changes may contribute to miscarriage, IUGR, and preterm birth (Aagaard et al., 2014). However, further studies are required to identify key bacterial metabolites in the female reproductive tract that have strong correlation with female reproductive disorders. Furthermore, it will be important to investigate the health status of the digestive tract and female reproductive health with special emphasis on the fluctuations of the composition and activity of the AA-metabolizing bacteria in the digestive tract (Offenbacher et al., 1996; Han et al., 2009; Suwabe et al., 2011; Yoshida et al., 2011). Here we propose the notion that the microbiota residing in the different niches of the female reproductive tract play unique roles in different events of reproduction. Further studies are warranted to uncover the role of bacteria originating from the digestive tract in modulating the metabolism and signaling of the vaginal epithelium, endometrium and the maternal-fetal interface, as well as their possible links to female reproductive health (Erlebacher, 2013).

### Important role of the gut microbiota in the mother-to-infant transfer of materials

After birth, the neonate grows rapidly. Development and maturation of the digestive and immune functions of the intestine is critical for supporting the survival, growth and health of the infant (Wu et al., 2012a). Environmental factors (e.g. delivery mode, maternal health conditions, diet, disease and medication) will shape the microbial diversity, as well as the abundance and metabolism of the developing gut microbiota (Matamoros et al., 2013). This will, in turn, affect the maturation of the innate immunity and metabolism of the infant (Konstantinov et al., 2013). The effect of maternal factors on the development of gut microbiota and metabolism of the neonate will be discussed in the following sections (Table I).

### Programming of the neonatal gut microbiota and metabolism

There are many stages and windows for the establishment of the gut microbiota in the neonate. The ‘programming’ of the neonatal gut microbiota and metabolism is not unique and can be altered before birth and in post-natal life (Clemente et al., 2012). Before birth, the gut of the fetus was thought to be sterile. However, recent findings have shown that the presence of bacteria, such as Escherichia coli, Enterococcus, Streptococcus, Staphylococcus, and Propionibacterium species, in the intrauterine environment (e.g. meconium, umbilical cord and amniotic fluid) may result from the translocation of the bacteria from the gut of the mother (Matamoros et al., 2013). At term, delivery mode affects the bacterial composition of the memenium of the neonate. Vaginally delivered infants have the gut microbiota that resemble, to a great extent, their mother’s vaginal microbiota which is dominated by Lactobacillus, Prevotella or Sneathia spp., whereas the Cesarean section infant harbors gut microbiota that is more similar to their mother’s skin microbiota which is dominated by Staphylococcus, Corynebacterium, and Propionibacterium spp. (Dominguez-Bello et al., 2010).

Physiological conditions of the mother can affect the establishment of the infant microbiota. As noted previously, overweight pregnant women have higher numbers of Bacteroides, Staphylococcus and Enterobacteria inceae in their gut microbiota (Collado et al., 2008). Accordingly, infants of overweight mothers have a higher abundance of Bacteroides and Staphylococcus in their fecal samples during the first 6 months after birth (Collado et al., 2010). Also, infants of normal-weight mothers and of mothers with a normal weight gain during pregnancy have a lower abundance of Akkermansia muciniphila, Staphylococcus, and Clostridium difficile in the gut, when compared with overweight or obese mothers (Collado et al., 2010). Studies with vaginally delivered neonates demonstrated that babies born large for gestational age (LGA) mainly from obese/overweight mothers had a higher abundance of Proteobacteria especially E. coli, when compared with babies with a normal birthweight for an appropriate gestational age (Bodnar et al., 2010; Karlsson et al., 2011).

The long-term effect of the short-term ‘programming’ of the neonatal and infant gut microbiota is largely unknown. The altered microbiota, especially the increased abundance of protein-degrading and AA-metabolizing bacteria in the mother’s gut and ‘inherited’ transfer into the gut of the infant, may modulate the metabolic status of the infant. Thus, an elevated abundance of Bacteroides is associated with increased SCFA production in the large intestine. This may stimulate fatty acid synthesis and storage, while inhibiting fatty acid oxidation through the regulation of the fasting-induced adipose factor (FIAF) and AMPK signaling pathways, respectively (Cani and Delzenne, 2009). The increased abundance of Gram-negative bacteria such as the Bacteroides and E. coli in the gut of overweight and obese mothers or LGA babies may lead to increased concentrations of lipopolysaccharide (LPS) in the gut and...
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<th>Subjects/target organs</th>
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<td>Digestive tract</td>
<td>First trimester*: <em>Fecalibacterium, Eubacterium</em>&lt;br&gt;Third trimester*: <em>Enterobacteriaceae</em> family and <em>Streptococcus</em> genus bacteria&lt;br&gt;Post-partum / early lactation*: <em>Streptococcus</em>. Overweight pregnant women*: bacteria belong to <em>Bacteroides, Staphylococcus, Enterobacteriaee</em></td>
<td>Metabolic re-programming during pregnancy: increased bacterial fermentation of carbohydrates and amino acid in the large intestine of perinatal women; increased concentrations of SCFA in the large intestine may induce greater adiposity and insulin insensitivity&lt;br&gt;Regulate immune and neuroendocrine function: production of bioactive molecules cell wall components and peptides; translocation of bacteria by dendritic cells and macrophages to other organs (breast, vagina, uterus, etc.); mediate the production of AA-derived neurotransmitters and gaseous signaling molecules</td>
<td>Collado et al. (2008), Cryan and Dinan (2012), Dai et al. (2011), Fernández et al. (2013), Jost et al. (2014), Konstantinov et al. (2013), Koren et al. (2012), Nast and LeDuc (1988), Santacruz et al. (2010) and Li et al. (2009)</td>
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<td>Breast</td>
<td>Dominant bacteria in breast milk: <em>Weisella, Leuconostoc, Staphylococcus, Streptococcus, Lactobacillus, Propionibacterium, Bifidobacterium</em>&lt;br&gt;Increased abundance of oral bacteria in breast milk with lactation: <em>Veillonella, Leptotrichia, Prevotella</em>&lt;br&gt;Overweight / obese mother: high numbers of total bacteria, <em>Lactobacillus, Staphylococcus, low number of Bifidobacterium</em></td>
<td>Regulate breast milk composition: composition of microbiota shift with different physiological conditions; utilize and transform AA, polysaccharides and other milk components into bioactive molecules such as polynamines&lt;br&gt;Help the development of infant gut microbiota: provide continues source of microbial ‘inocula’ for the baby</td>
<td>Cabrera-Rubio et al. (2012), Fernández et al. (2013), Gabrielli et al. (2011), Lo¨nnerdal (2014), Paliy et al. (2014), Plaza-Zamora et al. (2013) and Smilowitz et al. (2013)</td>
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<td>Uterus</td>
<td>Pathogens associated with endometritis: <em>Chlamydia trachomatis, Neisseria gonorrhoeae</em>&lt;br&gt;Associate with peri- and post-partum endometritis: <em>E. coli, enterococci, Streptococcus</em>, anaerobic bacteria such as peptococcus and <em>Bacteroides fragilis, Ureaplasma urealyticum</em></td>
<td>Regulate endometrium health and function: endocrine, transport of nutrients, pregnancy recognition, implantation of the embryo, maintenance of pregnancy, post-partum recovery, etc.&lt;br&gt;Mediate the occurrence of pregnancy complications (miscarriage, IUGR, preterm birth, stillbirth) and infertility</td>
<td>Aagaard et al. (2014), Cape et al. (2013), Cicinelli et al. (2009, 2012), Eckert et al. (2002), Erlebacher (2013), Haggerty et al. (2004), Savidisinsk et al. (2013) and Platt et al. (1979)</td>
</tr>
<tr>
<td>Conceptus</td>
<td>Dominant bacteria of the placenta: <em>E. coli, Bacteroides spp., Neissera lactomica, Staphylococcus epidermidis, Propionibacterium acnes</em>; presence of the oral-type bacteria (<em>Prevotella tannerae, non-pathogenic Neissera spp.</em>)&lt;br&gt;Bacteria present in the meconium, umbilical cord, and amniotic fluid: <em>E. coli, Enterococcus, Streptococcus, Staphylococcus, Propionibacterium</em>&lt;br&gt;Associated with antepartum infection: <em>Actinomycetales, Alphaproteobacteria, Streptococcus, Acinetobacter, Fusobacteria</em></td>
<td>Regulate material exchange at the maternal-fetal interface and fetus development: mediate amino acid metabolism; regulate immunity through bacterial cell components; participate in the metabolism and immunity of the fetus&lt;br&gt;Mediate the occurrence of pregnancy complications such as preterm birth, IUGR and stillbirth</td>
<td>Aagaard et al. (2014), Erlebacher (2013) and Matamoros et al. (2013)</td>
</tr>
</tbody>
</table>
cause metabolic endotoxemia, which promotes low-grade inflammation and metabolic disorders such as insulin resistance, oxidative stress, diabetes and obesity (Cani and Delzenne, 2009). On the contrary, production of other AA metabolites, such as vasodilators NO and H₂S, antioxidant glutathione, neurotransmitter serotonin, and polyamines, by both the gut bacteria and body tissues may be affected by the diversity, abundance and metabolism of the gut microbiota (Dai et al., 2010). This will, in turn, regulate whole-body metabolism and homeostasis (Jobgen et al., 2006; Wu, 2010; DelCurto et al., 2013). Therefore, it is necessary to ‘re-program’ the composition and activity of the gut microbiota from ‘obese-type’ microbiota in the gut of obese/overweight mothers and LGA babies to prevent the development of metabolic syndrome in young children and adolescents (Paly et al., 2014). However, one should bear in mind that both host genotype and environmental factors contribute to the shaping of the gut microbiota in either the short term or the long run (Zoetendal et al., 2001; Tims et al., 2013).

**Critical role of breast milk**

In normal conditions, breast milk is the major food for neonates. The composition of milk is complex and varies with stage of lactation and maternal nutritional status (Lei et al., 2012). Studies with the AA composition of milk in humans showed that the most abundant AA are glutamate, glutamine, aspartate, asparagine, proline and branched-chain AA (Davis et al., 1994a; Wu and Knabe, 1994). The pattern of free AA changes during transition from colostrum to mature milk, with concentrations of most free AA (including glutamate, glutamine, proline, methionine, isoleucine and lysine) increasing in mature milk (Davis et al., 1994b; Wu and Knabe, 1994). Of note, concentrations of free glutamine in human and sow’s milk increase progressively with advanced stages of lactation (Wu and Knabe, 1994; Zhang et al., 2013; Baldeon et al., 2014). Compared with term milk, preterm milk had higher concentrations of free valine, threonine and arginine (Zhang et al., 2013), but this observation is not confirmed in some studies (Baldeon et al., 2014). As physiologically important metabolites of arginine, concentrations of polyamines in the milk vary during lactation, depending on the types of polyamine and birth conditions of infants. For example, compared with mothers giving birth to term infants, mothers with preterm infants had higher concentrations of polyamines in their milk during the first month of lactation regardless of polyamine type. Concentrations of spermine decreased but concentrations of spermidine increased with increased days of lactation (Plaza-Zamora et al., 2013). Other components in milk, such as immunoglobulins (secretory immunoglobulin A), bioactive proteins (e.g. α-lactalbumin, lactoferrin, osteopontin and milk fat globule membrane proteins), oligosaccharides, and hormones (leptin, ghrelin, adiponectin, obestatin and resistin) are also vital for the health of infants (Lönnerdal, 2014). The concentrations of these substances in milk are also altered with different stages of lactation and with different physiological conditions of mothers (Gabrielli et al., 2011; Smilowitz et al., 2013; Paly et al., 2014). At present, not all components in the milk of humans and livestock have been identified.

Bacteria are present at 10³–10⁵ /mL in the breast milk. Studies on the composition of the breast milk microbiota have shown that the predominant bacteria in the colostrum and mature milk are Weisella and Leuconostoc (both from the order Lactobacillales), followed by Staphylococcus, Streptococcus, and Lactococcus (Cabrera-Rubio et al., 2012). This may explain the presence of agmatine in the milk of
nonruminants (e.g. 6.3 µmol/L in sow’s milk) that may be beneficial for polyamine synthesis in the small-intestinal mucosa (Dai et al., 2014). Likewise, the abundance of the oral cavity inhabitants, such as Veillonella, Lep-totrichia, and Prevotella, increases in milk samples obtained at 6 months of lactation, when compared with colostrum and milk samples obtained at 1 month of lactation (Cabrera-Rubio et al., 2012). Bacteria such as Lactobacillus, Propionibacterium and Bifidobacterium are also present in breast milk (Fernández et al., 2013). Of note, maternal body weight influences the composition of bacteria in breast milk. For example, compared with normal-weight mothers, obese mothers have a higher total number of bacteria (including Lactobacillus and Staphylococcus) and a lower number of Bifidobacterium in their milk over the first 6-months of breast-feeding (Cabrera-Rubio et al., 2012). Similarly, mothers with excessive weight gain during pregnancy have a higher number of Staphylococcus aureus in 1-month milk and a higher number of Lactobacillus but a lower number of Bifidobacterium in the 6-month milk (Cabrera-Rubio et al., 2012). Origin of the bacteria in the breast milk can be the translocation of the mother’s gut bacteria along with dendritic cells and macrophages, as well as bacteria from the skin of mother and oral cavity of the infant (Fernández et al., 2013). Macrophages comprise 40–60% of the cells in colostrum and up to 80–95% in mature milk (Wagner et al., 2008). These cells are resistant to low pH and can pass through the lumen of the stomach and reach the lumen of the small intestine in the infant (Wagner et al., 2008). Therefore, the composition of breast milk plus a sufficient load of bacteria in the milk that reach the small intestine guarantee the successful continuous ‘inoculation’ of the mother’s gut microbiota to the infant gut. However, formula-fed infants lack such a mechanism for the establishment of the normal gut microbiota and display an increased abundance of Enterobacteriaceae in the gut (Mata-moros et al., 2013).

Research has shown that breast-fed infants harbor different kinds of the gut microbiota, when compared with formula-fed infants (Favier et al., 2002). By tracing the succession of the fecal microbiota from newborn babies for up to 1 year using molecular fingerprinting techniques, these authors found that bacteria related to the genus of Rumino-coccus dominate in fecal samples from babies supplemented with formula milk after 17 days of age (Favier et al., 2002). Bifidobacteria remained dominant in fecal samples from breast-fed babies until weaning. Moreover, compared with breast-fed babies, composition of the fecal microbiota became more diverse in babies consuming formula milk (Favier et al., 2002). Other bacteria such as Bacteroides, Clostridia, Enterobacter, and Streptococcus were also present in the feces from both breast- and formula-fed babies (Mackie et al., 1999; Favier et al., 2002). It is noteworthy that bacteria belonging to Ruminococcus and Bifidobacteria have the ability to degrade different oligosaccharide chains of mucins, release mono- and disaccharides, and hydrolyze peptides to AA for utilization by other intestinal bacteria (Hoskins et al., 1985; Hoskins, 1993; Favier et al., 2002). This can help to explain the difference in the microbial composition and metabolism of the gut microbiota between breast-fed and formula-fed babies.

The presence of the maternal microbiota in breast milk may play an important role in the programming of the infant gut microbiota and metabolism through the following pathways. First, continuous ‘inoculation’ to the infant gut through breast-feeding helps establish the indigenous microbiota and its associated metabolic phenotypes. Second, conversion of nutrients in the milk into bioactive molecules (such as polyamines and glutathione) supports the maturation of the infant gut. Third, the presence of special communities of bacteria such as the Enterobacteriaceae in the neonatal gut modulates infant metabolism directly through the generation of AA metabolites and indirectly via the production of LPS or other bacterial products. The sequential changes in the composition of milk not only provide essential nutrients to support the growth of neonates but also facilitate the establishment of the indigenous gut microbiota, as well as body metabolism and the development of immune systems (Perez et al., 2007; Donnet-Hughes et al., 2010).

Bacteria and reproduction in the male

Male reproductive performance is inevitably related to the utilization and metabolism of AA. Adequate amounts of AA, especially arginine, in the circulation are essential for the generation, differentiation and maturation of spermatozoa, thereby affecting their quantity and quality (Eskiock et al., 2006; Wu et al., 2009). Early studies showed that dietary arginine deficiency in men for 9 days resulted in sharp decreases of the number and motility of sperms (Holt and Albanese, 1944). On the contrary, dietary supplementation with arginine-HCl (0.5 g/day) to infertile men for up to 2 months markedly increased sperm counts and motility, leading to successful pregnancies of their partners (Tanimura, 1967). Dietary arginine supplementation not only improves sperm quality but also enhances concentrations of polyamines, ornithine, arginine and proline in seminal fluid, which is crucial for fertilization ( Wu et al., 2007, 2009). An increase in the number of microbes (particularly those that express a high level of arginase) in the lumen of the small intestine will promote catabolism of dietary arginine and, therefore, contributes to low sperm viability.

Furthermore, studies over the last decades have identified an important role for gaseous metabolites of AA in male reproductive function (Li et al., 2009; Gratzeke et al., 2010; d’Emmanuele di Villa Bianca et al., 2011). As an important metabolite of arginine, physiological levels of NO stimulate penile erection through the activation of guanylyl cyclase to generate cyclic GMP (cGMP), which induces relaxation of vascular and cavernosal smooth muscles (Gratzeke et al., 2010; Wu, 2010). As another gaseous metabolite of sulfur-containing AA (e.g. cysteine and methionine), H₂S acts in concert with NO on the cGMP pathway to inhibit the activity of phosphodiesterase type 5 in smooth muscle cells and stimulate penile erection (d’Emmanele di Villa Bianca et al., 2011). NO and H₂S in the smooth muscle cells are derived from the cavernous nerve and endothelial cells of the penis. H₂S can also be produced within smooth muscle cells and from erythrocytes by the catabolism of cysteine, methionine or organic sulfate respectively (d’Emmanuele di Villa Bianca et al., 2011). Intestinal bacteria are a significant source of H₂S in animals (Gibson et al., 1993), and its excessive production may be deleterious to intestinal and reproductive tissues. Interestingly, in hypoxic conditions, H₂S is produced from cysteine in the human placenta and from thiosulfate in bovine and rat arterial blood vessels (Patel et al., 2009; Olson et al., 2013). These findings suggest an important role of H₂S in the pathophysiology of reproduction in both females and males. Thus, sulfur recycling may participate in whole-body H₂S signaling and in reproductive function.

Serotonin (a metabolite of tryptophan) is important for controlling ejaculation both centrally and peripherally (Giuliano and Clément, 2005; Berger et al., 2009). It has been reported that serotonin can
either increase or decrease ejaculatory latency through the activation of different 5-HT receptors in the brain or spinal cord (Giuliano and Clément, 2005). However, the net effect of serotonin is to prolong ejaculation and delay orgasm (Berger et al., 2009). More than 90% of serotonin in the body is released from the small intestine when animals consume food (Berger et al., 2009). Therefore, it is important to investigate the effect of food consumption and different nutrients on intestinal production and signaling of serotonin and its link to male reproduction.

As reported for females, the gut microbiota of males can produce all the AA metabolites mentioned in the preceding section (Table II). The utilization of AA by bacteria in the small intestine of men regulates not only AA supply to the reproductive organ for the maintenance of normal function, but also the synthesis of hormones and signaling molecules in the whole body. Likewise, the production of NO, H$_2$S, serotonin, dopamine and GABA by gut bacteria in the small and large intestine can participate in the signaling and regulation of male reproduction (Gratzke et al., 2010; Cryan and Dinan, 2012). The production and recycling of microbial AA metabolites in the body can regulate the conversion of H$_2$S to thiosulfate in the colonic cells and the regeneration of H$_2$S from thiosulfate in erythrocytes and arteries (Blachier et al., 2010; d’Emmanuele di Villa Bianca et al., 2011; Olson et al., 2013). Recent studies with mice showed that metabolites such as butyrate produced by Clostridium tyrobutyricum improved testis development by modulating the permeability of the blood-testis barrier and endocrine function of testis (Al-Asmakh et al., 2014). Furthermore, the presence of the gut microbiota in the male urogenital tract plays an important role in semen quality and male reproductive health through the production of important metabolites (Table III). These observations provide opportunities to uncover AA-related metabolic or hormonal interactions between gut bacteria and reproduction performance in men and in males of other species.

### Dietary interventions for sound gut ecology and optimal reproduction

Food is crucial for supporting normal reproduction in both men and women. The nutritional, metabolic and hormonal effects of diets are closely linked to the gut microbiota. Maintaining optimal conditions of the gut ecology (i.e. composition and activity of the gut microbiota, morphology and function) for the utilization of dietary AA is crucial for normal reproduction and the metabolic programming of the offspring. The effects of dietary supplementation with AA, prebiotics and probiotics on reproduction are highlighted in the following sections (Table IV).

#### Functional AA

Amino acids are not only nutritionally essential but are also functionally important (Wu, 2009, 2010, 2013). For example, AA (e.g. arginine,

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**Table II Important microbial metabolites of amino acids and their functions in reproduction.**

<table>
<thead>
<tr>
<th>AA</th>
<th>Metabolites</th>
<th>Function on reproduction</th>
<th>Key bacteria</th>
<th>Niches</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>Polyamines</td>
<td>Ovulation, ovarian steroidogenesis and oocyte quality; embryo implantation, survival, and growth; placental angiogenesis, growth, and function; spermatogenesis, sperm quality, and male fertility</td>
<td>Bacteroides thetaiotaomicron, Fusobacterium varium</td>
<td>Large intestine</td>
<td>Noack et al. (2000) and Wu et al. (2009)</td>
</tr>
<tr>
<td>NO*</td>
<td></td>
<td>Physiological vasodilator; angiogenesis; spermatogenesis; embryogenesis; fertility; modulate penile erection</td>
<td>Lactobacillus spp., Bifidobacterium spp., Staphylococcus aureus, Bocillus spp.</td>
<td>Small and large intestine</td>
<td>Gratzke et al. (2010), Sobko et al. (2006), Vermeiren et al. (2012) and Wu (2009)</td>
</tr>
<tr>
<td>Glutamate</td>
<td>GABA</td>
<td>Inhibit penile erection</td>
<td>Lactobacillus brevis, L. paracasei, Bifidobacterium dentium</td>
<td>Small and large intestine</td>
<td>Barrett et al. (2012), Gratzke et al. (2010) and Komatsuzaki et al. (2008)</td>
</tr>
<tr>
<td>Serine- and aspartate-families of AA, lysine</td>
<td>SCFA</td>
<td>Tight junction and paracellular permeability of umbilical vein; anti-inflammatory role in human labor</td>
<td>Clostridium spp., Peptostreptococcus spp.</td>
<td>Large intestine</td>
<td>Smith and Macfarlane (1997), Miyoshi et al. (2008) and Voltoinli et al. (2012)</td>
</tr>
<tr>
<td>Sulfur AA</td>
<td>H$_2$S</td>
<td>Physiological vasodilator; physiological control of penile tone</td>
<td>Escherichia coli, Salmonella enterica, Clostridium spp., Enterobacter aerogenes, Peptostreptococcus spp., Fusobacterium, Eubacterium, Desulfovibrio spp.</td>
<td>Large intestine</td>
<td>Blachier et al. (2010), d’Emmanuele di Villa Bianca et al. (2011) and Smith and Macfarlane (1997)</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>Serotonin</td>
<td>Inhibit penile erection and ejaculation</td>
<td>Streptococcus spp., Escherichia coli, Enterococcus spp.</td>
<td>Small and large intestine</td>
<td>Gratzke et al. (2010), Lyte (2011) and Park et al. (2011)</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Dopamine</td>
<td>Inhibit penile erection</td>
<td>Bocillus spp., Serratia spp.</td>
<td>Small and large intestine</td>
<td>Gratzke et al. (2010) and Lyte (2011)</td>
</tr>
</tbody>
</table>

*AA, amino acids; GABA, gama-aminobutyric acid; H$_2$S, hydrogen sulfide; NO, nitric oxide; SCFA, short chain fatty acids.

*Nitrate also contributes to the production of NO by bacteria in the gut lumen (Vermeiren et al., 2009).*
Table III  Possible links between gut bacteria and male reproduction.

<table>
<thead>
<tr>
<th>Target organs</th>
<th>Key bacteria</th>
<th>Possible mode of action</th>
<th>References</th>
</tr>
</thead>
</table>
| Digestive tract              | Not clear, possible bacteria affect male reproduction are: sulfur-producing bacteria (Table II); NO-producing bacteria (Table II); tryptophan-metabolizing bacteria (Table II); probiotic bacteria (*Clostridium Tyrobutyricum*, *Lactobacillus reuteri*) | Gut metabolism and health: reduce oxidative stress, promote butyrate production  
Modulation of immune and neuroendocrine function: production of bioactive molecules cell wall components and peptides; translocation of bacteria by dendritic cells and macrophages to reproductive organs; mediate the production of AA-derived neurotransmitters and gaseous signaling molecules thus regulate reproductive performance | Al-Asmakh et al. (2014), Poutahidis et al. (2014) and Li et al. (2009)  
Corynebacterium spp. were found in the semen of both fertile and infertile men; *Mycoplasma* spp. were found to be associated with infertility and HIV infection (Mändar, 2013; Liu et al., 2014). |
| Urogenital tract             | Testis: *Chlamydia trachomatis*, *E. coli*, *Brucella abortus*  
Urethra and coronal sulcus: *Lactobacillus*, *Corynebacterium* spp., *Streptococcus*, *Neathia* spp., *Bacteroides* spp., *Staphylococcus epidermidis*, *Gardnerella vaginalis*, etc.; uncircumcised: dominant bacteria belong to the families of *Clostridiales* XI and *Prevotellaceae*, *Peptoniphilis*; pathogens: *Chlamydia trachomatis*, *E. coli*, *Prevotella* spp., *Fusobacterium* spp., *Oxobacter*, *Aerovorax*, *Candida* sp. | Regulate testis development and pathogenesis: modulate permeability of blood-testis barrier and endocrine function of testis by bacterial metabolites such as butyrate; cause of necrosis and epididymo-orchitis  
Regulate spermatogenesis, sperm function and semen quality: chromatin integrity and apoptosis of spermatozoa, sperm motility, morphology; concentrations of zinc and fructose  
Mediate prostatitis: biofilm production  
Induce immune response and inflammation: expression of pathogen recognition receptors, Toll-like receptors and production of cytokines/chemokines of the urogenital tract cells | Filipiak et al. (2014), Hou et al. (2013), Liu et al. (2013, 2014), Lu et al. (2013), Mackern-Oberti et al. (2013), Mändar (2013), Moretti et al. (2009), Nelson et al. (2012), Price et al. (2010), Ron-Roman et al. (2012), Rybar et al. (2012), Sellami et al. (2014) and Türk et al. (2014)  
Corynebacterium spp. were found in the semen of both fertile and infertile men; *Mycoplasma* spp. were found to be associated with infertility and HIV infection (Mändar, 2013; Liu et al., 2014). |
Table IV  Effects of dietary interventions on gut bacteria and human reproduction.

<table>
<thead>
<tr>
<th>Subjects/Target organs</th>
<th>Key bacteria</th>
<th>Possible mode of action</th>
<th>References</th>
</tr>
</thead>
</table>
| Probiotics/prebiotics<sup>a</sup> | Commonly used probiotics: *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Bifidobacterium breve*, *Bifidobacterium bifidum*, *Bifidobacterium lactis*  
Male: *Clostridium Tyrobutyricum*, *Lactobacillus reuteri*  
Commonly used prebiotics to support the growth of probiotic bacteria: fructooligosaccharides, galactooligosaccharides | Pregnant women: modulate gut microbiota and immune function; reduce blood glucose concentration and increase glucose tolerance (summarized by Rautava et al., 2012)  
Infant: colonization in the intestine of infant; modulate gut microbiota and immune function; reduced risk of eczema  
Male: loss weight in overweight/obese subjects, reduce oxidative stress, promote butyrate production | Al-Asmakh et al. (2014), Gueimonde et al. (2006), Laitinen et al. (2009), Luoto et al. (2010), Marques et al. (2010), Mikami et al. (2009), Poutahidis et al. (2014), Rautava et al. (2012) and Sanz (2011) |
| Conceptus, Uterus | Consumption of probiotic bacteria: *Bifidobacterium lactis*, *Lactobacillus rhamnosus*, fermented milk and yogurt bacteria  
SCFA produced by gut bacteria: this event is regulated by the consumption of probiotic bacteria and prebiotics | Modulate the expression of Toll-like receptor genes in the placenta and fetal gut  
Prevention of genital infection and preterm labor: Regulate the expression of G protein-coupled receptors GPR43 and GPR41 and LPS-induced inflammatory processes in myometrium and fetal membranes | Othman et al. (2007), Rautava et al. (2012) and Voltoifini et al. (2012) |
| Testis | Consumption of probiotic bacteria: *Lactobacillus reuteri* | Increase seminiferous tubule cross-sectional profiles, spermatogenesis and Leydig cell numbers in aging mice | Poutahidis et al. (2014) |
| Amino acids/AA metabolites |  
Digestive tract | Small intestine: *Streptococcus* spp., *Megasphaera elsdenii*, *Mitsuokella* spp., *E. coli*, *Acidaminococcus fermentans*, *Lactobacillus*  
Large intestine (pollyamine-fed mice): *Bifidobacterium*, *Akkermansia*-like bacteria, *Lactobacillus—Enterococcus group bacteria* | Regulate the AA utilization and metabolism in small-intestinal bacteria: arginine, glutamine  
Regulate the microbiota abundance/composition in both the small and large intestine: arginine, polypeptide  
Shifts in the microbial abundance/composition and activity will change the availability of AA and their metabolites peripherally thus regulate reproductive health and performance | Dai et al. (2010, 2012a,b, 2013), Gómez-Gallego et al. (2012) and Ren et al. (2014a,b) |
|  
<sup>a</sup>Breast milk is the natural source of probiotics and prebiotics for infant (Marques et al., 2010; Fernández et al., 2013).  
AA: amino acids; LPS: lipopolysaccharide. |
glutamine, glycine, threonine, leucine, sulfur AA, taurine, tryptophan and tyrosine) are required to maintain gut integrity and function, stimulate intestinal protein synthesis and cell growth, sustain body metabolism, and support reproduction (Bazer, 2011; Wang et al., 2012; Wu et al., 2012b, 2013c). Considering the adverse effects of a high-protein diet on the metabolism and health of pregnant women and their offspring (Herrick et al., 2003; Thöne-Reineke et al., 2006; Andreasen et al., 2007; Lönnерdal, 2014), dietary supplementation with these functional AA in an adequate amount to avoid an excess of dietary protein can be beneficial for the reproductive performance of both humans and animals (Wu et al., 2009, 2013a; Wu, 2010). Because some of these AA have been reported to regulate AA metabolism in small-intestinal bacteria (Dai et al., 2012b, 2013), it can be surmised that their effects are mediated, in part, by the gut microbiota.

Growing evidence suggests that dietary supplementation of functional AA modulates gut ecology, AA metabolism and function (Dai et al., 2011). In vitro studies with the pig small-intestinal bacteria showed that different AA support the growth of different communities of bacteria (Dai et al., 2010). Bacteria such as Streptococcus sp., Megao- sphera elsdenii and Mitsuokella sp. were dominant in cultures containing lysine, threonine, arginine or glutamate, while Escherichia coli and Acidaminococcus fermentans were most abundant in cultures containing arginine, glutamate or histidine (Dai et al., 2010). The supplementation with arginine or glutamine to the cultures of small-intestinal bacteria decreased their AA utilization and changed the patterns of AA metabolism through different pathways (Dai et al., 2012a,b, 2013). Thus, functional AA can alter the composition and activities of AA utilization and metabolism by the small-intestinal bacteria. Furthermore, studies with mice indicated that dietary supplementation with 0.5% arginine increased the abundance of Bacteroidetes and decreased the abundance of Firmicutes in the small intestine, with the numbers of Lactobacillus and Streptococcus increasing in the jejunum and ileum, respectively (Ren et al., 2014a). The alteration of the gut microbiota in response to dietary arginine supplementation stimulated the innate immunity by activating several cell-signaling pathways involving Toll-like receptor (TLR), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), mitogen-activated protein kinase (MAPK), and phosphoinositide-3 kinase (PI3K)/PI3K-protein kinase B (Akt) (Ren et al., 2014a). The dietary intake of arginine also elevated concentrations of polyamines in the colon due to bacterial fermentation (Kibe et al., 2014). The altered levels of polyamines in the intestinal lumen will, in turn, modify the composition and abundance of the gut microbiota. Consistent with these reports, the abundance of the Bifidobacterium group of bacteria, Akkermansia-like bacteria, and the Lactobacillus—Enterococcus group of bacteria increased in the large intestine of mice fed infant formulas supplemented with polyamines (Gómez-Gallego et al., 2012). These findings suggest that dietary supplementation with arginine and other functional AA [e.g. glutamine (Chen et al., 2014; Ren et al., 2014b)] exerts beneficial effects on reproduction of human and other animals possibly through modulation of the composition, AA utilization and metabolism of the microbiota in the gut. The effects will be manifest under conditions of nutrient restriction, where the impact of intestinal bacteria on the supply of AA metabolites to reproductive organs versus dietary sources may be relatively greater, when compared with adequate feeding (Satterfield et al., 2013).

### Probiotics and prebiotics

Probiotics and prebiotics affect AA metabolism in the small intestine (Bergen and Wu, 2009). Studies of their effects on reproduction mainly focused on the health and metabolism of pregnant women and the subsequent programming in infants (Sanz, 2011). Many of the studies involved immunity and immune-related disease of infants (Sanz, 2011; Rautava et al., 2012). Some of them were focused on the development of the gut microbiota during the neonatal period (Gueimonde et al., 2006; Mikami et al., 2009). The others were concerned primarily about the metabolic programming in infants and its links to obesity (Laitinen et al., 2009; Luoto et al., 2010). The commonly used probiotics are Lactobacillus rhamnosus, Lactobacillus casei, Bifidobacterium breve, Bifidobacterium bifidum, and Bifidobacterium lactis. Prebiotics used to support the growth of these probiotic bacteria in the gut are mainly oligosaccharides, including fructooligosaccharides (FOS) and galactooligosaccharides (GOS). The use of probiotics, prebiotics or their combination showed beneficial effects on the health of pregnant women or infants in most studies (Marques et al., 2010; Sanz, 2011). However, the mechanisms responsible for the programming of the gut microbiota, immunity and metabolism in infants are largely unknown, and may include the following. First, dietary supplementation of probiotics and prebiotics modulate the gut ecology in pregnant women, thereby altering the composition and activity of the microbiota and the associated bacterial components (e.g. LPS, peptidoglycans and s-layer proteins) and metabolites (e.g. SCFA, NO, H₂S and polymamines) in the intestine. The altered gut ecology affects not only the immune and metabolic status of the pregnant women but also the environment of the conceptus, critical for early fetal programming (Rautava et al., 2012). Second, after birth, mother’s breast milk (the major naturally-made formula for neonates) programs the gut microbiota, immunity, and metabolism of the infant by providing the probiotic bacteria and prebiotic oligosaccharides to infants (Marques et al., 2010; Fernández et al., 2013). Many of the bacteria exert their probiotic role partially through AA metabolism directly or indirectly via bacteria-bacteria interactions and cross feeding (Table V).

To date, data on the effect of probiotics on male reproduction are rare. It was shown that dietary supplementation with probiotic bacteria Lactobacillus reuteri increased seminaliferous tubule cross-sectional profiles, spermatogenesis and Leydig cell numbers in aging mice (Pouthahidis et al., 2014). Improving metabolic status and reducing excessive white fat in overweight/obese male adults or infants (through metabolic programming) can enhance reproductive performance in the long run (Luoto et al., 2010; Aaltosen et al., 2011; Esposito and Giugliano, 2011; Kolotkin et al., 2012).

As an important group of metabolites produced from the fermentation of carbohydrates (including prebiotics) and AA by bacteria in the distal small intestine and large intestine, SCFA have been reported to play a critical role in the activation of inflammatory pathways and system immunomodulation, pathologies of pregnancy, and the onset of normal labor (Voltolini et al., 2012; Arpaia et al., 2013; Smith et al., 2013; Trompette et al., 2014). SCFA, individually or in combination, regulate the size and function of the regulatory T-cells pool in varieties of organs including colon, spleen and lymph nodes, while protecting the gut against colitis in a Ffar2 (G protein-coupled receptor GPR43)-dependent manner in mice (Arpaia et al., 2013; Smith et al., 2013). An increase in the circulating levels of SCFA through dietary
supplementation of pectin in mice has a protective effect against allergy inflammation in the lung (Trompette et al., 2014). Likewise, propionate enhances the generation of macrophage and dendritic-cell precursors and subsequent seeding of the lungs in mice, and its anti-allergic effects were GPR41-dependent (Trompette et al., 2014). Similarly, data on the study of human labor indicate that GPR43 and GPR41 mRNA levels are higher in myometrium and fetal membranes from women after the onset of labor (Voltolini et al., 2012). Interestingly, GPR43 protein is localized to the immune cells and vascular endothelium in the myometrium and epithelium of fetal membranes, and sodium propionate attenuates an LPS-induced increase in expression of GPR43 and inflammatory genes (Voltolini et al., 2012). At present, little is known about links between the intestine and plasma SCFA profiles, or about a role for probiotics and prebiotics in the immunity of the uterus-fetus interface or the onset of labor. More studies are warranted to investigate the role of G protein-coupled receptors in the SCFA-mediated immune response and function of reproduction organs in both men and women (Voltolini et al., 2012; Al-Asmakh et al., 2014; Poutahidis et al., 2014; Priyadarshini et al., 2014).

**Timing is critical**

With enhanced understanding of reproductive biology and metabolic syndrome (e.g. obesity and diabetes), critical windows for dietary intervention can be identified (Louis et al., 2008; Bazer, 2013; Cheong et al., 2013; Wu et al., 2013a). Studies with gestating gilts showed that dietary supplementation with L-arginine during Days 0 to 25 of gestation have strikingly different outcomes than during Days 14 to 25 of gestation. Compared with dietary arginine supplementation between Days 14 and 25 of gestation, supplementation of arginine immediately after breeding (day 0) reduced litter size (Li et al., 2010, 2014). These observations suggest that Days 0 to 14 of gestation and Days 14 to 25 are two critical windows for arginine supplementation to gestating gilts. It is possible that arginine may interact with intestinal microbes to affect pregnancy outcomes, and this possibility should be examined in future investigations.

In women with normal pregnancy, the composition of the gut microbiota during the first trimester is similar to that of non-pregnant women but changes dramatically during the third trimester (Koren et al., 2012). Increased abundances of Proteobacteria and Actinobacteria in the gut, as well as improved insulin sensitivity and reduced adiposity in the body, favor a maternal environment for embryonic/fetal survival and growth. However, in overweight/obese pregnant women, increased numbers of Proteobacteria and Actinobacteria during the first trimester or even before pregnancy result in early metabolic changes in the maternal body, which will have adverse effects on the fetus. Thus, early dietary intervention of the women to reduce the numbers of AA metabolizing bacteria in the gut may be of vital importance. Indeed, dietary supplementation of probiotics (Lactobacillus rhamnosus GG and Bifidobacterium lactis Bb12), coupled with dietary counseling at early pregnancy, reduced blood glucose concentration and this effect lasted from the first trimester to 12 months post-partum (Laitinen et al., 2009). The maintenance of blood glucose during pregnancy for overweight/obese or diabetic mothers will reduce the risk of related complications and have long-term benefits for the mother and the infant (Ostlund et al., 2003; Crowther et al., 2005; Cani and Delzenne, 2009). However, it is unknown whether dietary intervention with functional AA or probiotics and prebiotics is necessary for normal pregnant women. Nonetheless, there are suggestions that high rates of infertility and embryonic death in women and livestock during early gestation could be reduced by improving the uterine environment through dietary supplementation with functional AA (Bazer et al., 2014). Similarly, dietary interventions for several months before conception

### Table V Summary of the amino acid utilization and metabolism by intestinal bacteria.

<table>
<thead>
<tr>
<th>Types of utilization and metabolism</th>
<th>Amino acids*</th>
<th>Products/metabolites</th>
<th>Major intestinal site</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protein synthesis</strong></td>
<td>Not specific, major AA used for protein synthesis are: Lys, Thr, Glu, Gin, Ala, Val, Arg, Leu, Ile, Lys, Ser, Thr, and Pro</td>
<td>Bacterial cell proteins and small peptides</td>
<td>Jejunum, ileum, proximal colon</td>
<td>Dai et al. (2010, 2011, 2012a), Smith and Macfarlane (1998) and Yang et al. (2014)</td>
</tr>
<tr>
<td><strong>Catabolism</strong></td>
<td>Not specific, major AA used for catabolism are: Lys, Thr, Glu, Gin, Asp, Gin, Arg, Ser, Val, Leu, Ile, Pro, Trp, Phe, Met, and Cys</td>
<td>SCFA, BCFA, biogenic amine, polyamines, phenolic and indolic compounds, thiol, ammonia, NO, H2S, H2, CO2, and CH4</td>
<td>Jejunumb, ileumc, distal colon</td>
<td>Blachier et al. (2010), Dai et al. (2011, 2012a), Macfarlane and Macfarlane (2012), Smith and Macfarlane (1997, 1998) and Yang et al. (2014)</td>
</tr>
<tr>
<td><strong>Transformation</strong></td>
<td>Glu, Trp and Tyr</td>
<td>GABA, serotonin, dopamine and norepinephrine</td>
<td>Not clear</td>
<td>Cryan and Dinan (2012) and Dai et al. (2011)</td>
</tr>
<tr>
<td><strong>Cell–cell interaction</strong></td>
<td>Met, Leu, Ile and Phe</td>
<td>Peptidoglycan, S layer proteins, chemotactic peptides and AHL</td>
<td>Jejunum, ileum, proximal colon</td>
<td>Dai et al. (2011), Hernández et al. (2005), Konstantinov et al., (2008), Nast and LeDuc (1988) and Oyode et al. (2013)</td>
</tr>
<tr>
<td><strong>De novo amino acid synthesis</strong></td>
<td>Not required</td>
<td>Lys, Thr, Val, His, Pro, Glu, Ala and Gly</td>
<td>Ileum, proximal colon</td>
<td>Metges (2000), Metges and Petzke (2005) and Torrallardona et al. (2003a, b)</td>
</tr>
</tbody>
</table>

AA, amino acids; AHL, acyl homoserine lactones; BCFA, branched-chain fatty acids; GABA, gama-aminobutyric acid; H2S, hydrogen sulfide; NO, nitric oxide; SCFA, short chain fatty acids.

*Standard abbreviations of amino acids are used in the table.

bBoth the luminal and gut epithelium-attached bacteria may contribute to the utilization and metabolism of amino acids (Yang et al., 2014).

cPossible precursors used for the de novo synthesis of amino acid by intestinal bacteria are ammonia, urea and glucose (Metges 2000; Torrallardona 2014).
may be beneficial for overweight/obese men who plan to have babies (McKnight et al., 2011).

Critical window times for dietary intervention of the programming of gut microbiota and metabolism of the infant may start early, at the fetal stage (Aaltonen et al., 2011; Rautava et al., 2012; David et al., 2014). Bacteria translocated from the intestine, along with their AA metabolites in the placenta and fetal amniotic fluid, may alter the fetal intestinal innate immunity, which may act as a selection force for the colonization of gut microbiota. Several days and weeks after birth, consumption of breast milk may affect the gut microbiota and metabolic status of the infant. Dietary interventions (e.g., supplementation with functional AA or probiotics and prebiotics) through these windows may help to prevent abnormal AA metabolism in gut bacteria and, therefore, metabolic disorders (e.g., non-alcohol fatty liver disease, insulin resistance, glucose intolerance and erectile dysfunction) through the programming of the gut microbiota (Wolfe et al., 2012; Russell et al., 2013; Zhu et al., 2013; Brumbaugh and Friedman, 2014; Fujimoto et al., 2014; Vignozzi et al., 2014).

Conclusion and perspectives

Both the small intestine and the large intestine harbor large numbers of bacteria to metabolize dietary AA. Intestinal bacteria not only alter the pool and profile of AA that enter the bloodstream from the intestine, but also produce varieties of nitrogenous and sulfur-containing metabolites. Physiological levels of these microbial products may influence cellular signaling pathways and reproduction through the formation of gametes, penile erection, ejaculation, implantation of conceptus, placentation, delivery of newborns, and breast-feeding, as well as metabolic programming and re-programming during critical periods of the life cycle. Therefore, adequate dietary intakes of both ‘nutritionally essential AA’ and ‘nutritionally nonessential AA’ are required for optimal intestinal health and whole-body homeostasis (Wu, 2014). However, excessive production of microbial metabolites (e.g., ammonia, NO and H2S) is harmful to organisms. As a result, the traditional high-protein weight-losing diet may not be suitable for obese or overweight couples who plan to have babies (Westerterp-Plantenga et al., 2009). In this regard, the gut microbiota can be viewed as either a protector or an invader of the reproductive systems (Fig. 1).

Nutritionally, diet is a major factor that determines the composition and activity of the gut microbiota during pregnancy and in overweight/obese individuals. This, in turn, affects the metabolic status of the mothers and their offspring. The mother-to-baby mode of programming is mediated, in part, by the gut microbiota, especially AA-metabolizing bacteria. The balance of the AA-metabolizing bacteria in the gut is crucial for maintaining the metabolic homeostasis and reproductive

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**Figure 1** Schematic diagram of the links between amino acid metabolism in gut bacteria and reproduction in the host. AA, amino acids; NO, nitric oxide; 5-HT, 5-hydroxytryptamine (serotonin); GABA, gamma-aminobutyric acid; SCFA, short chain fatty acids; BCFA, branched-chain fatty acids; H2S, hydrogen sulfide.
performance of the host. This fetal or neonatal impact of nutrition may be inherited by the offspring through epigenetic modifications of the spermatozoa, oocyte and fetus by AA and their metabolites produced by tissues and intestinal bacteria. In addition, breast milk acts as a messenger that carries the metabolic signature from the gut microbiota of the mother to the intestine of infants, thereby playing a critical role in shaping the phenotype (e.g. metabolic profiles) of the offspring. Thus, it is important to uncover links between epigenetics and metabolic syndrome that impact reproductive function (Canani et al., 2011; Wang et al., 2012; DelCurto et al., 2013). Furthermore, functional redundancy of AA-metabolizing bacteria indicates that they can ‘produce’ varieties of active diet (e.g. plant-derived estrogen-like metabolites such as equol) when the host consumes plant-source foods (Jackson et al., 2011; Lozupone et al., 2012). This will further affect health and reproduction in both men and women and of their offspring.

In practice, dietary counseling and interventions for both men and women with metabolic syndrome are important to improve their reproductive performance. The same rule may also apply to infants consuming formula with high protein levels (Lo¨nnerdal, 2014). Personalized dietary supplementation with functional AA (e.g. arginine and glutamine), probiotics and prebiotics to restore the normal composition and activity of the gut microbiota may improve the reproductive performance in both males and females, while preventing the development of metabolic syndrome in offspring induced by fetal programming of the infant gut microbiota. Finally, integrating multi-disciplinary knowledge to understand how AA metabolism in the gut microbiota affects development and function of female and male reproductive organs will help in the development of new strategies to reduce infertility and improve pregnancy outcomes in mammals (e.g. humans and livestock) and other animal species.

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Z.D., Z.W. and G.W. composed and revised the manuscript. W.Z. and S.H. revised the manuscript.

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Conflict of interest
The authors declare that they have no conflict of interest.

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