Optimizing nutrition for early life development – from prenatal to toddler

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DuPont Nutrition & Health
Healthy maternal nutrition has a profound influence on the development of the immune system during fetal and postnatal periods.

Mothers transfer their immunity to a fetus via placenta and breast milk.

Microbial colonization of the digestive tract begins at birth – the mother playing a critical role.

Disruption of the development of the microbiota and immunity during this period has been linked to the development of allergies and eczema.
PRENATAL HEALTH AND WELL BEING

For some women, pregnancy brings ill health and discomfort through:

1. Increased susceptibility to bacterial vaginosis
2. Increased susceptibility to postnatal depression and anxiety
3. Increased susceptibility to gestational diabetes mellitus

Both mothers and their babies can benefit from probiotic dietary supplementation during pregnancy - HOWARU® Protect Prenatal+
HUMAN CLINICAL TRIAL RESULTS

The effect of supplementation with *Lactobacillus rhamnosus* HN001™ (6B) in pregnancy on cord blood and breast milk immune markers

<table>
<thead>
<tr>
<th>Marker</th>
<th>Study outcomes</th>
<th>Function in the immune system</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ</td>
<td>Mothers in HN001 group had higher levels of immune marker IFN-γ in their cord blood</td>
<td>Stimulation of Th1 immunity (IFN-γ) is associated with suppression of Th2 immune responses. Atopic (allergic) individuals have typically lower levels of Th1 cytokines (IL-12, IFN-γ)</td>
</tr>
<tr>
<td>IgA</td>
<td>In early breast milk, more mothers in the HN001 group (88%; p=0.011) from whom antibody immunoglobulin A (IgA) was detected, compared to placebo group (61%)</td>
<td>IgA antibodies in breast milk reflect maternally encountered antigens and provide passive protection to breast-fed infants</td>
</tr>
</tbody>
</table>

ORALLY CONSUMED PROBIOTICS COLONIZE THE VAGINAL TRACT

Study designs

> Randomized double blind placebo controlled

> A combination of *L. acidophilus* La-14 and *L. rhamnosus* HN001™ (10^{10} CFU/day) + lactoferrin or placebo for 14-15d

> 40 women (20+20)

> *Lactobacillus* species assessed by qPCR from vaginal swabs at different time points

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**DeAlberti et al. 2015**

- **Fold change from Day 0**
- **Treatment** vs **Placebo**
- **Species** detected:
  - *L. acidophilus* (7 days: 0, 14 days: 4, 21 days: 0)
  - *L. rhamnosus* (7 days: 9, 14 days: 9, 21 days: 9)

**Russo et al. 2018**

- **Baseline vs. end of treatment**
- **Species** detected:
  - *L. acidophilus* (7 days: 4, 14 days: 12*, 21 days: 16*)
  - *L. rhamnosus* (7 days: 16*, 14 days: 17*, 21 days: 17*)

* P < 0.001 compared to baseline

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*DeAlberti et al. (2015) Arch Gynecol Obstet; Russo et al. (2018) Arch Gynecol Obstet*
PROBIOTICS HELP IN RESTORING HEALTHY VAGINAL MICROBIOTA AND MAY ALLEVIATE VAGINAL SYMPTOMS

Nugent score returned to normal after 15 days of probiotic intervention

Significant improvement in self assessed vaginal symptoms after treatment (itching, discharge) compared to placebo

Russo et al. (2018) Arch Gynecol Obstet
EFFECT OF LACTOBACILLUS RHAMNOSUS HN001™ IN PREGNANCY ON POST PARTUM SYMPTOMS OF DEPRESSION AND ANXIETY AND GESTATIONAL DIABETES MELLITUS

Two-centre, double-blind, randomised, placebo-controlled parallel trial. Effect on GDM and depression and anxiety were secondary outcomes (primary outcome development of eczema & atopic sensitisation, Barthow et al. (2016) BMC Pregnancy and childbirth)

Pregnant women (less than 16 weeks gestation) were chosen for the study if either they or the father had a history of asthma, hay fever or eczema requiring medication

Study product: capsules with either HN001 (6x10⁹ CFU) or placebo (maltodextrin) once a day

Study length: From 14-16 weeks gestation to delivery and until 6 months post-partum if breastfeeding

<table>
<thead>
<tr>
<th></th>
<th>HN001</th>
<th>Placebo</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrolled</td>
<td>212</td>
<td>211</td>
<td>423</td>
</tr>
<tr>
<td>Analyzed outcomes</td>
<td>206</td>
<td>202</td>
<td>408</td>
</tr>
</tbody>
</table>
LACTOBACILLUS RHAMNOSUS HN001™ SUPPLEMENTATION MIGHT HELP IN REDUCING RISK FOR GDM

> HN001 might reduce the prevalence of GDM by 40%, if GDM defined with IADPSG guidelines, or by 68% using the New Zealand guidelines

- with New Zealand criteria (more specific), the prevalence was significantly lower in the HN001 group ($P=0.03$)

> HN001 seemed to prevent GDM especially in mothers older than 35 years old ($P=0.009$) and mothers with history of GDM ($P=0.004$)

> Fasting blood glucose levels were slightly lower in the HN001 group ($P=0.048$)
## PROBIOTICS IN PREGNANCY STUDY

<table>
<thead>
<tr>
<th></th>
<th>Treatment</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depression score</td>
<td>HN001</td>
<td>194</td>
<td>7.7*</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>187</td>
<td>9.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Anxiety score</td>
<td>HN001</td>
<td>192</td>
<td>12.0*</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>187</td>
<td>13.0</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Depression cut-off score for pathology: >12  
Anxiety cut-off score for pathology: >15  

Mothers taking HN001 reported significantly lower depression & anxiety scores than those in placebo

*S*p<0.05

Slykerman et al. (2017) Ebiomedicine
### PROBIOTICS IN PREGNANCY STUDY

<table>
<thead>
<tr>
<th></th>
<th>Treatment</th>
<th>Depressed</th>
<th>Not depressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depression</td>
<td>HN001</td>
<td>16.5%</td>
<td>83.5%</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>23.5%</td>
<td>76.5%</td>
</tr>
<tr>
<td>Anxiety</td>
<td>HN001</td>
<td>15.6%*</td>
<td>84.4%*</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>29.4%</td>
<td>70.6%</td>
</tr>
</tbody>
</table>

* *p<0.05

First double-blind RCT of probiotics that has evaluated symptoms of depression & anxiety in the postpartum period
ESTABLISHING A HEALTHY MICROBIOTA IN EARLY LIFE

> At birth, the infant acquires its microbiota from the mother

> Microbiota is transmitted vertically: vagina, gut, skin, and human milk

> It takes >2 years for a mature microbiota to develop

> Immune-system development is linked to that of the microbiota

> Microbiota varies according to birth mode, food source, and antibiotic use

Important for

✓ development and long-term health

✓ especially for immune development
MICROBIOTA OF INFANTS AND TODDLERS PROVIDE A WINDOW OF OPPORTUNITY FOR HOWARU® PROTECT EARLYLIFE

> Fetal and postnatal time up to 2-3 years of age are crucial for development of microbiota and immunity
  ▪ Microbiota reaches adult type at 3 years of age and immunity at 12 years of age

Source: Graph based on the publications: Yatsunenko et al. (2012) Nature; Kollmann et al. (2012) Immunity
ATOPIC DERMATITIS IN INFANTS AND TODDLERS
A HIGH AND INCREASING PREVALENCE

A UNIQUE STUDY: Prenatal & Postnatal Treatment

Mother

Infant

35 weeks
PREGNANCY

BIRTH
Between 2 & 16 days
POSTBIRTH
6 months if breastfeeding
POSTNATAL

12 months
Follow up
18 months
Follow up
2 years of age
Follow up
4 years of age
Follow up
6 years of age

Wickens et al. (2008); Wickens et al. (2012); Wickens et al. (2013)
POSITIVE EFFECT ON ALLERGIC DISEASES REMAINS AFTER 11 YEARS

<table>
<thead>
<tr>
<th>Outcomes present at 11 y</th>
<th>Placebo (N = 95)</th>
<th>HN001 (N = 94)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eczema</td>
<td>26.8%</td>
<td>12.4%</td>
<td>0.015</td>
</tr>
<tr>
<td>Hayfever</td>
<td>50.0%</td>
<td>36.5%</td>
<td>0.047</td>
</tr>
<tr>
<td>Asthma</td>
<td>29.0%</td>
<td>18.4%</td>
<td>0.068</td>
</tr>
<tr>
<td>Atopic sensitization</td>
<td>51.6%</td>
<td>43.6%</td>
<td>0.28</td>
</tr>
<tr>
<td>Wheeze</td>
<td>33.0%</td>
<td>25.9%</td>
<td>0.25</td>
</tr>
<tr>
<td>Rhinitis</td>
<td>51.8%</td>
<td>40.7%</td>
<td>0.10</td>
</tr>
</tbody>
</table>

The first early probiotic intervention to show positive outcomes on the spectrum of allergic disease to persist for 11 years

BREASTFEEDING IS THE GOLD STANDARD

Breast feeding promotes healthy growth and development in infants

Infant formula cannot mimic the composition of human milk

Commercially-prepared, fortified infant formulas, are considered appropriate food either to supplement or replace human milk during the first year of life
HUMAN MILK OLIGOSACCHARIDES (HMOs)

> HMOs are a group of unique oligosaccharides found in human milk.

> HMOs are the 3rd-largest solute in human milk.

> Over 130 different oligosaccharides have been identified in human milk, with 2’-fucosyllactose (2’-FL) being the most abundant, ~ 2.4 g/l.

> The types and levels of HMOs vary considerably among women, geographical regions, and stages of lactation.

> CARE4U™ - DuPont brand for 2’-FL.
HMOs HELP SHAPE A HEALTHY MICROBIOTA

HMOs are **important bioactives** that promote and maintain health from birth through early life.

- **HMOs are highly bifidogenic** and promote bifidobacterial-dominated microbiota.
- **HMOs strengthen** the gut barrier function and act as decoys for pathogens.
- **HMOs stimulate** the immune system.

Prevention of pathogens (*Campylobacter, E. coli*) adhesion to epithelia gut cells

Immune-modulating effects

Respiratory health effects

Prebiotic effects (affects human microbiota, bifidogenic)

Cognitive health

Anti-adhesion effect on norovirus (viral gastroenteritis), Influenza A

Reduction of colon motor contraction

Reduction of necrotizing enterocolitis (NEC) in preterm infants

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SUMMARY

Diet and lifestyle are key to a healthy pregnancy. Fetal and postnatal time up to 2-3 years of age are crucial for development of microbiota and immunity.

From the research with probiotics within HOWARU® Protect Prenatal+
- Helps maintain healthy vaginal pH
- Increases the number of beneficial bacteria
- Supports immune health of mother and baby
- Promotes mother’s happiness and calmness
- Promotes healthy blood glucose levels
- Proven safe and well tolerated
- Overall, helps keep mothers healthy during pregnancy

HOWARU® Protect EarlyLife; Helps to maintain a healthy, active immune system
- CARE4U™; HMOs are highly bifidogenic and act as decoy for pathogens
- CARE4U™; Different metabolites with HMO vs. lactose

Both mothers and their babies can benefit from probiotic dietary supplementation
- HOWARU® Protect Prenatal+
- HOWARU® Protect EarlyLife
DUPONT’S HUMAN MICROBIOME PLATFORM

PROBIOTICS
A leadership position
Large and growing market

HUMAN MILK OLIGOSACCHARIDES
Market entry in 2018
New market

NEXT-GEN MICROBIOME SOLUTIONS
Microbiome venture established
Future market opportunity

DUPONT PROBIOTICS
A DuPont Nutrition & Health business

DUPONT HMO INBIOSE PARTNERSHIP
Announced in 2016

DUPONT MICROBIOME VENTURE
Announced in 2017

SUPPORTED BY WORLD-LEADING CAPABILITIES IN MICROBIAL BIOTECHNOLOGY AND HEALTH & NUTRITION SCIENCE:

> Microbial product innovation: probiotics, starter cultures, antimicrobials, and specialty carbohydrates

> *In vitro* models, preclinical and GCP clinical trial capability, metagenomics and bioinformatics:
  > >100 clinical trials completed on probiotics

> Large-scale fermentation, separation, formulation, stabilization, and excipients technologies for live microbials and biomolecules

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Thank You