Effects of \textit{B. infantis} on the Maternal Restraint Model of Depression
Sarah Barker\textsuperscript{1}, Lori Driscoll\textsuperscript{1} \\
\textsuperscript{1}Colorado College, Colorado Springs, Colorado, 80903

Depression during pregnancy is a significant public health issue, with 18\% of pregnant women suffering from depressive symptoms. Prepartum depression is correlated with health complications in the mother, and with physical and behavioral abnormalities in the child. Psychotherapy provides relief to some, but access and compliance issues limit its efficacy, and the safety of antidepressant use during pregnancy has not been conclusively demonstrated. An alternative approach is to address the health of the gut brain axis, a bidirectional signaling pathway between gut microbiota and the central nervous system. Gut dysbiosis, an imbalance in gut microbe composition, is linked to psychiatric disorders, especially anxiety and depression. The consumption of probiotics, microbes beneficial to health, could potentially provide relief to pregnant women with depression and subsequently protect the child from deleterious effects of prepartum depression. \textit{Bifidobacterium infantis 35634 (B. infantis)} has been studied in the context of irritable bowel syndrome and reduces systemic inflammatory biomarkers. The present study explored the role of the gut microbiome in prenatal and early postnatal development by exploring the effects of \textit{B. infantis} supplementation on the maternal restraint model of depression in rats. Maternal restraint for 45 min three times per day, for six to seven days, significantly increased dams’ depressive behavior. Maternal restraint additionally increased offspring weights and depressive behavior. \textit{B. infantis} supplementation tended to rescue these deficits, although effects did not reach significance. Additional research with a larger sample size is warranted to elucidate the efficacy of this intervention in improving the physical and psychological health of mother and infant.

Abbreviations: CNS – Central Nervous System; FST – Forced Swim Test; SSRI – Selective Serotonin Reuptake Inhibitor

Keywords: Pre-Partum Depression; Gut Brain Axis; Probiotics

Introduction

Prepartum depression, characterized by somatic symptoms, difficulty concentrating, poor sleep and fatigue, diminished appetite, and suicidal thoughts, is a significant public health issue, with 18\% of pregnant women showing elevated depressive symptomology (Newport et al., 2007; Wichman and Stern, 2015; Yonkers et al., 2009) and 12.4\% of pregnant women experiencing a major depressive episode during pregnancy (Le Strat et al., 2011). Risk factors for perinatal depression include low socioeconomic status, poor social support, and general life stress (Lancaster et al., 2010). Despite the ubiquity of this phenomenon, approaches to reducing or alleviating pregnant women’s distress are lacking. Patients or clinicians may attribute the depressive symptoms to hormone fluctuations and fail to seek or provide help. Additionally, the safety of pharmacological treatment for depression during pregnancy is contested in the field (Stuart-Parrigon and Stuart, 2014). Even when women are screened and informed of their diagnosis, only 20\% choose to pursue treatment, and if they choose pharmacological treatment, they may choose to take subclinical doses in fear of harming the fetus (Marcus, 2009), thus subjecting the mother and the fetus to the
potential harmful effects of both depression and medication (Flynn et al., 2006).

Prepartum depression can result in health complications for the mother, including a higher risk of preeclampsia, in which high blood pressure in the mother necessitates early delivery (Qiu et al., 2007). Prepartum depression is also associated with inadequate pregnancy weight gain, increased substance abuse, poor nutrition, and underutilization of prenatal care (Allister et al., 2001; Barker et al., 2013; Marcus, 2009), which can affect the fetus. Biochemical sequelae include lower placental levels of nerve growth factor (Kaihola et al., 2015), decreased levels of monoamine oxidase A, which can lead to increases in serotonin in the fetus (Blakeley et al., 2013), and higher cortisol and norepinephrine levels and lowered dopamine levels (Lundy et al., 1999). These chemical changes can manifest in physical and physiological deficits in the newborn, such as premature birth, lower birth weight (Field et al., 2004; Hoffman and Hatch, 2000; Li et al., 2009; Steer et al., 1992), decreased head circumference, higher baseline fetal heart rate (Allister et al., 2001), and miscarriage (Arck, 2001; Michel-Wolfromm, 1968). Infants of depressed mothers also score lower on the Apgar test of physical condition, and demonstrate greater right frontal EEG asymmetry (Diego et al., 2004), an early marker of poor emotional and motivational health (Coan and Allen, 2004), than do infants of non-depressed mothers. Behavioral changes also manifest in infants born to mothers with depression. These infants score below normal on the Brazelton Scale, a test that assesses infants’ motor and interactive capacities, arousal control, and response to stress (AIs et al., 1977; Diego et al., 2004; Field et al., 2004). Short term irritability and affective problems are also more frequent in the offspring of depressed mothers (Yonkers et al., 2009); these manifest as increased stress behaviors compared to babies of mothers without depression and babies of mothers who suffered only from postpartum depression (Diego et al., 2005). This strongly suggests that depression during pregnancy, not just a depressed mother’s parenting after delivery, impacts the behavior of the child. These changes persist across development: the risk of depression is 4.7 times greater for offspring exposed to prepartum depression than for those who are not exposed (Pawlby et al., 2009).

Unfortunately, depression is very prevalent (15.4%) in women of reproductive age in general (Dawson et al., 2016), and this has implications for medication use when these women do become pregnant. In recent years, there has been an increase in the percentage of women who continue taking antidepressants during pregnancy, from 5.7% in 1999 to 13.4% in 2004 (Cooper et al., 2007). To wean off of or continue to take medication is a very difficult decision for doctors and patients, because data are contradictory regarding the risks of using antidepressants during pregnancy (Millard et al., 2017). The most current research available in 2014 suggests that there there have been no randomized clinical trials of antidepressant medication during pregnancy (Stuart-Parrigon and Stuart, 2014). In the non-randomized studies that have been conducted, some report that antidepressant use during gestation is associated with premature birth and low birth weight (Källén, 2004; Simon et al., 2002; Zeskind and Stephens, 2004), whereas others report no difference (Hendrick et al., 2003; Kulin et al., 1998). The data are also equivocal for cognitive and emotional outcomes, with some studies reporting impairments in infants exposed prenatally to a selective serotonin reuptake inhibitor (SSRI) (Casper et al. 2003; El Marroun et al., 2014; Gemmel et al., 2018; Hanley et al., 2013; Lattimore et al., 2005), and some reporting no effects (Nulman et al., 1997). Because the relationship between antidepressant use in pregnancy and child outcomes is unclear, many women discontinue medication. Of the women who discontinue treatment during pregnancy, 68% experience relapse of major depression compared to a 26% relapse rate in women who continued medication (Cohen et al., 2006). Due to the possible detrimental effects of both depression and antidepressants on the mother and the child, a safe and efficacious treatment is needed. Currently, psychotherapy is the most common treatment for prepartum depression. The most efficacious treatment for depression is a combination of psychotherapy and antidepressants, with an 85% response rate (Keller et al., 2000), which is problematic due to the potential risks of medication during
pregnancy. Additionally, psychotherapy can be prohibitively expensive and time-consuming.

Recent appreciation of the significant role of gut health in modulating mental health provides a potentially safe and effective treatment option. In particular, evidence for the link between gut and brain health is evident in comorbidities between disorders of the gastrointestinal tract, such as irritable bowel syndrome, and disorders of the central nervous system (CNS), such as depression and anxiety (Mayer et al., 2001). Communicating with the brain is the “second brain” of the enteric nervous system, coupled with the massive ecosystem of gut microbes.

The human gut contains one to two kilograms of microbes comprising 15,000-36,000 species. The gut microbiota (i.e., the collection of all microbial species in the gut) helps maintain bodily homeostasis, and its dysregulation has been implicated in obesity, inflammatory bowel syndrome, cancer, and neurological disorders (Lozupone et al., 2012). Microbiota in the gut communicate with the CNS through immune, endocrine, and neural pathways, and they have been shown to exert influence on CNS functioning (Lozupone et al., 2012). The immune system responds to microbiotic products such as histamine, serotonin, and corticotropin releasing factor, to send inflammatory signals to the CNS and the rest of the body (Mayer, 2011). Microbiota also communicate with the CNS by activating the hormonal hypothalamic-pituitary-adrenal (HPA) axis (Slyepchenko et al., 2014). This blood-borne pathway is bidirectional. Inducing stress in animals changes the composition of the microbiota in the gut (Bailey and Coe, 1999; Bendtsen et al., 2017), leading to increases in the permeability of the intestinal wall and dysregulation of the enteric nervous system (Gareau et al., 2008; Teitelbaum et al., 2008). In rodent models, stress during pregnancy disrupts the maternal microbiota (Jašarević et al., 2017). These changes in the maternal microbiota are also seen in humans and are passed to the child (Zijlmans et al., 2015). Detrimental changes in the microbiota are correlated with hyper-responsiveness of the HPA in rats (Golubeva et al., 2015) and with negative temperament changes in human children (Christian et al., 2015).

The abundance and diversity of gut microbes impacts emotion and behavior, and altering the composition of gut microbes using probiotics, capsules of viable and beneficial microorganisms, has been shown to impact both the brain and mental health (Schrezenmeir and de Vrese, 2001). Treatment with a probiotic in animal models of anxiety and depression restores brain norepinephrine and cortisone levels and rescues anxious and depressive behaviors (Bravo et al., 2011; Desbonnet et al., 2010; Marin et al., 2017; Messaoudi et al., 2011). This process is mediated, at least in part, by the vagus nerve, a mixed afferent and efferent cranial nerve that innervates the viscera, as animals with a severed vagus nerve do not show these probiotic effects (Bravo et al., 2011). Probiotics are also efficacious for improving depression and anxiety in humans. Probiotic administration decreases psychological distress (Messaoudi et al., 2011), improves Depression Anxiety and Stress Scale (DASS) scores (Mohammadi et al., 2016), and decreases symptoms of anxiety in patients with chronic fatigue syndrome (Rao et al., 2009). Therefore, probiotics might be an efficacious treatment for depression and anxiety in pregnant women, and the benefits may extend to the fetus.

In the current study, restraint-induced prepartum depression in a rodent model was treated with daily supplementation of Bifidobacterium infantis 35634 (B. infantis) during and after pregnancy. Restraint-induced prepartum depression creates direct stress in the mother, and an indirect influence on the fetus, but it does not induce miscarriage (Darnaudery and Maccari, 2008). B. infantis has been studied primarily in the context of inflammatory bowel syndrome, in which it has been demonstrated to change the gut microbiome, reduce visceral pain, and reduce systemic inflammatory biomarkers (Charbonneau et al., 2013; Groeger et al., 2013; Mckernan et al., 2010; Whorwell et al., 2006). Bifidobacterium produces neurotransmitters and neuropeptides and has been shown to normalize tryptophan and pro-inflammatory cytokine concentrations associated with depression (Desbonnet et al., 2008). In addition, this probiotic is readily available over the counter and is affordable, increasing accessibly to treatment. In the current study, we hypothesized that prepartum stress would produce increased...
depressive behavior in the forced swim test (FST, an assay for rodent depressive behavior) in the mothers and their offspring, and that this depressive behavior would be rescued by the probiotic. If *B. infantis* reduces the levels of depression in the mothers and offspring, this probiotic should be further explored as a viable treatment for depression during pregnancy.

**Material and Methods**

**Animals**

Animal protocols were approved by the Institutional Animal Care and Use Committee at Colorado College (Protocol 2017-004-LLD). Long Evans rats (*Rattus norvegicus*, Blue Spruce stock; Envigo, Inc.) were housed under a standard 12 hr:12 hr light cycle in a temperature range of 70-78° F and a humidity range of 30-40% in cages lined with Teklad Laboratory Grade 7090 Sani-Chips (Envigo, Inc.). Teklad 16% Protein Rodent Diet (Envigo, Inc.) and tap water were available ad libitum. Female rats (N=16) who met the criteria of weighing at least 250 g were paired for eight days with male rats (N=16). Pregnant females (N=16) were housed in individual cages, and they and their offspring were used in behavioral testing. Nine litters were selected to continue the experiment based on day of delivery. Pups were weaned and moved to individual housing on postnatal day 21 (PND21). Two to four pups from each litter were chosen for behavioral testing, to yield sufficient numbers of males (n=17) and females (n=15) in the total sample (N=32), with eight pups in each treatment condition.

**Probiotic Supplementation**

Supplementation with over-the-counter Align probiotic (Proctor & Gamble, Inc.) containing 1 x 10⁹ cfu of *B. infantis* began on the first day following breeding. Probiotic powder from one Align pill per rat was mixed into 1 ml of Snack Pack brand chocolate pudding and administered to dams via a plastic spoon suspended from the cage ceiling daily until pups were weaned at PND21. Half of the pregnant females received the probiotic mixed with chocolate pudding (n=8) and half received only chocolate pudding (n=8). After weaning, probiotic was directly administered to the pups in accordance with the treatment administered to their dams: half of the pups (n=16) received probiotic mixed with pudding, and half (n=16) received only pudding daily until the conclusion of the experiment. Pudding consumption was monitored daily, and rats consumed the pudding daily without exception. Treatment continued throughout offspring development to increase the power of the experiment.

**Prenatal Stress Procedure**

On approximately prenatal day 14, pregnant females in the stress group (n=8) were restrained in clear pastry bags (Wilton, Inc.) with the tip cut off to allow for breathing for 45 min, 3 times per day (9 A.M., 12 P.M., and 5 P.M.) in a lighted environment until delivery (i.e., for 6 to 7 days). This protocol, described by Ward and Weiss (1984), creates direct stress in the mother, and an indirect influence on the hyperactivity of the fetal HPA and on fetal levels of progesterone, testosterone, and corticosterone, but it does not induce miscarriage (Darnaudéry and Maccari, 2008).

**Weight**

Pregnant female rats were weighed daily throughout pregnancy. Litters were weighed together and average pup weight was calculated from PND0 to 21. After pups were weaned, sex was recorded and individuals were weighed every four days until completion of experiment.

**Forced Swim Test (FST)**

The FST is designed to assess depressive behavior in rats and is sensitive to anti-depressant manipulations (Slattery & Cryan, 2012). The dams were administered a modified FST, as detailed by Slattery & Cryan (2012), the day before breeding and again five days after delivery. A single FST session was conducted on the offspring between PND43 and PND48. The apparatus consisted of a clear cylindrical plexiglass tank with a diameter of 30.48 cm and a height of 45.72 cm. The tank was filled with 30 cm of tap water at 23-25 °C. Rats underwent a habituation session one day prior to the FST in which each rat swam in the tank for 15 min. The following day, the five-minute testing session
was recorded by a video camera mounted above the tank. Animals were gently lowered into the cylinder to begin testing. After completion, the animals were dried using towels, and returned to their home cage. Weight of the tank and a lack of a draining mechanism prevented changing the water between every trial. Instead, fecal boli were removed after each rat.

FST data were analyzed using the time sampling technique described by Slattery and Cryan (2012) by two independent raters who were blind to the experimental conditions with established inter rater reliability (Cronbach’s $\alpha = 0.87$ for percentage of time spent immobile). Each five second interval was rated for the most prominent behavior (immobility, swimming, or climbing). Immobility is characterized by the rat only moving to maintain its head above the water; immobility is a model of human “helplessness”. Swimming is characterized by horizontal movement around the tank. Climbing is characterized by vertical movement on the sides of the tank. Swimming and climbing behaviors are indicative of the rat actively operating on its environment to escape. The frequency of each behavior was converted to percent of total time for each behavior and averaged between the two raters.

A mixed ANOVA was performed comparing prepartum and postpartum immobility, swimming, and climbing percentages as a function of restraint and probiotic status for dams (restraint/ B. infantis, restraint/vehicle, no restraint/ B. infantis, and no restraint/vehicle). For pups, factorial ANOVAs were performed with factors of restraint, probiotic, and sex. Analyses were conducted using SPSS v.24 (IBM Corporation).

**Results**

**Maternal Outcomes**

Mixed ANOVAs were performed with between-subjects factors of restraint (yes, no) and probiotic (yes, no). There were no main effects of restraint, $F(1,9)=4.46$, $p>0.05$, or probiotic $F(1,9)=0.23$, $p>0.05$, and no significant interaction between restraint and probiotic, $F(1,9)=0.05$, $p>0.05$, on dam weight gain during pregnancy when controlling for litter size. There were no main effects of restraint, $F(1,9)=0.03$, $p>0.05$, or probiotic, $F(1,9)=2.60$, $p>0.05$, on litter size, nor was there an interaction between restraint and probiotic, $F(1,9)=0.09$, $p>0.05$.

Overall, dams spent half of the time immobile during the FST ($M=51.85\%, SD=17.57$). There was a significant main effect of restraint on postpartum FST immobile time, $F(1,9)=8.97$, $p=0.03$, with dams who underwent restraint spending more time immobile ($M=63.33\%, SD=8.74$) than dams who did not undergo restraint ($M=37.50\%, SD=15.06$). However, overall immobility was not influenced by probiotic treatment, $F(1,9)=0.59$, $p>0.05$, nor was there an interaction between restraint and probiotic, $F(1,9)=0.00$, $p>0.05$. As expected, there were no significant differences between prepartum and postpartum swimming and climbing times for any groups (See Figure 1).

![Figure 1](image.png)

**Figure 1:** Mean percentage of time spent immobile prepartum and postpartum in the FST in dams. Increased immobility indicates behavioral helplessness.

Prepartum and postpartum FST scores were compared in each group using descriptive statistics, due to small sample size, to assess the efficacy of the maternal restraint procedure in producing depressive behaviors. (see Table 1).

<table>
<thead>
<tr>
<th></th>
<th>Prepartum</th>
<th>Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Restraint/B. infantis</td>
<td>37.92 ± 9.46</td>
<td>62.92 ± 7.66</td>
</tr>
<tr>
<td>No restraint/B. infantis</td>
<td>38.75 ± 25.34</td>
<td>44.16 ± 1.18</td>
</tr>
<tr>
<td>Restraint/vehicle</td>
<td>51.67 ± 9.46</td>
<td>63.61 ± 11.10</td>
</tr>
<tr>
<td>No restraint/vehicle</td>
<td>22.50 ± 12.96</td>
<td>30.83 ± 22.39</td>
</tr>
</tbody>
</table>
Table 1. Descriptive statistics for pre and postpartum freezing percentages in the forced swim test in dams.

**Offspring Outcomes**

The postnatal data were broken into early development (PND1 to 9) and PND 10 to 21 because the pups were weight individually through PND 9 and averaged as a litter after that. A factorial ANOVA was performed with between-subjects factors of restraint (yes, no) and probiotic (yes, no) for offspring from PND0 to 21. After PND21, sex was also included as a factor for the offspring. Supplementation with *B. infantis* increased pup weights from PND1 to 9, with pups whose mothers received probiotics (*M* = 11.41 g, *SD* = 0.92) weighing more than pups whose mothers received the vehicle (*M* = 9.55 g, *SD* = 1.50; *F*(1,72) = 40.13, *p* < 0.001). Restraint stress also affected pup weights in an unexpected direction. On PND1 to 9, pups whose mothers underwent restraint had significantly greater weights (*M* = 11.00 g, *SD* = 1.12) than pups whose mothers did not undergo restraint (*M* = 9.96 g, *SD* = 1.84; *F*(1,72) = 12.54, *p* < 0.001; see Figure 2). However, there was no significant interaction between restraint and probiotic supplementation for PND1 to 9 pup weights, *F*(1,72) = 1.20, *p* > 0.05.

Later in the postnatal period (PND10 to 20), effects of restraint and probiotic stronger in male offspring. Probiotic supplementation...
M. ch is in accordance with those who underwent restraint and reduces litter size (Baker et al., 2008; Darnaudéry et al., 2004; Van den Hove et al., 2006). Neither of these effects were found in the present study, possibly due to differences in procedure: dams in the current study only underwent restraint for six days instead of the nine days utilized in the other studies.

The effects of maternal stress and probiotic supplementation in the offspring were not as pronounced as they were in the dams. _B. infantis_ supplementation significantly increased offspring weight from PND1 to 9. This is the first known report of _B. infantis_ effects on weight gain in pups of treated dams. Maternal restraint also significantly increased offspring weight from PND1 to 9, which is in accordance with some previous studies (Mueller and Bale, 2006), but not others (Barlow et al., 1978). Studies show that maternal stress may increase risk of offspring obesity, in accordance with these findings (Salsberry and Reagan, 2005; Tamashiro et al., 2009).

The above effects of maternal stress and probiotic supplementation diminished from PND9 to 21, in accordance with findings from previous studies (Mueller and Bale, 2006). Conversely, this diminished effect could also be due to a decrease in statistical power, as pups were weighed individually from PND1 to 9, but only average litter weights were recorded for PND9 to 21 due to personnel shortage in the colony during that time. However, the observed effect was still in the expected direction, with pups of restrained dams and pups receiving probiotic supplementation weighing more.

After weaning on PND21, weights were recorded individually. Males consistently weighed more than females, as expected. Effects of maternal restraint and probiotic supplementation continued to be nonsignificant. Although no statistically significant differences between offspring groups were found, the patterns in the data varied by sex. There was a significant effect of sex, with females showing more depressive behavior in the FST than males, consistent with previous research (Bogdanova et al., 2013). Male offspring showed consistent levels of depressive behavior across groups. However, in the females, the who underwent prenatal stress showed the highest amount of depressive behavior. This sex dependent FST behavior in prenatally stressed rats is generally more pronounced in males than in females, in contrast to the results from this study (Darnaudéry and Maccari, 2008; Morley-Fletcher et al., 2003; Van den Hove et al., 2006).

<table>
<thead>
<tr>
<th></th>
<th>% Freezing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Restraint/ <em>B. infantis</em></td>
<td>59.38 ± 14.58</td>
</tr>
<tr>
<td>No restraint/ <em>B. infantis</em></td>
<td>47.78 ± 10.30</td>
</tr>
<tr>
<td>Restraint/ Vehicle</td>
<td>66.88 ± 9.31</td>
</tr>
<tr>
<td>No restraint/ Vehicle</td>
<td>58.96 ± 8.29</td>
</tr>
</tbody>
</table>

Table 2. Descriptive statistics for female offspring percent freezing in the forced swim test in dams. 

\[ F(1,32)=5.73, p=0.03, \] with females spending more time immobile (\(M=58.64\%, SD=11.78\)) than males (\(M=45.34\%, SD=16.10\)) on average.

Additionally, the restraint and _B. infantis_ supplementation had a stronger effect on female offspring (see Table 2). However, these differences were not significant. No significant differences were seen between groups for time spent swimming and climbing.

**Discussion**

This study is the first to explore the effects of _B. infantis_ supplementation on prenatal stress in dams and their offspring. The restraint procedure was used to induce learned helplessness, a behavior indicative of depression, in the dams. As intended, dams who underwent restraint demonstrated increases in depressive behavior from the prepartum forced swim test to the postpartum forced swim test. This effect was not likely due to the stress induced by delivering pups, as postnatal dams and virgin controls have not been found to differ significantly in immobility scores in the FST (Craft et al., 2010). However, the probiotic did not rescue the depressive behavior. This is contrary to previous work in which _B. infantis_ had an antidepressant effect in the maternal separation model of depression (Desbonnet et al., 2010). However, the sample size in the current study was small (N=9), and therefore, the data must be interpreted with caution. _B. infantis_ had no effect on neurotypical (no induced depression) rats’ depressive behavior, as seen in previous studies (Desbonnet et al., 2008).

Previous studies found that maternal restraint reduces maternal weight gain throughout pregnancy and reduces litter size (Baker et al., 2008; Darnaudéry et al., 2004; Van den Hove et al., 2006). Neither of these effects were found in the present study, possibly due to differences in procedure: dams in the current study only
Effects of *B. infantis* on the Maternal Restraint Model of Depression

2018

*B. infantis* supplemented offspring showed the lowest amount of depressive behavior. The prenatally stressed female pups who were supplemented with the probiotic showed reduced depressive behavior compared to stressed female pups who received the vehicle.

There are several potential reasons why the offspring effects did not reach statistical significance. First, the small sample size for dams (N=9) and offspring (N=32) limited statistical power. Additionally, the maternal restraint procedure was only performed for 6 days, whereas most previous studies carried out this procedure for at least a week (Baker et al., 2008; Mueller & Bale, 2006). This likely reduced the impact of stress on both the dams and offspring. Additionally, the effects of prenatal stress exposure vary depending on the genetic background of the subjects (Boersma and Tamashiro, 2015). For example, rats can be bred for heightened or attenuated HPA-axis activity (Sternberg et al., 1992). Prenatal stress has no effect on FST behavior in rats bred for heightened HPA-axis activity, and it reduces immobility time in rats bred for attenuated HPA-axis activity (Stöhr et al., 1998). The rats used in the present study are not an inbred strain and could have had different levels of HPA-axis activity, possibly contributing to the large variability seen in the data.

It cannot be concluded whether the results did not reach significance due to insufficient power or an incorrect hypothesis. Further research into the cross generational effects of *B. infantis* on the maternal restraint model of depression is warranted. Previous research strongly supports the hypothesis that the effects of prenatal stress on depressive behavior are modulated by the gut-brain axis (Golubeva et al., 2015; Jašarević et al., 2015; O’Mahony et al., 2017; Zijlmans et al., 2015). Additionally, *B. infantis* has been shown to have antidepressant effects in rats that have experienced early life stress in the form of maternal separation (Desbonnet et al., 2008; 2010). In the current study, significant decreases in depressive behavior with *B. infantis* supplementation may have been found with a larger sample size, with the use of an inbred strain with known levels of HPA-axis activity, or with application of the maternal restraint procedure for at least seven to nine days. Future studies should also test for locomotive effects to ensure the FST results are due to alterations in depressive behavior and not motor function.

**Acknowledgements**

Research funded by a Venture Grant from Colorado College. Special thanks to Kris Erdal for all his work as colony keeper and to Nisha Venkateswaran and Nina Holley for assistance with supplementation and coding.

**Corresponding Author**

Sarah Barker
Colorado College
www.coloradocollege.edu
14 East Cache la Poudre St. Colorado Springs, CO 80903
Sarah.barker@coloradocollege.edu

**References**


Barker ED, Kirkham N, Ng J, Jensen SKG (2013) Prenatal maternal depression symptoms and


Effects of *B. infantis* on the Maternal Restraint Model of Depression

2018


