HOWARU® PROTECT PRENATAL+

Delivering immune, mood, and vaginal benefits for women during pregnancy and beyond

Clinically-documented targeted probiotics for prenatal health and beyond

Diet and lifestyle are key to a healthy pregnancy and healthy maternal nutrition has a profound influence on the development of the immune system during fetal and postnatal periods. Keeping mothers healthy during pregnancy is vital to the healthy development of the infant and child.

Microbial colonization of the digestive tract of the infant begins at birth where the mother becomes the primary source of bacterial species. Clearly, the mother's health plays a critical role at this stage and disruption of the development of the microbiota and immunity of the infant during this period has been linked to the development of allergies and eczema.

It has been shown in human clinical studies that both mothers and their babies benefit from probiotic dietary supplementation during pregnancy, birth, and beyond.

Your daily challenge

- Helping to keep women healthy during pregnancy
- Supporting immune health of mother and baby
- Promoting mother's happiness and calmness
- Helping to maintain healthy vaginal microbiota and pH
- Proven safe, well tolerated, clinically-documented probiotics
- Supporting healthy glucose levels*

The Probiotics in Pregnancy Study - HOWARU® Protect Prenatal+

The primary aim of this clinical study was to assess whether probiotic supplementation in pregnant women (n=423) with *Lactobacillus rhamnosus* HN001™ provided immune benefits for babies. The secondary aims focused on the health of the mothers and the effects of HN001™ supplementation on the risk of Gestational Diabetes Mellitus (GDM) and postnatal depression (PND) . In a two-center, randomized, double-blind, placebo-controlled trial, the probiotic HN001™ (6 x 10^9 CFU) was taken daily by pregnant women from 14 to 16 weeks of gestation to six months post-term, if breastfeeding.[1] The probiotic was administered in capsules to mothers, and the following evidence outlines and summarizes the major findings and health implications from this study.

Gestational Diabetes Mellitus (GDM)

GDM was assessed in the Probiotics in Pregnancy (PiP) Study by using both the International Association of Diabetes and Pregnancy Study Group (IADPSG) and New Zealand (NZ) guideline criteria from glucose tolerance test (GTT). GTT was taken by 373 mothers at 24- 30 weeks gestation following a 12 hour overnight fast.[6] The results indicated that there were significant differences between the HN001™ group and the placebo group - see Figure 1. Daily consumption of HN001™ was associated with a lower prevalence of GDM. Subpopulation analysis revealed that HN001™ supplementation may especially benefit those with higher risk of developing GDM during pregnancy due to higher maternal age (>35 y) at conception or previous GDM diagnosis.

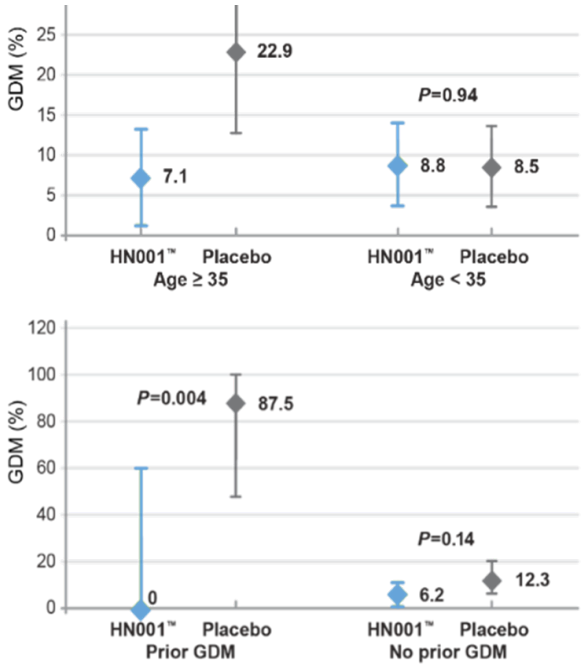


Figure 1 Treatment effects on the prevalence of GDM. Adapted from Wickens et al. 2017

Postnatal Depression and Anxiety

Mothers in the PiP Study were invited to fill out a questionnaire that considered their psychological well-being at 1-2 months after birth.[5] A summary of the results is given in Table 1. Mothers in the HN001™ group reported significantly lower depression and anxiety scores than those in the placebo group. Further statistical analysis showed that when more than one effect was considered (including infant colic and time since birth that questionnaires were completed) the scores in the HN001™ group remained significantly associated with reduced depression and anxiety.

Table 1. Depression and anxiety scores in the probiotic treatment (HN001™) and placebo groups. Adapted from Slykerman et al., 2017

	Treatment	Depressed	Not depressed
Depression	HN001	16.5%	83.5%
	Placebo	23.5%	76.5%
	Treatment	**Anxious**	**Not anxious**
Anxiety	HN001	15.6%*	84.4%*
	Placebo	29.4%	70.6%

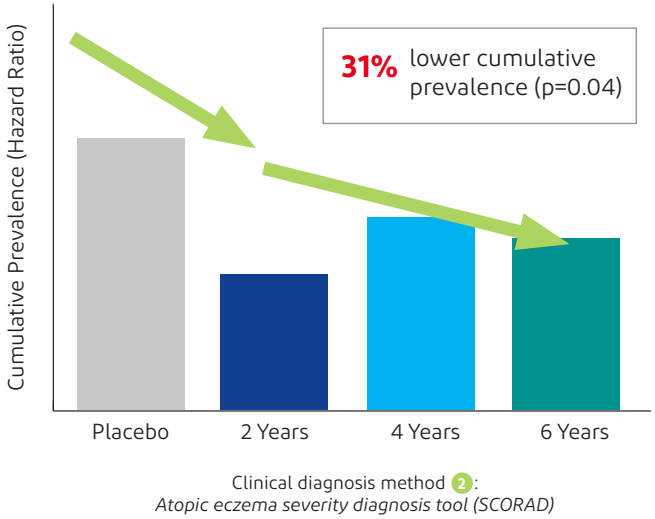
* $p < 0.05$

Immune Benefits for Expectant Mothers and Children

A clinical trial showed that HN001™ supports the immune health of pregnant women and their infants and children as well.[2] In a two-center, randomized, double-blind, placebo-controlled trial, the probiotic HN001™ (6 x 10^9 CFU) was taken daily by pregnant women from 35 weeks gestation to six months post-term, if breastfeeding. In addition, their infants consumed HN001™ (6 x 10^9 CFU) from birth until 2 years. In conjunction with the study, cord blood plasma and breast milk samples were collected from the women. The samples were then assessed for important markers related to immune health. The results showed mothers who had received the HN001™ supplement had higher levels of immune markers in cord blood and breast milk than those in the placebo group.[11]

The primary outcome of this study was the prevalence of eczema in infants. At ages two, four, and six, fewer instances of eczema - as measured by the 'UK Working Party's Diagnostic Criteria Atopic Dermatitis and SCORing atopic dermatitis - were reported in the HN001™ group compared to the placebo group- see Figure 2.[2,3,4] Fewer instances of allergic sensitization were also reported at 6 years old (p=0.04). Furthermore, the positive effect of HN001™ on allergic diseases remained at eleven years of age (p= 0.047).[9]

* Not an implied structure/function claim



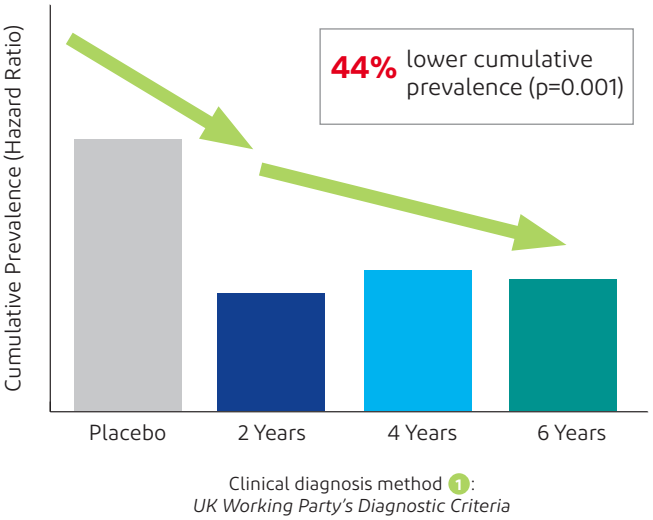


Figure 2: Approx 150 infants /children /treatment group
Source: Wickens et al. 2008; Wickens et al. 2012; Wickens et al. 2013

Balanced Vaginal Microbiota

A randomized, double-blind, placebo-controlled trial has shown that after taking two probiotic capsules (5 x 10^9 CFU) once daily of HOWARU® Protect Prenatal+ containing *L. rhamnosus* HN001™ and *L. acidophilus* La-14® plus lactoferrin for 14 days that there was a colonization of the vagina of healthy women with these microbes and that the colonization persisted at least one week after intervention compared to the placebo (Figure 3).[7] Vaginal pH remained in the healthy range throughout the study.

In another clinical trial 40 women with an intermediate Nugent score, indicating a perturbed vaginal microbiota, and with signs/symptoms of vaginitis/vaginosis orally consumed twice daily either a proprietary mixture of 5 x 10^9 CFU of *L. acidophilus* La-14® and *L. rhamnosus* HN001™ in combination with 50mg of bovine lactoferrin RCX™ (Respecta®) or a placebo for 15 days. *Lactobacillus* species were analyzed by RT-PCR from vaginal swabs at baseline and in the end of the 15-day probiotic/placebo treatment.[8]

The study showed that both species were detected in significantly higher levels from the vaginal samples at the end of intervention as compared with baseline and placebo. In addition, the Nugent score of women in the probiotic group significantly improved from intermediate at baseline to normal (P=0.0004) after 15 days of supplementation. In contrast, the Nugent score in the placebo group remained unchanged. Self-assessed vaginal symptoms (itching, discharge) decreased significantly at the end of 15d treatment compared with placebo (P<0.001) as well. Significant results from the clinical study are shown in Table 2.

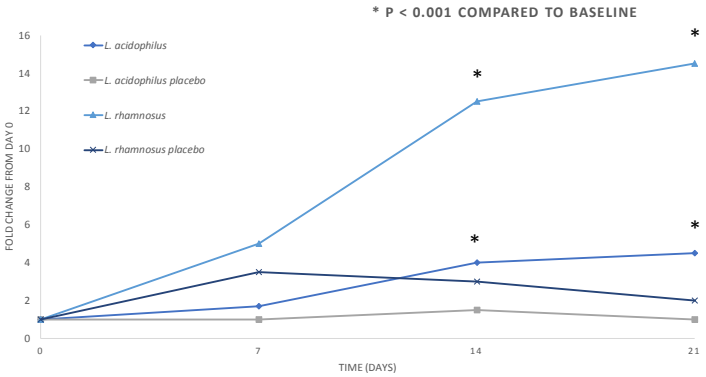


Figure 3: Vaginal recovery of healthy bacteria Adapted from DeAlberti et al. 2015. Arch Gynecol Obstet (2015) 292:861-867 DOI 10.1007/s00404-015-3711-4 .

Bacterial Vaginosis

Bacterial vaginosis (BV) is one of the most common types of vaginal infections faced by women. It is characterized by a depletion of beneficial bacteria (lactobacilli) and an overgrowth of atypical bacteria. Typically between 10-30% of women experience BV during pregnancy.

A randomized, double-blind, placebo controlled trial was conducted in Romania to investigate the effectiveness of probiotics in the treatment of BV.

Table 2: Significant results from the clinical trial comparing probiotic + lactoferrin group with the placebo group (Russo et al., 2018a)

Evaluation (15 days)	Result
Vaginal colonization of *L. acidophilus* La-14® and *L. rhamnosus* HN001™	• There was vaginal colonization of both species • The bacterial counts in the probiotic group was significantly higher than the placebo group and baseline
Nugent score	• There was a significant improvement from intermediate to normal score in the probiotic group from baseline (P=0.0004) • No changes from the baseline in the placebo group • Significantly lower for the probiotic group compared with the placebo group (P=0.011) at the end of the study
Vaginal symptoms (itching/discharge)	• Symptoms decreased significantly in the probiotic group compared to the placebo group (P<0.001)
Vaginal pH changes	• No change in pH between groups

In the clinical trial, 48 women with symptomatic BV infections were treated with metronidazole (500 mg twice daily) for seven days and were randomly assigned to take simultaneously orally either the *L. acidophilus* La-14®, *L. rhamnosus* HN001™ and lactoferrin formulation or placebo (two capsules/day for five days followed by one capsule/day for 10 consecutive days; induction phase).[10] The administration of study products (one capsule/day for 10 consecutive days) was repeated monthly (maintenance phase) during the six months of follow-up starting the first day of menstrual cycle as the menstrual blood increases the vaginal pH and contributes to an increased the risk of BV recurrences. The clinical cure rate of BV symptoms, microbiological cure rate (Nugent score), and BV recurrence was evaluated during the 6-month study period.

The results showed that BV-associated symptoms (vaginal discharge and itching) and the Nugent score were significantly improved, as well as BV recurrence rate was significantly reduced by the probiotic treatment group containing *L. acidophilus* La-14® and *L. rhamnosus* HN001™ in association with lactoferrin when compared with the placebo. A significant regression of Nugent score and resolution of symptoms were seen both during the induction phase (15 days) and maintenance phase (after 6-month follow-up). This alternative approach has been shown to represent a safe and effective adjunct for the restoration of healthy vaginal microbiota and in reducing vaginal symptoms associated with bacterial vaginosis. Significant results from the clinical study are shown in Table 3.

Table 3 Significant results from the clinical trial comparing probiotic + lactoferrin group with the placebo group (Russo et al., 2019a)

Evaluation (6 monts)	Result
Itching	• Women in the intervention group compared to the placebo without itching were 70.5% vs 20.8%.
Vaginal discharge	• Women in the intervention group remained vaginal discharge free in 70.8% of cases vs 33.3% in placebo group.
Overall cure rate	• Women in the intervention group vs placebo who were cured (absence of any symptoms, neither itching nor vaginal discharge, and Nugent score >7) was 83.3% vs 37.5%.
Recurrence Rate	• Women in the intervention group had a significant reduction is the recurrence rate in comparison to that in the placebo group.

Vulvovaginal Candidiasis

A randomized, double-blind, placebo-controlled clinical trial involving 48 adult women assessed the clinical cure rate of vulvovaginal candidiasis (VVC) symptoms, overall cure rate, as well as the recurrences during a follow-up period of six months.[12]

In the study, women with symptomatic acute episodes of VVC and a documented anamnestic history of recurrences were treated with a topical clotrimazole (100 mg) for seven days and were randomly assigned to simultaneously orally take either the probiotic mixture plus lactoferrin (active) or a placebo (two capsules/day for five days followed by one capsule/day for 10 consecutive days; induction phase). The active or placebo administration (one capsule/day for 10 consecutive days) was repeated monthly (maintenance phase) during the six months of follow-up in the premenstrual phase or luteal phase as during this time, hormonal and immunological reasons make the vagina more vulnerable to pathogens and pose a higher risk of VVC recurrences.

The results showed that after three months, symptoms of VVC (vaginal discharge and itching) were significantly improved and the recurrence rate was significantly reduced in the participants taking the probiotic-lactoferrin combination in comparison to that of the placebo until the end of the study (six months). This approach may represent a safe and effective adjunct to clotrimazole therapy for supporting and maintaining vaginal health following VVC.

Why Choose HOWARU® Protect Prenatal +?

- 15 Billion CFU of clinically proven strains
- Supports the immune health of expectant mothers and infants
- Promotes mother's happiness and calmness
- Helps maintain a healthy vaginal microbiota and pH
- Increases the number of beneficial bacteria
- Proven safe and well tolerated
- Supports healthy glucose levels*

Why Choose DuPont?

- Leader in probiotic science
- Broadest range of clinically-documented probiotics
- Unrivalled dietary supplement formulation expertise
- Robust regulatory support
- Marketing support and industry insights to help you successfully position your products

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