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The impact of oral probiotics on vaginal group b streptococcal colonisation rates in pregnant women: a pilot randomised control study

Paula Grace Olsen
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WOLLONGONG**



School of Nursing

**The Impact of Oral Probiotics on Vaginal Group B Streptococcal
Colonisation Rates in Pregnant Women: A Pilot Randomised
Control Study**

Paula Grace Olsen

**This thesis is presented as part of the requirement for the
Award of the Degree of Master of Midwifery (Research) of the
University of Wollongong**

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ABSTRACT

Aim

The aim of this study was to complete a pilot project to ascertain if the research design was appropriate to determine whether a daily oral dose of probiotics can reduce the rate of vaginal group B streptococcal (GBS) colonisation in pregnancy.

Methodology

A pilot randomised controlled trial was performed which recruited 34 GBS-positive women who were approximately 36 weeks pregnant. The participants were randomly allocated to the control group, who continued with standard antenatal care, or to the intervention group, which continued with standard antenatal care and received a daily oral dose of probiotics for three weeks or until the birth of their infant. A lower vaginal swab to detect the presence of GBS was collected three weeks post consent or when a participant was in labour.

Results

No significant difference was found in vaginal GBS rates between the control and intervention groups. Only seven of 21 women in the intervention group completed the entire 21 days of probiotics. A subgroup analysis, including only those who had completed 14 days or more of probiotics (n=16), also showed no significant difference in vaginal GBS when compared to the control. As a secondary finding of the analysis did show significantly more vaginal commensals in the probiotics group (p=0.048).

Discussion

There are five possible reasons for the lack of significant results:

- The length of the intervention was too short.
- The dosage of the probiotics was too low.
- The wrong strains of probiotics were used.
- The sample size was inadequate.
- Oral probiotics are ineffective in impacting vaginal GBS.

Implications

The secondary finding of a significant increase of vaginal commensals (normal vaginal flora, including Lactobacilli) in women who completed 14 days or more of probiotics supports the potential of probiotics to impact GBS in pregnancy. The presence of commensals should be included as an indicator in future research projects. This pilot project has provided no evidence that probiotic use in later pregnancy is unsafe. Many possibilities remain for future research to further investigate the use of probiotics to impact vaginal GBS.

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1 INTRODUCTION

1.1 Background

Group B streptococcus (GBS) is a bacterium that colonises the vaginas of 15 to 25 per cent of pregnant Australian women (McIlwaine et al., 2006; Royal Australian and New Zealand College of Obstetricians and Gynaecologists, 2012). This bacterium is the leading cause of infections in newborns in the developed world (Centers for Disease Control and Prevention, 2010; Columbo et al., 2006; Ohlsson & Shah, 2013). It can be passed from a woman to her newborn during the process of labour and birth and has the potential to result in pneumonia, septicaemia and meningitis in the infant (Centers for Disease Control and Prevention, 2010; Columbo et al., 2006; Hassan et al., 2011; Jones et al., 2006; Matsubura et al., 2007; Ohlsson & Shah, 2013; Valkenburg-van den Burg et al., 2006).

Presently across Australia, prenatal GBS screening and prophylaxis are widely practised, though there is considerable variation in strategies between hospitals (Angstetra et al., 2007; Connellan & Wallace, 2000; Hiller et al., 2005; May et al., 2005). These strategies aim to identify women who are at risk of transmitting GBS to their infants. These at-risk women are given intravenous antibiotics in labour to inhibit the transmission (Royal Australian and New Zealand College of Obstetricians and Gynaecologists, 2012; Reingold et al., 2007). As a result of intrapartum antibiotic prophylaxis, the incidence of early onset neonatal GBS disease in Australasia fell from 1.43 per 1000 live births in 1993 to 0.25 per 1000 live births in 2001 (Daley & Isaacs, 2004). However, there are disadvantages associated with the use of intravenous antibiotics.

The disadvantages of giving intravenous antibiotics in labour include the development of antibiotic resistance in GBS and other bacteria, and the disruption of the early growth of good bacteria in the newborn's gut (Baltimore, 2007; Centers for Disease Control and Prevention, 2010; Cheng et al., 2006; Daley & Isaacs, 2004; Edwards, 2006; Grimwood et al., 2002; Ohlsson & Shah, 2013; Pattern et al., 2006; Rautava et al., 2012; Russel & Murch, 2006). In the United States and Canada, 20 to 35 per cent of labouring women now receive intravenous antibiotic prophylaxis (Baltimore, 2007; Centers for Disease Control and Prevention, 2010; Chen et al., 2005; Glasglow et al., 2005; Pattern et al., 2006).

It has been proven by many research studies that women with higher vaginal colonisations of *Lactobacillus* are more likely to have no detectable vaginal GBS colonisations (Altoparlak et al., 2004; Donders et al., 2000; Kubota et al., 2002; Takeyoshi et al., 2002; Whitney et al., 2004). This finding generates the hypothesis: could increasing *Lactobacillus* colonisation rates in pregnant women's vaginas decrease GBS colonisation rates? One possible means of increasing the *Lactobacillus* colonisation in women's vaginas may be through the use of probiotics.

1.2 Aim

The aim of this study was to complete a pilot to ascertain if the research design was appropriate to determine whether a daily oral dose of probiotics can reduce the rate of vaginal group B streptococcal (GBS) colonisation in pregnant women.

1.3 Overview of thesis

The intent of this thesis is to give a succinct and understandable description of the research that was performed into the impact of oral probiotics on vaginal GBS colonisation rates and to provide a clear discussion of the implications of the

completed study. The introductory chapter of this thesis provides background information on the study being presented and an overview of what can be anticipated in the following chapters.

The literature review in Chapter 2 describes the search strategies used to amass the scientific information available pertaining to GBS, probiotics and vaginal health. As probiotics are an emerging area of scientific interest, the published data on previously completed research studies was limited. No previous clinical trials were discovered that investigated the impact of probiotic use on GBS colonisation rates. In reaction to this, the search field was broadened to include completed investigations into the impact of probiotics on other vaginal health concerns, such as bacterial vaginosis. The review also correlates the published information on the impact of probiotic use by pregnant women, specifically addressing the area of the safety of probiotic use in pregnancy.

Chapter 2 also identifies a gap in the available published scholarly information on the impact of probiotics on GBS vaginal colonisation rates in pregnancy. It highlights the need for well-designed, well-powered randomised controlled trials in the area of probiotics and vaginal health.

The methodology and study design of the project are presented in Chapter 3. In this chapter the theoretical framework of a pragmatic approach within a pilot randomised controlled trial is described and justified. The chapter also describes and details the methods used to conduct the research, including a description of the study site, study population, recruitment strategies, sample size, intervention and observations. A discussion of the data analysis methods utilised and the ethical considerations pertaining to this study is also included in Chapter 3.

The research findings are presented in Chapter 4. The SPSS computer program was utilised as a tool in this chapter to assist with the analysis of the raw data. Two significant findings emerged from the analysis, pertaining to the absence of adverse events with the use of probiotics in later pregnancy and the positive potential for further studies into the use of oral probiotics to impact vaginal GBS colonisation rates. However, due to the small sample size, the potential for Type 1 errors is increased.

In Chapter 5, the major findings are highlighted and discussed. The aim of this study was to determine the appropriateness of the research design used in this pilot project. The discussion chapter focuses on the implications of the results generated by this study for future research designs. It also highlights the strengths of the study and the information it has contributed to the growing body of knowledge regarding probiotic use to impact the vaginal micro environment.

Finally, Chapter 6 concludes the thesis. In this chapter, the aim of the study and the major findings are reiterated. The findings are then reflected upon in light of their implications for future research projects and clinical practice.

2 LITERATURE REVIEW

2.1 Introduction

This chapter reviews the current literature pertaining to group B streptococcal (GBS) vaginal colonisation and probiotics in pregnancy. Due to the limited availability of information on this topic evidence surrounding probiotics and bacterial vaginosis (BV) in both pregnant and non-pregnant women is also included in this review.

A search was performed of the electronic data bases: Cochrane Library, Expanded Academic, Health Reference Centre Academic, Health Sciences, Meditext, Medline, Nursing and Allied Health Source, Popline, ProQuest, Sage, Science Direct, Springer and Wiley Interscience. The search terms used were ‘probiotics’, ‘pregnan*’, ‘urogenital’, ‘Group B Streptococc*’, ‘Streptococcus Agalactiae’, and ‘Lactobacillus’, as found in the abstract. Additional journal articles were then procured by identifying relevant studies in the body and reference lists of articles found through the database search.

The inclusion criteria for the review were any articles pertaining to probiotics and pregnancy, probiotics and GBS, or probiotics and BV. Studies were excluded if they focused on urinary tract infections or in vitro experiments. Electronic auto alerts were instigated throughout the duration of the study to allow for regular updates of emerging literature in this area.

The bacterium GBS is the leading cause of infections in newborns in the developed world (Centers for Disease Control and Prevention 2010; Columbo et al., 2006; Hassan et al., 2011; Ohlsson & Shah, 2013). Fifteen to 25 per cent of pregnant Australian women carry GBS in their vaginas (McIlwaine et al., 2006; Royal Australian and New Zealand College of Obstetricians and Gynaecologists, 2012).

The bacteria are passed from a woman to her newborn during the process of labour and birth and may result in pneumonia, septicaemia and meningitis in the infant (Centers for Disease Control and Prevention, 2010; Columbo et al., 2006; Jones et al., 2006; Matsubura et al., 2007; Ohlsson & Shah, 2013; Valkenburg-van den Burg et al., 2006). In Australia, approximately one per cent of maternal GBS carriers will infect their infants at birth; of these infants, six per cent will die and many others will sustain permanent neurological damage (Centers for Disease Control and Prevention, 2010; Connellan & Wallace, 2000).

As stated in the Introduction chapter, due to the severity of neonatal GBS infection, a variety of strategies which aim to identify women at risk of transmitting GBS to their infants are practised around Australia (Angstetra et al., 2007; Connellan & Wallace, 2000; Hiller et al., 2005; May et al., 2005). These at-risk women are given intravenous antibiotics in labour to assist in inhibiting the transmission of GBS to the neonate (Reingold et al., 2007; Royal Australian and New Zealand College of Obstetricians and Gynaecologists, 2012). However, there are disadvantages associated with the use of intravenous antibiotics.

The disadvantages of giving intravenous antibiotics in labour include the development of antibiotic resistance in GBS and other bacteria, and the disruption of the early growth of good bacteria in the newborn's gut (Baltimore, 2007; Centers for Disease Control and Prevention, 2010; Cheng et al., 2006; Daley & Isaacs, 2004; Edwards, 2006; Grimwood et al., 2002; Gronlund et al., 2011; Ohlsson & Shah, 2013; Pattern et al., 2006; Rautava et al., 2012; Russel & Murch, 2006).

Many studies are available which have shown that women with higher vaginal colonisations of *Lactobacillus* are more likely to have no detectable vaginal GBS

colonisations (Altouparlak et al., 2004; Donders et al., 2000; Kubota et al., 2002; Takeyoshi et al., 2002; Whitney et al., 2004). These findings form the basis of this study. Can we impact the vaginal colonisation of GBS by manipulating the *Lactobacilli* colonisation? One means of facilitating this manipulation process may be through the use of probiotics.

This review of the literature has identified that there is a gap in the research on the impact of probiotic usage on GBS vaginal colonisation in both pregnant and non-pregnant individuals. As a result, this review focuses mainly on related studies investigating the impact of different probiotic strains on the incidence of bacterial vaginosis, which, like GBS, is a bacterial infection of the vagina. The review also summarises the available research surrounding the safety of probiotic use in pregnancy. Safety is an important issue which must be addressed prior to undertaking research due to its obvious implications for future research, particularly research into the impact of probiotics in pregnancy on GBS vaginal colonisation rates. The next section defines the term ‘probiotic’ in order to assist in clarifying the use of this word in the literature as opposed to the broader colloquial term used in mainstream marketing.

2.2 Probiotics

Probiotics are defined as ‘live microorganisms which, when administered in adequate amounts, confer a health benefit to the host’ (World Health Organisation, 2002). Probiotics are readily available ‘over the counter’ in Australia. They are contained in many dairy products, such as yoghurt, consumed daily by pregnant women. Anecdotally, probiotics have been used by midwives for many years to manage GBS in pregnancy, though no research studies have been performed to determine their

effectiveness (Murry, 2002). Additionally, there are many websites extolling the benefits of yoghurt with live cultures and probiotics for vaginal health.

Lactobacilli are one such probiotic microorganism. *Lactobacilli* are the primary organisms in the vaginas of healthy women. Their colonisation of the vagina has been shown to reduce the incidence of bacterial vaginosis, yeast vaginitis, urinary tract infections and sexually transmitted diseases (Ehrstrom et al., 2010; Marcone et al., 2010; Reid, 2008; Reid & Bocking, 2003; Zarate & Nader-Macias, 2006). Similar to many urogenital infections, *Lactobacilli* colonise the vagina mainly through ascension across the perineum from the rectum to the vagina (Reid & Bocking, 2003; Lagenaur et al., 2011a).

In contrast to the advantages of naturally occurring *Lactobacillus*, many of the current commercially available strains of probiotics raise concerns regarding their efficiency in producing purported health benefits. The documented concerns relating to this category of probiotics are that companies can make unsubstantiated claims, the products can contain dead or unreliable probiotic contents, they can contain inadequate numbers of probiotic strains, and they can have a poor shelf life (Reid, 2008; Reid et al., 2003; Senok, 2005). In order for an oral probiotic to be effective, it must be able to survive passage through the gastro-intestinal tract. It must be able to proliferate and colonise the digestive tract. It must be safe and effective, and it must maintain its effect for the duration of the shelf life of the product (Barrons & Tassone, 2008, Senok et al., 2005; Zarate et al., 2005).

In addition to these concerns, the effects of probiotics have been shown to be strain specific; therefore, the use of an individual micro-organism strain is only justified in procuring the specific benefits that it has been proven to convey (Martinez et al.,

2009; Pham et al., 2008, World Health Organisation, 2002). For example, a specific strain of *Lactobacilli* which has been proven to improve travellers' diarrhoea cannot also be assumed to improve eczema. Each strain and its benefits need to be determined individually. Another challenge surrounding probiotics is that in vitro experiments do not necessarily translate to real-life health benefits in human and animal models. As a result, it is necessary for individual specific strains to have been proven in clinical trials in order to confirm their ability to produce specific health outcomes in humans before beneficial claims can be made (Reid, 2008; World Health Organisation, 2002).

2.3 Probiotics and bacterial vaginosis

There was no literature found specifically investigating the impact of probiotics on the vaginal colonisation rate of GBS in either pregnant or non-pregnant women. One area which has been investigated is the impact of probiotics on bacterial vaginosis. Bacterial vaginosis (BV) is an overgrowth in the vagina of various anaerobic bacterial species. It is associated with endometriosis, pelvic inflammatory disease, complications of pregnancy and an increased risk of sexually transmitted diseases (Reid et al., 2004; Wang et al., 2010). The studies discovered have shown promising results regarding the impact of different strains of *Lactobacilli* on the incidence of BV, both as a therapy in themselves and as an adjunct to antibiotic treatment. The following section of this review presents the available research on probiotics and BV, as classified by the specific probiotic strains presented in the literature.

2.3.1 *Lactobacillus rhamnosus* GR-1 and *fermentum/reuteri* RC-14

The combination of probiotic strains *Lactobacillus rhamnosus* GR-1 (GR-1) and *Lactobacillus fermentum/reuteri* RC-14 (RC-14) has undergone much research with

regard to BV. Four trials have been completed that investigated the oral effectiveness of GR-1/RC-14 to alter vaginal flora. One study by Reid et al. (2001) involving 42 healthy female participants randomly assigned each woman to one of four different treatment groups. There was no control in this study. Three groups were given GR-1/RC-14 in different dosages. The fourth group was given a different strain of probiotics, *Lactobacillus rhamnosus* GG. It was found that a daily oral dose of more than 10^8 culture-forming units (cfu) of GR-1/RC-14 restored and maintained healthy vaginal micro flora. No improvements were noted in the *L. rhamnosus* GG group (Reid et al., 2001). Two other studies randomly allocated 64 (Reid et al., 2003) and 59 (Reid et al., 2004) healthy women respectively to receive either a daily oral dose of GR-1/RC-14 ($>10^9$ cfu) or a placebo for 60 days. Both studies showed significant improvement in the vaginal micro flora in the intervention group, with increased *Lactobacilli* colonisation and decreased yeast and coliform infections compared to the control (Reid et al., 2003; Reid et al., 2004). A fourth study (Anukam et al., 2006a) randomly allocated 40 participants diagnosed with BV either to insert vaginally two gelatine capsules of GR-1/RC-14 (10^9 cfu) daily for five days or to apply the antibiotic Metronidazole in vaginal cream twice daily for five days. Post follow-up on days six, 15 and 30, this study showed BV was cured in significantly more probiotic-treated participants compared with the antibiotic-treated participants (Anukam et al., 2006a). These studies indicate that the *Lactobacillus* strains RC-14 and GR-1 have the potential to impact vaginal health. The drawbacks of each of these studies were the small sample sizes and the lack of reporting of power calculations in each of the journal articles.

Two studies (Anukam et al., 2006b; Martinez et al., 2009) have also been undertaken to demonstrate the effectiveness of GR-1/RC-14 (10^9 cfu) as an adjunct treatment to

antibiotic therapy for BV. In the first study by Anukam et al. (2006b), which involved 106 women diagnosed with BV, all the participants were given an oral dose of the antibiotic Metronidazole twice daily for seven days. The participants randomised into the treatment group were then given oral GR-1/RC-14 twice daily for 30 days and the control group was given a placebo twice daily for 30 days. After 30 days, 88 per cent of the treatment group no longer had BV. It was interesting to note that in contrast, 40 per cent of the placebo group no longer had BV.

Similarly, a study by Martinez et al. (2009) involving 64 women diagnosed with BV treated every participant with a single dose of Tinidazole. They were then randomly assigned to the treatment or control group. The treatment group was given two oral capsules of GR-1/RC-14 (10^9 cfu) daily for 28 days. The control group, on the other hand, was given two placebo capsules daily for 28 days. On day 28, there was an 87.5 per cent cure rate for BV in the treatment group versus a 50 per cent cure rate in the control group. These studies indicate that the use of these probiotic strains can aid in the prevention of relapses of BV post antibiotic treatment. These results are in addition to their potential as a treatment for BV independent of antibiotics.

2.3.2 *Lactobacillus acidophilus*

Another strain of *lactobacillus* that has been investigated with regard to BV is *Lactobacillus acidophilus*. One study (Hallen et al., 1991) randomly allocated 57 participants with BV to receive *L. acidophilus* (10^8 to 10^9 cfu) in vaginal suppositories two times daily for six days, or a placebo. Follow-up immediately after treatment found that 57 per cent of the probiotic group had normal findings while the entire placebo group still had BV. No further follow-up was possible, as 22 of the

participants who still had BV required treatment with antibiotics after the first follow-up visit (Hallen et al., 1991).

A study by Drago et al. (2007) treated 40 BV-infected women with an *L. acidophilus* douche (10^9 cfu/ml) for six days. There was no control group in this study. It found that, post treatment, 30 of the participants had improved vaginal *Lactobacilli* counts, while three participants remained unchanged and seven had decreased *Lactobacilli* counts. Twenty to 23 days after the completion of treatment, it was found that the increased *Lactobacilli* counts persisted in 29 of the participants. A third study also claimed a clear increase in the colonisation of the rectum and vagina with *L. acidophilus* and a significant reduction in the occurrence of BV after women ingested culture-containing yoghurt for two months versus ingesting pasteurised yoghurt for two months (Shalev et al., 1996). The validity of these claims needs to be viewed with caution due to the extremely high attrition rate experienced in the study. Another study (Delia et al., 2006), without a control group, administered vaginal suppositories with *L. acidophilus* to 60 women with suspected or confirmed BV. This study found vaginal *Lactobacilli* to be a successful treatment for BV. Though these studies trend towards indicating that *Lactobacillus acidophilus* has the potential to positively impact vaginal *Lactobacilli* colonisation, their lack of control groups, high attrition rates and small sample sizes bring each of the individual findings into question.

A final study (Ozkinay et al., 2005) involving *L. acidophilus* recruited 360 women with vaginal infections. After receiving anti-infective treatment, the participants were randomly allocated to receive either a vaginal tablet containing a minimum of 10^7 cfu of *L. acidophilus* and Oestriol daily for six days, or a placebo. This study

found a significantly higher improvement in the vaginal ecology of participants in the probiotics group versus the control group. This study adds to the argument of the aforementioned research that *Lactobacillus acidophilus* is another strain of probiotics with the potential to impact the micro flora of the vagina.

2.3.3 Other strains of *Lactobacillus*

In contrast to the positive findings of the studies mentioned, a study by Eriksson et al. (2005) found no significant difference in outcome rates between its probiotic and placebo groups. This study randomly assigned women with BV to the intervention group or to the control group after a course of Clindamycin. The intervention group used tampons impregnated with the *Lactobacilli gasseri*, *casei* and *fermentum* (10^6 to 10^8 cfu) during menstruation. The control group used a placebo. No significant improvement was noted between the two groups. Another study (Larsson et al., 2008) was performed which showed no significant difference in outcome rates between the probiotics and placebo groups. In this study, women with BV were randomly assigned, after a course of Clindamycin, to use vaginal capsules containing *Lactobacilli gasseri* and *rhamnosus* (10^8 to 10^9 cfu) for 10 days or a placebo. Both these studies highlight that clinical results are specific to individual probiotic strains. This emphasises the importance of choosing the appropriate strains and dosages of probiotics when trying to elicit a specific health benefit in the micro environment of the vagina.

A final study (Ronnqvist et al., 2006) is of note because it is the only one to make mention of GBS. In this study, 176 healthy, fertile women were randomly assigned to wear vapour-permeable panty liners impregnated with *Lactobacillus plantarum* ($>5 \times 10^8$ per panty liner) 24 hours a day for four consecutive menstrual cycles or to

use a placebo. This study found that, post treatment, *Lactobacilli* were found in the labial samples of 86 per cent and in the vaginal samples of 54 per cent of women in the probiotics group, but it did not show any significant decrease in the presence of microbes. The study population had an extremely high prevalence of GBS among its participants, with a 43 per cent GBS carrier rate. It reported that women with a high number of *Lactobacillus* in their vaginas had a 17 per cent prevalence of GBS but the use of panty liners impregnated with *Lactobacillus plantarum* did not lower the GBS carrier rate. This is the only study that reported on the impact of such an intervention on GBS. The results of this study highlight the need for further research to identify whether a strain of *Lactobacillus* exists that would be effective in reducing GBS colonisation rates in women, specifically in pregnancy.

The following section reports on the research conducted involving probiotic use in pregnancy. It focuses mainly on the use of probiotics in pregnancy to impact BV and the safety of probiotic use in pregnancy.

2.4 Probiotics in pregnancy

The clinical trials that have been completed investigating the impact of probiotics in pregnancy have focused mainly on the use of oral probiotics to prevent the development of atopic eczema in infants. No published studies were identified that reported on the impact of probiotics on GBS vaginal colonisation rates in pregnancy. In a related field, four studies (Krauss-Silva et al., 2011; Neri et al., 1993; Nishijima et al., 2005; Thiagarajan, 1998) have investigated the impact of a daily dose of probiotics, administered either orally or vaginally, on rates of bacterial vaginosis specifically in pregnant women.

One of these studies (Nishijima et al., 2005) randomly assigned 24 pregnant women to receive either a daily dose of *Lactobacillus jonsonii* (10^9 cfu) or a placebo milk drink for two weeks. The study discovered that the consumption of this probiotic drink significantly increased the number of vaginal *Lactobacilli* and showed a trend towards a decrease in BV pathogens. Another study (Thiagarajan, 1998) randomly assigned 381 pregnant women with BV in their first trimester of pregnancy to receive either an intravaginal dose of yoghurt twice daily for one week or an intravaginal placebo. This study found that yoghurt was two-thirds as effective as antibiotics for treating BV, as measured by the persistence or absence of BV indicators. A study by Neri et al. (1993) randomly assigned 84 women with BV in their first trimester of pregnancy to receive either yoghurt vaginal douching (*L. acidophilus* $>10^8$ /ml) or acetic acid tampons twice daily for seven days. This study showed an 87.5 per cent clinical improvement rate at one and two months post treatment in the yoghurt group versus a 37.5 per cent clinical improvement rate in the acetic acid group.

A final study by Krauss-Silva et al. (2011) randomised 644 women in their second trimester of pregnancy to receive orally either two capsules of GR-1/RC-14 (10^9 cfu) or a placebo for six to 12 weeks. Sixty-two per cent of the women recruited completed the study. The aim of the study was to determine the impact of administering oral probiotics to women in early pregnancy with asymptomatic BV on premature delivery rates. Unfortunately, the trial was unable to be completed due to resource restraints and had an insufficient study sample to estimate any statistically significant effects. However, this study did not report any safety concerns with the use of GR-1/RC-14 in its pregnant participants.

These studies indicate the positive potential of probiotics to impact the vaginal micro ecology in not only non-pregnant women but pregnant women as well. When broaching the area of the therapeutic use of probiotics in pregnancy safety is an obvious concern. The following section addresses this concern, presenting the available research pertaining to probiotic use and safety considerations in pregnancy.

2.5 Safety considerations

Are probiotics safe for the mother and the developing foetus when used in pregnancy? Probiotics are strain dependent and their safety needs to be determined on a strain by strain basis (Liong, 2008). Infections caused by *Lactobacilli* are extremely rare in the general non-pregnant population. They have been implicated in approximately 0.05 to 0.4 percent of combined cases of infective endocarditis and bacteraemia. Most of these infections occurred in individuals with chronic diseases or debilitating conditions such as recent surgery, organ transplant, valvulopathy, diabetes mellitus, AIDS, acute pancreatitis and cancer (Barrons & Tassone, 2008; Besselink et al., 2008; Boyle et al., 2006; Liong, 2008; Pham et al., 2008; World Health Organisation, 2002). In light of this, it is necessary to monitor all strains for potential adverse side-effects (Allen et al., 2010).

The studies mentioned earlier in this review, involving both healthy non-pregnant and pregnant women, all reported mild, transient and rare adverse effects from probiotic use. The most serious side-effect reported involved two cases of persistent headache for the first three days of probiotic treatment in a sample of 106 non-pregnant participants (Anukam et al., 2006b).

When specifically considering the safety of probiotic use in pregnancy, there have been other studies performed investigating probiotic use in pregnancy in relation to

blood glucose control and the development of atopic eczema in infants. None of these studies reported any adverse side effects in their total combined 984 pregnant participants (Doege et al., 2012; Kalliomaki et al., 2001; Lahtinen et al., 2009; Leiten et al., 2008; Luoto et al., 2010; Schultz et al., 2004; Wickens et al., 2008). One study (Allen et al., 2010) traced adverse outcomes post-probiotic administration in pregnancy until the infants were six months of age. In this study, pregnant women in their last trimester of pregnancy were randomly allocated to receive *Bifidobacterium* and *Lactobacillus salivarius* and *paracasei* (10^9 cfu) daily or to receive a placebo. This study found that, of the 220 mother-infant dyads in the treatment group, the reported adverse events did not differ significantly from the placebo group. (Allen et al., 2010).

In order to further investigate the safety of probiotic use in pregnancy, Dugoua et al. (2009) performed a meta-analysis of the available randomised controlled trials to ascertain specifically the safety of *Lactobacillus*, *Bifidobacterium* and *Saccharomyces* spp. during pregnancy. In total, eight studies met the inclusion criteria. The meta-analysis indicated that administering certain strains of *Lactobacillus* spp. and *Bifidobacterium* spp. did not have any effect on caesarean section rate, birth weight, gestational age or birth malformations. There was not enough evidence to determine the safety of *Saccharomyces* spp. in pregnancy.

In summary, the studies to date have shown no major adverse events in healthy non-pregnant and pregnant participants. It is imperative to stress that each strain is specific in its health benefits and health concerns. Therefore, it is particularly important to continue monitoring for the adverse effects of each specific strain of

probiotics, particularly when involving vulnerable population groups such as pregnant women.

2.6 Conclusion

In conclusion, this review has shown that there is adequate evidence in the literature to indicate the potential for different probiotic strains to positively impact vaginal ecology. Group B streptococcus is a bacterium that causes major health risks for birthing infants when it colonises women's vaginas in pregnancy. Through a review of the literature, it is apparent that a gap in the evidence exists regarding the impact of individual probiotic strains on the GBS vaginal colonisation rates of pregnant women. The discovery of such a beneficial strain would contribute to the development of strategies for the prevention GBS vaginal colonisation in pregnant women, thereby reducing the need for intravenous antibiotics in labour while ensuring the safety of birthing infants. In this quest to discover an appropriate strain of probiotic to combat GBS, it remains important to recognise the potential for adverse side-effects of probiotics and to be vigilant in monitoring for any safety issues that may arise while investigating probiotic use in pregnancy.

3 RESEARCH DESIGN

3.1 Introduction

As stated in the literature review chapter, research has consistently shown that women with higher vaginal colonisations of *Lactobacillus* are more likely to have no detectable vaginal GBS colonisations (Altouparlak et al., 2004; Donders et al., 2000; Kubota et al., 2002; Takeyoshi et al., 2002; Whitney et al., 2004). One potential means of manipulating *Lactobacillus* concentrations in order to attempt to impact the GBS colonisation rates is through the use of probiotics. The aim of this study was to complete a pilot project to ascertain whether the research design was appropriate to determine if a daily oral dose of probiotics can reduce the rate of vaginal group B streptococcal (GBS) colonisation in pregnancy.

The following chapter outlines and justifies the theoretical frameworks and the research design used while conducting this research project. It also explains the ethical issues surrounding the research methods and the intervention used.

3.2 Theoretical framework for the methodology

This section outlines and justifies the decision to utilise a pilot randomised controlled trial (RCT) framework as the major method for this study. It also describes and explains how a pragmatic approach was implemented within the RCT design in order to increase the applicability and comprehensibility of the study results.

3.2.1 Quantitative methods

A pilot RCT was chosen as the research method for this study. The main aim of the study was to ascertain whether the research design would be appropriate to explore potential causal relationships between oral probiotic use and GBS vaginal colonisation rates in pregnant women. The RCT has been identified as the research

method which provides the highest level of evidence for determining the presence of causal relationships and the effects of an intervention (Peat, 2001; Steen & Roberts, 2011). The features of an RCT include random allocation to control and intervention groups, double blinding as possible and identical treatment for both groups except for the intervention being tested (Hoffmann et al., 2013; Sibbald & Roland, 1998).

The key aspect of RCT is the randomisation process. This process serves to allocate each participant to a group in an unbiased manner, thereby limiting the influences of selection bias and known and unknown confounders (Hoffmann et al., 2013; Peat, 2001). In this study, once a participant had met the inclusion criteria and had consented to involvement in the study, the participant was randomly allocated to either the control or intervention group. Randomisation assisted in controlling for any confounding factors that may have been present in the two different groups of participants.

Another aspect to an RCT that assists in controlling for bias is the process of blinding (Hoffmann et al., 2013; Sibbald & Roland, 1998). In this study, there was no placebo; therefore, the researcher and the participants both knew the arm of the study to which the participants belonged. However, the pathologists analysing the swabs were blinded to each participant's allocation in the trial. This ensured that there was no bias in the formulation of the results of the vaginal swabs. The fact that the pathologists analysing the swabs were unaware of the group allocation alleviates concerns regarding bias in the study and contributes further confidence to the final results generated.

Both the control and intervention groups continued to receive standard care in the antenatal clinic throughout the duration of the study. The only difference that existed between the care received was that the intervention group was given a daily oral dose

of probiotics for three weeks. Since both groups were receiving comparable care, any differences noted in the final analysis could be justifiably attributed to the intervention.

Finally, a pilot study was undertaken for this project. Pilot studies are used to test study appropriateness and feasibility (Peat, 2001). The decision to perform a pilot study was influenced by the scope and resource limitations of the project. A pilot study served to determine the appropriateness of the study design and of the recruitment strategy prior to committing the time and resources to a large RCT.

3.2.2 Pragmatic study design

This study, in addition to the RCT model, embraced a pragmatic approach to research. This approach is based on the fact that the ‘traditional criteria for scientific validity do not in themselves guarantee usefulness to practitioners’ (Worren et al., 2002, p. 1228). Even if results can be produced in a laboratory, these results do not necessarily translate into knowledge that is applicable in real-life settings and useful for improving clinical practice. A pragmatic approach is used to determine whether an intervention will be successful when implemented under normal circumstances (Steen & Roberts, 2011; Zwarenstein et al., 2008). Research with probiotics has shown that results in vitro do not necessarily translate to intervention successes in clinical trials (Reid, 2008; World Health Organisation, 2002). This emphasises the importance of ensuring the effectiveness of probiotic interventions in real-life clinical settings prior to declaring their health benefits.

The influences of a pragmatic approach can be identified in many details of the trial design. The researcher did not collect the vaginal swabs for the study but the women collected their own swabs, which is the standard means of GBS vaginal swabbing at the research site. To aid in achieving a level of standardisation in swabbing

techniques, each woman received the same instructions on how to collect a lower vaginal swab. Each participant collected both her own pre- and post-intervention vaginal swabs, which ensured a degree of internal control in swabbing techniques.

Another design detail where a pragmatic approach was applied was in the pathology technique used for determining the presence of GBS. The standard pathology methods for measuring GBS at the study site were used. One limitation of the standard pathology tests is they do not give a quantified amount for the GBS colony counts per vaginal swab. The results only specify the presence of a light, moderate or heavy growth of GBS. These results are not sensitive enough to determine whether a slight reduction in GBS colonisation has occurred due to probiotic use but they would indicate whether GBS had been eradicated in a woman's vagina. The swabbing and pathology testing techniques used in this study replicated standard procedures at the study site, making the results of this study immediately practically applicable in the clinical setting.

3.3 Conducting the research

This pilot randomised controlled trial was performed from April 2011 to August 2011, with a follow-up telephone survey performed from November 2011 to March 2012. Funding for this project was assisted by a \$2000 contribution from the New South Wales Nurses and Midwives' Association's Edith Cavell Trust. The following section describes the details of the recruitment and data collection processes to conduct the research, including a description of the study site, study population, recruitment strategies, sample size, intervention and observations.

3.3.1 Study site

The Sutherland Hospital was selected as the study site. The Sutherland Hospital is a low-risk public birthing unit in the southern suburbs of Sydney, Australia. At this

hospital all women attending the antenatal clinic routinely self-perform a lower vaginal swab at 36 weeks gestation. If a woman's swab result is GBS positive, she then receives intravenous antibiotics in labour. The vaginal GBS rate at the Sutherland Hospital for 2008 was approximately 21 per cent (The Sutherland Hospital Obstetrix database, retrieved June 2009).

3.3.2 Study population

The selected study population for this research was GBS-positive women attending the antenatal clinic at the Sutherland Hospital. The GBS status of these women was determined by the routine, self-collected lower vaginal swab performed at 36 weeks gestation.

The following inclusion criteria were applied: age over 18 years, overall good health and a normal, uncomplicated pregnancy. The exclusion criteria and their rationales were as follows:

- Diabetes: women with gestational diabetes have been shown to have an increased risk of being GBS positive in pregnancy (Hakansson & Kallen, 2008).
- A previous history of endocarditis/valvular heart disease: non-pregnant individuals with these conditions have been shown to be at an increased risk of developing sepsis with probiotic use (Boyle et al., 2006).
- Any medical condition or those who were undergoing any treatment that would cause their immune systems to be compromised: non-pregnant individuals with these conditions have been shown to be at an increased risk of developing sepsis with probiotic use (Boyle et al., 2006).

If a woman developed an infection at any time during the study, she was withdrawn from participation but continued having data collected.

In addition, women who had any language barrier that hindered their ability to read and understand the provided information, which was in English, were excluded from participation. Women with a language barrier were excluded from the study because the resource limitations of the study did not make it possible to engage an interpreter for such situations. As such, it would have been impossible to ensure consent was informed, making it unethical to recruit such women.

3.3.3 Recruitment

Prior to commencing recruitment, it was important to inform the staff of the pending research project. The obstetric team, antenatal and delivery suite midwives and antenatal support staff were informed through on-site educational sessions and through one-on-one conversations. These sessions assisted in increasing the awareness of the staff, which helped them to understand the role of the researcher and to be able to answer questions from women in the antenatal clinic about the research.

In order to inform pregnant women of this study, posters (Appendix A) were displayed in the antenatal clinic at the Sutherland Hospital. When women attending the antenatal clinic were approximately 33 weeks pregnant, they were given a participant information sheet and a brochure describing the study and informing them of a possible future invitation to participate in the study (Appendices B and F).

The recruitment process commenced once a woman who met the inclusion criteria was determined to be GBS positive at 36 weeks. The woman then received a phone call from the researcher with a formal invitation to participate. If a woman agreed to be involved in the research, she was approached by the researcher the following week at her antenatal appointment. At this time informed consent was obtained

(Appendix E). The woman was then randomly assigned to receive both standard care and a daily oral dose of probiotics for three weeks, or to continue with standard care with the staff in the antenatal clinic. The randomisation process was computer generated by an online site. For the women who were assigned to be in the intervention group, the probiotics were provided at the time of consent.

Women were recruited based on the results of their standard lower vaginal swab at 36 weeks gestation. This decision capitalised on the results of this standard procedure, avoiding additional expenses due to the resource limitations of the study.

3.3.4 Sample size and randomisation

A power calculation performed to determine the sample size necessary for a RCT determined that a sample size of 217 participants per arm of the study would be required to reach a 90 per cent significance rate. To perform the power calculation, the vaginal GBS colonisation rate was assumed to be 21 per cent based on the vaginal GBS rate at the study site in 2008 (The Sutherland Hospital Obstetrix database, retrieved June 2009). The effect size used was determined G. Reid as 50 per cent (personal communication, 19 August 2009). The power calculation was based on detecting the difference between two proportions using a normal distribution approximation. Such a study was unrealistic due to scope and resource limitations. A pilot project was utilised to assist in becoming the basis for future power calculations and to determine the feasibility of the research design. This pilot project chose to recruit as many women as possible within the three-month recruitment time frame, with a minimum aim of 30 participants. A total of 34 women were recruited during this time period.

Since the exact number of women who would be recruited was uncertain, a simple randomisation method was applied using an online randomisation site to create a table which randomised 100 potential participants (Kang et al., 2008). The numbers on the table were covered until a woman gave informed consent to the researcher to participate in the study. At this point the researcher revealed whether the woman was assigned to the intervention or control group. The allocation ratio was intended to be 1:1. Unfortunately, since only 34 women were recruited but 100 numbers had been randomised, the allocation process was weighted heavily towards the intervention group. As a result, the study ended up with 13 women in the control group and 21 women in the intervention group. For a copy of the CONSORT flow diagram, refer to Appendix H.

Neither the participants nor the researcher were blinded to the group allocation.

3.3.5 Intervention

The women who consented to be involved in the research were randomly allocated to receive both standard care and a daily oral dose of probiotics *Lactobacillus rhamnosus* GR-1 (GR-1) and *Lactobacillus fermentum/reuteri* RC-14 (RC-14) in a dose of 10^8 viable strains for three weeks or to continue with standard care. The probiotic strains GR-1 and RC-14 were chosen for use in this study because they are the strains that have undergone the most clinical trials with respect to urogenital health in women. They have been found to survive passage through the gastrointestinal tract and to colonise the vagina after oral administration (Gardiner et al., 2002; Morelli et al., 2004). These strains have been shown to colonise the vagina during the second week of oral administration (Morelli et al., 2004). The

justification of a three-week intervention period to impact *Lactobacillus* colonisation of the vagina was based on this finding.

3.3.6 Observations

Three weeks post consent and post intervention, the GBS-positive women who were recruited into the study self-collected a repeat lower vaginal swab. This swab was given to the researcher or to the health practitioner at their appointment in the antenatal clinic. These swabs were tested in the pathology department, which was blinded to group allocation. If a participant commenced labour or spontaneously ruptured her membranes prior to completion of the study, a second lower vaginal swab was self-collected upon admission to the delivery suite and analysed by the pathology department.

The researcher also collected demographic data about each woman at the time of consent. These data were to assist in controlling for confounding factors. In order to protect the privacy of the participants, all the data collected was de-identified at the time of collection. The medical record numbers of each participant were documented with the data in order to allow for verification of any missing or ambiguous information upon completion of the trial. The information collected about each participant at the time of consent can be categorised into demographics and pregnancy details. This information is as follows:

Demographics

- ethnicity
- age

Pregnancy details

- body mass index (BMI)
- number of pregnancies
- number of children

- expected date of birth
- number of weeks pregnant upon entry into the study
- past history with GBS colonisation
- the research group to which the woman was allocated.

At the end of the three-week intervention period, each woman was asked whether she had taken any antibiotics during the trial. The women who received the probiotics were also asked how often they had been able to take their daily dose of probiotics and whether they had had any adverse reactions or side-effects during the trial.

After women in the trial had given birth, further information was collected. This information can be categorised into maternal labour and birth details and neonatal details. It is as follows:

Maternal labour and birth details

- the development of pregnancy complications (which included the development of infections)
- the type of birth
- the occurrence of antibiotic administration in labour
- the final lower vaginal swab result

Neonatal details

- the infant's birth weight.

Please refer to the appendix for a diagram of the research design (Appendix C) and a copy of the list of the additional data (Appendix D) that was collected about each participant.

Six months post birth, a follow-up telephone survey of the participants was performed. This survey was a requirement of the ethics board for ethics approval. The intention of this survey was to aid in collecting further data on the safety of these specific probiotic strains in pregnancy. The survey specifically addressed whether the infant had any concerns at birth, which included neonatal GBS infection.

Appendix G lists the questions asked at the follow-up telephone survey. The post-birth questions were structured following the indicators used in two systematic reviews conducted on the safety of probiotic use in pregnancy (Allen et al., 2010; Dugoua et al., 2009).

3.4 Data analysis

The final vaginal swab results were not revealed until after each woman had given birth. The results of this study, therefore, did not impact on the current management of the women participating in the study. These GBS-positive women still received intravenous antibiotics in labour, as was hospital policy. Once the data collection phase was complete, all the raw data, including the final vaginal swab results, was brought together and entered into the SPSS statistical computer program by the researcher. This process occurred in two stages: firstly, after the initial data collection phase was complete and, secondly, after the six-month follow-up telephone survey was complete. The data were then analysed using the SPSS statistical program. This process was assisted by the statistical consulting department of the University of Wollongong.

In order to test whether any differences existed between the intervention and control groups, an independent t-test was applied to the continuous demographic data, such as age and BMI. A t-test hypothesis states that the difference in the mean of a variable when comparing two groups is equal to zero. The null hypothesis in a t-test is that the difference in the means is NOT equal to zero. In this analysis it was decided that if a t-test produced a significance level of greater than 0.05 then the null hypothesis could be rejected, indicating that there was no difference between the means of a variable when comparing the control and intervention groups. Therefore, if the significance level was >0.05 it would indicate that, with regard to a specific

demographic variable, both the probiotic group and standard care group were similar (McDonald, 2009). In order to apply an independent t-test it was assumed that the data were normally distributed. The small sample size made tests of normality, such as the Shapiro-Wilk test, in the analysis unreliable. In order to confirm the use of the independent t-test on the continuous data in these findings, the Mann-Whitney test was applied to each continuous variable. The Mann-Whitney test is a non-parametric test. All of the Mann-Whitney tests which were applied to the continuous variables in the analysis had non-significant results, as did the independent t-test applied to the same variables. The Mann-Whitney tests therefore verify the results of the t-tests in this study (Batterham, personal communication, 2015; Newton & Rudestam, 2013). To determine whether associations existed between the two groups, a Fisher's exact test was applied to the categorical data, such as the presence of vaginal GBS. Normally a chi square test is used to test correlations but due to the small sample size of this study the Fisher's exact test was deemed more appropriate for this data set. With this statistical test, the null hypothesis states that the two variables are independent of each other and the alternative hypothesis states that they are NOT independent. If the test applied to the data set produces a significance value of less than 0.05 then the null hypothesis can be rejected and it can be assumed that a relationship exists between the two variables (McDonald, 2009).

3.5 Ethics

Within a description of the methodology used, it is also important to discuss the ethical considerations surrounding the study. This discussion justifies the research methods from an ethical point of view, thereby ensuring that the study was performed in a way that protected the integrity of the participants and their unborn infants. The following section discusses the ethical issues pertaining to this study

and its design. These issues have been categorised into safety considerations, risk of coercion, informed consent and confidentiality.

Ethics approval for this study was granted by the University of Wollongong's Human Research Ethics Committee (HE 10/306). Site-specific approval was granted through the South Eastern Sydney Local Health Network (SSA/11/STG/39).

3.5.1 Safety considerations

The primary ethical concern in this project surrounded the issue of safety (Beauchamp & Childress, 2009). Could daily oral use of probiotics result in any harm to the mother or the foetus? Probiotics comprise organisms that are identical to those found in the human gastrointestinal tract and in the vagina. The available research indicates that the risk to pregnant women of developing any infections as a result of probiotic use is low and there is no expected risk to the developing foetus (Kalliomaki et al., 2001; Lahtinen et al., 2009; Leiten et al., 2008; Liong, 2008; Pham et al., 2008; Schultz et al., 2004; Wickens et al., 2008; World Health Organisation, 2002). For a discussion of the issue of probiotic safety in pregnancy, please refer to section 2.5, 'Safety considerations'.

In order to maintain the safety of the participants, the exclusion criteria for this study state that any woman with diabetes, a history of valvular heart disease/endocarditis or any condition or treatment causing immune system compromise was unable to participate in the study. These are all conditions that have been identified in the literature as putting an individual at higher risk of developing infections with probiotic use (Boyle et al., 2006). In addition, the research site was a low-risk birthing unit. Therefore, women with major health concerns, including those concerns that would put women at higher risk of developing an infection with

probiotic use, would have their care transferred to a tertiary hospital. As a result, the potential risk of harm to the study population was minimised.

Based on the above literature and the low-risk status of the birthing unit where the study was performed, the anticipated risk to participants was minimal. In order to further evaluate the safety of the probiotics used in the study, pregnancy details, maternal labour and birth details, and neonatal details were collected from each participant. These details assisted to audit whether the intervention negatively impacted on any of the participants. A follow-up telephone survey was also performed involving all the participants at six months post-partum in order to generate further information on the safety of probiotic use in later pregnancy.

3.5.2 Risk of coercion

The researcher was a midwife employed in the antenatal clinic and delivery suite at the study site. To reduce the risk of coercion, in the first three months of the study, during the recruitment and initial data collection phases, the researcher did not work in the antenatal clinic. This reduced the chance of the researcher being directly involved with the care of potential participants. The result was that researcher did not care for any participants during the antenatal stage of their pregnancy. After these three months, the women naturally moved through their pregnancies and were admitted to the delivery suite to give birth to their babies. There was a risk that the researcher be involved in the care of participants when they were admitted to delivery suite but by this stage in the research, the risk of coercion was minimal because recruitment was already completed.

In addition to not being involved in the antenatal care of potential participants, the initial contact of potential participants with the researcher was through a telephone call. During this phone call, women who had been determined to be GBS positive

were invited to participate in the study. The telephone call allowed a woman a degree of anonymity if she decided to decline participation which would not be present in a face-to-face encounter. If a woman declined to participate, she was not approached again by the researcher to participate in the study.

It was also specified in the consent form and reiterated verbally at the time of consent that women were able to withdraw participation consent at any time during the study without ramifications for the care they received throughout their pregnancies. All these factors combined to ensure the risk of coercion to potential participants was minimised.

3.5.3 Informed consent

In this study, the participants were required to sign a consent form. All pregnant women were given an information sheet when they were 33 weeks pregnant (Appendices B and F). GBS-positive women who indicated an interest in participating in the study over the telephone were approached the following week in the antenatal clinic by the researcher. At this time, informed consent was sought (Appendix E). The delay between obtaining the information sheet, expressing an interest in participation over the telephone and providing informed consent gave women the opportunity to think about entry to the study and discuss it with their partners prior to officially consenting.

The researcher's direct involvement in the consenting process helped to facilitate informed choice by allowing women the opportunity to ask any questions or to clarify any issues prior to signing their consent. The information letter and the consent form outlined all the foreseeable risks and benefits of the research and contained the contact telephone numbers and email addresses of the researchers.

This gave women the opportunity to contact the researchers if they had any questions or concerns about the study. It also specified in the consent form that women were able to withdraw their consent to participate at any time without ramifications to the care they received throughout their pregnancies.

The information provided and the availability of the researchers for further questions ensured that the participants were able to acquire all the information they required to make an informed choice about involvement in the study.

3.5.4 Confidentiality

In order to protect the privacy of the participants, all the data collected was de-identified at the time of collection. The medical record numbers of each participant were documented with the data in order to allow for verification of any missing or ambiguous information upon completion of the trial. Once the study was completed, all identifiable information, including medical record numbers, was deleted.

The researcher was the only staff member at the study site with access to the raw data. For the duration of the data collection phase, the information was stored in a locked cabinet at the research site. Once data collection was completed, the raw data were transferred to a locked cabinet in the School of Nursing, Midwifery and Indigenous Health at the University of Wollongong. The de-identified data were entered in the SPSS database by the researcher to allow for data analysis. The data were saved and stored on a password-protected laptop computer. The de-identified state of the data has been preserved at any time when the data has been presented in poster, written or oral format.

All of these actions have ensured the safeguarding of participants' privacy with respect to their involvement in the study.

3.5.5 Ethics summary

As has been demonstrated above, all attempts were made to ensure the women and the unborn infants participating in the study were protected physically, psychologically and emotionally for the duration of the research. This can be seen through the attention given not only to physical safety considerations but also to the rights of women to make a free, uncoerced, informed choice and to have their information remain private and confidential. In summary, the attention paid to all these different aspects demonstrate that this study was performed in an ethical manner.

3.6 Conclusion

In order to determine the appropriateness of this study design to address whether the use of oral probiotics in pregnancy can impact vaginal GBS colonisation rates, a pilot RCT was performed. This chapter has described the justification for the research design used in this study. It has achieved this, firstly, by describing the methodological frameworks of a pilot RCT and a pragmatic trial, which were the underlying foundations of the study. Secondly, it has detailed the design by describing the study site, the study population, the recruitment strategy, the sample size and randomisation process, the intervention, the observations and the data analysis utilised in the study. Lastly, it explained the ethical concerns arising from the study and the means by which the design addressed these concerns.

The following chapter describes the findings that were generated through the research design detailed here.

4 FINDINGS

4.1 Introduction

This chapter presents the statistical findings that were generated by the study. The first group of findings has been organised into a comparison of the antenatal demographics, a comparison of the final vaginal swab results and a comparison of the final vaginal swab results of a subgroup of the participants. The second group of the findings presents comparisons of the variables pertaining to safety considerations within the study. These variables include antenatal, immediately postnatal and six months postnatal safety considerations. These are presented in order to determine the absence of adverse events associated with the intervention.

Between April 2011 and July 2011, 34 GBS-positive pregnant women experiencing uncomplicated pregnancies were recruited into this study. All the participants were in the late stages of their pregnancies at the time of recruitment. The average mean gestation was 35 weeks and 5 days at the time when the first vaginal swab was collected. A pregnancy is considered to be at term at 37 weeks. Due to the uncontrollable nature of labour and birth and the late stages of the participants' pregnancies at the time of recruitment, a high attrition rate was anticipated. A three-month recruitment phase was performed with an anticipated minimum of 30 participants. In total, 34 women were recruited to participate in the study during this time frame.

Of these 34 participants, 21 women were randomly allocated to the intervention group and 13 to the control group (for a complete explanation of the uneven participant allocation, please refer to p 25-26). Of the 21 women in the intervention group, seven women were able to complete the entire 21 days of daily oral probiotics. This was mainly due to participants birthing prior to completion of the

21-day intervention. Seventeen women were successful in consuming at least 14 days of oral probiotics. Two participants did not collect their second lower vaginal swab. This occurred due to the precipitous nature of their individual births. Both of these women were allocated to the intervention group.

A research study conducted by Morelli et al. (2004) found that *Lactobacilli* colonised the vagina after two weeks of a daily oral dose of GR-1 and RC-14 in a dose of $>10^8$ culture-forming units. For the purpose of the data analysis, and based on this research, the participants were further limited to a subgroup which included any women who had completed 14 days or more of probiotics. This further reduced the participants to 17 women in the intervention group and 13 women in the control group. Of the 17 woman in the intervention group, one woman did not complete the final lower vaginal swab due to the precipitous nature of her birth. This further reduced the available final swab results in the subgroup to 16 in the intervention and 13 in the control groups.

A follow-up telephone survey of the participants was performed when their infants were approximately six months old. The purpose of this survey was to collect further data to ensure the absence of adverse events with the intervention. All 34 women participated in this survey. No participants were lost to follow-up.

The following sections present the findings of this research project. It is important to frame these findings within the context of its original design. This project was set out to be a pilot. The aim was to ascertain the appropriateness of the design to guide the development of future research projects. As such, the sample size was small and lacks power. This increases the potential for Type 1 or 2 errors in the statistical results (Borbasi & Jackson, 2008). Therefore, while the findings from this study are

intended to guide future research, assertions made on the basis of these findings alone should be considered carefully.

The small sample size made tests of normality for the continuous data in the analysis unreliable. In order to verify normal distribution and justify the use of the independent t-test on the continuous data in this analysis, the Mann-Whitney test was applied. All of the Mann-Whitney tests applied to the continuous variables in this study had results which were non-significant, as were the results of the independent t-tests when applied to the same variables. The Mann-Whitney test is a non-parametric test. The results of the Mann-Whitney tests support the results of the t-tests in the findings (Batterham, personal communication 2015; Newton & Rudestam, 2013).

The findings of the study have been classified into antenatal demographics, final lower vaginal swab results, subgroup analysis of final lower vaginal swab results, safety considerations and the six-month follow-up telephone survey.

4.2 Antenatal demographics

Upon entry to the study, antenatal demographic information was collected from each participant. This information included age, ethnicity, BMI, parity and previous history of GBS. Null parity refers to a woman who is currently experiencing her first pregnancy, which she has carried past the age of viability (Wong & Perry, 1998). Due to the small sample size, information collected on ethnicity could not accurately be statistically analysed.

In order to compare the demographic variables between the probiotic and standard care groups, independent sample t-tests and Fisher's exact tests were used. Using the independent sample t-test, it was shown that the participants in the probiotics and standard care groups did not differ significantly with respect to age (Probiotic:

M=32, SD=4; Standard: M=30, SD=4.3, $t(df)=1.7$, $p=0.1$) and body mass index (BMI) (Probiotic: M=23.4, SD=3.8; Standard: M=23.7, SD=4.3, $t(df)=-0.2$, $p=0.8$). When a Fisher's exact test was applied to the variables 'Nulliparous' and 'Previous history of GBS', the results were also found to be non-significant ($p=0.5$ and 0.6 respectively, FET). This indicates that both the probiotics and standard care groups in this specific group of participants have a degree of homogeneity with respect to age, BMI, null parity and history of GBS. Therefore, these results aid to increase the confidence that these factors, with potential confounding effects, should not influence the findings of the study. Since the two groups are similar in these areas prior to the intervention, it justifies the comparison of these two groups following the intervention.

4.3 Final lower vaginal swab results

The aim of this study was to complete a pilot project to ascertain if the research design was appropriate to determine whether a daily oral dose of probiotics can reduce the rate of vaginal group B streptococcal (GBS) colonisation in pregnancy. To determine whether a relationship existed in this study between oral probiotic consumption and GBS-negative vaginal swab results, a Fisher's exact test was applied to the data.

4.3.1 Timing between the first and second vaginal swabs

In order to ensure consistency between the two groups and control for possible confounding factors, the time between collecting the first vaginal swab and second vaginal swab for the probiotic and standard care groups was compared. If one group had a significantly longer time period between performing their first and second vaginal swabs, this would have the potential to impact the number of swabs spontaneously reverting to a negative result independent of the intervention. This

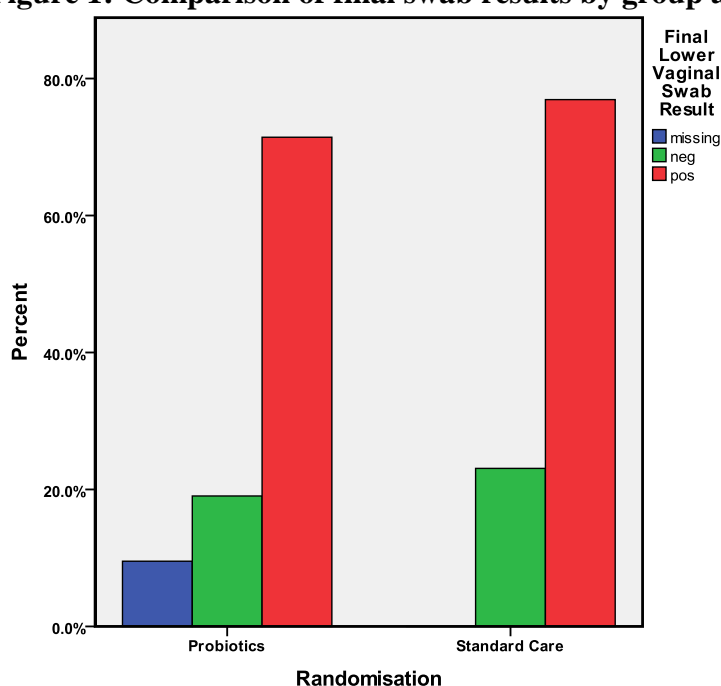
comparison was performed using an independent sample t-test. In this analysis the mean number of days between collection of the first and second vaginal swab for both the probiotic and control groups was 25 days, producing a non-significant result (Probiotic: SD=5.3; Standard: SD=9.7, $t(df)=0.3$; $p=0.8$). This indicates that the number of days between collecting the two swabs was similar for both the probiotic and standard care groups. In this analysis, it was assumed that the two participants who missed collecting their second swabs due to precipitate labours, if it had been possible, would have collected their swabs on the same day that they gave birth.

4.3.2 Comparison of final lower vaginal swab results

It has been verified that similarity exists between the intervention and control groups in the aforementioned variables. Therefore, since it has been determined that a degree of homogeneity exists between the probiotic and standard care groups in this specific sample, the comparison of the final lower vaginal swab results between the two groups is justified. In the control group, involving 13 women, it was found that three women were GBS negative when the final swab was collected. In the probiotics group, involving 21 women, two of the participants had no final swab collected. Of the remaining 19 women in the probiotics group, four were determined to be GBS negative, as indicated by their final lower vaginal swab. Using the Fisher's exact test, it was discovered that the group of women consuming an oral daily dose of probiotics did not have a significant decrease in GBS in their vaginas, as indicated by their final swab result when compared with the control ($p=0.7$, FET). A comparison of the final swab results has been summarised in Table 1 and Figure 1.

Table 1: Comparison of final swab results by group allocation

Intervention status	Final Lower Vaginal Swab Result (N)		
	Negative	Positive	Total
Probiotics	4	15	21
Standard	3	10	13
Total	7	25	34

Figure 1: Comparison of final swab results by group allocation

4.4 Subgroup analysis of final vaginal swab results

4.4.1 Subgroup analysis of GBS results

The number of days that women in the intervention group took the probiotics varied from six days to 21 days, with an average mean of 17 days. Only eight of these participants completed 19 or more days, with the missing days being attributed to forgotten doses. The reason why participants completed fewer than 19 days of probiotics was that they birthed prior to completion of the course of probiotics. The

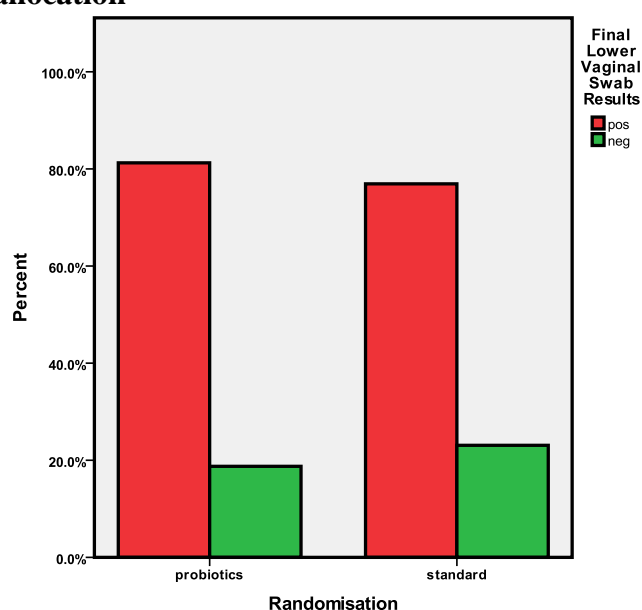
foundation for the decision to perform a three-week intervention was the finding that the probiotics RC-14/GR-1 in the dose of $>10^8$ culture-forming units can colonise the vagina after 14 days of oral consumption (Morelli et al., 2004). The premise of the current study was that women with higher colonisation of *Lactobacilli* in their vaginas have a greater probability of being GBS negative. Therefore, if previous research has shown that these probiotics can colonise the vagina after two weeks of oral use, then, in the current study, it would not be expected for the women's GBS statuses to be impacted until at least 14 days of probiotic use. In light of this, a further subgroup analysis of the final swab results was performed including only the women in the intervention group who had completed at least 14 days of oral probiotics.

The subgroup limited the intervention group to 17 women and the control group to 13 women. In the intervention subgroup, one woman did not have a final lower vaginal swab collected due to precipitous labour, further reducing the intervention group to 16 women. When the Fisher's exact test was applied to this subgroup, the results remained above significance ($p=1.0$, FET), indicating no difference in the rate of GBS-negative final swabs between the intervention and control when including only the women in the intervention who had completed at least 14 days of oral probiotics. These results are summarised in Table 2 and Figure 2.

Table 2: Comparison of the subgroup analysis of final swab results from the intervention and control groups

Intervention status	Final Lower Vaginal Swab Result (N)		
	Negative	Positive	Total
Probiotics	3	13	16
Standard	3	10	13
Total	6	23	29

Figure 2: Comparison of subgroup analysis of final swab results by group allocation



4.4.2 Subgroup analysis comparing the presence of commensals

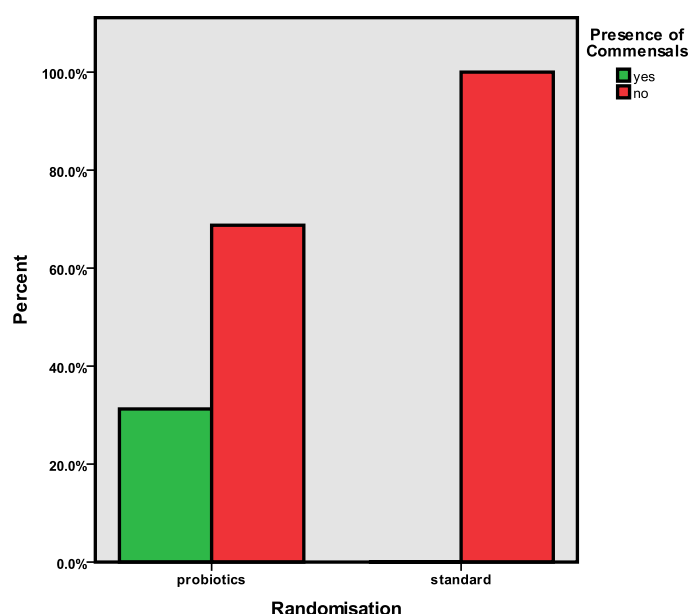
A secondary, unexpected finding in this study was that the lower vaginal swab results indicated the presence of commensals in a number of women's vaginas. At the study site, the pathology department routinely reported on both the presence of GBS and the presence of commensals. Commensals are normal vaginal flora (Barrons & Tassone, 2008; Lagenaur et al., 2011b; Rose et al., 2012). In this study, the presence of commensals was reported in final vaginal swabs among the women who took a daily dose of probiotics for at least 14 days: five women in the probiotics group had commensals and no women in the standard care group had commensals. Of these five women, none had had the presence of commensals reported in their vaginas at the initial 36-week swab. When the Fisher's exact test was applied to determine the presence of an association between consuming oral probiotics and the presence of commensals, a significant result was obtained ($p=0.048$, FET). This result indicates that the women in this study consuming a daily oral dose of

probiotics for 14 days or more had a significantly increased probability of having commensals in their vaginas in comparison to the women allocated to the standard care group. These findings have been summarised in Table 3 and Figure 3.

Table 3: Comparison of the presence of commensals by group allocation

Intervention status	Presence of Commensals (N)		
	No	Yes	Total
Probiotics	11	5	16
Standard	13	0	13
Total	24	5	29

Figure 3: Comparison of the presence of commensals by group allocation



4.5 Safety considerations

One of the major concerns surrounding probiotic use in pregnancy is the assurance of safety for both the mother and the infant. A more detailed discussion of this topic can be found in the literature review chapter. This section presents the findings of the study pertinent to the safety of probiotic use in pregnancy. The safety indicators for this section and for the questions in the six-month follow-up telephone survey

have been adapted from two systematic reviews conducted on the safety of probiotic use in pregnancy (Allen et al., 2010; Dugoua et al., 2009). Furthermore, the indicators for allergy-related concerns in the six-month follow-up telephone survey were adapted from a systematic review of the impact of probiotic use in pregnancy on eczema (Kalliomaki et al., 2001).

4.5.1 Antenatal concerns

Data were collected on each participant regarding the emergence of complications in the pregnancy after entry to the study. Two women were found to develop complications, one with pregnancy-induced hypertension and one with pruritic urticarial papules and plaques of pregnancy (PUPPS). Both of these women had been allocated to the standard care group.

4.5.2 Side-effects

After the second vaginal swab was collected from each participant, information was collected documenting the self-reported side-effects women in the intervention group experienced from the probiotics. Two out of the 21 women in the probiotics group stated having side-effects. One woman reported that she experienced an itchy throat. She was uncertain whether this was due to the probiotics. One other woman reported that the probiotics assisted her with her constipation. None of the remaining 19 women reported any side-effects from the probiotic usage.

4.5.3 Birth outcomes

In order to further explore the area of probiotic safety in pregnancy, data pertaining to a number of birth outcomes was collected. This included birth weight, gestation at the time of birth, and mode of birth/delivery.

4.5.4 Birth weight and gestation at the time of birth

The mean birth weight for the 21 infants born to the women in the intervention group was 3,545 grams. The mean birth weight for the 13 infants born to women in the standard care group was 3,542 grams. An independent sample t-test was applied to the birth weights to determine if there was a significant difference between the two groups with regard to birth weight. No significant difference was found (Probiotics: SD=379; Standard: SD=370, $t(df)=0.02$, $p=0.98$).

The mean pregnancy gestation at the time of birth was also compared between the two groups. This information was to aid in determining whether probiotics could impact the time at which a woman may go into labour. It was calculated that the mean gestation at the time of birth for the women in the probiotics group was 39.7 weeks and in the standard care group was 39.9 weeks. The results of the independent sample t-test verified that there was no significant difference between the two groups (Probiotics: SD=1.2; Standard: SD=1.0, $t(df)=-0.7$, $p=0.5$).

4.5.5 Mode of birth

The final safety indicator investigated with regard to birth outcomes in this study was mode of birth. The four identified modes of birth were normal vaginal birth, instrumental birth, elective lower segment caesarean section (LSCS) or emergency LSCS. An instrumental birth indicates the need for a vacuum or forceps delivery. An elective LSCS refers to a caesarean section that has been planned in advance. An emergency LSCS refers to a caesarean section that has been unplanned and has occurred because of an unforeseen event, such as 'foetal distress' or 'failure to progress'.

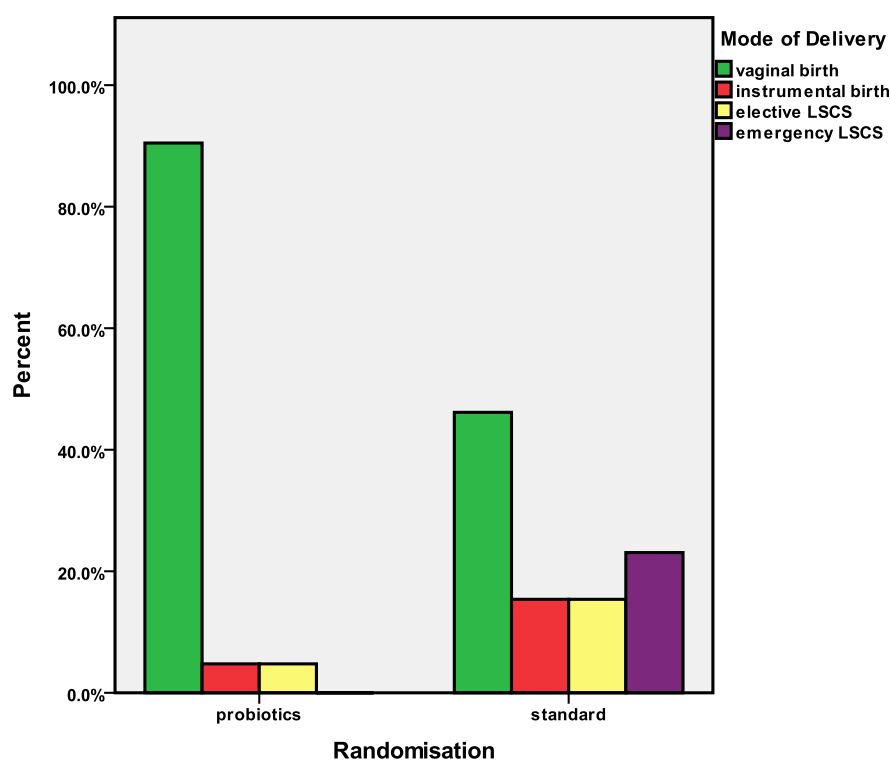
Of the 21 women in the probiotics group, 19 experienced a normal vaginal birth, one underwent an instrumental birth and one underwent an elective caesarean. Of the

13 women in the standard care group, six women experienced a normal vaginal birth, two underwent an instrumental birth, two underwent an elective caesarean and three underwent an emergency caesarean. A summary of these findings can be found in Table 4 and Figure 4.

Table 4: Summary of the modes of delivery for the intervention and control groups

Intervention status	Mode of Delivery (N)				
	Vaginal Birth	Instrumental Birth	Elective LSCS	Emergency LSCS	Total
Probiotics	19	1	1	0	21
Standard	6	2	2	3	13
Total	25	3	3	3	34

Figure 4: Mode of birth by group allocation



In order to compare these groups further, instrumental birth and emergency LSCS were grouped together into assisted delivery. Elective LSCSs were not included in this group, as they were planned in advance and were, therefore, births that did not require an emergency intervention. This categorisation further reduced the data to one woman in the probiotics group requiring an assisted delivery and five women in the standard care group requiring an assisted delivery. When a Fisher's exact test was applied to this data, the women in the standard care group were shown to have significantly more assisted deliveries than the women in the probiotics group ($p=0.02$, FET). These results have been summarised in Table 5.

Table 5: Comparison of assisted delivery rates by group allocation

Intervention status	Assisted Delivery (N)		
	No	Yes	Total
Probiotics	20	1	21
Standard	8	5	13
Total	28	6	34

These findings imply that probiotics may have a protective property when consumed in later pregnancy. One possible confounding variable that may impact these findings is the fact that women who are undergoing their first labour and birth experience (nulliparous) have a greater probability of experiencing an assisted delivery (Baskett et al., 2008). If there were more nulliparous women in the standard care group as compared to the probiotics group, this could skew the data. In response to this, the data were further limited to the 18 nulliparous women participating in the study. Within this category, one out of 10 nulliparous women in the probiotics group underwent an assisted delivery and five out of eight nulliparous

women in the standard care group underwent an assisted delivery. A Fisher's exact test indicated that the significance remained despite limiting the data to nulliparous women ($p=0.04$, FET). A summary of the data can be found in Table 6. Due to the small sample size, it would be unreasonable to conclude that probiotics have a protective effect on birth outcomes, but these results do support the evidence that probiotic use in later pregnancy does not produce negative birth outcomes.

Table 6: Comparison of assisted delivery rates in nullips by group allocation

Intervention status	Assisted Delivery (N)		
	No	Yes	Total
Probiotics	9	1	10
Standard	3	5	8
Total	12	6	18

4.5.6 Pre-labour spontaneous rupture of membranes

One unexpected outcome was the number of women who experienced a pre-labour spontaneous rupture of membranes (SROM) in the study. Four out of the 21 women in the probiotics group and one out of the 13 women in the standard care group had a pre-labour SROM. When analysed further with a Fisher's exact test, a comparison of the SROM rates for the two groups was not statistically significant ($p=0.6$, FET). However, this may be an area to be alert to for future studies. A summary of these results can be found in Table 7.

Table 7: Comparison of pre-labour SROM rates by group allocation

Intervention status	Prelabour SROM		
	No	Yes	Total
Probiotics	17	4	21

Standard	12	1	13
Total	29	5	34

4.6 Follow-up telephone survey

A follow-up telephone survey was performed approximately six months after birth for all the women involved in the study. The participation rate for the telephone survey was 100 per cent: all 34 participants were contacted and interviewed. The purpose of the survey was to collect further data on the safety of probiotic use in later pregnancy and to determine whether the probiotic use had had any impact on the development of eczema, asthma or allergic rhinitis in the infants of the participants.

The survey collected some demographic information to aid in controlling for confounding variables. This information included the method and duration of infant feeding, the presence of smokers or pets in the home, the infant's exposure to childcare, and the family history of allergies, eczema and asthma. The survey then collected information about the infant regarding the number of doctors' visits, presentations to the emergency department and hospitalisations. It also collected information on whether the infant had had any concerns at birth, such as neonatal GBS infection, and whether the infant had shown any signs of eczema, allergies, asthma or allergic rhinitis. Finally, the mother was asked to rate the health of her infant as 'very healthy', 'occasionally unwell' or 'nearly always unwell', and given the opportunity to comment further on the health of her infant. Refer to Appendix G to review a copy of the survey.

4.6.1 Demographic information

The demographic information collected by the survey was: method of infant feeding, duration of exclusive breastfeeding, the presence of smokers in the house, the

presence of pets in the house, the infant's attendance at childcare, and the family history of allergies, asthma or eczema. These are all variables that may contribute to the overall health of the infant. These variables were compared statistically between the probiotic and standard care groups of participants. An independent sample t-test was applied to the variable length of exclusive breastfeeding (Probiotic: M=4.2 months, SD=2.2; Standard: M=3.5 months, SD=2.6, $t(df)=0.9$, $p=0.4$) and a Fisher's exact test was applied to the variables: presence of smokers ($p=1.0$, FET), presence of pets ($p=0.7$, FET), attendance at childcare ($p=1.0$, FET) and family history ($p=1.0$, FET). Based on these statistical analyses, no significant differences were found for any of the variables.

The final demographic variable that was considered was the method of infant feeding at six months of age. Of the 21 women in the probiotics group, 11 of their infants were breastfed, nine infants were formula fed and one infant received a combination of breast and formula feeds. Of the 13 women in the standard care group, seven of their infants were breastfed, five infants were formula fed and one infant received a combination of breast and formula feeds. A Fisher's exact test cannot be applied to an analysis with three variables. A chi square test was not appropriate due to the small sample size. Therefore, no tests of significance were applied to this data. A summary of these results can be found in Table 8.

Table 8: Comparison of infant feeding methods at six months between the intervention and control groups

Intervention status	Method of Feeding at 6 Months (N)			
	Breast feeding	Formula	Combination	Total
Probiotics	11	9	1	21
Standard	7	5	1	13
Total	18	14	2	34

4.6.2 Infant well-being at six months of age

The remaining sections of the survey collected data relating to the well-being of the infant. The indicators used to ascertain this were: health concerns at birth, number of visits to the doctor, number of visits to hospital emergency departments, number of hospitalisations, use of medications, and presence of asthma, allergies, rhinitis or eczema. The participants were also asked to rate their infants' health as 'very healthy', 'occasionally unwell' or 'nearly always unwell'. Finally, the participants were given the opportunity to comment further on health concerns relating to their infants.

4.6.3 Concerns at birth

Among the participants, two women in the probiotics group and one woman in the standard care group reported that their infants had concerns at birth. The two concerns noted in the probiotics group were low Apgar scores and clicky hips. The one concern noted in the standard care group was transient tachypnoea of the newborn. None of the participants reported neonatal GBS infections in their infants. All of these concerns resolved after the immediate postpartum period. The Fisher's exact test indicated that there was no association between group allocation and concerns at birth ($p=1.0$, FET).

4.6.4 Encounters with the medical system

The second group of indicators collected information on the number of encounters the infant had had with the medical system in the first six months of life. These encounters were broken down into visits to the doctor, visits to emergency departments and hospitalisations. The routine vaccination visits to the GP were not

included in the tally of doctors' visits. The mean number of visits to the doctor for the probiotics group was 1.5 visits. The mean number of visits to the doctor for the standard care group was 1.9 visits. The maximum number of visits for one infant in the probiotics group was 10 visits. These visits were for an infant with reflux who had also contracted rubella in the first six months of life. The maximum number of visits to the doctor in the standard care group was 12 visits. These visits were for an infant who had developed a haemangioma post discharge from the hospital. He had required fortnightly visits to a specialist at the Sydney Children's Hospital. By six months of age, these visits had been reduced to every second month. An independent sample t-test indicated no difference in means between the two groups with respect to their number of doctors' visits (Probiotics: SD=2.8; Standard: SD=3.2; $t(df)=-0.3$, $p=0.8$).

The mean number of visits to the emergency department for the probiotics group was 0.2. The mean number of visits to the emergency department for the standard care group was 0.2. The probiotics group had four presentations to emergency; the standard care group had two presentations. In the probiotic group, two of the presentations were for the same infant who had begun projectile vomiting when commenced on formula. This infant was later diagnosed with cow's milk intolerance. One presentation was due to constipation when commenced on formula. The final presentation was due to pyloric stenosis. This infant was hospitalised and required surgery at Sydney Children's Hospital. The two presentations in the standard care group were for a virus and bronchiolitis. The infant with bronchiolitis was admitted and treated in hospital for the infection. An independent sample t-test indicated that no significant difference existed between the mean number of

presentations to the emergency department between the probiotics and standard care group (Probiotics: SD=0.5; Standard=0.4, $t(df)=0.2$, $p=0.8$).

One infant in the probiotics group and two infants in the standard care group required hospitalisation in their first six months of life. The infant in the probiotics group was admitted to hospital for pyloric stenosis. The two infants in the standard care group were admitted to hospital for bronchiolitis and haemangioma. A Fisher's exact test indicates that there was no association between incidence of hospitalisation and group allocation ($p=0.5$, FET).

In this study, no significant association existed between the group allocation and encounters with the medical system in the first six months of life. This further supports the absence of adverse events in this study with the use of probiotics in later pregnancy.

4.6.5 Medications

The incidence of infant medication use was also an indicator for infant well-being. The most frequently reported medication taken by the infants in this study was Losec. This is an anti-reflux medication. In the probiotics group three infants were taking Losec and in the standard care group two infants were taking Losec. The only other medication reportedly used was Propranolol, a beta blocker, which was being used to treat one infant with haemangioma. A Fisher's exact test statistic showed no association between group allocation and incidence of medication use ($p=0.7$, FET).

4.6.6 Allergy-related health concerns

Information was also collected on the incidence of allergy-related health concerns that had developed in the infants. The indicators used to determine these health concerns were the occurrence of asthma, eczema, allergic rhinitis or allergies in the

infants. Many research studies have indicated a trend in a reduction in allergy-related illness in infants whose mothers used probiotics in later pregnancy (Kalliomaki et al., 2001). This information aimed to discover whether any such trend could be seen in this research project.

Only one woman reported having an infant with possible asthma. This woman was in the probiotics group. Her infant had had some incidences of wheezing and was currently under investigation with a paediatrician for asthma. One woman also reported having an infant with rhinitis. This woman was in the probiotics group. She described her infant as having a continuously runny nose. Both of her other children and her husband were asthma sufferers and they also had other allergy-related health concerns. Only one woman also reported having a child with allergies. This woman was in the probiotics group. Her infant had been diagnosed with cow's milk intolerance after commencing on formula. These frequencies were too low to permit reliable statistical analysis.

The final allergy-related concern that was explored was the incidence of eczema. Three women reported that their infants experienced mild skin rashes or irritations but were uncertain whether this was eczema. Two of these women were in the probiotics group and one woman was in the standard care group. One woman in the probiotics group and three women in the standard care group reported eczema in their infants. Of this total of seven women, all but one quantified their infant's skin concerns as mild or slight. When all the uncertain answers were assumed to be yes and the Fisher's exact test was applied to this variable, no significant association was shown to exist between incidence of eczema and group allocation ($p=0.4$, FET). For a summary of these findings, see Table 9.

Table 9: Summary of incidence of eczema by group allocation

Intervention status	Incidence of Eczema (N)		
	No	Yes	Total
Probiotics	18	3	21
Standard	9	4	13
Total	27	7	34

4.6.7 Overall health

The final indicator used to assist in determining the overall well-being of the infants of the study participants at six months of age was a scale which asked women to describe their baby's health as: 'very healthy', 'occasionally unwell' or 'nearly always unwell'. Only one woman described her infant as 'occasionally unwell'; all the other participants described their infants as 'very healthy'. This woman was in the standard care group. Her infant had been diagnosed with haemangioma at three weeks of age and at six months required twice daily medication and frequent follow-up with doctors. These results were too low to permit for reliable statistical analysis. As a conclusion to the telephone survey, women were asked whether there were any other health concerns that they would like to mention regarding their baby. Three participants offered further responses to this question. One woman in the probiotics group reported that her infant was seeing a physiotherapist once a month because the infant preferred to turn her head to one side. Another woman in the probiotics group reported that her infant had had one episode of diarrhoea in the past six months. Finally, one woman from the standard care group reported that her infant was a poor sleeper.

4.6.8 Conclusion to six-month follow-up telephone survey

The study lost no participants to follow-up. All 34 participants were contactable for the six-month follow-up telephone survey. The data provided by the six-month follow-up study found that no significant differences existed between the confounding variables in the probiotics and the standard care groups. The study also found no differences between the two groups relating to the overall health of the infants of the study participants at six months of age. These findings further support the lack of adverse events with probiotic use in later pregnancy in healthy women experiencing uncomplicated pregnancies.

4.7 Conclusion

In summary, this study found that no differences existed between the probiotics and standard care groups with regard to demographic information collected both at the time of recruitment and at the time of the six-month follow-up survey.

Further, no significant difference was found between the two groups with regard to most of the safety indicators investigated in the study. The only difference that was discovered was in mode of delivery, where women in the standard care group were found to have a greater incidence of assisted deliveries. This difference remained even when the data were limited to nulliparous participants. These findings further support the lack of adverse events with probiotic use in later pregnancy in healthy women experiencing uncomplicated pregnancies.

The aim of this study was to ascertain the appropriateness of the research design to determine whether a daily oral dose of probiotics could impact a pregnant woman's GBS status, as indicated by a lower vaginal swab. The women in the probiotics group did not show a reduction in the incidence of GBS in their vaginas in comparison to the standard care group. A subgroup analysis, including only women

in the intervention group who had taken at least 14 days of probiotics, also found no reduction in the incidence of vaginal GBS in comparison to the standard care group. But an unexpected, secondary finding did show that the subgroup had a significant increase in the presence of commensals in the vaginas of the women in the probiotics group. This finding has ramifications for future studies, as will be explored further in the discussions chapter.

5 DISCUSSION

5.1 Introduction

The following chapter discusses the multiple findings that have been generated by this research project. The hypothesis of the study asked the question: if *Lactobacillus* colonisation rates can be increased in pregnant women's vaginas, could this result in a decrease in group B streptococcal (GBS) colonisation rates? One potential means of manipulating *Lactobacillus* concentrations in order to attempt to impact GBS colonisation rates is through the use of probiotics. The aim of this study was to complete a pilot project to ascertain if the research design of this study was appropriate to determine if a daily oral dose of probiotics can reduce the rate of vaginal group B streptococcal (GBS) colonisation in pregnancy.

This research project did not discover any significant difference in vaginal GBS colonisation rates in women who had consumed daily oral doses of probiotics as compared to women who had not. This chapter discusses the possible explanations for the lack of positive results in this study. It also presents the limitations of the study. Finally, it presents the strengths of the study with an emphasis on the information that has been generated to aid in the development of future research designs.

5.2 Possible reasons for the lack of results relating to probiotic use and vaginal GBS

The aim of this study was to complete a pilot project to ascertain if the research design was appropriate to determine whether a daily oral dose of probiotics can reduce the rate of vaginal group B streptococcal (GBS) colonisation in pregnancy. In this study, only seven of the 21 participants in the intervention group succeeded in completing the full 21 days of probiotics. In order to manage this problem, a

subgroup analysis was performed including participants in the intervention group who had completed 14 days or more of probiotics. This decision was based on research which has shown that *Lactobacilli rhamnosus* GR-1 (GR-1) and *fermentum/reuteri* RC-14 (RC-14) can colonise the vagina after 14 days of oral consumption (Morelli et al., 2004; Reid et al., 2001). The subgroup analysis found no significant difference in the rate of vaginal GBS post intervention between the control and probiotic groups.

As this was a pilot study, one of the purposes of the study was to analyse the results in the light of the study design in order determine areas that require improvement for future designs. There are numerous potential explanations for the lack of positive findings in this study. These explanations include: inadequate length of intervention, incorrect strain of probiotic, inadequate dosage of probiotic, and possible ineffective treatment. The following sections address the possible explanations for the lack of positive results in further detail, with the intention of aiding the design of future research projects.

5.2.1 Inadequate length of intervention

The initial expected length of the intervention period was three weeks of probiotic consumption. As stated earlier, due to the late gestation of 36 weeks at which the participants were recruited and due to the unexpected nature of labour and birth, only seven of the 21 participants allocated to the intervention group were successful in completing the entire three-week intervention. In response, a subgroup analysis was completed including the participants in the intervention group who had completed 14 or more days of probiotics. A subgroup analysis examines the effects of the study on different subgroups within the study after analysing the main hypothesis of the study (Peat, 2001). This limited the participants in the intervention group to 17. One

of these participants was unable to collect a final vaginal swab due to the precipitous nature of her labour. This further limited the subgroup analysis to 16 participants in the intervention group.

The late gestation at recruitment—and the resulting large number of participants unable to complete the intervention—is an obvious shortfall of the study design. It was decided to recruit women in the final weeks of their pregnancies in order to utilise the vaginal GBS screening that was already being routinely performed at the study site at 36 weeks gestation. This choice was purely one of economic rationalisation. This study has made it obvious that this gestation was too late to commence the intervention.

Previous research which procured positive results with respect to bacterial vaginosis with the use of the same probiotic strains as the current study had an intervention period of between 28 and 60 days (Krauss-Silva et al., 2011; Reid et al., 2003; Reid et al., 2004; Anukam et al., 2006b; Martinez et al., 2009). This further emphasises that future research will need to intervene at an earlier gestation and aim for at least a four-week intervention period.

In addition, a secondary, unexpected finding of the study was that women who had taken daily probiotics for at least 14 days were found to have significantly more commensals in their vaginas in the probiotics group versus the control group. Commensals are normal vaginal flora, such as *Lactobacilli* (Barrons & Tassone, 2008; Lagenaur et al., 2011b; Rose et al., 2012). This finding suggests that possibly the probiotics had begun colonising the vaginas of the women in the intervention group with *Lactobacilli* but the colonisation was not significant enough to impact on the colonisation of GBS. As such, this finding sways the reason for the lack of

positive results towards a problem with the length of the intervention, with the dosage of the intervention or with both.

5.2.2 Inadequate probiotic dosage

Another possible explanation why the probiotics did not impact on the vaginal GBS colonisation rates of the participants in this study is that the probiotic dosage may have been too low. The women randomly assigned to the intervention group consumed a daily oral dose of the probiotics *Lactobacillus rhamnosus* GR-1 (GR-1) and *Lactobacillus fermentum/reuteri* RC-14 (RC-14) in a dose of 10^9 viable strains. Reid et al. (2003, 2004) report that this dosage positively impacted the vaginal micro flora of their participants when given daily for 60 days. Alternatively, Anukam et al. (2006b) and Martinez et al. (2009) both found GR-1 and RC-14 to positively impact the cure rate for bacterial vaginosis post-antibiotic treatment when given in a dosage of 10^9 viable strains *twice* daily for 30 days in its non-pregnant participants. These research projects would support the consideration of a twice daily dose, instead of a once daily dose, of probiotics if using a 30-day intervention period for future studies. The one challenge that may arise from increasing the probiotic dosage from once daily to twice daily in future studies would hedge around safety issues. In order for ethics approval to be obtained, it would be necessary to adequately prove to the ethics board that this increased dosage would not cause harm to the pregnant woman or her unborn child. The ethics process for this study took one year due to the justifiably weighty concern placed on the safety of women and their unborn infants by the ethics board when exploring emerging treatments in pregnancy. One stipulation placed on this study by the ethics board prior to approval was that a follow-up survey be completed six months after each participant's birth in order to further explore the issue of safety. Based on this experience, it can be anticipated

that an increase in dosage would also come under high levels of scrutiny by the ethics board.

5.2.3 Incorrect strain of probiotic

Another potential explanation for the lack of a significant change in vaginal colonisation rates in the participants who consumed daily oral probiotics is the possibility the study used the incorrect strain of probiotics (Lamont et al., 2011). The probiotic strains *Lactobacillus rhamnosus* GR-1 and *reuteri/fermentum* RC-14 were chosen because they have been shown to survive passage through the gastro-intestinal tract and to colonise the vagina after oral consumption (Morelli et al., 2004; Reid et al., 2001; Reid et al., 2003; Reid et al., 2004). This feature of these strains made them a justifiable choice for use in this study.

Recently, two separate studies have emerged which have performed in vitro experiments specifically investigating the impact of different strains of *Lactobacillus* on GBS (Bodaszewska-Lubas et al., 2012; Ruiz et al., 2012). The study by Ruiz et al. (2012) found that the *Lactobacillus* strains *rhamnosus* L60 and *fermentum/reuteri* L23 have probiotic potential for the control of vaginal GBS colonisation. The strains used by Ruiz et al. (2012) and the strains used in the present study are similar to the species level. The strains used in the present study are currently manufactured and readily available within the Australian market. All these factors continue to support the use of RC-14 and GR-1 in future studies.

On the other hand, probiotics have been shown to have strain-specific health benefits (Martinez et al., 2009; Pham et al., 2008; World Health Organisation, 2002). This makes room for the possibility that the probiotic strains used in this study, though they colonise the vagina, may not have the ability to impact on GBS colonisation. The second in vitro study by Bodaszewska-Lubas et al. (2012) found *Lactobacillus*

plantarum C11 to have the strongest antibacterial properties against GBS. In addition, Lamont et al. (2011), in their review on impacting genital tract flora using molecular based techniques, recommend that ‘future research should concentrate on the *Lactobacilli* that are prevalent in the vagina, rather than on species such as *L. fermentum* and *L. rhamnosus*’ (p. 538). These authors recommend focusing on the *Lactobacillus* strains *L. crispatus*, *L. iners*, *L. jensenii* and *L. gasseri* when conducting research to impact vaginal health. The use of *L. jensenii* can further be supported by research done using monkeys (Lagenaur et al., 2011a&b).

Since this is an emerging area of research, many new studies are being published each year investigating the impact of probiotics on the vaginal ecology of women. These studies are being completed using a variety of strains of *Lactobacilli* and using both oral and vaginal administration. Studies continues to support the positive potential of probiotics to modulate the vaginal microbiota. It will be interesting to discover which strain or combination of strains emerge as the best choice for vaginal health through future research (Ehrstrom et al., 2010; Marcone et al., 2010; Stojanovic et al., 2012; Vitali et al., 2012; Wang et al., 2010).

In summary, if an increase in length of intervention time and an increase in probiotic dosage did not improve the results of this study, it would be justifiable to explore the impact of other strains of probiotics on vaginal GBS colonisation prior to rejecting the hypothesis.

5.2.4 Ineffective treatment

The final possibility for the lack of significant results pertaining to the use of oral probiotics to impact vaginal GBS colonisation rates is that probiotics may be an ineffective treatment. Before arriving at this final conclusion, the other possible

explanations that have arisen from the study design need to be explored further with future research projects.

5.2.5 Conclusion to possible reasons for the lack of positive results

There is a very real possibility that the strains chosen for this study may not be the most effective probiotics for impacting vaginal GBS or that this may be an ineffective treatment for the control of vaginal GBS. However, the research, at this stage, indicates that the most logical explanations for the lack of positive results are the length of intervention and the dosage. Once these areas have been further investigated and no impact noted, a change of probiotic would then be justified. After all these avenues have been explored, it would be prudent to reject oral probiotics as a possible treatment for vaginal GBS.

5.3 Limitations to the study design

As stated earlier, this was a pilot project. It was intended to be the preliminary testing to determine the viability of the study design's ability to address the study hypothesis. The proposed hypothesis was that probiotics will decrease vaginal GBS colonisation rates in pregnant women. The fact that this study was a pilot project and that it was based on a pragmatic approach to research generates limitations regarding the results. This section expands on these limitations.

5.3.1 Sample size

One of the limitations present in this study was the small sample size. In total, 34 women were recruited over a three-month period. Initially, a power calculation was performed to determine the sample size necessary for a large randomised controlled trial (RCT). It was calculated that a sample size of 217 participants per arm of the study would be required to reach a 90 per cent significance rate. A pilot

study, with a small sample size, was utilised to determine the feasibility of the study design and of the recruitment strategy prior to committing the time and resources to a large RCT.

The small sample size in the study gives rise to the increased risk for Type 1 or 2 errors in the statistical results. A Type 1 error is when a true hypothesis is rejected and a Type 2 error is when a false hypothesis is accepted (Peat, 2001). Therefore, despite the small sample size, the findings of this study can serve to guide future research. The small sample size limits the generalisability of the results. It also means that the magnitude of the results needs to be cautiously interpreted in the context of their original purpose. This purpose was to serve as a guide for future study designs.

5.3.2 Uneven distribution of random allocation to control and intervention groups

Another complication that the small sample size contributed towards was the uneven distribution of the random allocation to the control and the intervention groups. A three-month data collection time frame was set, with an anticipated minimum number of 30 participants and an uncapped maximum number of participants to be recruited within this time frame. A simple randomisation strategy was utilised in the study (Kang et al., 2008). The randomisation process was computer generated by an online site. One hundred possible participants were randomised into group 1 or group 2. The participants allocated to group 2, the intervention group, were heavily weighted at the beginning of the randomised series. Therefore, of the 34 women recruited, 21 were randomised to the intervention group and only 13 to the control group. Though unplanned, this uneven distribution worked in well with the research because, when the results were limited to only the participants in the intervention

group who had completed 14 or more days of probiotics and who had completed the final vaginal swab, only 16 participants remained. The subgroup analysis, therefore, ended up with 13 women in the control group and 16 women in the intervention group.

In addition, despite the uneven distribution, when the two groups were statistically compared they were found to be similar in their antenatal demographics, length of time between first and second vaginal swabs, and gestation at birth. Since the two groups were comparable in these areas, this minimised the concern about external confounding factors that may have impacted the final vaginal swab results (Peat, 2001). The comparison of the final vaginal swab results was therefore justified.

Even though the uneven distribution did not appear to adversely impact the study, it did not produce ideal circumstances for data analysis. Simple randomisation strategies have been noted to be problematic in trials with small sample sizes, as they can produce unequal numbers of participants between groups. A block randomisation technique would have been more appropriate to control for equal distribution of participants between groups in this study (Kang et al., 2008). Alternatively, a larger sample size of greater than 100 participants would have eradicated the limitation produced by a simple randomisation method.

5.3.3 Timing of intervention

As discussed in detail in section 5.2.1, ‘Inadequate length of intervention’, the timing of the intervention was a glaringly obvious limitation of the study. The late gestation at which the participants were recruited and at which the intervention was commenced resulted in only seven of the 21 participants in the intervention group completing the entire 21 days of probiotics. This accounts for a 66 per cent attrition

rate. For future research studies, it is imperative that the recruitment and intervention commence much earlier in the third trimester of pregnancy.

5.3.4 Pragmatic design

A pragmatic approach is based on the fact that the ‘traditional criteria for scientific validity do not in themselves guarantee usefulness to practitioners’ (Worren et al., 2002, p. 1228). As such, even if results can be produced in a laboratory, these results do not necessarily translate into knowledge that is applicable in real-life settings and useful for improving clinical practice. A pragmatic approach is used to determine whether an intervention will be successful when implemented under normal circumstances (Peat, 2001; Steen & Roberts, 2011). This study design adopted a pragmatic approach to research.

Initially, in the planning stages of this study, consultation was completed with the microbiology department of the St. George/Sutherland hospitals. During this consultation period, a variety of swab collection and testing techniques were explored to determine the level of GBS and *Lactobacilli* counts in women’s vaginas. One option that was explored was testing vaginal swabs with polymerase chain reaction (PCR). PCR is a technique that amplifies the DNA sequences of a sample to accurately identify the presence of specific organisms (Edwards et al., 2008). This would be the most sensitive and accurate form of testing to investigate the micro flora present in a woman’s vagina. The major drawback of this testing is the cost. This method would also have required the researcher to collect every vaginal swab from every participant. This would have been logistically very difficult to achieve. It also would have increased the invasiveness of the study sampling methods, as research indicates that women prefer to collect their own vaginal swabs (Mercer et

al., 1995; Price et al., 2006). Despite these drawbacks, PCR still does remain a possible option for future research studies.

After the microbiology consultation, and in light of the drawbacks, a more basic, pragmatic approach was agreed upon. It was decided to utilise the study site's standard collection and testing techniques for GBS vaginal swabbing for the research project. By doing so, the project was able to take advantage of the GBS vaginal swab results routinely collected by all pregnant women at the study site at approximately 36 weeks gestation.

The concerns that arose through using this approach revolved around the concepts of consistency and specificity. The routine practice at the study site was that each pregnant woman collected her own vaginal swab at a gestation of approximately 36 weeks. Self-collection is considered less invasive and has been shown to be preferable to women (Mercer et al., 1995; Price et al., 2006). This called into question the consistency of the swabbing collection techniques, since each woman would collect her swab in a slightly different way. One manner in which this issue was addressed was through consistent information distribution. In the antenatal clinic, an informative brochure about GBS, including instructions on how to collect a vaginal swab, was given to every woman when it was necessary for them to complete the swab. This information allowed for a degree of consistency in swabbing techniques with the different participants.

In addition to consistent information distribution, each participant not only collected her routine vaginal swab at 36 weeks but also self-collected her final vaginal swab for the purposes of the study. This procedure allowed for a degree of internal consistency because each individual participant collected both her own swabs.

The second concern with the pragmatic approach was the specificity of the testing techniques. The results of the standard vaginal swab testing at the microbiology labs of the study site only specify the presence of a light, moderate or heavy growth of GBS. They do not give numerical quantities. They also only report on the presence of commensals, or normal vaginal flora, without specifying the identity of the flora. This lack of specificity did not allow for the study to monitor any subtle changes in the colonisation rates of vaginal GBS in the participants.

In defence of this choice, clinically, medical professionals are only concerned with the presence or absence of GBS. They are not concerned with the specific quantities. Therefore, in order to make the results of this study immediately relevant in the clinical setting, which is the basis of a pragmatic approach, the study also only focused on the presence or absence of GBS and the presence or absence of commensals.

Although the pragmatic approach raised concerns regarding the consistency of swab collection techniques among the participants and regarding the specificity of the GBS results, it did utilise the standard processes undertaken at the study site. Since these standard processes were used, it made the results immediately applicable, relevant and understandable in the clinical setting to the clinicians who would be utilising such information. As such, though the pragmatic approach limits the results of the study, this approach can also be considered a strength of the research design. The design flexed to work within the context of the clinical setting, instead of manipulating the clinical setting to accommodate the study.

5.4 Strengths of the study

Despite the lack of positive results and the limitations of this research, strengths also exist. The major strength of this study is its contribution to the growing body of

knowledge supporting the safety of probiotic use in later pregnancy. The other strengths are that it continues to justify the further investigation of the benefits of probiotic use on vaginal GBS in pregnancy and it aids in the development of future research designs. The following sections discuss these points in further detail.

5.4.1 Safety considerations

The lack of adverse events noted in this study with probiotic use in the third trimester of uncomplicated pregnancies is a major strength of this study. The safety indicators utilised in this study were adapted from the indicators used in two systematic reviews conducted on the safety of probiotic use in pregnancy (Allen et al., 2010; Dugoua et al., 2009).

The results showed that there were no differences between the intervention and control groups with respect to the safety indicators investigated at and around the time of birth. These safety indicators included birth weight, gestation at birth, mode of delivery, pre-labour spontaneous rupture of membranes and infant concerns post birth. The only significant result that was produced in the statistical analysis was that women in the control group underwent assisted deliveries more often than women in the probiotics group. Assisted deliveries include instrumental deliveries and emergency caesarean sections. The significance remained even when the participants were limited to nulliparous women, who research indicates have a greater incidence of assisted delivery (Baskett et al., 2008). As mode of delivery was one of the safety indicators, the higher probability of achieving a normal birth in the probiotics group further supports the safety of the intervention.

The second set of data collected investigating the safety of the intervention was through a follow-up telephone survey at approximately six months after the birth of each of the participant's infants. All 34 of the participants participated in the survey.

Not one was lost to follow-up. The survey found that no differences existed between the two groups relating to the overall health of the infants of the study participants at six months of age. These findings further support the evidence that probiotic use in later pregnancy produces no adverse events in healthy women experiencing uncomplicated pregnancies.

5.4.2 Justification for further research

Another strength of this study is it suggests further research in this area may be justified. Commensals can be defined as normal vaginal micro flora, such as *Lactobacilli* (Barrons & Tassone, 2008; Lagenaur et al., 2011b; Rose et al., 2012). The significant presence of commensals in the vaginas of participants who had taken daily probiotics positively reflects on the potential of probiotics to impact vaginal GBS. The justification for this study was based on multiple, consistent research findings which found that women with higher vaginal colonisations of *Lactobacillus* are more likely to have no detectable vaginal GBS colonisations (Altöparlak et al., 2004; Donders et al., 2000; Kubota et al., 2002; Takeyoshi et al., 2002; Whitney et al., 2004). This finding from the current study supports the possibility that the consumption of oral probiotics can result in the colonisation of the vagina with *Lactobacillus*. This increases the potential then, in turn, to reduce or eradicate the presence of GBS from the vagina. The door is still wide open for further research on the use of probiotics to impact vaginal GBS.

5.4.3 Assistance in the development of study designs

This study's significant finding of commensals in the vaginas of women who had consumed probiotics, as discussed in the previous section, leads us on to the study's third strength. This strength is the information that this research provides in aiding

the development of future study designs. Since this study had the unexpected finding of showing an increase of vaginal commensals with probiotic use, future design recommendations would include the presence of commensals as a research indicator. They would also revolve around strategies for increasing the commensal colonisations to a point where they can compete with and impact upon vaginal GBS. These strategies, as discussed earlier in this chapter, include increasing the length of the intervention, increasing the dose of the intervention and possibly changing the species of *Lactobacillus* used.

This study was always intended to be a pilot project. It was developed to be the initial research attempt for the purpose of testing the viability of this study design for future research. As such, this project fulfilled its purpose. It was successful in generating valuable information to help guide the actions of future researchers in their design attempts.

5.5 Conclusion

In conclusion to the discussion chapter, the aim of this study was to complete a pilot project to ascertain if the research design was appropriate to determine whether a daily oral dose of probiotics can reduce the rate of vaginal group B streptococcal (GBS) colonisation in pregnancy. This research study has contributed valuable information that has added to the growing body of knowledge around probiotic use in pregnancy. It did generate multiple avenues of exploration for future research. These avenues include research designs which incorporate longer intervention times, higher probiotic doses and different probiotic strains. These future designs may also incorporate larger sample sizes, different sampling techniques and different swab testing techniques. Many possibilities for future research are apparent from this pilot project.

In addition, a major strength of the current study was that its findings have provided further support for the safety of probiotic use in later pregnancy by healthy women experiencing normal pregnancies. All the findings of this study coincide to declare that the opportunity still remains for future research to uncover the preventative potential of probiotics for vaginal GBS in pregnancy.

6 CONCLUSION

6.1 Introduction

The final chapter of this thesis reiterates the findings of the study in light of the original aim of the study: to complete a pilot project to determine if the research design of this study was appropriate to determine if a daily oral dose of probiotics can reduce the rate of vaginal group B streptococcal (GBS) colonisation in pregnancy. This chapter also reflects on the study's success at achieving this aim. It summarises the potential reasons for the lack of a reduction in vaginal GBS with oral probiotic use that was seen in this study. This chapter also presents the implications this study has for future research projects as well as for clinical practice.

6.2 Summary of findings

The main finding of the study was that there was no significant difference in vaginal GBS colonisation rates in women who had consumed a daily oral dose of probiotics as compared to women who had not. This lack of significant difference remained even when a subgroup analysis was performed including only those participants who had consumed 14 days or more of probiotics. One unexpected finding from the subgroup analysis was that significantly more women who had taken probiotics had commensals colonising their vaginas ($p=0.048$). Commensals are normal vaginal flora, such as *Lactobacilli* (Barrons & Tassone, 2008; Lagenaur et al., 2011b; Rose et al., 2012).

Another significant finding was that there were no differences between the probiotics group and the standard care group with respect to the safety indicators investigated. This lack of difference in the safety indicators was present throughout all stages of the research trajectory, including the antenatal period, labour and birth, the postnatal period and six months post birth.

6.3 Achievement of study aims

The hypothesis of the study asked the question: if *Lactobacillus* colonisation rates can be increased in pregnant women's vaginas, could this result in a decrease in group B streptococcal (GBS) colonisation rates? The aim of the study was to pilot the appropriateness of this research design to determine whether a daily oral dose of probiotics can reduce the rate of vaginal group B streptococcal (GBS) colonisation in pregnant women.

As stated in section 6.2, 'Summary of findings', no significant difference was found in vaginal GBS colonisation between the participants who consumed daily oral doses of probiotics and those who were in the control group. Even though there was a lack of positive results, this study still attained its aim. The study was investigating the appropriateness of this specific research design in addressing the question raised by the hypothesis. The investigation has shown that this is not an effective study design to trial the impact of oral probiotics on vaginal GBS colonisation.

However, this research, through its design, has laid foundational groundwork for future research. The main drawback of the study design was the length of intervention. Only seven of the 21 women recruited to the probiotics group were successful in completing the entire three-week intervention. This limitation was due to the uncontrollable nature of labour and birth and the late gestation at which women were recruited. This is one obvious area which has the opportunity to be addressed with future research and already the findings have been disseminated through national and international conferences (Appendix I). Other areas that may have contributed to the lack of positive results were a potentially inadequate probiotic dosage and a potentially incorrect probiotic strain.

6.4 Recommendations for future research

The finding of significantly more women who had consumed 14 or more days of probiotics with vaginal commensal colonisation when compared to the control group continues to support the potential of oral probiotics to impact the vaginal micro environment. Future research possibilities exist investigating the impact of probiotics with longer intervention times, higher doses of probiotics and different strains of probiotics. Future research should plan to recruit women at an earlier gestation in their pregnancies. Future designs may also incorporate larger sample sizes, different sampling techniques and different swab testing techniques. Many possibilities for future research are apparent from this pilot project.

6.5 Implications for clinical practice

The major implication for clinical practice that has arisen through this research is it has provided further evidence to support the lack of adverse events with probiotic use in later pregnancy by healthy women experiencing normal pregnancies. Even though no significant positive results were obtained through this specific research study, the potential of probiotic use in pregnancy remains. This project continues to reinforce the use of probiotics by pregnant women if they choose to pursue this avenue of health. Due to the results of this study, clinicians can support women in these choices with reduced concerns about the safety of probiotic use in later pregnancy.

6.6 Final remarks

Although this pilot project did not succeed in providing evidence that oral probiotic usage in pregnancy can impact vaginal GBS colonisation, it did achieve its piloted aim, which was to determine the validity of the study design used. This aim was achieved in this thesis by highlighting the limitations in the study design, which will aid in guiding the development of future research designs. The results of this study

did not close the door on the potential use of probiotics to impact vaginal GBS, but rather opened the opportunity for future research in this field. The opportunity still remains for research to uncover the preventative potential of probiotics for vaginal GBS in pregnancy.

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8 APPENDICES

8.1 Appendix A: Antenatal promotional poster



The hospital is doing research to try and answer this question:

Can Probiotics Improve the Bacteria in your Vagina?

- ❖ 20% of pregnant Australian women have a bacteria in their vaginas called Group B Streptococcus (GBS)
- ❖ GBS does not hurt the women but babies who are infected during labour and birth can become seriously ill
- ❖ We are seeking to find out if a daily oral dose of probiotics can prevent this infection
- ❖ You can be involved if you are over 18 yrs old, are having a normal healthy pregnancy, and have been found to have GBS in your vagina at 36 weeks pregnant
- ❖ If you are interested in participating in this research, then please talk to your doctor or midwife

8.2 Appendix B: Participant Information Sheet



University of Wollongong

PARTICIPATION INFORMATION SHEET FOR PREGNANT WOMEN

Title: Study Investigating the Impact of Oral Probiotic Use on Vaginal Group B *Streptococcal* Rates in Pregnant Women: A Pilot Randomised Controlled Study

Purpose of the Research

This is an invitation to participate in a study conducted by a Master's Research Student (Paula Olsen), under the supervision of experienced researchers, at the University of Wollongong. The purpose of this research is to determine if taking a certain strain of oral probiotics can decrease the number of pregnant women with a bacterial colonisation called Group B *Streptococcus* (GBS) in their vaginas. Probiotics are live micro-organisms that pass on a health benefit to the person taking them. GBS infections may cause serious illness in some newborn babies. If this study is successful it will show that there may be a way to prevent GBS colonisation of the vagina. It could then become the basis of a larger research study in order to prove the effectiveness of probiotics in treating GBS vaginal colonisation.

Investigators

Paula Olsen	Dr Moira Williamson	Professor	Don
Iverson	Dr Chris Georgiou		
Registered Midwife/Masters Student	Health & Behavioural Sciences	Pro Vice-	
Chancellor (Health)	IHMRI/Grad School Medicine		
	(02) 4221- 3381	(02)	4221-
4677	(02) 4222-5000		
Pgc589@uow.edu.au	moiraw@uow.edu.au		daynah@uow.edu.au
georgiou@uow.edu.au			

Demands on Participants

If you decide to be involved in the research you will be randomly chosen to either continue with your standard antenatal care or continue with your standard care plus to take the oral probiotics *Lactobacillus* Rhamnosus GR-1 and *Lactobacillus* Reuteri RC-14 daily for three weeks from about the thirty-sixth week of your pregnancy.

You will also be required to participate in a telephone survey when your baby is 6 months old. This survey should take about 10 minutes of your time.

Possible Risks, Inconveniences and Discomforts

If you are randomly chosen to be in the probiotics group, you will have the inconvenience of taking oral probiotics daily for three weeks. The probiotics will be provided to you free of charge. All participants will be required to collect an additional vaginal swab at about 39 weeks pregnant.

There is a small chance that probiotics can cause some infections; these infections generally only occur in people who have underlying chronic illnesses. There is no expected risk to your baby. Research studies have been done testing probiotics in pregnancy. Most of these studies have been different from this current study because they have investigated whether taking probiotics in pregnancy can reduce the chances of infants developing allergies. None of these studies have reported any adverse events from the probiotics. The specific strains of probiotics to be used in this study have not previously been tested in pregnancy. They have been tested in healthy women who are not pregnant and have resulted in no adverse events. These strains are theoretically safe for use in pregnancy but their absolute safety has not yet been proven through research studies. The follow-up telephone survey will help to prove the safety of these probiotics by providing the researchers with the chance to ask questions about the well-being of yourself and your baby after being involved in the study.

It is current hospital policy that all women found to have GBS in their vaginas receive intravenous antibiotics in labour. This is an initial study to test if probiotics have any impact on GBS in vaginas. As a result, all women participating in the research will still be encouraged to have intravenous antibiotics in labour in accordance with current hospital policy. Any results from this study will not alter the treatment you will receive during your pregnancy or your labour.

Your involvement is completely voluntary and you are free to withdraw your participation and your information at any time. If you choose not to participate or choose to withdraw from participation this will not impact the care you receive during your pregnancy at the Sutherland Hospital. All information gathered throughout the research will be kept strictly confidential.

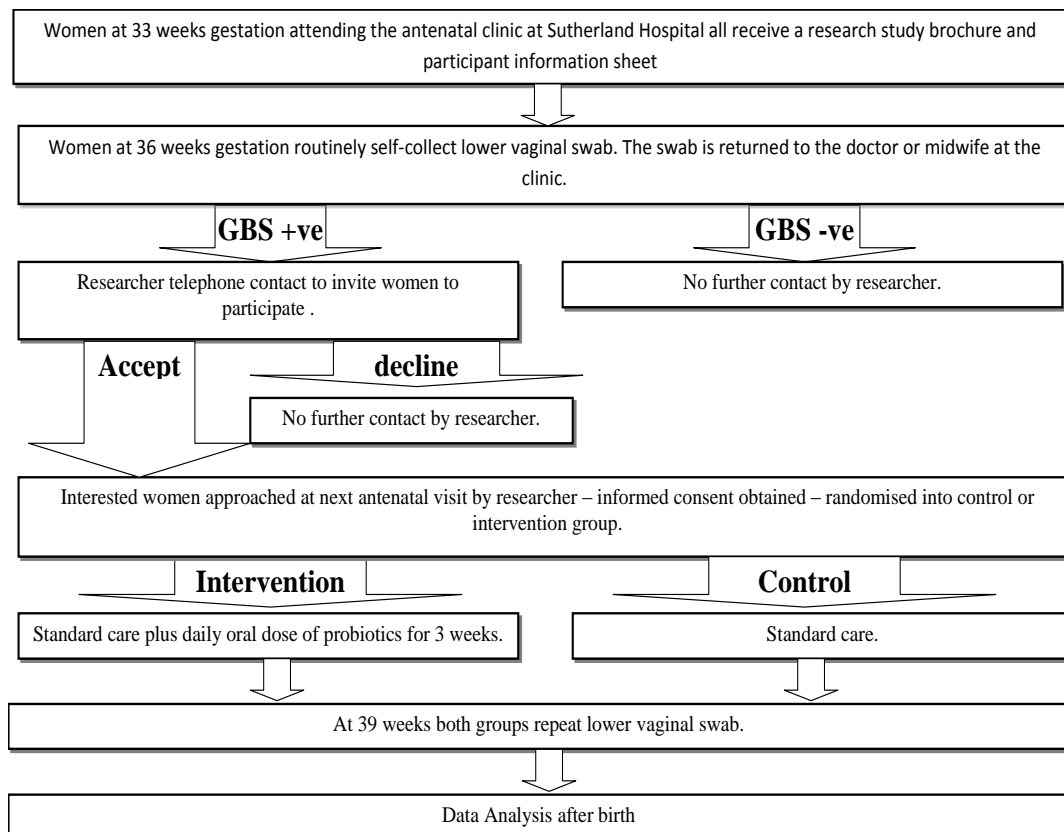
Funding and Benefits of the Research

The funding for this research is being provided by the Edith Cavell Trust. If probiotics are found to reduce the rate of GBS in pregnancy this will give women a possible strategy to prevent GBS vaginal colonisation. It also may decrease the number of women receiving intravenous antibiotics in labour as a result reducing the number of newborns exposed to this bacterium. This study will provide preliminary evidence on which to base a larger, more definitive research study.

Ethics Review and Complaints

This study has been reviewed by the Human Research Ethics Committee of the University of Wollongong. If you have any concerns or complaints regarding the way this research has been conducted, you can contact the University of Wollongong's ethics officer on (02) 4221-4457.

8.3 Appendix C: Diagram of research design



8.4 Appendix D: Participant data collection sheet

Probiotics and GBS Research Form

Coding # _____
 Date of entry _____
 MRN _____
 EDC _____
 Gestation on entry _____
 Parity _____
 BMI _____
 Ethnicity _____
 Age _____
 Past hx of GBS _____

RESEARCH GROUP (Please Circle)

❖ **STANDARD CARE PLUS PROBIOTICS**

❖ **STANDARD CARE**

Did you take any antibiotics during the trial period? For how long?

Were you able to take the probiotics every day? How many days were missed?

Did you have any adverse side effects to the probiotics? Provide details.

Pregnancy & Birth

Complications in pregnancy?

What kind of birth and why?

Given antibiotics while in labour (why or why not)?

What was the baby's birth weight?

Final Swab Result

8.5 Appendix E: Consent form



University of Wollongong

CONSENT FORM FOR PREGNANT WOMEN

The impact of oral probiotic use on vaginal Group B *Streptococcal* rates in pregnant women: a pilot randomised controlled study

Researcher: Paula Olsen

I have been given information about “*The impact of oral probiotic use on the vaginal Group B Streptococcal rates in pregnant women*” and discussed the research project with the researcher. I understand that Paula Olsen is conducting this research as part of a Master of Midwifery (Research) degree and is being supervised by Dr Moira Williamson, Professor Don Iverson and Dr Chris Georgiou from the University of Wollongong.

I have been advised of the potential risks and burdens associated with this research, which includes the possible inconvenience of taking a daily dose of oral probiotics for three weeks. It has been explained to me that there is an extremely low risk of developing some form of infection from the probiotics and these infections generally only occur in people with underlying chronic illness. There is no anticipated risk to myself or my developing infant. I have had the opportunity to contact Paula Olsen with any questions I may have about the research and my participation.

I understand that my participation in this research is voluntary, I am free to refuse to participate and I am free to withdraw from the research at any time. My refusal to participate or withdraw consent will not affect the care I receive during my pregnancy at Sutherland Hospital in any way.

If I have any enquiries about the research, I can contact Paula Olsen on and Dr Chris Georgiou on (02) 4222-5000 or if I have any concerns or complaints regarding the way the research is or has been conducted, I can contact the Ethics Officer, Human Research Ethics Committee, Office of Research, University of Wollongong on 4221 4457.

By signing below I am indicating my consent to:

- being randomly chosen to either continue with the standard antenatal care or to receive standard care plus taking orally the probiotic strains *Lactobacillus Rhamnosus* GR-1 and *Lactobacillus Reuteri* RC-14 for three weeks from approximately 36 weeks gestation.

I understand that the data collected from my participation will be used for a thesis, journal articles, conference presentations and a larger research study. I consent for it to be used in these manners. I understand that my identity will not be disclosed through any of these uses.

Signed

Date

.....

...../...../.....

Name (please print)

.....

8.6 Appendix F: Information brochure

Did you know...



- The Sutherland hospital is doing research, in collaboration with IHMRI, into whether oral probiotics can prevent Group B *Streptococcus* from colonising pregnant women's vaginas
- If your vaginal swab result at 36 weeks shows that you have Group B *Streptococcus* you may receive a phone call from a researcher to see if you would be interested in being involved in this study.
- If you receive a phone call and do **NOT** want to be involved just tell the researcher. You will not be contacted again and it will not impact the care you receive at the clinic in any way.
- If you would like to be involved, the researcher will meet with you the next time you are at the hospital. You will be able to ask the researcher any questions you may have at this time.
- Women who would like to be involved will be randomly chosen either to continue with the usual antenatal care or to continue with the usual antenatal care & receive an oral dose of probiotics daily for three weeks. All women who are involved will then recollect their own vaginal swab at 39 weeks into the pregnancy.

8.7 Appendix g: Follow-up telephone survey at six months

Follow-up Telephone Survey at Six Months

Preamble

Hello, may I speak to _____ please? It's Paula Olsen calling. You were involved in a research project at the Sutherland Hospital during your pregnancy. I am just calling to ask some questions about you and your baby's health over the past six months. These questions should take about 10 minutes to answer. Is this a convenient time or would another time be better for you?

Background Information

How is the baby fed?

- Breast
- Formula
- Combination

How long was the baby exclusively breastfed for?

Are there any smokers in the house?

Do you keep any pets in the house?

Does the baby go to child care?

Do you have any family history of allergies, asthma or eczema?

Infant

Did the baby have any health concerns at birth?

How many times have you taken the baby to the GP?

Has the baby ever been hospitalised?

Have you given any medications to your baby?

Has the baby had any signs of allergies? Eczema? Asthma? Allergic Rhinitis?

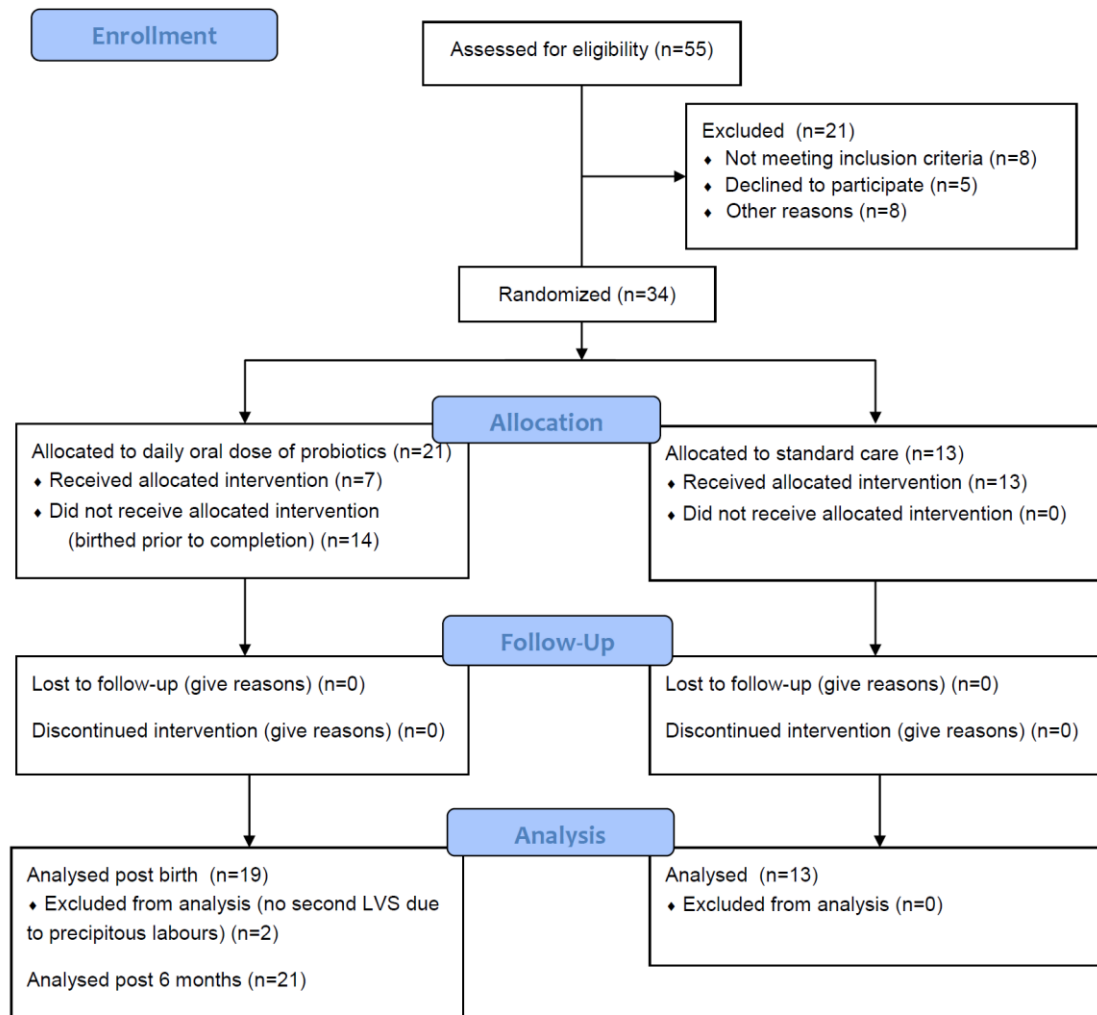
How would you describe your baby's health?

- Very healthy
- Occasionally unwell
- Nearly always unwell

Any other health concerns that you would like to mention regarding your baby?

8.8 Appendix H: Consort Flow Diagram

CONSORT 2010 Flow Diagram



8.9 Appendix I: Successful Conference Abstracts

1. Asia Pacific midwives conference 2009, Hyderabad, India—oral presentation

Developing Natural Ways for Preventing Group B Streptococcal Vaginal Infections in Pregnancy

Group B Streptococcus (GBS) is an organism that has been recognized in the developed world since the 1970s as the leading cause of neonatal sepsis. GBS infection is vertically transmitted from an asymptomatic mother to her infant during labour and birth. Considering the serious consequences of neonatal GBS infection, strategies for prevention have so far focused on administration of antibiotics to women in last week of pregnancy or administering antibiotics during labour.

The disadvantages of these strategies are the development of antibiotic resistance in GBS and non-GBS pathogens, the disruption of the colonization of the neonates' gut with the appropriate flora, the risk of maternal anaphylaxis, the deficit in maternal knowledge regarding GBS and the medicalization of birth. It is commonly understood that administering intravenous antibiotics in labour to prevent neonatal GBS is only an intermediate solution until better solutions are developed.

In response to these facts and the seriousness of GBS infection, it becomes apparent that future research is necessary to determine natural ways of increasing Lactobacillus colonisations in women's vaginas in order to decrease GBS colonisation rates and to protect their infants from exposure to GBS infections. This presentation will discuss natural ways in which GBS vaginal infections can be prevented.

2. Australian College of Midwives National Conference 2011, Sydney, Australia— Poster presentation

The impact of oral probiotic use on vaginal Group B *Streptococcal* colonisation rates
in pregnant women: A pilot randomised controlled study

Background

Group B *Streptococcus* (GBS) is the leading cause of bacterial infections in the neonate in the developed world. This bacterium is passed from a woman's vagina to her new-born during the process of labour and birth, potentially causing pneumonia, septicaemia and meningitis in the infant. In order to prevent neonatal GBS infections most hospitals have protocols in place that identify pregnant women at risk of infecting their new-borns and give these women intravenous antibiotics in labour to inhibit the transmission. The disadvantages of intravenous antibiotics in labour are the development of antibiotic resistance in GBS and non-GBS pathogens, the inhibition of the colonisation of the new-born's gut with the appropriate flora and the medicalisation of childbirth.

It has been shown that women with higher colonisation of vaginal *Lactobacillus* are more likely to have no detectable vaginal GBS. This raises the hypothesis: Would increasing the colonisation rates of *Lactobacillus* in pregnant women's vaginas result in a decrease in GBS colonisation rates? A pilot randomised controlled trial is proposed to determine if oral probiotics may be a strategy for decreasing GBS vaginal colonisation rates by increasing vaginal *Lactobacillus* rates.

Methods

A sample of thirty GBS positive pregnant women will be recruited. The GBS status of these women will be determined by the routine lower vaginal swabs that are self-collected by all women in the antenatal clinic at the proposed hospital site at thirty-six weeks gestation. These women will be randomised into control and intervention groups. The control group will continue with standard care; the intervention group will receive standard care and take a daily dose of oral probiotics for three weeks. After three weeks both groups will repeat the self-collection of a lower vaginal swab.

It is anticipated that the data collection phase will take approximately three months. A telephone survey will be performed at six months postpartum in order to verify the safety of the intervention.

Expected Outcomes

It is expected that a significant number of women in the intervention group will be GBS negative after three weeks of oral probiotics when compared with the control. A positive outcome would then become the basis of a larger randomised control trial.

3. Breathing New Life into Maternity Care conference 2012, Melbourne, Australia—poster presentation

The impact of oral probiotic use on vaginal Group B *Streptococcal* colonisation rates in pregnant women: A pilot randomised controlled study

Background

Group B *Streptococcus* (GBS) is the leading cause of bacterial infections in the neonate in the developed world. This bacterium is passed from a woman's vagina to her new-born during the process of labour and birth, potentially causing pneumonia, septicaemia and meningitis in the infant. In order to prevent neonatal GBS infections most hospitals have protocols in place that identify pregnant women at risk of infecting their new-borns and give these women intravenous antibiotics in labour to inhibit the transmission. The disadvantages of intravenous antibiotics in labour are the development of antibiotic resistance in GBS and non-GBS pathogens, the inhibition of the colonisation of the new-born's gut with the appropriate flora and the medicalisation of childbirth.

It has been shown that women with higher colonisation of vaginal *Lactobacillus* are more likely to have no detectable vaginal GBS. This raises the hypothesis: Would increasing the colonisation rates of *Lactobacillus* in pregnant women's vaginas result in a decrease in GBS colonisation rates? A pilot randomised controlled trial is proposed to determine if oral probiotics may be a strategy for decreasing GBS vaginal colonisation rates by increasing vaginal *Lactobacillus* rates.

Methods

Thirty-five women found to have GBS colonisation in their vaginas at 36 weeks gestation were recruited into the study. These women were randomised into control and intervention groups. The control group continued with standard care and the intervention group continued with standard care in addition to receiving a daily oral dose of probiotics. Three weeks after recruitment or while in labour, depending on which occurred first, a lower vaginal swab was collected. These swabs were sent to pathology to be tested for the presence of GBS. Six months post the birth of their infants, the participants then engaged in a follow-up telephone survey to determine the safety of the intervention.

Outcomes

The data analysis phase of the project is ensuing at the moment. The results of these findings will be presented. These results will include an emphasis on the safety of probiotic use in pregnancy, an area of great current interest in obstetrics.

4. International Confederation of Midwives conference 2014, Prague, Czechoslovakia—oral presentation

The impact of oral probiotic use on vaginal Group B *Streptococcal* colonisation rates in pregnant women: A pilot randomised controlled trial

Aim

The main hypothetical question was: Can a daily oral dose of probiotics reduce the rate of vaginal Group B Streptococcal (GBS) colonisation in pregnancy? The aim was to test the viability of this specific study design to address the hypothesis.

Methodology

A pilot randomised controlled trial was performed which recruited 34 GBS positive women at approximately 36 weeks pregnant. The participants were randomly allocated to the control group, which continued with standard antenatal care, or to the intervention group, which continued with standard antenatal care and received a daily oral dose of probiotics for 3 weeks or until the birth of their infant. A lower vaginal swab, to determine the presence of GBS, was collected 3 weeks post consent or when a participant was in labour.

Results

No significant difference was found in vaginal GBS between the control and intervention groups. Only 7 of 21 in the intervention group completed the entire 21 days of probiotics. A sub-group analysis, including only those who had completed 14 days or more of probiotics, also showed no significant difference in vaginal GBS when compared to the control. It did show significantly more vaginal commensals in the probiotics group.

Discussion

There are 4 possible reasons for the lack of significant results:

- The length of the intervention was too short.
- The dosage of the probiotics was too low.
- The wrong strain of probiotics was used.
- Oral probiotics are ineffective at impacting vaginal GBS.

Implications

The significant increase of vaginal commensals (normal vaginal flora, including Lactobacilli) in women who had completed 14 days or more of probiotics continues to support the potential of probiotics to impact GBS in pregnancy. This pilot project supports the safety of probiotic use in later pregnancy. Many possibilities remain for future research to further investigate the use of probiotics to impact vaginal GBS.