Pre and postnatal maternal distress and infant gut immunity in the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort

by

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Abstract

Introduction: Secretory immunoglobulin A (sIgA) plays a critical role in gut mucosal immunity and is a marker of immune maturation in early life. Delayed IgA production is associated with increased risk of allergic diseases. Animal models of stressful events before birth and during infancy show changes in the maternal vaginal microbiome and in intestinal microbial composition of offspring. A human study found infants born to mothers with greater prenatal stress more likely to have gut dysbiosis, imbalance in microbial composition, but there is a gap in literature on stress-microbiome-immunity pathways in humans. This study investigated differences in infant fecal sIgA levels according to the depression and stress status of the mother pre and postnatally.

Methods: Data were obtained from a sub-sample of 403 term infants from the Vancouver, Edmonton and Winnipeg sites of the general cohort in the Canadian Healthy Infant Longitudinal Development (CHILD) Study. Mothers of the infants were enrolled during pregnancy and were asked to report stress and depressive symptoms through scored-scales administered to the general CHILD cohort at several time points throughout pregnancy and postpartum. Center for Epidemiologic Studies Depression (CES-D) Scale ascertained depressive symptoms, and Perceived Stress Scale (PSS) determined perceived stress. Infant stool samples were collected at a mean age of 3.8 months, and fecal sIgA levels were measured using the Immundiagnostik sIgA ELISA kit. Using IBM SPSS version 24, Mann-Whitney U-tests detected median differences in IgA levels according to maternal distress status, and logistic regression models estimated the odds of lowest and highest quartile, lowest tertile and below median sIgA in infants.

Results: About 12% of women had prenatal depressive symptoms, 9% had symptoms postpartum and 9% had symptoms both pre and postnatally. With perceived stress scores in the highest quartile, 16.7% of mothers fell in this category at prenatal only, 10.2% for postnatal only and 18.7% for both pre and postnatal. Mothers with any depressive symptoms had infants with significantly lower sIgA compared to mothers without symptoms (p=0.004). Median sIgA levels are 6.3 (IQR=3.6 – 12.4) mg/g feces, 5.2 (IQR = 2.0 – 9.8) mg/g feces, 5.7 (IQR = 2.6 – 8.6) mg/g feces, 4.4 (IQR = 2.4 – 8.0) mg/g feces for exposure to less symptoms (below cut-off), prenatal, postnatal, both pre and postnatal symptoms respectively, which are mutually exclusive categories. Median sIgA levels with higher stress were not significantly different compared to the reference group, but after stratification for the presence of other children and pets at home, significant differences were seen when the mother had both pre and postnatal higher stress. The odds of low fecal sIgA was 3.07 times higher when the mother had both pre and postnatal symptoms (95% CI: 1.25 – 7.55) compared to less symptoms (reference group), after controlling for infant age, antibiotics exposure, maternal asthma or allergy, prenatal SSRI use, pets, breastfeeding and gravida. In the same model, the odds ratio for prenatal only depressive symptoms was 2.44 (95% CI: 1.07 – 5.57). No significant odds ratios were found with higher perceived stress.

Conclusion: Infants born to mothers with higher levels of depressive symptoms and perceived stress appear to have lower fecal sIgA levels independent of various study covariates. Due to the association with lower sIgA and possibly changes in the infant gut microbiome, maternal distress may put infants at higher risk of later development of allergic diseases.

Preface

This thesis is an original work by Liane Joo Kang. The thesis was written in a traditional thesis format according to the guidelines of the Faculty of Graduate Studies and Research at the University of Alberta. Another version of this thesis is in preparation for submission to the Journal of Allergy and Clinical Immunology titled as "Maternal perceived stress and depressive symptoms is associated with reduced secretory immunoglobulin A levels in Canadian infants".

Chapter 1 is the introduction that consists of a literature review on maternal distress, infant gut immunity and potential covariates between the association of distress and gut immunity, study objectives and hypotheses and sample size calculations.

In Chapter 2, the relevant methods of the CHILD study and the information on variables used for the study on distress and gut immunity are outlined. Chapter 3 includes the results of this quantitative study. The final chapter (Chapter 4) contains the discussion and conclusions. This chapter contains the main findings and interpretation of the findings from Chapter 3, the strengths and limitations of the study, the clinical significance and implications for future research.

Dedication

Dedicated to my family, friends, mentors and my heavenly Father

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I would like to thank my mentor and supervisor, Dr. Anita Kozyrskyj, for accepting me as her student and for supporting and teaching me in every step during my Master's program. Also, I would like to thank my supervisory committee members, Dr. Eytan Wine and Dr. Gerald Giesbrecht for guiding me and providing their knowledge and expertise.

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Table of Contents

List of Tables and Figures

Chapter 1: Introduction

1.1 Distress, Asthma and Allergic Diseases

The prevalence of asthma and allergies is increasing worldwide, and these conditions remain as a public health concern in both children and adults (1-3). Although various factors may lead to the development of these diseases, many studies show a relationship between distress (stress, depression and anxiety) and allergic diseases (4-7). A meta-analysis of 10 studies on prenatal maternal stress (higher perceived stress, stress exposure or difficult life circumstances) and child asthma and wheezing, where four studies were on asthma, reports a significant OR of 1.56 for respiratory morbidity with the exposure of prenatal stress, supporting the negative impact of maternal psychological stress on the respiratory health of the child (4). Andersson's systematic review of 16 studies, primarily prospective cohort studies, found support for the association between maternal stress during pregnancy and atopic diseases in children, but there were diverse measures for the exposure and outcome (5). Many questions still need to be answered about the mechanistic links between distress and allergy and asthma development. One of the possible links is through the development of the infant gut microbiome, as early changes in the infant microbiome have been linked to asthma and allergies. This thesis will observe the associations between pre and postnatal distress (stress and depression) in mothers and gut immunity in infants.

1.2 Maternal Distress

1.2.1 Prenatal distress

Major depression during pregnancy affects approximately 10% of women based on a systematic review of 28 studies from developed countries (8). However, the prevalence of

psychosocial stress during pregnancy is not known but has been reported to be common in an ethnically diverse sample of pregnant women (9). A small minority of pregnant women receives care for when they have depression (10), and stress is not routinely measured in obstetric care (9). Distress may be occurring despite the fact that both pre and postnatal distress are detrimental to the cognitive, socio-emotional and psychomotor development in the infant (11). Valid psychometric measures of stress and anxiety are needed for clinical care; however, Bann et al. (12) was able to identify the Perceived Stress Scale, Pregnancy Experience Scale and State Trait Anxiety Inventory – Trait-Anxiety Subscale as reliable and valid measures of higher levels of stress and anxiety in nulliparous pregnant women.

1.2.2 Prenatal distress impact on fetal development

According to the DSM-5, the symptoms during a depressive episode include depressed mood for the majority of the day almost every day, clinically significant weight loss or gain, inability to sleep or oversleeping, loss of energy, feelings of worthlessness or inappropriate guilt, diminished ability to think or concentrate and lower interest in activities (13). These symptoms, occurring at clinically significant levels for a pregnant woman, may pose health risks on both the pregnant mother and the developing fetus. Lack of nutritional foods in the mother's diet could lead to poor transfer of nutrients from placenta to fetus, affecting fetal development, and lead to iron deficiency, oxidative stress, chronic inflammation and smaller birth size in the children (14). Increased stress may elevate glucocorticoid transfer between mother and fetus via the placenta due to maternal distress having the potential to downregulate enzymes that prevent transfer (14, 15). Increased fetal exposure to glucocorticoids may impact the responsivity of stress

neuroendocrine and immunological systems in the child later by affecting density of receptor sites and epigenetic programming (14, 15).

1.2.3 Postnatal distress

Overall prevalence of postpartum depression is 19%, but prevalence differs from country to country (8, 16). Like prenatal depression, majority of affected women do not seek treatment for their symptoms of sadness and anxiety, reduced energy leading to less activity, loss of enjoyment and interest, changes in appetite and sleeping patterns, and feelings of inadequacy when caring for their infant (10, 17, 18). Mothers with prenatal distress or with a history of mental disorders are more likely to develop postpartum depression (19). However, the impact of distress differs depending on the time period. New mothers may be at higher risk because of the stress that comes with the initiation of parenting (20). A study found that there is a common tendency for depressive symptoms in the postpartum period to peak after the first month of the infant's life (20). It should also be acknowledged that maternal depression during the perinatal period is rather heterogeneous as several different symptom profiles and trajectories have been found with different risk factors and child outcomes, but there is limited information as to which specific symptoms and combinations contribute to the heterogeneity (21).

1.2.4 Postnatal distress and mother-infant interactions

Mothers who are depressed are less likely to interact with their infants most likely due to the symptoms of low self-esteem and loss of concentration (22). Maternal depression has been associated with greater instances of abuse and neglect on the child (23). This lack of interaction with the child affects the mother-child bonding and attachment and is associated with both short-

term and long-term child health outcomes (24). In 2-4 month-old infants raised by mothers with depressive symptoms, there were reduced odds of parental behaviours that are important for child development, such as playing and conversing with the child, and there was lower likelihood and reduction of breastfeeding, the gold standard for infant feeding (22, 25). Maternal distress has been shown to affect the stress reactivity of the infant through affected maternal behaviours potentially leading to epigenetic changes in the infant (26).

Postpartum maternal distress may also affect the environment in which the child develops. Santos et al. (27) reported significant differences in the quality of the home environment between mothers with high and lower levels of distress based on social-emotional and stimulation characteristics, but this study was focused on preterm infants. Stressful environments during the infants first year of life may impact the HPA axis and immune development of the infants (28, 29).

1.2.5 Long-term outcomes to mother and child

Some studies have found that toddlers did not appear to be affected behaviourally or developmentally by maternal depression (30). However, child developmental delays in language and cognitive function and poor child mental health have been seen with maternal depression (23, 31). A recent systematic review of 16 studies on adolescent outcomes found that adolescents exposed to postnatal depression at infancy had higher rates of attentional difficulties and conduct problems but found inconsistent reports for psychosocial outcomes (23). The review also included two studies from a research group that found higher depression incidence at age 16 and

cortisol levels at age 13 with postnatal depression, mediated by postnatal depression and insecure attachment during infancy.

Common co-morbidities with depression, asthma and allergic diseases, have been found in children that were born to or raised by distressed mothers (5, 32). Caregiver stress is a major predictor and mediating risk factor of wheeze in children (33, 34). A recent publication on the association between postpartum distress and asthma in a nationally representative sample of Canadian infants (7), found the association diminished with child age, which may suggest for less concern for mothers that are worried about the effects of their distress on their child's health. The directionality and causality of the association between poor mother-infant relationships and asthma have yet to be determined due to the lack of empirical studies (28).

1.3 Gut Microbiome Development in Early Life

The early gut microbiota composition in infants, rather than later composition, is considered important to the development of the immune system (35). The *in-utero* fetal gut microbiome development may allow for the development of the gut immune system in the fetus during pregnancy (36). Microbial seeding in the infant gut is currently thought to start *in-utero* through sources from the placental and maternal gut microbiome that can be transferred through the bloodstream and swallowed by the infant in amniotic fluid (36), as shared microbial composition features are found between the placental and amniotic samples with infant meconium (37).

The newborn gut is first colonized by aerobes and facultative anaerobes that are tolerant to the aerobic environment in the newborn gut, and these colonizers create an anaerobic environment for other microbes, obligate anaerobes, to thrive in the gut (36). Initially, the infant

gut has lower microbial diversity and greater variability between individuals than the adult gut. Eventually at around one year of age, the infant gut microbiome shows some similarities with the adult gut (36). Differences in infant gut microbiome composition in the first few months of life have been shown between vaginal and C-section births, breastfed and not breastfed and with environmental factors. Infants born from vaginal births more likely have maternal vaginal and fecal bacteria colonized in their gut; whereas, those born via C-sections have maternal skin and hospital bacteria colonized in the gut (36). Breastmilk also contributes to the establishment of gut microbiome in infants, as breastfeeding is linked to reduced gut microbial diversity in 3-month infants (38), due to the human milk oligosaccharides (HMOs) that favour higher abundance of microbes from the genera *Bifidobacteria* and *Lactobacillus* (39). Formula-fed infants have higher gut microbial diversity and have microbes from the genera *Bacteriodes, Veillonella* and *Clostridium* (36). Environmental factors, such as the presence of older siblings and pets, have been shown to affect the infant gut microbial composition (40).

1.4 Infant Gut Secretory IgA

1.4.1 Role in gut immunity

Secretory immunoglobulin A (sIgA) is an antibody that is produced by plasma B cells in the Peyer's patches, lamina propria and cecal patches in the gut, and IgA response against antigens are thought to involve both T cell-dependent and independent mechanisms (41). Secretory IgA is also present in other secretions, such as breastmilk, tears and mucus. The structure of sIgA differs from serum IgA, and plays a more decisive role in mucosal immune defense (42). As the first line of defense in the gut, sIgA plays a critical role in the gut mucosal immune system in early life because it prevents pathogens from entering the gut serum through

immune exclusion and allows for the induction of oral tolerance to commensal gut bacteria at early life (42). Secretory IgA can change the expression of surface molecules in commensal bacteria to reduce inflammation, but the exact mechanisms have yet to be established (43). On the other hand, sIgA may allow for transcytosis of pathogenic microbial species via Microfold cells (M cells) so that they can be degraded by other immune cells (43). Animal studies show that supplementing sIgA in mice that are deficient in sIgA can adequately restore the microbiota composition, which suggests a possibility of the development of immunoglobulin-based therapies that can restore optimal microbiota composition (44, 45). Therefore, sIgA seems to play a key role in shaping the gut microbiota composition in early life, and because of its significant role, sIgA is considered a marker of immune maturation in infants (46).

The initial infant gut microbiota composition is also thought to be important to the development of the immune system (35). Germ free mice, mice that do not have any microbes, are lacking in sIgA-producing B cells (47). However, when there is colonization of microbes in these mice, sIgA plasma cells are seen again. Certain microbial species can also stimulate sIgA production in the gut, and thus factors affecting gut microbial composition may also affect the production of sIgA. This suggests interplay between gut microbiota composition and gut immunity. Mice provided with lactic acid bacteria, such as *Lactobacillus* and *Bifidobacterium* species, had increased both serum and secretory IgA production as well as increased the number of Peyer's patches (48). Proteobacteria, for instance pathogenic *Escherichia coli* and *Salmonella enteritidis*, have been reported to promote sIgA production in the intestine (49, 50).

1.4.2 Breastmilk sIgA

Newborns have limited production of sIgA in the first few weeks of life (42, 51); therefore, breastmilk provides sIgA to protect the child from pathogens during this vulnerable period. Breastfeeding has had significant associations with IgA levels in later months of infancy (52). Six-month old infants when breastfed secreted higher levels of salivary sIgA than formula-fed infants, which suggests breastfeeding may help stimulate the production of sIgA in infants (53). In addition, after the cessation of breastfeeding, changes in microbiota composition in 12-month infants indicate the importance of sIgA in breastmilk in shaping and driving the maturation of the infant gut microbiome (54). An experimental study on sIgA-deficient dams found changes in gut microbiota composition in the pups that lasted to adulthood as well as a compromised gut epithelial barrier at early life that may prevent sensitization to microbe antigens from an immature immune system (55). This study suggests that sIgA from breastmilk given to the infant may influence the development of the gut microbiome and immune system that could influence later health.

1.4.3 sIgA Production in Infants

Peyer's patches and B cells develop at 11 and 12-16 weeks gestation respectively, and IgA H chain transcripts have been found in cord blood at 27 weeks gestation (47). Although endogenous IgA is thought to start producing several months after birth in humans (56), fecal total IgA have been found in exclusively formula-fed infants as early as 3 months of age (52). Endogenous IgA production slowly increases over the first few years after birth (56).

1.4.4 Importance of gut immunity at early life

According to data from the World Health Organization (WHO), about 1.1 million newborns in the world die from early infections every year (57). The exposure to a myriad of

pathogenic microbes in their new environment after birth necessitates newborn infants to transition from *in-utero* protection via their mother's immune system to dependence on their own immunity, which is relatively immature at birth. The reason as to why some newborns are able to survive this transition is still not known. Since infants are not able to produce sIgA until as early as one month after birth, they rely on sIgA derived from maternal breastmilk to obtain passive immunity during early life. There are several reports of breastfeeding and its protective role against infections (47).

Delayed immune maturation and production of serum and intestinal IgA in early life is associated with increased risk of allergic diseases in later childhood (46, 58, 59). Higher total infant fecal IgA at 3 months has been associated with reduced *Clostridium difficile* colonization, and early colonization of this microbial species in infants have been associated with increased risk of allergic diseases (60). Another study on secretory IgA found that total fecal sIgA measured at 1 month and 12 months of age were not significantly different between healthy and allergic/asthmatic groups of children at age 7, but the sample sizes for both groups were under 30 (61). However, the microbe-sIgA interactions at 1 year of age were significantly different in the allergic and asthmatic groups compared to the control group, notably lower sIgA binding to *Bacteriodes* genus and *Escherichia* species in allergic and asthmatic children. Different IgA responses to gut microbiota are preceding the development of allergic conditions; therefore, screening sIgA responses may provide more insight about allergic diseases. Secretory IgA has many links to asthma and allergies and may potentially reveal more about pathway links to maternal distress.

1.5 Potential Covariates

1.5.1 Maternal asthma and allergies

Higher rates of depression and stress are present in children and adults with asthma and allergies, and animal and human research studies indicate chronic stress disrupts physiological systems that increase risk of allergies and asthma (6, 62). Stress in the mother also exacerbates allergen responses in infants (63). Corticosterone, a glucocorticoid that is increased during pregnancy and can pass through the placenta, seems to enhance Th2 cytokine responses, and therefore, it seems to be a mediator that is transferred from mother to fetus and increases allergy responsiveness in the child (63). In addition, studies seem to suggest that maternal transfer of allergy risk to offspring may occur prenatally (63). Th2 responses in mothers with allergies, such as antibody depletion of maternal T cells, seem to modulate the allergen responsiveness development in the infants. Differences in allergen responsiveness appear to be induced by maternal alterations in the dendritic cells of offspring in the murine model (63), and these cells are important for sIgA production (41).

1.5.2 Pregnancy overweight

Women who are depressed have a higher risk of becoming overweight and obese (64). Depression is directly related to overweight during pregnancy, but this association is multidirectional as the concern of overweight can increase depressive symptoms and depression prior to pregnancy can increase risk of excessive gestational weight gain, while the relationship seems to stronger for the latter (64, 65). Alternatively, maternal stress is not associated with overweight during pregnancy. Maternal obesity during pregnancy has several health risks on

both the mother and infant, such as gestational diabetes and higher likelihood of having a C-section in the mothers and increased infant birth weight and likelihood of fetal defects (66).

In addition, overweight in mothers after birth may impact the mother-infant relationship as according to Bergmann et al. (67), obese mothers score significantly lower in emotional availability, in terms of sensitivity to child's signals, appropriate guidance of child activity, non-hostility and the child's response and involvement in the interaction with the mother. Lower emotional availability has been previously reported to predict childhood obesity. Interestingly, depressive symptoms in the Bergmann study did not have strong associations with emotional availability perhaps due to depressive symptoms not reaching clinical significance in the sample.

Pregnancy overweight is linked to changes in infant gut microbiome. One seminal study based on a prospective birth cohort looked at maternal pre-pregnancy and pregnancy weight and infant fecal microbiota composition. This study found lower abundance of *Bacteriodes* and *Bifidobacterium* at one month and higher abundance of *Clostridium histolyticum* at six months with higher pre-pregnancy weight and excessive weight gain during pregnancy (68). Some of these microbiota composition changes may influence IgA production in the gut. Whether maternal overweight directly affects IgA production in the infant gut is yet to be determined, but lower salivary sIgA have been found in obese children (69). A recent review suggests that maternal obesity is associated with immune dysregulation in the mother, which may affect the fetal immune development and postnatal immunity (39). One study compared sIgA levels in colostrum and found significantly higher levels in the colostrum of overweight and obese mothers (70). Although immunological changes were observed, the group did not find support for the negative impact of obesity on immune protection through breastfeeding. One point to add is that obese mothers are less likely to initiate and have shorter duration of breastfeeding (66),

which may be one explanation to the reduced *Bifidobacterium* abundance in infants who are born to overweight mothers. This may suggest infants of overweight women may be provided with less immune protection in the early months of life due to reduced breastfeeding.

1.5.3 Delivery mode

Studies on the delivery mode and pre and postnatal distress have conflicting results as to whether the association is significant possibly due to different tools used to measure distress and demographic differences. A longitudinal cohort study of over 55 000 women found no association between postpartum emotional distress and mode of delivery after adjusting for confounding variables, but this study used a distress measure that is not commonly used for diagnosing depression due to low specificity (71). However, this study suggests that there is a strong association between prenatal emotional distress and the occurrence of scheduled/elective C-sections. While a much smaller Canadian cohort used the Edinburgh Postnatal Depression Scale (EPDS), they found no significant associations suggesting that the place of birth and other confounding factors may explain the co-occurrence of delivery mode and depression (72).

The studies that did find significant associations postpartum distress and mode of delivery found higher incidence of depression with C-sections (73, 74) as well as primary C-sections or first C-section for the mother (75). Delivery mode preference of pregnant women prior to delivery may be a factor in the stress response of women to the actual delivery mode. Garthus-Niegel et al. (76) found increased post-traumatic stress symptoms in women who preferred delivery by C-section but delivered vaginally compared to women who both preferred and delivered vaginally with depression and anxiety being significant covariates. The same effect

was not seen in mothers who preferred vaginal delivery but delivered via C-section. The psychological factors in mothers may be more relevant to the performance of scheduled C-sections rather than vaginal and emergency C-sections.

A population-based study in Denmark found caesarean section associated with diseases involving immune function and mucosal surfaces in the infants: asthma, gastroenteritis, ulcerative colitis, celiac disease, and differences were seen between elective and acute caesarean sections (77). An older study found 3-, 6-, 12-month total fecal IgA was lower in the vaginally-delivered babies compared to the babies delivered through caesarean section, but this study did not look at elective and emergent caesarean section separately that have different circumstances (78). Early colonization of microbiota in infant gut is widely known to be affected by the mode of delivery (36, 40), and therefore mode of delivery may influence the immune response and development in the infant gut.

1.5.4 Gravidity

A preliminary study on a pilot cohort of the CHILD study by Bridgman et al. (52) found associations between parity and total fecal IgA levels in 3-4-month-old infants. First-borns had higher total fecal IgA than those with older siblings, and this may be because first-borns were more likely to be breastfed in this study. A study on breastfeeding durations among women with postpartum depressive symptoms found greater prevalence of clinically significant depressive symptoms when the mother was a new parent (79). These new mothers were less likely to breastfeed up to the recommended period. Bascom et al. (79) reports a common reason for early cessation of breastfeeding in women with depressive symptoms was the burden of too many

household duties. This reason along with sleep problems and fatigue that are symptoms of depression may affect the mother-infant interactions and reduce breastfeeding.

1.5.5 Small-for-gestational-age (SGA)

Prenatal stress and depression increases risk of small-for-gestational-age (SGA) during the second trimester, and this risk appears to be higher in male infants (80). The use of antidepressants during pregnancy also appears to increase SGA risk (81), which may be part of explanation to the association between distress and birth weight. Epigenetic evidence in placenta supports the link between distress and SGA, which shows lower methylation levels for the transcription site of a leptin receptor in SGA births and when the mother had active depression that was not treated (82). Leptin is considered to have an important regulatory role in fetal nutritional intake and growth. Low birth weight and SGA are associated with greater risks of neonatal hyperbilirubinemia, which is linked to three to four times greater risk of asthma and allergic rhinitis (39). Whether SGA affects IgA production has not be studied thus far. SGA infants may have more IgA as higher IgA content has been found in the breastmilk of mothers that gave birth to a low birth weight and small-for-date infant (83).

1.5.6 Antibiotics exposure

Antibiotics have a profound effect on gut microbiota. Although there is no conclusive evidence on the impact of prenatal antibiotics use on maternal microbial transmission to the infant gut (84), antibiotics usage during the delivery and the first few months of life appear to cause major perturbations in the infant gut microbial composition. Distressed women during pregnancy have higher usage of antibiotics during pregnancy possibly attributable to the

increased susceptibility of infections (85), which may affect the composition of the maternal vaginal microbiome. Infants born to depressed mothers have more infections in the early weeks of postpartum (86), and thus they may have increased use of antibiotics that can alter the gut microbiota establishment. Antibiotics taken by the mother have been found in breastmilk in small concentrations, but even the small amounts can change the gut microbiota in the infant (87). Intrapartum antibiotics use for vaginal delivery and C-sections have been studied in the CHILD study, and it has been reported that with intrapartum antibiotics exposure there is a lowering of microbiota richness and *Bacteriodetes* abundance in 3-month infant gut (38).

1.5.7 Selective serotonin reuptake inhibitors (SSRI)

Pregnant women may take antidepressants, selective serotonin reuptake inhibitors (SSRIs) being the first choice, if they do not respond to psychological therapies alone when treating for moderate to severe depression (18). These medications sometimes have negative effects on the developing fetus and cause immunomodulatory effects (18, 88, 89). In addition, short-lived neonatal adaptation syndrome occurs with SSRI use (18). Antidepressants are taken to prevent the risk of relapse of depression, which counterbalances the pharmacological risks on the fetus (90). A recent review on antidepressant use during pregnancy, discusses the favouring of preventing depressive symptoms over risks of antidepressant use due to currently published studies that reveal statistically significant but minor clinical outcomes. However, there are no clinical trials that confirm the safety of antidepressants that are used during this critical developmental period (91).

1.5.8 Breastfeeding

Breastfeeding seems to be the main covariate between distress and infant gut IgA levels. According to some studies (79, 92), mothers are less likely to interact and continue breastfeeding their infants when depressed starting at early postpartum, but the directionality of this association is yet to be determined as some evidence show difficulty breastfeeding can precede the development of postpartum depression. Reasons for breastfeeding cessation in depressed mothers are lack of social support, the need to go back to work, self-consciousness about breastfeeding in public and other medical reasons (30, 79). Infants cared by depressed mothers may be provided with less sIgA from breastmilk. Lower breastfeeding could reduce the amount of exposure to antidepressants taken by the mother during lactation, but usually the levels of SSRIs are low and undetectable in breastmilk and few adverse effects have been reported in infants (81). Increased levels of breastfeeding have been associated with increased levels of total fecal IgA (52). Whether breastfeeding induces sIgA production is still not known because of the inability to differentiate between maternal-derived and infant-produced sIgA of breastfed infants.

Breastmilk favours greater abundance of *Bifidobacteria* and *Lactobacillus* because of the presence of bioactive ingredients like the human oligosaccharides (36, 39). Infants supplemented with probiotics that include microbial species belonging to the two genera are likely to have higher fecal IgA than infants without supplementation. This altering of colonization may impact the secretion of IgA, since certain microbial species may be stimulatory of sIgA production. However, evidence is still not clear as to whether breastfeeding has protective abilities against later development of allergy and asthma (39, 93).

1.5.9 Smoke exposure

As distress in the perinatal time period is linked with higher levels of smoking (94-96), smoke exposure to the infant pre and postnatally may have impact on the development of infant gut immune system. Maternal smoking may disturb the establishment of the gut microbiota in early life as smoking cessation has been linked to major changes in proportions of *Firmicutes* and *Bacteriodetes* in the adult human gut (97). Mothers who are smokers or were exposed to environmental tobacco smoke (ETS) gave birth to infants with tobacco smoke metabolites in the meconium, the first stool of a newborn (98). Another study on 20 human meconium samples reported maternal smoking during the entire pregnancy had no meconium samples with predominantly lactic acid bacteria (99), which are stimulatory of IgA production (46).

Conflicting results have been shown for the association between maternal smoking and infant salivary IgA levels, but these differences may be due to difference in age and other covariates. Four to six year old children who live with a smoker tended to have lower salivary sIgA (100). Total salivary IgA in 12-month infants was higher when born to mothers who smoked during pregnancy and during the first year of life, which may be due to the increased vulnerability to infections from smoking since IgA was significantly associated with chest infections (101). The same study did not find differences in common respiratory pathogen-specific IgA with maternal smoking status. This study also found that mothers who smoked were more often stopping breastfeeding earlier, and other studies have reported the same association along with lower rates of breastfeeding initiation (102-104). Breastmilk from mothers who smoked during lactation tended to have lower sIgA levels, but this result was not significant (105). Currently, there are no studies on tobacco smoke and gut sIgA.

1.5.10 Home environment

Interactions with animals appear to have beneficial effects on psychological stress in humans. A review by Beetz et al. (106) summarized that human-animal interactions were seen with an improvement of social attention, interpersonal interaction and mood as well as a reduction of self-reported anxiety. However, there is limited evidence on the association between human-animal interactions and reduction of stress hormones, epinephrine and norepinephrine. The review proposes that the interactions with animals may work through the oxytocin system and reduce psychological stress, as increased oxytocin levels are seen with pet ownership (106). Animal-assisted therapy has been used in conjunction with psychotherapies for various psychological disorders (107). Although patients report effectiveness of this therapy, evidence-based support is still needed. The absence or presence of siblings at home in relation to maternal distress may be similar to the relationship between gravida and distress as discussed in Section 1.5.4.

The presence of pets and other children at home are considered because of reported changes in gut microbiome. Martin et al. (40) reported significantly lower *Lactobacillus reuteri* species in the 6-month old infant gut with pet ownership and if there were older siblings. Based on a systematic review of 6 studies that looked at the gut microbial profile of children with eczema, increased *Lactobacilli* and *Bacteriodes* have been found with increased number of siblings, while a lack of older siblings was associated to a rapid colonization of *Clostridium* (108). It is generally assumed that pets and older siblings increase exposure to environmental microbes in early life, which may help the development of the infant gut microbiome and immune system according to the "hygiene hypothesis" (40, 109). Two preliminary studies on the CHILD cohort found increased microbiota richness and diversity when the infant lived with pets, greater

representation of *Clostridiaceae* and *Veillonella* and lower representation of *Bifidobacteria* when living with pets and pet exposure modifying the association between total fecal IgA and breastfeeding (52, 110).

1.5.11 Infant allergic eczema

Infant allergic eczema was considered due to the possibility of reverse causation of postpartum maternal stress. Caregiving an infant with eczema may increase stress on the parents. There is very little literature on the parental experience of caregiving an infant with eczema. One report by Faught et al. (111) was able to find increased stress levels in mothers who were caring for a child with eczema of higher severity at less than six years of age. A more recent study in the Korean population showed lower family quality of life and more negative emotions in the parents associated with increased severity of atopic dermatitis (112). Possible reasons for the increased stress are the child's behaviours and demands when having eczema that increase pressure on parenting, as well as the financial burden for caring moderate to severe eczema (113). Due to the small sample size, large variations of age and possibilities of selection bias in the sample in the two studies, this association requires further investigation.

Infants with allergic conditions have different levels of immune factors. Tendency of lower concentrations of 6-month fecal IgA and other inflammation markers was found in infants with IgE-associated eczema at two years of age, but the difference was not statistically significant (46). A recent study on IgA interactions with gut microbiota explored the differences between allergic or asthmatic children and healthy children, and they found significantly more IgA-bound fecal bacteria in the healthy children at 12 months of age (61).

1.6 Distress and Infant Gut Development

1.6.1 Distress and infant gut development

An animal study by Martinez-Carrillo et al. (114) found reduced plasma B cell counts in Peyer's patches with the administration of dexamethasone, a corticosteroid, which suggests that distress may affect the development of the fetal gut immune system if greater than normal levels of glucocorticoids are passed through the placenta. Maternal glucocorticoids are tightly regulated from passing the placental barrier by the 11β-hydroxysteroid dehydrogenase enzyme. However, in the presence of higher maternal stress levels, some maternal corticosterone can cross the placenta and affect fetal corticosterone levels (115). Mice models of chronic stress reveal alterations of the vaginal microbiome in pregnant mice (116). *Lactobacillus* abundance was decreased in the vagina of pregnant mice after exposure to chronic stress, and this vaginal abundance was positively correlated to the gut *Lactobacillus* abundance in the offspring. This lowering of abundance *Lactobacillus* was also seen in pregnant rhesus monkeys (117). A possible reason for this change is that stress during pregnancy increases susceptibility to vaginal infections due to the immunosuppression (118). These infections can lead to responses that deplete the *Lactobacillus* in the vagina, and potentially affect the early colonization of microbiota in infants when vaginally delivered. However, studies on the impact of prenatal distress on vaginal microbiome in humans are still lacking. Increased stress in the mother may increase infection susceptibility and increase antibiotic use during pregnancy, which may affect microbiome seeding in the infant gut before and during birth (119). Whether prenatal antibiotics use has a sole major impact on seeding is yet to be determined since many metabolic and immune changes occur during pregnancy (84).

1.6.2 Distress and gut immunity

Infant immunity differences have been found when the mother was distressed during pregnancy (120). Therefore, maternal stress and depression may have an impact on the development of the infant gut immune system at early life. Zijlmans et al. (121) was the first to report, based on longitudinal data of 56 infants, human infants born to mothers with greater prenatal anxiety and stress more likely to have gut dysbiosis, an imbalance in gut microbiome composition. Altered infant gut microbiome composition has been associated with the later development of asthma and allergies (119). Although this first report on humans found that stress predisposes allergic symptoms in early life, this study did not have enough power to investigate the influence of breastfeeding, an important covariate, and did not look at immune factors that may lead to changes in gut microbiome composition (121). Since distress has been shown to affect the gut microbiome, maternal distress has potential to affect the gut immunity and IgA levels in infants.

Jarillo-Luna et al. (122) reported lower secretory intestinal IgA concentrations in chronically stressed mice when compared to the control group. Adrenalectomy was performed to determine the role of adrenal glands on IgA levels, and it confirmed adrenal stress hormones, catecholamines and glucocorticoids, play a role in reducing intestinal IgA levels when chronically stressed. A later study from the same group on repeated stress found lower sIgA-producing B cells in the Peyer's patches (114). This suggests that distress may have an influence on the production and release of immune factors in the gut. Currently, there is a paucity of literature on connections between distress and gut immunity in humans.

1.6.3 Summary of gaps in literature

Few studies have investigated the association between distress and gut immunity. Gut microbiome studies in animal models and humans show various links in changes in early life gut composition with prenatal distress that could possibly lead to alterations in infant immunity (116,121). However, the animal models are not representative for the depression and stress that humans experience. A commonly used animal model for depression is the olfactory bulbectomized rat that is used to test antidepressants and other treatments for depression-like symptoms (123), but this model has not been used in the animal studies that investigated gut immunity and microbiome differences with distress. Instead, the animal studies used restraint stress when testing for differences in intestinal immunity (114, 122), which may not be applicable to humans. The animal studies also looked at effects of direct stress and not maternal stress effects on the infant gut immunity.

No studies have investigated the impact of postnatal distress on infant gut microbiome and immunity in humans. Although Zijlmans et al. (121) controlled for postnatal distress in their analyses for prenatal distress and infant gut microbiome composition, the impact of postnatal distress on the infant gut was not tested. This study also did not measure maternal depression, but focused on prenatal stress and anxiety. A recently published study on rat pups by Moussaoui et al. (124) reports differences in plasma corticosterone levels, intestinal permeability and microbial diversity and composition in pups that were exposed to postnatal chronic stress (limited nesting room and brief maternal separation), suggesting that stressful environments postnatally may affect the stress response and gut microbiome development in offspring. There is a gap in literature on the potential effects of maternal pre and postnatal distress specifically on human infant gut immunity, which is an important contributor of gut microbiome development that may

assist the prevention of allergies and asthma. Therefore, this thesis will provide some insight on the impact of pre and postnatal maternal distress on infant gut immunity in the first few months of life.

1.7 Hypothesis and Objectives

The hypothesis for this study is that infants born from mothers that self-report having stress or depressive symptoms during and after pregnancy will have lower fecal secretory IgA than infants born from mothers that experience lower levels of stress or depression. Having longer exposure to distress (during both pre and postnatal periods) may greatly affect sIgA levels. Secondary hypotheses are that distress in the prenatal only period or postnatal only period will also affect gut immunity by lowering sIgA levels, and differences in effects may be observed between pre and postnatal distress exposure.

The objective of this study is to determine whether infants born from mothers that self-report stress and depressive symptoms during and after pregnancy have lower fecal sIgA than infants born from non-distressed mothers. We are testing this association while controlling for the effects of breastfeeding status, antidepressant use, delivery mode, antibiotics exposure, gravidity, maternal asthma/allergy and overweight status, depression history, tobacco smoke exposure, infant allergic eczema and number of pets and children at home.

1.8 Sample Size Calculation

In an animal study by Jarillo-Luna et al. (122), the stressed group had intestinal IgA concentrations of 1μg/ml; whereas, the control group had 3μg/ml of IgA.

Percent decrease:

$\bar{X}_1$_= 3μg/ml $\bar{X}_2$= 1μg/ml

[(3 – 1)μg/ml / 3μg/ml] ×100% = 66.67%

Mean IgA of infants from non-depressed mothers in human sample = 10.74mg/g of protein

Estimated IgA of infants from depressed mothers in human sample = 10.74 – (10.74mg/g × 0.6667) = 3.5809mg/g of protein

Pooled SD = $\sqrt{\frac{(n_1-1)s_1{}^2+(n_2-1)s_2{}^2}{n_1+n_2-2}} = \sqrt{\frac{(88-1)(7.0991)^2+(25-1)(5.7116)^2}{88+25-2}}$ = 6.8230mg/g of protein

To determine the sample size with a 2-sided α of 0.05 and β of 0.20 (power=80%), the Power Index (PI) will be: 1.96 + 0.84=2.80

N per group = [PI * SD/clinical difference]^2

= $[2.80*\frac{6.8230mg/g}{(10.74-3.5809)mg/g}]^2 = 7$

The minimum required number of participants per group is seven.

Chapter 2: Methods and Materials

2.1 Methods overview

This chapter describes the methods and materials used in this thesis study. This study is a quantitative study based on data from sub-sample of the Canadian Healthy Infant Longitudinal Development (CHILD) study, a nationally representative prospective longitudinal birth cohort study.

2.2 Study population

The CHILD study has 3623 enrolled pregnant mothers and full-term infants recruited between 2008 and 2012 from four Canadian cities: Vancouver, Edmonton, Manitoba and Toronto (125). Mothers were followed throughout pregnancy and children are clinically assessed at birth, at three months, and at 1-, 3-, and 5-year visits. Moraes et al. (125) documented the inclusion and exclusion criteria for this cohort study. Pregnant women who are 18 years and older, who lives in residence in reasonable proximity to the delivery hospital and plans to give birth at a designated recruitment center participating hospital, who is able to read, write and speak English and who provides informed consent to the study and cord blood donation were recruited. Their infants were born at or after 35 weeks and the families were able to provide name, address and telephone numbers of two alternate contact individuals. The exclusion criteria for this cohort are children born with major congenital abnormalities or respiratory distress syndrome, expectation of moving away within one year, children of multiple births, children resulting from in vitro fertilization, children who will not spend at least 80% of nights in the home and children born before 35 weeks.

The subsample of 403 infants for this study used data from three of the four study sites (excluding Toronto), and these infants were selected based on availability of the stool samples. Although home births were originally meant to be excluded from the study, some infants born from home births were initially included in our sample. Sensitivity analyses show that including these infants had a major effect on our results, and therefore, these infants (n=12) were excluded from our sample (Appendix D; Table D1). Late preterm infants (35-37 weeks gestation) were also meant to be excluded from the CHILD study, but they were included in our sample because of the low impact on our results (Table D3).

2.3 Exposures

Maternal distress data collected at recruitment (mean 27 weeks) and 36 weeks gestation, and at 6 months and 12 months of child age were used for this study. Mothers with scores above the cut-offs only at 27 weeks or 36 weeks were grouped in the Prenatal group, mothers with higher scores only at 6 months or 12 months of child age were grouped into the Postnatal group and mothers with higher scores in both time periods were group into the Both group. The groups were mutually exclusive.

Perceived Stress

Maternal stress was assessed using the 10-item version of the Perceived Stress Scale (PSS) (126). The items measured how frequent mothers had found their lives stressful, unmanageable and overwhelming in the past month, and the items were scored on a scale ranging from zero (never) to four (very often). The scores for the items are summed, and the higher sums indicate greater stress. Since there are no established criteria when evaluating perceived stress, we used the highest quartile of perceived stress scores to indicate higher stress

and the cut-off was 17. A preliminary analysis used the PSS mean in the entire CHILD cohort (12.96) as the cut-off for higher level of perceived stress, which is close to the mean score of females in the general population of USA (127). However, associations with infant sIgA were not significant with this cut-off (see Appendix B).

Depressive Symptoms

Pre and postnatal depressive symptoms were determined using the 20-item Center for Epidemiologic Studies Depression (CES-D) Scale (128). Mothers reported the frequency of experiencing various depression behaviors, cognitions and affect during the past one week using scores ranging from zero (none of the time or less than one day) to three (most or all the time or five to seven days). The scores were summed, and sums of 16 and greater reflect clinically significant levels of depressive symptoms.

2.4 Definition of potential covariates

Covariates investigated in this study were infant age at stool collection, breastfeeding status at time of stool collection, antidepressant (SSRI) use, tobacco smoke exposure, antibiotic exposure to infant from birth to three months, delivery mode, gravidity, number of children and pets at home, small-for-gestational-age (SGA), pregnancy overweight, maternal asthma or allergy status, depression history and infant allergic eczema. These variables were created from data obtained from the CHILD general cohort questionnaires.

Depression history was determined from mother reports of having depression before pregnancy. *Pregnancy overweight* indicated whether the mother was overweight or obese based on maternal BMI during pregnancy. This information was available for only 304 mothers in our

sample, and so separate analyses on the 304 mother-infant dyads were conducted with pregnancy overweight (Appendix A). *Maternal asthma or allergy status* was based on mother reports of having asthma or allergies during pregnancy. *Antidepressant (SSRI) use* indicated if mothers took selective serotonin reuptake inhibitors (SSRIs) based on prenatal and postnatal maternal medication questionnaires.

Antibiotic exposure from birth to three months determined whether infant was exposed to antibiotics at any time from birth to 3 months of age based on questionnaire data (caesarean section, antibiotics during vaginal delivery, newborn given antibiotics, antibiotics used before the 3-month visit). *Delivery mode* information came from birth chart questionnaires and had three categories: emergency caesarean section (C-section), scheduled caesarean section and vaginal delivery. *Gravida* indicated whether the mothers were primigravida or not. *Small-for-gestational-age (SGA)* was defined as born with birthweight below 10^{th} percentile for corresponding gestational age and gender based on Canadian birthweight for gestational age chart.

Breastfeeding status at time of stool collection indicated whether the infant was exclusively breastfed (exclusively breastfed at time of collection), partially breastfed (breastfed and formula-fed) or had zero breastfeeding (formula-fed). *Infant age at stool collection* was the infant age in weeks during the time the stool samples were collected for the CHILD study 3-month visits. *Prenatal smoke exposure* was determined from recruitment questionnaires on maternal health and home environment, and was defined as mothers who smoked during pregnancy or had a household member smoking inside the house. *Postnatal smoke exposure* was defined as infants living with a household member that smokes inside the house based on the 3-month home environment questionnaire. *Number of children and pets at home* living with the

infant before the 3-month visit was determined from home environment questionnaires. *Infant allergic eczema* was determined if there was rash in more than one area excluding diaper rash (face, inside elbow, ankle, back of knee, wrist/hand, scalp, other) or if there was a physician diagnosis of atopic dermatitis as reported by the mother.

2.5 Stool collection and storage

Infant stool was collected during 3-month CHILD study visits. Stool samples (5-10g) were collected from a freshly soiled diaper using a sterile spatula, divided into aliquots and stored at -80ºC. Freezing of stool only caused marginal decreases in sIgA levels as reported by Forrest et al. (129), and so freezing does not have a significant impact on the quantification of sIgA.

2.6 Extraction and analysis of sIgA

The sIgA ELISA kit from Immundiagnostik AG is used to measure the amounts of fecal sIgA in mg per gram of protein. Immundiagnostik AG is a diagnostics company operating globally to develop and produce immunoassays and other analytical detection methods for medical research (130). After thawing the stool samples, sIgA is extracted with IDK Extract extraction buffer. The stool samples are diluted 1:125 in wash buffer. The diluted patient samples, controls, and 100µl standards are put into a microtiter plate, washed and incubated at 15-30ºC. After the wells are aspirated and washed, the microtiter plate is tapped on absorbent paper. 100µl of conjugate is added, and then the samples are incubated at 15-30ºC and shaken on a horizontal mixer. After the final washing step and adding TMB substrate, the samples are incubated in a dark room for 10-20 minutes at 15-30ºC. Absorption is determined with an ELISA reader at

450nm against 620nm as a reference. The results of the microplate reader were multiplied by the dilution factor of 12500. Standards with known concentrations that were provided in the kit were used to create standard curves and determine sIgA concentrations.

2.7 Statistical analyses

The sIgA concentrations were highly skewed, and so nonparametric tests, Mann Whitney U-tests and Kruskal-Wallis tests, were used to detect differences in sIgA medians according to the distress status of the mother. Bonferroni sequential correction was used for critical p-values for multiple comparisons when comparing distressed groups with the reference group. Mothers with both pre and postnatal distress was compared to a critical p-value of 0.05 as determined a priori since our primary hypothesis is to see if distress at both time periods affect immunity (131). Prenatal only and postnatal only comparisons with the reference group were adjusted for two pairs of interest using Bonferroni sequential correction (132). Since the prenatal only group infants had exposure to distress before the time of stool collection whereas the postnatal only group had exposure measure during or after stool collection, it was considered the next hypothesis to be tested and was compared to an adjusted critical p-value of 0.05/2 = 0.025. The postnatal only group comparisons with the reference group had p-values compared to the adjusted critical p-value of 0.05/1 = 0.05. Logistic regression models used sIgA binary outcome variables (yes/no) of lowest quartile, highest quartile, lowest tertile and below median. Models were created using purposeful selection of covariates. Statistical analyses were conducted using IBM SPSS 24 and figures were created using GraphPad Prism 6.

Chapter 3: Results

3.1 Sub-sample Characteristics

The 403 sub-sample did not differ significantly for the majority of potential covariates than all the infants in the three sites of the CHILD cohort (Table 1). Breastfeeding at 3 months was slightly higher in the CHILD cohort compared to the sub-sample; however, 38 infants in the CHILD cohort were missing information for breastfeeding status at 3 months, which may have contributed to the difference in proportions. About 12.2% of women in our sample had clinically significant prenatal only depressive symptoms, 8.7% had symptoms postpartum only and 9.2% had symptoms both pre and postnatally (Table 1). For PSS scores higher than the CHILD mean, 19.5% of all mothers in the sample had stress scores above the mean for prenatal only, 14.7% had postnatal only and 37.4% had both pre and postnatal stress scores above the mean (Table B2). With PSS scores in the highest quartile, 16.7% of mothers fell in this category at prenatal only, 10.2% for postnatal only and 18.7% for both pre and postnatal (Table 1).

Although the stool samples were meant to be collected at three months of infant age, some infants had their stool collected earlier or much later than the proposed time point. The mean of stool collection time was 3.8 months, while the range was 2-8 months. Thirty infants had their stool collected at greater than six months of age, but sensitivity analyses indicate including these infants did not affect our results (Appendix D; Table D2), and thus these infants were included in our sample. Instead of excluding infants, we controlled for age at stool collection in the logistic regression models as shown in Section 3.2.4.

Table 1. Proportion of depressive symptoms, higher perceived stress and potential covariates in 403 sub-sample and CHILD cohort

	sIgA Sample % (95% CI) N=403	CHILD Cohort (Three Sites*) % N=2373
Depressive Symptoms		
Less (below cut-off)	69.8 (65.3 – 74.3)	66.6
Prenatal Only	12.2 (9.0 – 15.4)	13.8
Postnatal Only	8.7 (5.9 – 11.5)	8.6
Both Pre and Postnatal	9.2 (6.4 – 12.0)	10.9
Higher Perceived Stress (highest quartile)		
Less (below cut-off)	54.4 (49.5 – 59.3)	51.5
Prenatal Only	16.7 (13.0 – 20.4)	18.8
Postnatal Only	10.2 (7.2 – 13.2)	12.1
Both Pre and Postnatal	18.7 (14.9 – 22.5)	17.6
Depression History		
Yes	20.0 (16.1 – 23.9)	19.8
No	80.0 (76.1 – 83.9)	80.2
Maternal Asthma/Allergy		
Yes	63.9 (59.2 – 68.6)	65.4
No	36.1 (31.4 – 40.8)	34.6
Child Sex		
Male	55.8 (51.0 – 60.6)	52.3
Female	44.2 (39.4 – 49.0)	47.7
Gravida		
Primigravida	38.1 (33.3 – 42.9)	35.8
Multigravida	61.9 (57.1 – 66.7)	64.2
Birth Mode		
Caesarean Section	28.8 (24.4 – 33.2)	24.0
Vaginal	71.2 (66.8 – 75.6)	76.0
Antibiotics Exposure		
Yes	56.4 (51.5 – 61.3)	51.3
No	43.6 (38.7 – 48.5)	48.7
Breastfeeding at 3 Months		
Yes	78.6 (74.6 – 82.6)	84.8**
No	21.4 (17.4 – 25.4)	15.2**
Prenatal Smoke Exposure		
Yes	6.1 (3.7 – 8.5)	7.1
No	93.9 (91.5 – 96.3)	92.9
Postnatal Smoke Exposure		
Yes	16.0 (12.4 – 19.6)	15.7
No	84.0 (80.4 – 87.6)	84.3
Pets at Home		
Yes	46.4 (41.5 – 51.3)	45.2
No	53.6 (48.7 – 58.5)	54.8
Infant Allergic Eczema		
Yes	10.8 (7.7 – 13.9)	13.4
No	89.2 (86.1 – 92.3)	86.6

*Since the sub-sample came from three sites, only infants from the three sites of the entire CHILD cohort were used to compare with the sub-sample.

**Significant difference since percentage is not within the 95% CI calculated for the sub-sample proportions

Table 2. Median infant fecal sIgA levels and percentage distribution of maternal depressive symptoms and lowest quartile sIgA in relation to potential covariates (n=403)

	Less symptoms (69.8% overall)	Prenatal only (12.2% overall)	Postnatal only (8.7% overall)	Both (9.2% overall)		Fecal sIgA		Lowest quartile sIgA (% yes)	
	N (%)	N (%)	N (%)	N (%)	p-value (χ^2)	median (IQR)	p-value	N (%)	p-value (χ^2)
Child sex									
Male	159 (71)	25 (11.2)	20 (8.9)	20 (8.9)	0.89	6.1 (3.3 – 12.0)	0.58	54 (24)	0.67
Female	121 (68.4)	24 (13.6)	15 (8.5)	17 (9.6)		5.6 (3.1 – 10.4)		46 (25.8)	
Depression History									
Yes	40 (50.6)	18 (22.8)	10 (12.7)	11 (13.9)	**<0.001**	6.2 (3.3 – 11.4)	**0.04**	25 (31.6)	0.13
No	236 (74.9)	29 (9.2)	25 (7.9)	25 (7.9)		5.1 (2.3 – 9.3)		74 (23.4)	
Maternal asthma/allergy during pregnancy									
Yes	175 (69.2)	33 (13)	18 (7.1)	27 (10.7)	0.19	6.0 (3.3 – 11.0)	0.61	61 (24.1)	0.59
No	101 (71.1)	15 (10.6)	17 (12)	9 (6.3)		5.7 (3.0 – 11.1)		38 (26.6)	
Delivery mode									
Vaginal	198 (70.2)	30 (10.6)	30 (10.6)	24 (8.5)	0.16	6.1 (3.2 – 11.2)	0.50	70 (24.6)	0.56
Scheduled C-section	47 (69.1)	8 (17)	4 (8.5)	3 (6.4)		5.6 (3.5 – 10.9)		19 (27.9)	
Emergency C-section	32 (68.1)	11 (16.2)	1 (1.5)	9 (13.2)		5.4 (3.0 – 10.5)		9 (19.1)	
Antibiotics exposure up to 3 months									
Yes	155 (70.5)	31 (14.1)	16 (7.3)	18 (8.2)	0.28	5.6 (3.1 – 11.2)	0.28	58 (26.2)	0.43
No	120 (70.6)	15 (8.8)	18 (10.6)	17 (10)		6.1 (3.3 – 12.0)		39 (22.8)	
Gravida									
Primigravida	112 (73.7)	19 (12.5)	12 (7.9)	9 (5.9)	0.32	5.1 (2.9 – 10.0)	0.077	43 (28.3)	0.18
Multigravida	165 (67.3)	30 (12.2)	23 (9.4)	27 (11)		6.4 (3.5 – 12.1)		55 (22.3)	
Small-for-gestational-age									
Yes	20 (74.1)	4 (14.8)	2 (7.4)	1 (3.7)	0.77	7.5 (4.5 – 16.0)	0.063	3 (11.1)	0.09
No	256 (69.6)	45 (12.2)	33 (9)	34 (9.2)		5.7 (3.1 – 10.8)		95 (25.7)	
Prenatal SSRI use									
Yes	5 (27.8)	8 (44.4)	0 (0)	5 (27.8)	**<0.001**	3.6 (2.5 – 7.0)	0.12	6 (33)	0.42
No	269 (71.9)	40 (10.7)	34 (9.1)	31 (8.3)		6.0 (3.2 – 11.4)		93 (24.8)	
Postnatal SSRI use									
Yes	10 (43.5)	8 (34.8)	1 (4.3)	4 (17.4)	**0.002**	4.6 (3.3 – 7.4)	0.18	6 (26.1)	0.89
No	266 (71.9)	40 (10.8)	31 (8.4)	33 (8.9)		6.0 (3.2 – 11.5)		92 (24.8)	
Prenatal smoke exposure									
Yes	10 (62.5)	3 (18.8)	2 (12.5)	1 (6.3)	0.78	5.1 (1.8 – 12.3)	0.39	6 (37.5)	0.24
No	203 (72)	32 (11.3)	26 (9.2)	21 (7.4)		6.0 (3.2 – 11.1)		69 (24.4)	
Postnatal smoke exposure									
Yes	31 (67.4)	7 (15.2)	2 (4.3)	6 (13)	0.26	4.9 (2.8 – 9.6)	0.25	14 (30.4)	0.37
No	184 (72.2)	29 (11.4)	25 (9.8)	17 (6.7)		6.1 (3.2 – 11.5)		62 (24.4)	
Pets at home									
Yes	127 (68.6)	22 (11.9)	13 (7)	23 (12.4)	0.20	5.3 (2.7 – 10.4)	**0.031**	55 (29.7)	**0.045**
No	151 (70.9)	27 (12.7)	21 (9.9)	14 (6.6)		6.4 (3.7 – 12.0)		45 (21)	
Children at home									
Yes	135 (71.4)	20 (10.6)	17 (9)	17 (9)	0.79	6.5 (3.6 – 11.9)	0.11	43 (22.6)	0.34
No	144 (68.9)	29 (13.9)	17 (8.1)	19 (9.1)		5.3 (3.1 – 10.7)		56 (26.8)	
Breastfeeding at stool collection									
Zero	64 (64)	15 (15)	11 (11)	10 (10)	0.30	3.4 (2.0 – 5.9)	**<0.001**	47 (47)	**<0.001**
Partial	108 (72)	13 (8.7)	16 (10.7)	13 (8.7)		5.5 (3.2 – 10.0)		39 (26)	
Exclusive	99 (75.6)	15 (11.5)	6 (4.6)	11 (8.4)		10.1 (5.6 – 16.2)		11 (8.4)	
Infant allergic eczema									
Yes	28 (65.1)	4 (9.3)	4 (9.3)	7 (16.3)	0.38	5.9 (3.4 – 11.2)	0.84	9 (20.9)	0.55
No	251 (71.1)	44 (12.5)	28 (7.9)	30 (8.5)		5.8 (3.1 – 11.0)		89 (25.1)	

Table 3. Median infant fecal sIgA levels and percentage distribution of maternal higher perceived stress and lowest quartile sIgA in relation to potential covariates (n=403)

	Less stress (54.4% overall)	Prenatal only (16.7% overall)	Postnatal only (10.2% overall)	Both (18.7% overall)		Fecal sIgA		Lowest quartile sIgA (% yes)	
	N (%)	N (%)	N (%)	N (%)	p-value (χ^2)	median (IQR)	p-value	N (%)	p-value (χ^2)
Child sex									
Male	125 (55.8)	33 (14.7)	22 (9.8)	44 (19.6)	0.64	6.1 (3.3 – 11.7)	0.58	54 (24)	0.67
Female	93 (52.5)	34 (19.2)	19 (10.7)	31 (17.5)		5.6 (3.2 – 10.4)		46 (25.8)	
Depression History									
Yes	31 (39.2)	17 (21.5)	8 (10.1)	23 (29.1)	**0.009**	6.1 (3.3 – 11.4)	**0.04**	25 (31.6)	0.13
No	184 (58.4)	49 (15.6)	32 (10.2)	50 (15.9)		5.1 (2.3 – 9.0)		74 (23.4)	
Maternal asthma/allergy during pregnancy									
Yes	136 (53.8)	44 (17.4)	24 (9.5)	49 (19.4)	0.88	6.0 (3.2 – 12.0)	0.49	66 (24.7)	0.99
No	79 (55.6)	22 (15.5)	16 (11.3)	25 (17.6)		5.7 (3.2 – 9.8)		32 (24.8)	
Delivery mode									
Vaginal	157 (55.7)	41 (14.5)	31 (11)	53 (18.8)	0.26	5.6 (3.6 – 10.4)	0.50	70 (24.6)	0.56
Scheduled C-section	26 (55.3)	7 (14.9)	4 (8.5)	10 (21.3)		5.3 (2.9 – 10.4)		19 (27.9)	
Emergency C-section	33 (48.5)	19 (27.9)	5 (7.4)	11 (16.2)		6.2 (3.2 – 11.4)		9 (19.1)	
Antibiotics exposure up to 3 months									
Yes	123 (55.9)	41 (18.6)	16 (7.3)	40 (18.2)	0.25	5.6 (3.1 – 11.0)	0.28	58 (26.2)	0.43
No	92 (54.1)	25 (14.7)	22 (12.9)	31 (18.2)		6.1 (3.4 – 12.0)		39 (22.8)	
Gravida									
Primigravida	85 (55.9)	27 (17.8)	16 (10.5)	24 (15.8)	0.72	5.2 (3.0 – 10.0)	0.077	43 (28.3)	0.18
Multigravida	131 (53.5)	40 (16.3)	24 (9.8)	50 (20.4)		6.4 (3.5 – 12.0)		55 (22.3)	
Small-for-gestational-age									
Yes	14 (51.9)	7 (25.9)	3 (11.1)	3 (11.1)	0.52	7.5 (4.7 – 15.7)	0.063	3 (11.1)	0.09
No	201 (54.6)	60 (16.3)	37 (10.1)	70 (19)		6.0 (3.2 – 10.9)		95 (25.7)	
Prenatal SSRI use									
Yes	4 (22.2)	6 (33.3)	1 (5.6)	7 (38.9)	**0.012**	3.8 (2.7 – 6.7)	0.12	6 (33)	0.42
No	208 (55.6)	61 (16.3)	39 (10.4)	66 (17.6)		6.0 (3.2 – 11.0)		93 (24.8)	
Postnatal SSRI use									
Yes	7 (30.4)	7 (30.4)	2 (8.7)	7 (30.4)	0.062	4.7 (3.2 – 7.1)	0.18	6 (26.1)	0.89
No	208 (56.2)	59 (15.9)	38 (10.3)	65 (17.6)		6.1 (3.2 – 11.4)		92 (24.8)	
Prenatal smoke exposure									
Yes	7 (29.2)	7 (29.2)	3 (12.5)	7 (29.2)	0.072	5.1 (1.8 – 12.3)	0.39	6 (37.5)	0.24
No	208 (56.1)	59 (15.9)	37 (10)	67 (18.1)		6.0 (3.2 – 11.1)		69 (24.4)	
Postnatal smoke exposure									
Yes	30 (46.9)	11 (17.2)	5 (7.8)	18 (28.1)	0.17	4.9 (2.8 – 9.6)	0.25	14 (30.4)	0.37
No	188 (56)	56 (16.7)	36 (10.7)	56 (16.7)		6.1 (3.2 – 11.5)		62 (24.4)	
Pets at home									
Yes	96 (51.9)	31 (16.8)	18 (9.7)	40 (21.6)	0.46	5.3 (2.7 – 10.4)	**0.031**	55 (29.7)	**0.045**
No	121 (56.8)	36 (16.9)	23 (10.8)	33 (15.5)		6.4 (3.7 – 12.0)		45 (21)	
Children at home									
Yes	105 (55.6)	28 (14.8)	21 (11.1)	35 (18.5)	0.76	6.5 (3.6 – 11.9)	0.11	56 (26.8)	0.34
No	112 (53.6)	39 (18.7)	20 (9.6)	38 (18.2)		5.3 (3.1 – 10.7)		43 (22.6)	
Breastfeeding at stool collection									
Zero	49 (49)	17 (17)	13 (13)	21 (21)	0.67	3.3 (2.0 – 6.0)	**<0.001**	47 (47)	**<0.001**
Partial	88 (58.7)	19 (12.7)	14 (9.3)	29 (19.3)		5.4 (3.2 – 9.8)		39 (26)	
Exclusive	76 (58)	22 (16.8)	12 (9.2)	21 (16)		10.0 (5.6 – 16.1)		11 (8.4)	
Infant allergic eczema									
Yes	20 (46.5)	9 (20.9)	4 (9.3)	10 (23.3)	0.62	5.7 (3.4 – 11.4)	0.84	9 (20.9)	0.55
No	196 (55.5)	57 (16.1)	37 (10.5)	63 (17.8)		6.0 (3.2 – 11.2)		89 (25.1)	

Tables 2 and 3 report the frequency distribution of the exposure, maternal distress, and the outcome of infant fecal sIgA across putative confounding factors. Depressive symptoms and high perceived stress were significantly more common in women with a history of for depression and among those treated with SSRIs during or after pregnancy. Four percent of women with minimal depressive symptoms took SSRIs, while this was 18.8% in the prenatal group, 3.2% in the postnatal group and 16.7% in the pre and postnatal group (Appendix E; Table E1). Significantly lower median fecal sIgA levels were observed in infants born to mothers having no depression history compared to those with history of depression ($p=0.04$), living with pets compared to those without pets ($p=0.03$), and formula feeding or partially breastfeeding their infant compared to no breastfeeding at stool collection time ($p<0.001$; Table 2 and 3). Borderline significant differences were observed in median sIgA for gravida and small-for-gestational-age (SGA), where infants born to primigravidas and not SGA infants had lower sIgA concentrations. Percentage of those in the lowest quartile for sIgA was significantly different with the pet ownership and breastfeeding status at stool collection time.

3.2 Maternal Distress and Infant sIgA

Due to the skewness of the sIgA data, comparisons between the distress groups and the reference group were conducted for median sIgA levels. Infant sIgA levels were significantly lower in the infants exposed to both pre and postnatal depressive symptoms compared to the reference group ($p=0.033$; Figure 1). Median sIgA levels were 6.3 (IQR=3.6 – 12.4) mg/g feces for exposure to less depressive symptoms (reference group) and 4.4 (IQR = 2.4 – 8.0) mg/g feces for exposure to both pre and postnatal symptoms (see Appendix E). Infants in the prenatal only group had median sIgA levels of 5.19 (IQR=2.02 – 9.78) mg/g feces, and those in the postnatal only group had median levels of 5.69 (IQR=2.62 – 8.56) mg/g feces; however, statistically

significant differences in these levels were not detected in relation to the reference group. There were no significant differences in sIgA levels among the perceived stress groups.

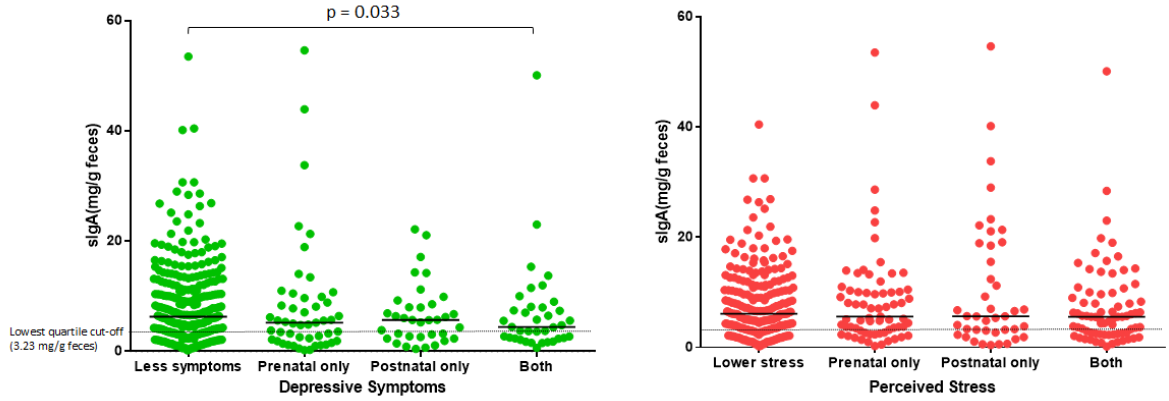

Figure 1. Infant fecal sIgA levels depending on distress status of mother. Secretory IgA levels were significantly lower in the group exposed to both pre and postnatal depressive symptoms (green) compared to the group exposed to lower symptoms (not clinically significant). No significant differences were seen in sIgA with maternal higher perceived stress status (red). Lowest quartile cut-off for sIgA used in logistic regression models was at 3.23 mg/g feces (dotted line).

Sections 3.2.1 to 3.2.3 contain the median comparisons for sIgA with exposure to both pre and postnatal (primary question), prenatal only and postnatal only maternal distress respectively after stratifying for covariates. The final section shows the simple and multiple logistic regression analyses using lowest quartile sIgA (yes or no) as the outcome (Table 4 and 5). Logistic regression analyses using highest quartile, lowest tertile and below median sIgA cut-offs are listed in Appendix C.

3.2.1 Infant sIgA with exposure to both pre and postnatal distress (primary question)

After stratifying for breastfeeding status at stool collection time, significantly lower sIgA levels were seen in formula-fed infants with exposure to both pre and postnatal depressive symptoms compared to the reference group (p=0.033; Figure 2). No significant differences were seen in the breastfed infants between the depressive symptoms groups and the reference group. Breastfed infants not exposed to clinically significant depressive symptoms had higher sIgA than formula-fed infants born to mothers experiencing both pre and postnatal depressive symptoms (p<0.001; Figure 2). Median sIgA was not significantly different between perceived stress groups after breastfeeding stratification, but similar trends were seen (Table E4).

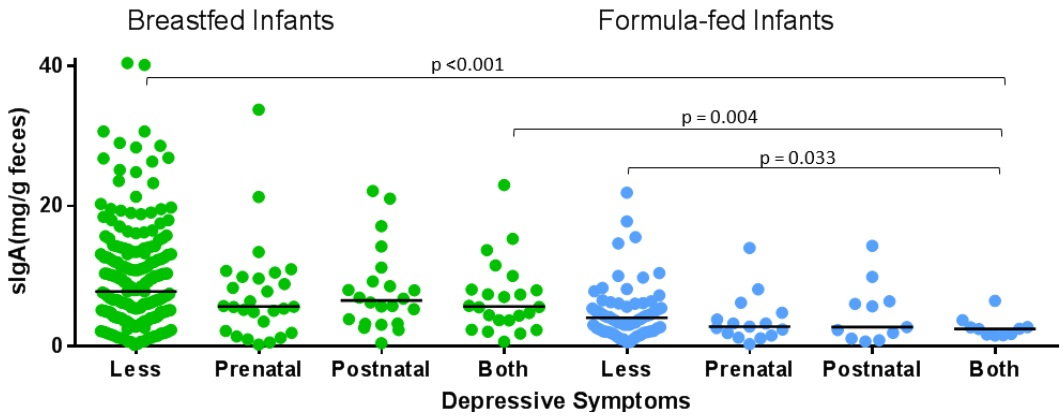

Figure 2. sIgA levels among breastfed and formula-fed infants at time of stool collection for different depressive symptoms groups. Breastfed infants (green) did not have significant differences in sIgA levels based on distress levels, while formula-fed infants (blue) had significantly lower sIgA when exposed to both pre and postnatal depressive symptoms. Formula-fed infants had significantly lower sIgA levels than breastfed infants for all depressive symptoms groups.

After stratifying by other covariates, there were many instances of significantly lower infant sIgA levels when the mother experienced both pre and postnatal distress. This was the case when the infant was exposed to tobacco smoke after birth (p=0.005), exposed to antibiotics (p=0.027), born to a mother with no depression history (p=0.021), born to a multigravida (p=0.022) and living in a home with other children (p=0.038; Figure 3). Figures 5 and 6 in Section 3.2.2 and 3.2.3 also show statistically significant differences between the both group and the reference group. The presence of pet ownership (p=0.022; Figure 5) and maternal asthma or allergy during pregnancy (p=0.032; Figure 6) showed the same lowering of infant sIgA in the presence of both pre and postnatal depressive symptoms. Interactions for the previously mentioned covariates were significant except for antibiotics and maternal asthma or allergy (Table E1). Subsequent stratifications are listed in Appendix E.

Lower median sIgA levels were found with the presence of higher perceived stress in mothers when the dyad was living with other children (p=0.004) and pets at home (p=0.041; Figure 4). Interactions were significant for both covariates in the association between sIgA and higher stress (Table E4). With subsequent stratifications listed in Table E4, lower sIgA was seen with the exposure of both pre and postnatal stress in breastfed infants that live with other children (p=0.025) and in infants exposed to antibiotics that live with pets (p=0.006). With or without antibiotics exposure, having other children in the household had infants with significantly lower sIgA levels after exposure to both pre and postnatal stress than the reference group (Table E4). In the absence of pets, infants living with other children had lower sIgA levels (p=0.037), while infants living without other children had higher sIgA levels when their mom experienced higher pre and postnatal stress (p=0.015; Table E4).

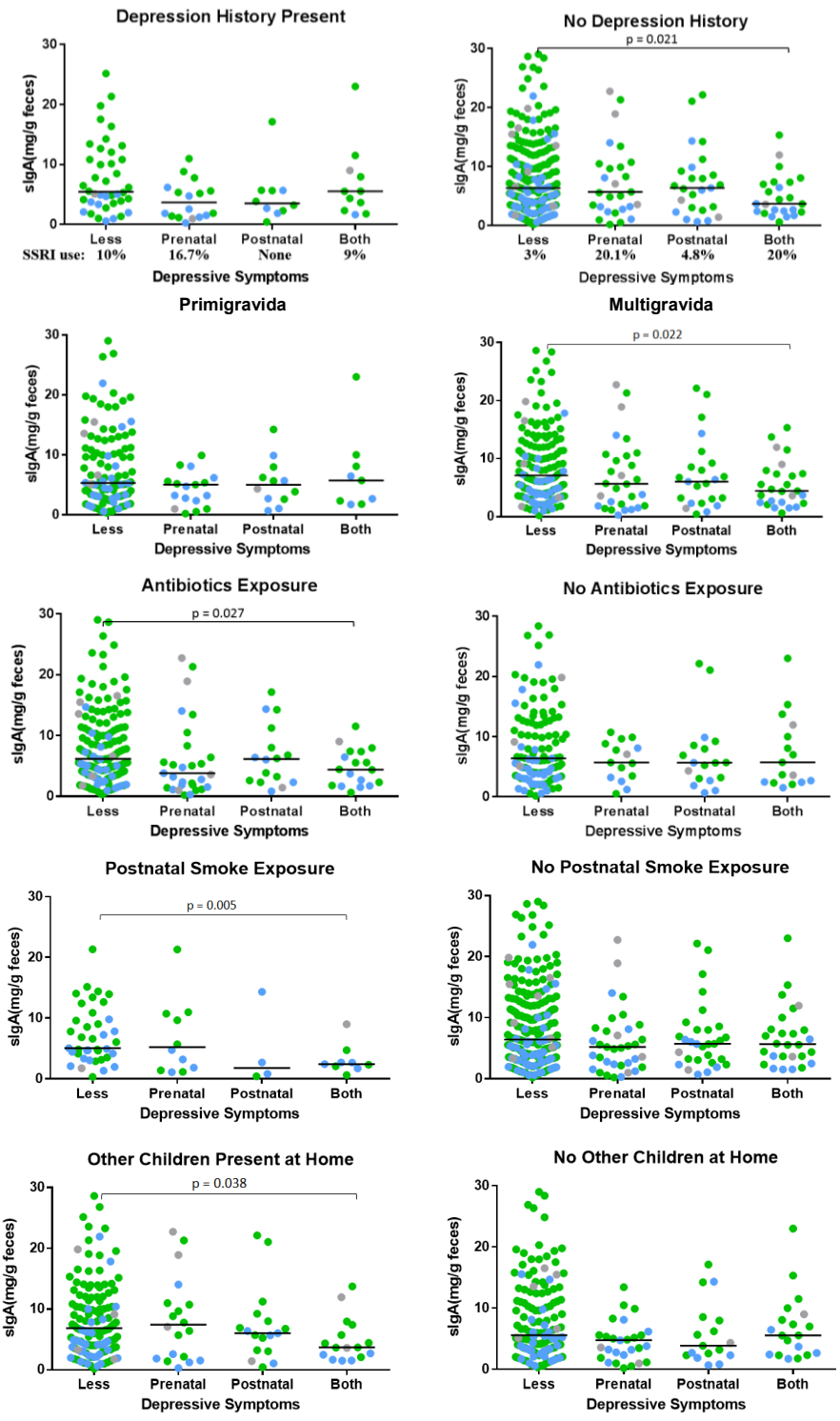

Figure 3. sIgA levels among depressive symptoms groups after stratification for potential covariates. Breastfed infants are in green and exclusively formula-fed infants are in blue. 2-10 points were omitted in each graph for having an sIgA level greater than the scale.

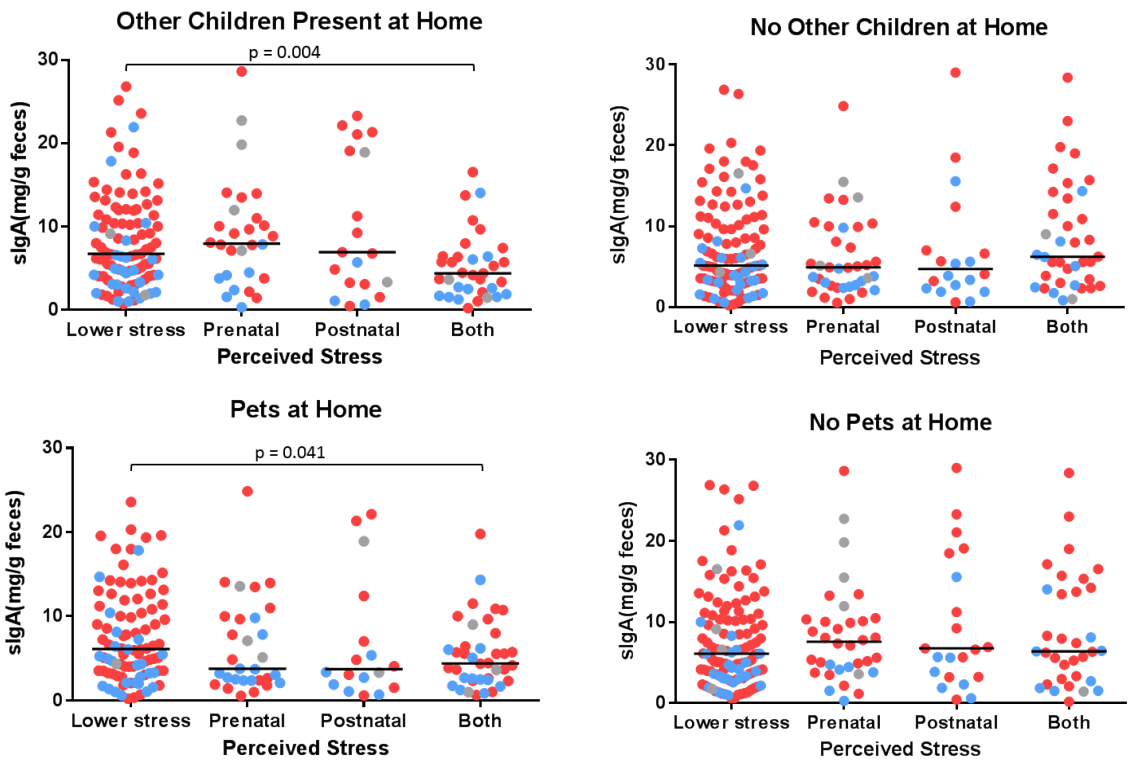

Figure 4. Infant sIgA levels among higher perceived stress groups after stratification for other children and pets living at home. Statistically significant differences were seen between infants exposed to both pre and postnatal higher maternal stress compared to the lower stress group (reference group). Breastfed infants are in red, exclusively formula-fed infants are in blue and infants with unknown breastfeeding status at time of stool collection are in gray. 4-7 points were omitted in each graph for having sIgA greater than the scale.

3.2.2 Infant sIgA with exposure to only prenatal distress

For the group of mothers who experienced clinically significant depressive symptoms during the prenatal period only, a significantly lower median value of sIgA than the reference group was seen with pet ownership, and significance remained in this group of infants when exposed to antibiotics and born to a primigravida (Figure 2, Table E1). Significant interactions

were found with pet ownership (Table E1). In infants born to mothers that reported perceived stress in the highest quartile, no significant differences in sIgA levels were seen compared to the reference group (Table E4).

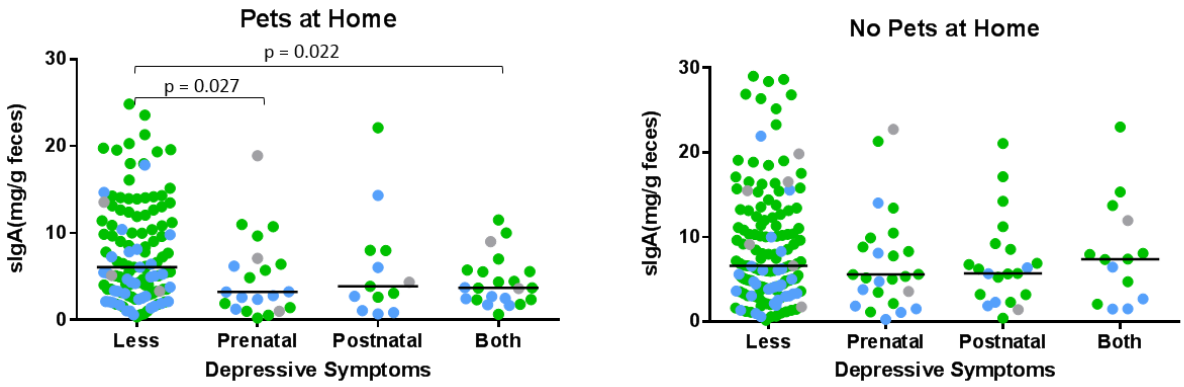

Figure 5. Infant sIgA levels based on maternal depressive symptoms groups after stratification for pet ownership. With pet ownership, prenatal only and both pre and postnatal groups had significantly lower sIgA levels than the reference group. No differences were seen among the depressive symptom categories with the absence of pets. Green data points indicate breastfed infants during the time of stool collection, blue indicates exclusively formula-fed infants and gray indicates unknown breastfeeding status. 2-10 points were omitted for having sIgA greater than the scale.

3.2.3 Infant sIgA with exposure to only postnatal distress

Sex differences were seen with the exposure to only postnatal depressive symptoms. Figure 6 shows significantly lower infant sIgA levels with exposure to postnatal depressive symptoms compared to the reference group in male infants (median (IQR)=4.56 (2.00 – 7.99) mg/g feces versus 6.62 (3.53 – 13.15) mg/g feces), but this difference was not observed in female infants. After stratifying by antibiotics exposure up to 3 months of age, the male infants with no antibiotics exposure still showed this significant difference (p=0.037; Table E1); however, the number of males in this category was small. Additionally, when the mother had asthma or allergy during pregnancy, a statistically significant difference was seen in infant sIgA levels with lower levels after exposure to postnatal depressive symptoms (p=0.041; Figure 6). Infants born to mothers with asthma or allergy continued to have significantly lower sIgA levels with exposure to postnatal depressive symptoms when they had antibiotics exposure (p=0.02) and were living with pets (p=0.014; Table E1). Interestingly, the differences with depressive symptoms were not seen with higher perceived stress (Table E4). Higher sIgA concentrations were seen with exposure to postnatal higher stress when the infant was breastfed and had antibiotics, when exposed to antibiotics and pets and when the mother was primigravida and living with pets (Table E4). Since the counts were small in these stratification groups, these results should be taken with caution.

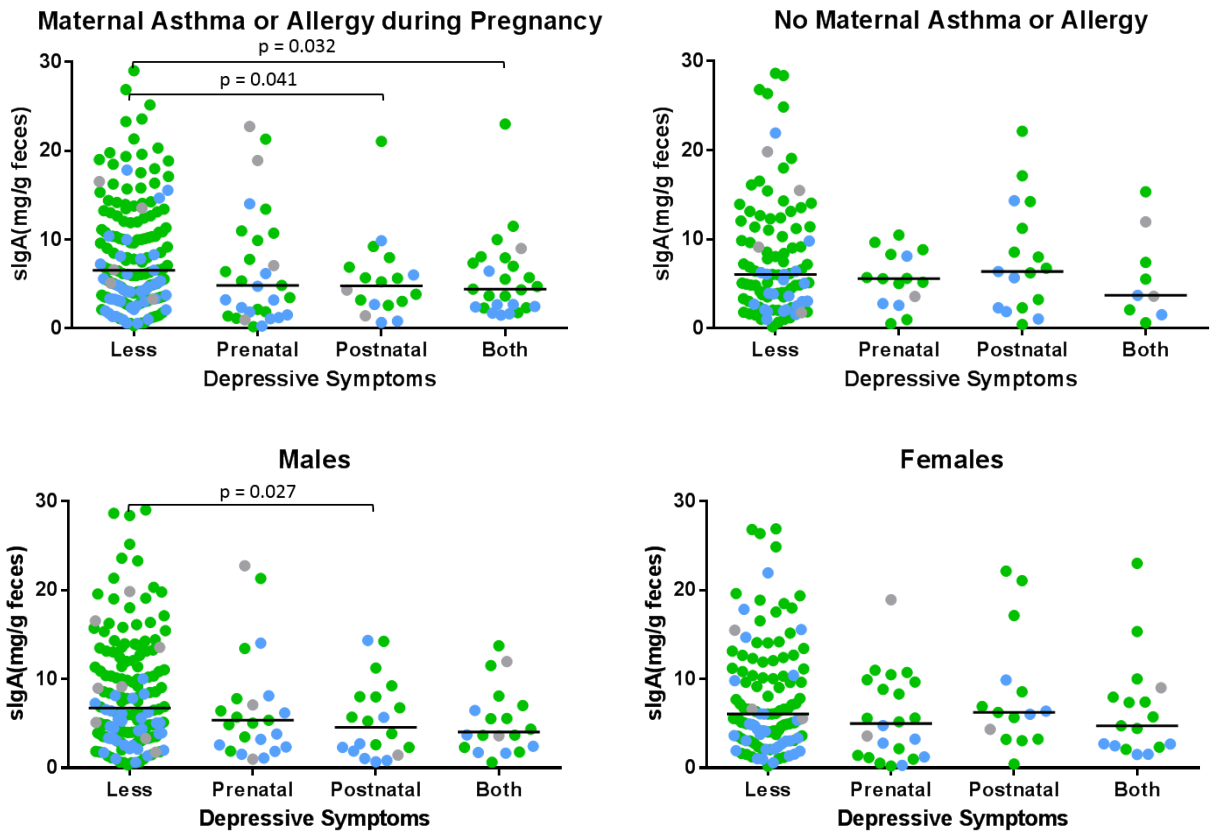

Figure 6. Infant sIgA levels based on maternal depressive symptoms groups after stratification for maternal asthma or allergy during pregnancy and child sex. In infants born to mothers with asthma or allergy and in male infants, the postnatal only group had significantly lower sIgA levels than the reference group. No differences were seen among the depressive symptom categories in the absence of maternal asthma or allergy and in female infants. Green data points indicate breastfed infants during the time of stool collection, blue indicates exclusively formula-fed infants and gray indicates unknown breastfeeding status. 2-10 points were omitted for having sIgA greater than the scale.

3.2.4 Simple and multiple logistic regression analyses with lowest quartile sIgA

A preliminary logistic regression analysis, shown in Appendix C, looking at the odds of being below median for sIgA levels showed significant odds ratios with only prenatal depressive symptoms when adjusting for antibiotics exposure (1.96, 95% CI (1.03 – 3.73)) and collectively adjusting for antibiotics, breastfeeding, pets and gravida (2.57, 95% CI (1.17 – 5.65); Table C1). Highest quartile IgA was also used as the outcome variable in, and the only significant OR was found after controlling for breastfeeding status at time of stool collection with the exposure of prenatal depressive symptoms (Table C2). With depressive symptoms during the prenatal period only, there was a 68% reduction in odds of having infant sIgA levels in the highest quartile (Table C2). Although a first look at the sIgA data suggested more differences detected between higher distress and lower distress when categorizing sIgA into tertiles (Table B1), the final regression models using lowest tertile did not detect many significant odds ratios (Table C4).

Instead of using lowest tertile of sIgA for the outcome, we used lowest quartile for the main regression analyses, which looks at the odds of the infant having sIgA levels in the lowest quartile depending on the maternal distress status. The crude OR for both pre and postnatal depressive symptoms is 2.12 (95% CI: 1.02 – 4.42; Table 4), which means with the exposure of maternal depressive symptoms, the likelihood of the infant having sIgA levels in the lowest quartile is 2.12 times greater than when there are less symptoms (below cut-off). The OR lost significance after single adjustment for the pet ownership, postnatal smoke exposure and prenatal SSRI use. After including covariates of confounding influence and statistically significance at $p<0.20$ in various combinations in the multiple logistic regression models, the final models were created. Final regression model for low sIgA showed a 3.07 times increase in the odds of having fecal sIgA in the lowest quartile with the exposure of higher pre and postnatal depressive

symptoms than less symptoms after multiple adjustment for infant age, antibiotics exposure, maternal asthma or allergy, prenatal SSRI use, pets, breastfeeding and gravida (95% CI: 1.25 – 7.55; Table 4).

Table 4. Crude and adjusted odds ratios (OR) of low sIgA for exposure to maternal depressive symptoms

(Ref: less depressive symptoms)	**OR (95% CI) for pre and postnatal**	**OR (95% CI) for prenatal only**	**OR (95% CI) for postnatal only**
Crude	**2.12 (1.02 – 4.42)**	**2.08 (1.08 – 4.01)**	**2.31 (1.10 – 4.87)**
Adjusted for age (quartiles)	**2.35 (1.11 – 4.97)**	1.88 (0.96 – 3.67)	2.12 (0.99 – 4.55)
Adjusted for maternal asthma or allergy during pregnancy	**2.27 (1.08 – 4.76)**	**2.18 (1.12 – 4.21)**	**2.28 (1.08 – 4.82)**
Adjusted for antibiotics exposure	**2.39 (1.13 – 5.05)**	**2.30 (1.18 – 4.49)**	**2.22 (1.03 – 4.78)**
Adjusted for gravida (primigravida or not)	**2.44 (1.15 – 5.16)**	**2.17 (1.12 – 4.20)**	**2.46 (1.16 – 5.21)**
Adjusted for birth characteristics (gravida, antibiotics)	**2.56 (1.20 – 5.45)**	**2.36 (1.20 – 4.62)**	**2.29 (1.06 – 4.94)**
Adjusted for pets at home	1.96 (0.93 – 4.12)	**2.08 (1.08 – 4.03)**	**2.51 (1.18 – 5.35)**
Adjusted for home environment (pets and children at home)	1.81 (0.85 – 3.86)	**2.05 (1.06 – 3.96)**	**2.50 (1.17 – 5.34)**
Adjusted for prenatal smoke exposure	**2.15 (1.01 – 4.55)**	1.96 (1.00 – 3.96)	**2.33 (1.10 – 4.95)**
Adjusted for postnatal smoke exposure	2.00 (0.95 – 4.21)	**1.96 (1.01 – 3.81)**	**2.48 (1.16 – 5.26)**
Adjusted for breastfeeding at stool collection	**2.39 (1.06 – 5.39)**	**2.21 (1.05 – 4.65)**	1.78 (0.79 – 4.01)
Adjusted for prenatal SSRI	2.11 (1.00 – 4.48)	1.86 (0.94 – 3.70)	**2.36 (1.12 – 5.01)**
Adjusted for postnatal SSRI	**2.11 (1.01 – 4.41)**	**2.15 (1.10 – 4.21)**	2.01 (0.92 – 4.41)
Adjusted for postnatal SSRI and breastfeeding	**2.41 (1.06 – 5.48)**	**2.37 (1.10 – 5.10)**	1.52 (0.64 – 3.58)
Adjusted for breastfeeding and prenatal smoke	**2.42 (1.06 – 5.52)**	**2.26 (1.05 – 4.83)**	1.81 (0.80 – 4.09)
Adjusted for age, breastfeeding, antibiotics, postnatal SSRI, maternal asthma/allergy, pets, gravida (final model for postnatal only)	**3.33 (1.36 – 8.19)**	**2.82 (1.23 – 6.47)**	1.43 (0.57 – 3.60)
Adjusted for age, antibiotics, maternal asthma/allergy, pets, breastfeeding, gravida, prenatal SSRI (final model for prenatal only and both)	**3.07 (1.25 – 7.55)**	**2.44 (1.07 – 5.57)**	1.93 (0.80 – 4.68)
Final model for prenatal only and both with interaction term for antibiotics	**5.04 (1.32 – 19.26)**	0.70 (0.16 – 3.06)	3.13 (0.83 – 11.78)

With the same model, the OR for prenatal depressive symptoms only was 2.44 (95% CI: 1.07 – 5.57). Adjusting for most of the study covariates individually did not affect the statistical significance of the odds ratios for prenatal depressive symptoms, but when adjusting for prenatal SSRI use and smoking in mothers and infant age, significance was lost (Table 4). The interaction term between depressive symptoms and antibiotics exposure from birth to three months of age was significant (Table E3), and so when the interaction term was added to the model, the OR for prenatal only lost significance. However, the OR for both pre and postnatal raised to 5.04 (95% CI: 1.32 – 19.26) with the addition of the interaction in the final model. With the exposure of only postnatal depressive symptoms, we found a crude odds ratio of 2.31 (95% CI: 1.10 – 4.87), which means infants have 2.3 times greater odds of having sIgA levels in the lowest quartile. The odds ratio maintained significance until we controlled for breastfeeding and postnatal SSRI use in the mothers (Table 4). The final model did not have a significant odds ratio for postnatal depressive symptoms.

The crude OR for pre and postnatal higher stress (highest quartile) in mothers was not statistically significant (OR=1.65, 95% CI: 0.92 – 2.98; Table 5). The odds ratio only became significant with the adjustment of gravida and antibiotics exposure (OR=1.87, 95% CI: 1.03 – 3.41). Therefore, after controlling for gravida and antibiotics, the odds of having low sIgA is 1.87 times greater when having higher stress during the perinatal period than having lower stress (reference group). The final multivariate model that adjusts for infant age, breastfeeding, antibiotics, postnatal SSRI and maternal asthma or allergy did not have a significant odds ratio. The crude and adjusted odds ratios for low sIgA were not statistically significant with the exposure to higher prenatal only stress and higher postnatal only stress (Table 5).

Table 5. Crude and adjusted odds ratios (OR) of low sIgA for exposure to maternal higher perceived stress

(Ref: lower stress)	OR (95% CI) for pre and postnatal	OR (95% CI) for prenatal only	OR (95% CI) for postnatal only
Crude	1.65 (0.92 – 2.98)	1.37 (0.73 – 2.58)	1.74 (0.83 – 3.62)
Adjusted for child age	1.44 (0.80 – 2.58)	1.27 (0.63 – 2.54)	1.28 (0.61 – 2.71)
Adjusted for maternal asthma or allergy during pregnancy	1.63 (0.90 – 2.97)	1.42 (0.75 – 2.67)	1.82 (0.87 – 3.81)
Adjusted for antibiotics exposure	1.82 (1.00 – 3.30)	1.40 (0.74 – 2.65)	1.59 (0.73 – 3.46)
Adjusted for gravida (primigravida or not)	1.76 (0.97 – 3.19)	1.39 (0.87 – 2.22)	1.63 (0.77 – 3.46)
Adjusted for birth characteristics (gravida, antibiotics)	**1.87 (1.03 – 3.41)**	1.40 (0.74 – 2.65)	1.59 (0.73 – 3.48)
Adjusted for pets at home	1.64 (0.90 – 2.98)	1.36 (0.72 – 2.56)	1.74 (0.83 – 3.63)
Adjusted for home environment (pets and children at home)	1.57 (0.86 – 2.86)	1.33 (0.70 – 2.51)	1.74 (0.83 – 3.63)
Adjusted for prenatal smoke exposure	1.75 (0.97 – 3.17)	1.43 (0.76 – 2.71)	1.82 (0.87 – 3.81)
Adjusted for postnatal smoke exposure	1.51 (0.82 – 2.76)	1.35 (0.72 – 2.55)	1.77 (0.84 – 3.69)
Adjusted for breastfeeding at stool collection	1.44 (0.76 – 2.74)	1.72 (0.86 – 3.44)	1.70 (0.77 – 3.78)
Adjusted for prenatal SSRI	1.52 (0.83 – 2.78)	1.30 (0.69 – 2.45)	1.73 (0.83 – 3.63)
Adjusted for postnatal SSRI	1.62 (0.89 – 2.96)	1.38 (0.73 – 2.61)	1.38 (0.73 – 2.61)
Adjusted for postnatal SSRI and breastfeeding	1.45 (0.74 – 2.81)	1.70 (0.84 – 3.44)	1.46 (0.65 – 3.31)
Adjusted for breastfeeding and prenatal smoke	1.53 (0.80 – 2.94)	1.87 (0.93 – 3.79)	1.76 (0.79 – 3.93)
Adjusted for breastfeeding and postnatal smoke	1.38 (0.71 – 2.67)	1.69 (0.84 – 3.41)	1.71 (0.77 – 3.79)
Adjusted for age, breastfeeding, antibiotics, postnatal SSRI, maternal asthma/allergy (final model for perceived stress)	1.39 (0.73 – 2.65)	1.26 (0.59 – 2.69)	0.97 (0.40 – 2.32)

Chapter 4: Discussion and Conclusions

This final chapter will summarize the findings that were mentioned in Chapter 3 and interpret the results to see how it relates to other studies. The strengths and limitations of the study design mentioned in Chapter 2, as well as clinical relevance and directions for future research are also outlined in this chapter.

4.1 Summary and interpretation of the findings

4.1.1 General overview

We tested for possible associations between pre and postnatal maternal distress and infant fecal sIgA in humans based on a sub-sample of 403 infants from a prospective birth cohort. We found that after higher exposure or clinically significant exposure to maternal perceived stress and depressive symptoms during both the pre and postnatal period, infant fecal sIgA levels in the first few months of life were significantly lower compared to the reference group. This result is consistent with the sole animal study that found that chronic restraint stress lowered total intestinal IgA in mice (122). Maternal prenatal stress affects the maternal gut and vaginal microbiome, which are speculated to affect the colonization of microbiota in the infant gut during the pregnancy and birth process (15). Although a first report on humans has shown prenatal maternal stress associated with gut dysbiosis in infants linked to the development of increased gastrointestinal symptoms and allergic reactions (121), whether the stress was also affecting gut immunity in infants has not been previously investigated. Our results show that distress may lower infant fecal sIgA levels, which is a vital component in the first-line of immune defense and development of the gut microbiota composition in early life. A possible reason for the low levels of sIgA is that distress may affect the gut immune system development

during pregnancy. Along with this change, delivery or factors that co-occur with distress in the perinatal period may affect colonization of microbes that favour sIgA production, such as an altered stress response seen in infants of distressed mothers.

4.1.2 Time-specific distress effects

Median comparisons with covariate stratifications suggest interaction effects of covariates with time and type of distress, such as pet ownership for prenatal only depressive symptoms, maternal asthma or allergy and infant sex for postnatal only depressive symptoms and breastfeeding status, depression history, gravida, antibiotics, tobacco smoke exposure, other children and pets for both pre and postnatal distress. The different results found with the time period and duration of distress emphasizes that stress and depression effects on infant gut health have time-dependent variations, and other factors related to the association that co-occur with distress during a specific period may affect the association between distress and infant gut immunity. Lee et al. (133) found dose-response relationships between maternal stress and childhood asthma for prenatal and postnatal periods respectively in a prospective birth cohort and found that higher stress in both pre and postnatal periods increased the odds of diagnosis of asthma in girls. Our results support the temporal-specific effects of distress on infant gut immunity, which suggest a need for greater understanding on the different mechanisms in different perinatal periods and how the prenatal stress combined with postnatal stress works to affect infant immunity and risk of developing allergic diseases.

4.1.3 Pre and postnatal distress effects on infant sIgA levels

In the logistic regression analyses, the odds ratio for higher exposure to both pre and postnatal depressive symptoms lost significance after controlling individually for pets, other children, postnatal smoke exposure and prenatal SSRI use, but only pets and prenatal SSRIs stayed in the final model. Breastfeeding, when combined with postnatal tobacco smoke exposure or SSRI use, made the smoke and SSRI variables have little influence on the odds ratios, suggesting that breastfeeding status may explain for the influence of tobacco smoke exposure and SSRI use on the association. Smoke and SSRIs may affect the breastmilk composition or breastfeeding status or duration due to their associations with distress. The final model collectively controlled for infant age, antibiotics, maternal asthma or allergy, breastfeeding, pets and gravida, suggests that despite controlling for the many study covariates, there appears to be a unique association between pre and postnatal depressive symptoms and low infant sIgA. For instance, although being born to a mother with asthma or allergy could cause immunological differences and allergic responsiveness in the infant as shown in Section 1.5.1, regardless of this status infants still appear to have higher risk of low sIgA when their mom has clinically significant perinatal depressive symptoms.

The odds ratio for higher perceived stress both pre and postnatally in mothers was only significant after controlling for gravida and antibiotics, suggesting that these covariates may mask the association between perinatal stress and infant IgA. In other words, if the mother-infant dyads had the same gravida status and antibiotics exposure status, a significant association between higher stress and low sIgA would be observed. No other odds ratios were found with higher perceived stress and low sIgA, even for the prenatal only and postnatal only odds ratios. This may have happened because of the higher levels of sIgA seen with higher stress in some of

our median comparisons. Perhaps more infections occurred in the infants of these groups, which may elevate the fecal IgA levels (101), but the number of infections was not determined in our study. Another explanation may be other physiological processes occurring with stress, and some animal models of acute stress have shown upregulation of immune cells (134). Restraint stress in rats have shown increased levels of IgA and increased mRNA expression of pIgR, the polymeric immunoglobulin receptor that is important for sIgA transcytosis into the gut lumen (134).

The combination of the prenatal distress affecting fetal stress response and gut immune development along with environmental circumstances co-occurring with distressed mothers during the postpartum period may have greater impact on the infant gut immunity than having only prenatal distress or only postnatal distress in the first few months of life. Maternal stress appears to affect the programming of the fetal hypothalamic–pituitary–adrenal (HPA) axis, as maternal stress seems to stimulate corticotrophin-releasing hormones that elevate fetal secretion of glucocorticoids to excess levels (135). One possible mechanism for this programming may be from methylation of fetal glucocorticoid receptors (136). This change in infant stress response may affect the infant immune response to environmental factors that occur with postpartum distress. Maternal distress usually does not end at birth and continues during postpartum (15), possibly impairing the interaction between mother and infant and the stress and immune response of offspring (22, 137). Offspring have elevated cortisol levels when the mother is distressed during the early months in both animal and human studies (135).

Depressed mothers have been known to interact less with their infant and are less likely to breastfeed their child, which may make the infant more vulnerable to infections (79). No significant differences in sIgA were observed in breastfed infants among the maternal depressive symptoms groups compared to reference but differences were seen in formula-fed infants. This

may indicate the protective effects of breastfeeding against the impact of depressive symptoms on infant gut immunity. Breastfeeding has been associated with higher levels of total fecal IgA in the infant (52), and is associated with the reduced risks of allergic disease (39, 59). Interventions encouraging breastfeeding in depressed mothers may allow for sufficient maturation of the infant immune system and reduce the risk of developing allergic diseases. However, it should be acknowledged that breastfeeding is not a simple task, and it could cause more stress and frustration on mothers. In addition, breastfeeding was controlled in the final logistic regression models for lowest quartile sIgA, which suggests that depressive symptoms still affect infant gut immunity regardless of the infant's diet in the early months.

4.1.4 Prenatal only distress effects on infant sIgA levels

For the prenatal only analyses, we found that exposure to depressive symptoms at prenatal only increased the crude odds of having low fecal sIgA in infants by 2.1 times. Prenatal SSRI use in mothers was the only covariate that made the odds ratio lose significance, suggesting that serotonin levels in mothers during pregnancy may affect the fetal development of the gut immune system. This confirms the suggested immunomodulatory effects of SSRIs as previously discussed in Section 1.5.7. Serotonin has a negative association with inflammation in depressed patients, and there are serotonin receptors in blood cells involved in immunity, B cells, T cells, and dendritic cells, which are involved in the class switching and secretion of sIgA (138). However, the multiple subtypes of serotonin receptors may cause serotonin to have various inhibitory or stimulatory effects on the gut immune system. The final logistic regression model was the same as the model for both pre and postnatal depressive symptoms, and the statistically significant odds ratio indicates 2.4 times greater likelihood of having low sIgA after higher

exposure during the prenatal period. Even after taking into account the SSRI use and other covariates, the association between higher prenatal depressive symptoms and gut sIgA levels remained significant, suggesting that the physiology of depressed mothers may have influence on the fetal gut immunity development. Murine models have shown lower plasma B cell counts after direct exposure to restraint stress due to the effects of glucocorticoids and catecholamines (114), and these hormones were responsible for the reduction of intestinal IgA (122). Although catecholamines have not been associated with maternal perceived distress (stress and anxiety) (139), one particular glucocorticoid that is associated with maternal stress, corticosterone, at elevated levels is a strong Th2 cytokine inducer and increases allergic responses in the offspring after it crosses the placenta (63).

An interesting finding was the interaction between prenatal depressive symptoms and antibiotics exposure from birth to 3 months of infant age. In another study, there was a significant reduction in the odds ratio for prenatal distress and childhood asthma at age seven when adjusting for antibiotics use during infancy (140). Once the interaction was added to our final logistic regression models, the prenatal only OR greatly reduced and lost statistical significance, while the opposite occurred for the pre and postnatal depressive symptoms OR. This result may suggest the difference in gut immunity of infants born to a mother that experiences prenatal only and both pre and postnatal depressive symptoms. Antibiotics use for those in the prenatal only group may explain for the association between prenatal distress and low sIgA, but this is not the case for the both pre and postnatal group.

4.1.5 Postnatal only distress effects on infant sIgA

The odds ratio for only postnatal depressive symptoms became non-significant after individually controlling for breastfeeding, postnatal SSRI use and tobacco smoke exposure, indicating that postnatal depressive symptoms seem to not have a unique association with fecal sIgA after the effects of the covariates are removed. The final model for postnatal depressive symptoms, which adjusted for infant age, antibiotics exposure, maternal asthma/allergy, postnatal SSRI, breastfeeding and gravida, was not statistically significant. The odds ratios for postnatal higher stress remained non-significant for both crude and adjusted.

SSRI use and living with a household member that smokes, both which are more common with maternal depression (141), may be the factors that affect gut immunity. SSRIs can be passed through the breastmilk and have been related to some neonatal health outcomes (18), and our model shows that it may be part of the explanation for the lower sIgA levels with postnatal depressive symptoms. Not only does serotonin affect the immune system, but it also works closely with the gut microbiota, since some gut microbes contribute to the serotonin system and the serotonin system can alleviate the cognitive impairment effects of antibiotic-induced gut dysbiosis (142, 143). Percent SSRI use may be an explanation for the differences seen with depression history (see Figure 3). Lower percent SSRI use was seen in women with less depressive symptoms who did not have depression history compared to women with less symptoms who did have depression history. The number of women who did take SSRIs pre and postnatally was low ($n<30$); therefore, median comparisons could not be made with stratification of SSRI use. The findings with SSRIs should be taken with caution, as inadequate adjustment of SSRI use may have occurred in the regression models due to the low number of SSRI use. In addition, the effect of tobacco smoke exposure was not significant when breastfeeding status was

added to the model. Women living with a smoker may be more likely to be depressed, which may affect their breastfeeding status, or tobacco smoke may change the breastmilk content and sIgA levels (105). Therefore, breastfeeding status may be the primary explanation for why there are lower fecal sIgA levels in infants that are exposed to postnatal depressive symptoms only.

4.1.6 Birth characteristics and home environment impacting on association of interest

As we hypothesized, birth characteristics and the environment appeared to affect the relationship between distress and fecal sIgA. Although new mothers have been documented to have greater depressive symptoms (20, 79), we found significantly lower sIgA after depressive symptoms exposure in multigravidas. Other birth characteristics, antibiotic exposure and non-SGA were also associated with lower sIgA levels possibly due to the gut dysbiosis caused by antibiotics (144) and increased levels of IgA secreted in breastmilk in SGA infants (145). The presence of other children in the house is related to the number of pregnancies of the mother, but we were able to see separate effects on sIgA from the presence of children in the house after exposure to maternal stress with the median comparisons. Even though pets are seen as beneficial for psychological health in adults (146), there were significant associations between depressive symptoms and lower infant sIgA levels in the presence pets. This is contrary to the "hygiene hypothesis" and studies that revealed pets and other children increasing sIgA levels in breastmilk and exposure to pets inducing total IgA production in non-breastfed infants to similar levels of IgA as breastfed infants (52, 59, 109). This may suggest that the presence of pets and other children may not have protective effects against maternal distress affecting infant gut immunity. The increased exposure to microbes in early life may cause competition and reduce the abundance of microbes that are stimulatory of sIgA. Lower *Lactobacillus reuteri* and

Bifidobacteria have been found with pet ownership (40,110), which are stimulators of sIgA. However, most of these variables were controlled for in the logistic regression analyses, and we still found significant associations between maternal depressive symptoms and infant gut sIgA.

4.2 Strengths of study

Although stress and gut microbiome and immunity have been studied in animal models, this is the first study to ever report associations between maternal distress and the development of gut immunity in humans. Since animal models are beneficial to determine mechanisms but sometimes not applicable to humans, our study greatly adds suggestions to the literature on the links between maternal psychosocial health and the gut immune maturation in early life. As having a trial testing this association would violate ethics, the prospective cohort design of the CHILD study allows us to investigate the exposure-outcome relationship of our question of interest in humans.

Although causality cannot be concluded with our study design, the prospective CHILD birth cohort can support the temporality of the relationship between exposure of perinatal distress and outcome of infant sIgA levels in the first few months of life. The cohort is a nationally representative birth cohort for Canada, and our sub-sample seems to be representative of the entire cohort based on Table 1. This sufficient representation of the country makes our sample more generalizable to the Canadian population. Since the cohort study was able to recruit over 3500 mothers, we were able to investigate with a sizeable sample and conduct various stratification analyses with potential covariates. The CHILD cohort obtained multifarious

information on home environment, maternal medications, maternal and infant health, birth characteristics and breastfeeding that allowed our study to look into many covariates.

Many previous studies used negative or stressful life events as a measure of parental distress. In our study, mothers reported perceived stress and depressive symptoms, which would indicate whether life stressors did affect the mother's psychosocial health, as opposed to relying on reports of negative life events that have uncertainty as to whether there was an experience of stress. Our distress measures were both from validated questionnaires that have been used widely in the research and clinical community (126, 128, 147).

We used single and multiple logistic regression analyses to statistically control for potential confounding variables and covariates for our question of interest. Since stress and depressive symptoms may co-occur with various other factors that affect infant gut immunity, such as smoking, antidepressant use, less breastfeeding and lower quality of relationship between mother and child, controlling for covariates may strengthen the validity of the association that was studied. In the final multivariate models, we were able to still find significant adjusted odds ratios when the mother had prenatal only depressive symptoms and when the mother had both pre and postnatal depressive symptoms.

4.3 Limitations of study

One limitation is that our stool samples were analyzed based on availability from the three study sites, which may have led to selection bias in our sample. Although our sub-sample was representative of the rest of the CHILD cohort for most of the covariates that we analyzed, there is the possibility that residual confounding that was not controlled. For instance, the

pregnancy overweight variable was only available for 304 infants in the sub-sample, and so we could not control for this variable in the final model. Covariates self-reported by mothers may be subject to reporting bias, such as maternal distress, maternal asthma and allergy, medications, smoke exposure, breastfeeding, depression history and the home environment. We did not have access to information about whether the infant was experiencing stress, if there was supplementation of prebiotics and probiotics, the quality of relationship and interactions between mother and child, the cleanliness of the home, the availability of social support for the mothers and the use of psychological treatments for distressed mothers (*e.g.,* Cognitive Behavior Therapy).

Our distress measure is not without limitations. The concept of distress includes anxiety along with depression and stress, but our study only measured perceived stress and depressive symptoms. A measure for maternal anxiety was not included in the CHILD study despite the strong associations with asthma (148, 149). In addition, the CES-D scores themselves cannot confirm the diagnosis of depression in the mothers (147).

Another limitation is that there is uncertainty as to whether the ELISA is detecting both free and bound sIgA. The manufacturing company claims that the kit partially separates sIgA from the bacteria, and therefore, the antibodies detect both free and bound sIgA. One study that looked at total sIgA using ELISA did not find significant differences in 1-month and 12-month total fecal sIgA between infants that developed allergy and did not develop allergy but found less sIgA-coated bacteria in the allergy group, which was determined by flow cytometry (61). This may suggest the need for further studies on sIgA and the interaction with gut microbiota in relation to maternal distress.

Some 3-month visits of the CHILD study were conducted when the infant was 2-8 months of age due to difficulties in communication. As no set timeframe was used to exclude infants from our sample, this wide range of infant age may have increased the variation of sIgA levels in our sample, and perhaps lower sIgA was observed because of the younger and older ages (61). However, there were also many variations in sIgA levels within 3-4 months of age. In the logistic regression analyses, we controlled for infant age, but still obtained significant results.

Although stool collected greater than 6 months had significantly lower sIgA levels than stool collected less than 6 months, sensitivity analyses show that having these differences did not have a significant impact on our results. Having these infants in our sample may help with the comparisons for postnatal depressive symptoms since the depression information was collected at 6 months of child age, which leads to another limitation about the postnatal measure for distress being based on time points that come after the proposed time of stool collection. One study on the EDEN mother-child cohort reported depressive symptom trajectories with measures at 4 months and 8 months postpartum that had very small changes in symptom scores between the two time points for both prenatal and postnatal trajectories (150). McCall-Hosenfield et al. (151) also reported postpartum depressive symptom trajectories with time points at 1 month and 6 months, and no major changes were observed.

Lastly, we detected few significant associations between higher perceived stress and low sIgA, and sometimes with the presence of stress, sIgA levels were higher than the reference group. Although we used a validated questionnaire for perceived stress, we did not have a recommended cut-off for very high levels of stress but instead used the highest quartile cut-off for all CHILD mothers, which may have affected our detection of an association between maternal stress and infant fecal sIgA. In addition, immigrant families were included in this study,

and so the questions in the maternal distress surveys may not capture the variations of the concept of stress depending on the culture.

4.4 Clinical relevance

Asthma and allergic diseases are the most common chronic diseases among children worldwide, and the prevalence is increasing (3, 152). This study will greatly contribute to the scholarly literature on the impact of maternal stress and depression on the immune system maturation in human infants. In addition, determining the existence of gut immunity changes in infants of mothers with pre and postnatal distress will provide insight on potential infant biomarkers to predict development of common chronic conditions in young individuals. The findings may encourage interventions in reducing distress during pregnancy and after birth to become more valuable to mothers and their families, as they have the potential to reduce the risk of future chronic diseases in newborns.

Encouraging breastfeeding without inflicting more stress on distressed mothers or having prebiotic or probiotic treatments for mother and infant may improve the infant gut health. Our results show that breastfeeding may have protective effects against distress lowering infant fecal sIgA levels. Several studies have found supplementation of prebiotics and probiotics containing microbes that are stimulatory of sIgA can increase sIgA levels in the gut (46, 48, 153). Prebiotics and probiotics also show benefits for reducing stress, depressive and anxiety-like symptoms (154-156). These may be future interventions to allow for sufficient establishment of the infant gut microbiota and prevent many chronic illnesses that are related to alterations in early gut microbiome composition.

4.5 Implications for future research

More studies on the mechanisms of maternal distress impacting infant gut immunity are needed. Still it is not known whether stress and depression have similar mechanisms. The compounds in tobacco smoke and the use of SSRIs in depressed women may be some of the factors that affect infant gut health, but this could not be directly tested with our study design. It would be beneficial to know which sIgA-microbe interactions distress affects as well as the optimal level of sIgA that is needed for infants to have healthy gut microbiome compositions. These various ideas may assist with the future developments of interventions that allow for the development of healthy infant gut microbiome and immunity.

4.6 Conclusion

This thesis investigated the impact of perinatal maternal distress on infant gut immunity in the first few months of life. We found that when the mother experiences both pre and postnatal depressive symptoms, the infants are more likely to have lower sIgA in their gut, even after controlling for various covariates. With population-level findings, this study highlights the importance of psychosocial health in mothers during the pre and postnatal period as it may contribute to the health of children in the early months of life and in later life.

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Appendices

Appendix A: Preliminary analyses with 304 sample and pregnancy overweight

Table A1. Infant fecal sIgA levels and percentage distribution of potential covariates in relation to prenatal maternal depressive symptoms and lowest quartile sIgA (n=304)

	Lower symptoms (71.2% overall)	Prenatal only (11.9% overall)	Postnatal only (9.3% overall)	Both (7.6% overall)		Fecal sIgA		Low sIgA	
		N (%)	N (%)	N (%)	p-value (χ^2)	median (IQR)	p-value	N (%)	p-value
Child sex									
Male	123 (73.7)	17 (10.2)	15 (9.0)	12 (7.2)	0.71	5.7 (3.1 – 11.2)	0.60	43 (25.6)	0.79
Female	92 (68.1)	19 (14.1)	13 (9.6)	11 (8.1)		6.1 (3.2 – 11.0)		33 (24.3)	
Depression History									
Yes	27 (50.9)	11 (20.8)	8 (15.1)	7 (13.2)	**0.004**	5.1 (2.2 – 8.7)	0.073	16 (30.2)	0.35
No	186 (75.9)	24 (9.8)	20 (8.2)	15 (6.1)		6.2 (3.3 – 11.5)		59 (24)	
Maternal asthma/allergy during pregnancy									
Yes	135 (71.8)	21 (11.2)	15 (8)	17 (9)	0.37	5.7 (3.3 – 11.0)	0.64	44 (23.4)	0.38
No	78 (70.9)	14 (12.7)	13 (11.8)	5 (4.5)		5.7 (2.8 – 11.2)		31 (27.9)	
Pregnancy overweight/obese									
Yes	85 (68.5)	19 (15.3)	11 (8.9)	9 (7.3)	0.54	5.1 (3.0 – 10.4)	0.11	35 (28.2)	0.28
No	128 (73.6)	17 (9.8)	15 (8.6	14 (8)		6.3 (3.5 – 12.0)		40 (22.7)	
Delivery mode									
Vaginal	152 (71.7)	23 (10.8)	23 (10.8)	14 (6.6)	0.26	6.3 (3.2 – 11.4)	0.51	51 (23.8)	0.36
Scheduled C-section	23 (71.9)	3 (9.4)	4 (12.5)	2 (6.3)		5.2 (3.7 – 11.2)		17 (31.5)	
Emergency C-section	37 (68.5)	10 (18.5)	1 (1.9)	6 (11.1)		5.1 (2.8 – 10.1)		6 (18.8)	
Antibiotics exposure up to 3 months									
Yes	112 (72.3)	22 (14.2)	11 (7.1)	10 (6.5)	0.23	5.5 (2.9 – 11.0)	0.38	42 (26.9)	0.40
No	98 (72.1)	11 (8.1)	16 (11.8)	11 (8.1)		6.1 (3.4 – 12.1)		31 (22.6)	
Gravida									
Primigravida	84 (73.7)	15 (13.2)	8 (7)	7 (6.1)	0.61	5.1 (2.7 – 10.0)	**0.046**	35 (30.7)	**0.058**
Multigravida	128 (69.6)	21 (11.4)	20 (10.9)	15 (8.2)		6.4 (3.5 – 12.2)		39 (21)	
Small for gestational age									
Yes	16 (69.6)	4 (17.4)	3 (13)	0	0.43	7.5 (3.6 – 15.6)	0.20	3 (13)	0.22
No	196 (71.3)	32 (11.6)	25 (9.1)	22 (8)		5.7 (3.1 – 10.9)		71 (25.6)	
Prenatal SSRI use									
Yes	4 (28.6)	6 (42.9)	0	4 (28.6)	**<0.001**	3.9 (2.4 – 6.7)	0.14	5 (35.7)	0.36
No	209 (73.3)	30 (10.5)	28 (9.8)	18 (6.3)		6.0 (3.2 – 11.3)		71 (24.8)	
Postnatal SSRI use									
Yes	7 (41.2)	6 (35.3)	1 (5.9)	3 (17.6)	**0.005**	5.3 (3.0 – 8.7)	0.59	4 (23.5)	0.89
No	205 (73.2)	30 (10.7)	25 (8.9)	20 (7.1)		5.7 (3.1 – 11.4)		71 (25.3)	
Prenatal smoke exposure									
Yes	10 (62.5)	3 (18.8)	2 (12.5)	1 (6.3)	0.77	5.1 (1.8 – 12.3)	0.39	6 (37.5)	0.24
No	203 (72)	32 (11.3)	26 (9.2)	21 (7.4)		6.0 (3.2 – 11.1)		69 (24.4)	
Postnatal smoke exposure									
Yes	31 (67.4)	7 (15.2)	2 (4.3)	6 (13)	0.26	4.9 (2.8 – 9.6)	0.25	14 (30.4)	0.37
No	184 (72.2)	29 (11.4)	25 (9.8)	17 (6.7)		6.0 (3.2 – 11.9)		62 (24.2)	
Pets at home									
Yes	106 (72.1)	18 (12.2)	8 (5.4)	15 (10.2)	0.089	5.1 (2.7 – 10.0)	**0.045**	43 (29.3)	0.12
No	108 (70.6)	18 (11.8)	19 (12.4)	8 (5.2)		6.4 (3.6 – 12.3)		33 (21.4)	
Other children at home									
Yes	101 (73.2)	12 (8.7)	15 (10.9)	10 (7.2)	0.33	6.4 (3.7 – 11.0)	0.16	30 (21.6)	0.22
No	114 (70.4)	24 (14.8)	12 (7.4)	12 (7.4)		5.3 (2.7 – 11.0)		45 (27.8)	
Breastfeeding at stool collection									
Zero	59 (72)	9 (11)	8 (9.8)	6 (7.3)	0.60	3.7 (2.1 – 6.0)	**<0.001**	36 (43.6)	**<0.001**
Partial	79 (70.5)	10 (8.9)	14 (12.5)	9 (8)		5.6 (3.3 – 10.3)		27 (24.1)	
Exclusive	65 (74.7)	12 (13.8)	4 (4.6)	6 (6.9)		10.4 (5.6 – 18.1)		10 (11.5)	
Infant allergic eczema									
Yes	20 (57.1)	4 (11.4)	4 (11.4)	7 (20)	0.025	5.9 (3.2 – 11.2)	0.90	8 (22.9)	0.76
No	194 (73.5)	32 (12.1)	22 (8.3)	16 (6.1)		5.7 (3.1 – 11.0)		67 (25.3)	

Table A2. Median comparisons of infant sIgA between maternal depressive symptoms groups when mothers are overweight/obese and normal weight (N=304)

sIgA (mg/g feces) Median (IQR)	Reference group											Interaction
	N	Lower symptoms	N	Prenatal only	p-value	N	Postnatal only	p-value	N	Both	p-value	p-value
Depressive symptoms	215	6.42 (3.52 – 12.40)	36	5.27 (2.64 – 10.29)	0.22	28	5.70 (2.64 – 9.06)	0.28	23	3.70 (1.78 – 8.09)	**0.027***	-
Pregnancy overweight/obese	85	5.46 (3.28 – 11.27)	19	4.77 (2.37 – 9.66)	0.35	11	5.66 (3.07 – 11.23)	0.73	9	2.30 (1.61 – 8.70)	**0.047***	0.95
Normal weight	128	7.20 (3.58 – 13.14)	17	5.59 (3.29 – 10.74)	0.52	15	6.04 (2.62 – 9.23)	0.36	14	5.14 (2.54 – 8.32)	0.15	

*Significant at alpha of 0.05 determined a priori

Table A3. Logistic regression analyses of lowest quartile sIgA and maternal depressive symptoms (N=304)

(Ref: Less depressive symptoms)	N for lowest quartile sIgA	OR for depressive symptoms	OR for covariate	Decision
Crude OR	76	1.38 (0.62 – 3.05) 1.69 (0.72 – 3.99) 2.75 (1.13 – 6.67)		
Adjusted for child sex	M-43 F-33	1.39 (0.62 – 3.09) 1.70 (0.72 – 4.01) 2.77 (1.14 – 6.71)	0.91 (0.53 – 1.54)	Out
Adjusted for maternal asthma/allergy	Y-44 N-31	1.44 (0.64 – 3.22) 1.67 (0.71 – 3.96) 3.17 (1.28 – 7.84)	0.75 (0.43 – 1.29)	In
Adjusted for pregnancy overweight	Y-35 N-40	1.31 (0.59 – 2.93) 1.56 (0.64 – 3.82) 2.73 (1.13 – 6.64)	1.32 (0.77 – 2.24)	Out
Adjusted for depression history	Y-16 N-59	1.41 (0.63 – 3.18) 1.68 (0.71 – 3.98) 2.94 (1.18 – 7.30)	1.17 (0.59 – 2.31)	Out
Adjusted for gravida	Y-35 N-39	1.42 (0.63 – 3.18) 1.89 (0.79 – 4.51) 3.30 (1.32 – 8.21)	1.75 (1.02 – 3.02)	In ($p<0.20$)
Adjusted for birth mode	El-6 Em-17 V-51	1.37 (0.61 – 3.06) 1.88 (0.79 – 4.48) 2.99 (1.21 – 7.41)	1.44 (0.73 – 2.82) 0.72 (0.28 – 1.87)	In
Adjusted for SGA	Y-3 N-71	1.47 (0.66 – 3.28) 1.80 (0.76 – 4.28) 2.95 (1.19 – 7.28)	0.45 (0.13 – 1.58)	In (marginally significant)
Adjusted for antibiotics exposure	Y-42 N-31	1.54 (0.68 – 3.49) 1.60 (0.65 – 3.90) 3.40 (1.35 – 8.53)	1.29 (0.75 – 2.23)	In
Adjusted for breastfeeding at time of stool collection	E-10 P-27 Z-36	1.52 (0.62 – 3.70) 1.38 (0.54 – 3.50) 3.43 (1.30 – 9.07)	6.25 (2.80 – 13.96) 2.45 (1.10 – 5.46)	In (always in model)
Adjusted for pets at home	Y-43 N-33	1.37 (0.61 – 3.05) 1.94 (0.81 – 4.66) 2.59 (1.06 – 6.31)	1.51 (0.88 – 2.58)	In ($p<0.20$)
Adjusted for other children	Y-30 N-45	1.32 (0.59 – 2.94) 1.85 (0.78 – 4.39) 2.48 (0.99 – 6.16)	0.72 (0.42 – 1.23)	In
Adjusted for prenatal SSRI use	Y-5 N-71	1.31 (0.58 – 2.97) 1.68 (0.71 – 3.96) 2.83 (1.13 – 7.11)	1.28 (0.38 – 4.27)	Out
Adjusted for postnatal SSRI use	Y-4 N-71	1.40 (0.62 – 3.15) 1.56 (0.64 – 3.82) 2.78 (1.14 – 6.79)	0.75 (0.23 – 2.47)	Out
Adjusted for prenatal smoke exposure	Y-6 N-69	1.42 (0.63 – 3.18) 1.70 (0.72 – 4.01) 3.04 (1.23 – 7.50)	1.83 (0.63 – 5.28)	In
Adjusted for postnatal smoke exposure	Y-14 N-62	1.36 (0.61 – 3.02) 1.82 (0.77 – 4.34) 2.67 (1.10 – 6.50)	1.31 (0.65 – 2.65)	Out
Adjusted for infant allergic eczema	Y-8 N-67	1.41 (0.64 – 3.15) 1.97 (0.82 – 4.73) 3.02 (1.22 – 7.50)	0.71 (0.30 – 1.70)	In
Adjusted for birth characteristics (gravida, birth mode, antibiotics,	73	1.59 (0.69 – 3.67) 1.81 (0.72 – 4.51) 3.37 (1.32 – 8.62)		

SGA)				
Adjusted for gravida, antibiotics, SGA	73	1.64 (0.72 – 3.75) 1.74 (0.70 – 4.32) 3.43 (1.35 – 8.72)		Keep birth mode out
Adjusted for gravida and antibiotics	73	1.57 (0.69 – 3.57) 1.71 (0.69 – 4.21) 3.60 (1.42 – 9.13)		Keep SGA out
Adjusted for home environment (pets and children)	75	1.31 (0.59 – 2.93) 2.00 (0.83 – 4.81) 2.34 (0.94 – 5.87)		In
Adjusted for home environment and breastfeeding	72	1.40 (0.57 – 3.46) 1.69 (0.64 – 4.44) 2.88 (1.06 – 7.83)		
Adjusted for other children and breastfeeding	72	1.42 (0.58 – 3.51) 1.57 (0.61 – 4.06) 3.04 (1.12 – 8.25)		Keep pets out
Adjusted for pets and breastfeeding	73	1.49 (0.61 – 3.65) 1.65 (0.63 – 4.30) 3.22 (1.21 – 8.56)		Keep children in
Adjusted for breastfeeding and maternal asthma/allergy	72	1.70 (0.69 – 4.18) 1.34 (0.52 – 3.46) 3.76 (1.40 – 10.10)		Keep asthma/allergy in
Adjusted for breastfeeding and pregnancy overweight	72	1.47 (0.60 – 3.60) 1.23 (0.46 – 3.27) 3.40 (1.28 – 9.03)		Keep overweight in
Adjusted for breastfeeding and prenatal smoke exposure	72	1.66 (0.67 – 4.11) 1.39 (0.54 – 3.57) 3.53 (1.33 – 9.40)		Keep smoke out
Adjusted for breastfeeding and postnatal smoke exposure	73	1.52 (0.62 – 3.71) 1.52 (0.59 – 3.92) 3.44 (1.29 – 9.12)		In
Adjusted for breastfeeding and prenatal SSRI	73	1.42 (0.57 – 3.54) 1.36 (0.53 – 3.46) 3.10 (1.12 – 8.57)		In
Adjusted for breastfeeding and postnatal SSRI	72	1.60 (0.65 – 3.96) 1.24 (0.46 – 3.30) 3.67 (1.36 – 9.95)		Out
Adjusted for breastfeeding and infant allergic eczema	72	1.53 (0.63 – 3.74) 1.62 (0.62 – 4.19) 3.70 (1.36 – 10.11)		In
Adjusted for gravida, antibiotics, breastfeeding	70	1.81 (0.71 – 4.63) 1.29 (0.48 – 3.48) 4.82 (1.68 – 13.84)		In
Adjusted for gravida, antibiotics, breastfeeding, maternal asthma/allergy	69	2.02 (0.78 – 5.24) 1.28 (0.47 – 3.49) 5.18 (1.79 – 15.03)		In
Adjusted for previous and overweight	68	1.95 (0.76 – 5.09) 1.13 (0.39 – 3.26) 5.15 (1.77 – 15.03)		Keep overweight out
Adjusted for gravida, antibiotics, breastfeeding, asthma/allergy, other children	69	2.01 (0.77 – 5.23) 1.45 (0.52 – 4.02) 4.56 (1.53 – 13.62)		In
Adjusted for antibiotics, breastfeeding, asthma/allergy, other children	68	1.93 (0.75 – 4.99) 1.38 (0.50 – 3.80) 4.34 (1.46 – 12.93)		Keep gravida out
Adjusted for previous &	68	1.73 (0.65 – 4.59)		In

prenatal SSRI		1.37 (0.50 – 3.77) 3.55 (1.13 – 11.13)		
Adjusted for previous & postnatal smoke	68	1.75 (0.66 – 4.66) 1.35 (0.49 – 3.73) 3.56 (1.14 – 11.16)		Keep smoke out
Adjusted for previous & prenatal smoke	69	1.69 (0.63 – 4.49) 1.34 (0.48 – 3.71) 3.54 (1.12 – 11.14)		Keep smoke out
Adjusted for antibiotics, breastfeeding, other children, maternal asthma/allergy, prenatal SSRI, infant allergic eczema	67	1.75 (0.66 – 4.62) 1.47 (0.53 – 4.08) 3.83 (1.19 – 12.36)		Keep infant eczema out
Adjusted for antibiotics, breastfeeding, other children, maternal asthma/allergy, prenatal SSRI	68	1.73 (0.65 – 4.59) 1.37 (0.50 – 3.77) 3.55 (1.13 – 11.13)		Final Model (No significant interactions)

Appendix B: Preliminary analyses with perceived stress cut-off at mean score of CHILD cohort (12.96)

Table B1. Quartile and tertile medians for sIgA (N=403)

sIgA (mg/g feces) Median (IQR)	All infants	Lower depressive symptoms	Higher depressive symptoms (above 16 for prenatal, postnatal or both)	Lower perceived stress	Above CHILD mean stress (for prenatal, postnatal or both)
1st quartile (lowest)	1.88 (1.15 – 2.55)	1.89 (1.16 – 2.60)	1.87 (1.11 – 2.48)	1.57 (1.07 – 2.10)	2.01 (1.18 – 2.58)
2nd quartile	4.46 (3.80 – 5.12)	4.33 (3.81 – 5.04)	4.81 (3.74 – 5.59)	4.33 (3.61 – 4.90)	4.73 (3.86 – 5.29)
3rd quartile	8.00 (6.62 – 9.67)	8.00 (6.61 – 9.82)	7.99 (6.87 – 9.34)	8.00 (6.64 – 9.62)	8.00 (6.62 – 9.80)
4th quartile	15.82 (13.44 – 21.64)	15.71 (13.21 – 20.08)	18.03 (13.81 – 22.94)	15.43 (13.15 – 25.17)	15.97 (13.48 – 21.32)
1st tertile (lowest)	2.32 (1.42 – 3.24)	2.46 (1.50 – 3.35)	2.23 (1.27 – 3.00)	2.09 (1.27 – 3.35)	2.33 (1.51 – 3.21)
2nd tertile	5.97 (4.97 – 7.11)	5.79 (4.87 – 6.83)	6.19 (5.45 – 7.60)	6.10 (4.86 – 7.20)	5.96 (5.08 – 7.07)
3rd tertile	14.06 (11.02 – 19.41)	14.01 (11.11 – 19.03)	14.14 (10.80 – 21.94)	14.26 (11.03 – 19.58)	13.95 (11.01 – 19.22)

Note: There is a suggestion that a greater difference between greater distress and lower distress groups with the tertiles. Logistic regression analyses were tested for both lowest tertile and lowest quartile (yes/no).

Table B2. Infant fecal sIgA levels and percentage distribution of potential covariates in relation to maternal perceived stress above the CHILD cohort mean and lowest quartile sIgA (n=403)

	Lower stress (28.4% overall)	**Prenatal only stress (19.5% overall)**	**Postnatal only stress (14.7% overall)**	**Both (37.4% overall)**		**Fecal sIgA**		**Low sIgA**	
		N (%)	N (%)	N (%)	p-value (χ^2)	median (IQR)	p-value	N (%)	p-value
Child sex									
Male	66 (29.5)	45 (20.1)	33 (14.7)	80 (35.7)	0.88	6.1 (3.3 – 11.7)	0.58	54 (24)	0.67
Female	48 (27.1)	33 (18.6)	26 (14.7)	70 (39.5)		5.6 (3.2 – 10.4)		46 (25.8)	
Depression History									
Yes	19 (24.1)	15 (19)	6 (7.6)	29 (49.4)	**0.056**	6.1 (3.3 – 11.4)	**0.04**	25 (31.6)	0.13
No	93 (29.5)	62 (19.7)	51 (16.2)	109 (34.6)		5.1 (2.3 – 9.0)		74 (23.4)	
Maternal asthma/allergy during pregnancy									
Yes	78 (29.3)	50 (18.8)	32 (12)	106 (39.8)	0.17	6.0 (3.2 – 12.0)	0.49	66 (24.7)	0.99
No	34 (26.6)	27 (21.1)	25 (19.5)	42 (32.8)		5.7 (3.2 – 9.8)		32 (24.8)	
Delivery mode									
Vaginal	85 (30.1)	52 (18.4)	43 (15.2)	102 (36.2)	0.64	5.6 (3.6 – 10.4)	0.50	70 (24.6)	0.56
C-elective	12 (25.5)	11 (23.4)	4 (8.5)	20 (42.6)		5.3 (2.9 – 10.4)		19 (27.9)	
C-emergent	15 (22.1)	15 (22.1)	12 (17.6)	26 (38.2)		6.2 (3.2 – 11.4)		9 (19.1)	
Antibiotics exposure up to 3 months									
Yes	62 (28.2)	43 (19.5)	33 (15)	82 (37.3)	0.99	5.6 (3.1 – 11.0)	0.28	58 (26.2)	0.43
No	50 (29.4)	34 (20)	24 (14.1)	62 (36.5)		6.1 (3.4 – 12.0)		39 (22.8)	
Gravida									
Primigravida	46 (30.3)	27 (17.8)	25 (16.4)	54 (35.5)	0.70	5.2 (3.0 – 10.0)	**0.077**	43 (28.3)	0.18
Multigravida	66 (26.9)	51 (20.8)	34 (13.9)	94 (38.4)		6.4 (3.5 – 12.0)		55 (22.3)	
Small for gestational age									
Yes	8 (29.6)	8 (29.6)	2 (7.4)	9 (33.3)	0.45	7.5 (4.7 – 15.7)	**0.063**	3 (11.1)	**0.09**
No	103 (28)	70 (19)	57 (15.5)	138 (37.5)		6.0 (3.2 – 10.9)		95 (25.7)	
Prenatal SSRI use									
Yes	0	3 (16.7)	0 (0)	15 (83.3)	**<0.001**	3.8 (2.7 – 6.7)	0.12	6 (33)	0.42
No	109 (29.1)	75 (20.1)	59 (15.8)	131 (35)		6.0 (3.2 – 11.0)		93 (24.8)	
Postnatal SSRI use									
Yes	2 (8.7)	5 (21.7)	2 (8.7)	14 (60.9)	**0.056**	4.7 (3.2 – 7.1)	0.18	6 (26.1)	0.89
No	109 (29.5)	72 (19.5)	56 (15.1)	133 (35.9)		6.1 (3.2 – 11.4)		92 (24.8)	
Prenatal smoke exposure									
Yes	3 (12.5)	9 (37.5)	3 (12.5)	9 (37.5)	0.087	5.1 (1.8 – 12.3)	0.39	6 (37.5)	0.24
No	109 (29.4)	68 (18.3)	54 (14.6)	140 (37.7)		6.0 (3.2 – 11.1)		69 (24.4)	
Postnatal smoke exposure									
Yes	12 (18.8)	13 (20.3)	7 (10.9)	32 (50)	0.084	4.9 (2.8 – 9.6)	0.25	14 (30.4)	0.37
No	102 (30.4)	65 (19.3)	52 (15.5)	117 (34.8)		6.1 (3.2 – 11.5)		62 (24.4)	
Pets at home									
Yes	49 (26.5)	36 (19.5)	29 (15.7)	71 (38.4)	0.86	5.3 (2.7 – 10.4)	**0.031**	55 (29.7)	**0.045**
No	64 (30)	42 (19.7)	30 (14.1)	77 (36.2)		6.4 (3.7 – 12.0)		45 (21)	
Children at home									
Yes	54 (28.6)	39 (20.6)	28 (14.8)	68 (36)	0.95	6.5 (3.6 – 11.9)	0.11	56 (26.8)	0.34
No	59 (28.2)	39 (18.7)	31 (14.8)	80 (38.3)		5.3 (3.1 – 10.7)		43 (22.6)	
Breastfeeding at stool collection									
Zero	23 (23)	21 (21)	14 (14)	42 (42)	0.57	3.3 (2.0 – 6.0)	**<0.001**	47 (47)	**<0.001**
Partial	50 (33.3)	28 (18.7)	19 (12.7)	53 (35.3)		5.4 (3.2 – 9.8)		39 (26)	
Exclusive	40 (30.5)	23 (17.6)	23 (17.6)	45 (34.4)		10.0 (5.6 – 16.1)		11 (8.4)	
Infant allergic eczema									
Yes	10 (23.3)	5 (11.6)	9 (20.9)	19 (44.2)	0.28	5.7 (3.4 – 11.4)	0.84	9 (20.9)	0.55
No	103 (29.2)	72 (20.4)	50 (14.2)	128 (36.3)		6.0 (3.2 – 11.2)		89 (25.1)	

Table B3. Median sIgA depending on maternal perceived stress above CHILD cohort mean after stratifying for potential covariates

sIgA (mg/g feces) Median (IQR)	Reference group											
	N	Less stress (1.8% SSRI use)	N	Prenatal only (6.4% SSRI use)	p-value	N	Postnatal only (3.4% SSRI use)	p-value	N	Both pre and postnatal stress (12% SSRI use)	p-value	p-value (K-W)
Perceived stress	114	6.17 (3.48 – 12.01)	78	6.16 (3.43- 11.02)	0.92	59	5.53 (3.16 – 11.40)	0.59	150	5.68 (2.71 – 10.78)	0.43	0.78
Depression history	19	6.48 (3.53 – 12.06)	15	5.19 (1.99 – 10.84)	0.64	6	2.01 (0.54 – 4.18)	0.009**	39	5.07 (2.31 – 7.96)	0.15	0.052
No depression history	93	6.10 (3.30 – 11.31)	62	6.69 (3.43 – 11.52)	0.57	51	6.62 (3.86 – 12.70)	0.61	109	6.07 (3.10 – 11.13)	0.97	0.88
Maternal asthma or allergy	78	6.30 (3.28 – 13.14)	50	7.90 (3.43 – 11.19)	0.68	32	5.51 (3.47 – 12.50)	0.94	106	5.64 (2.92 – 11.74)	0.69	0.86
No maternal asthma or allergy	34	6.12 (3.75 – 10.53)	27	5.19 (3.02 – 9.80)	0.77	25	6.42 (1.92 – 10.63)	0.60	42	5.70 (2.47 – 9.87)	0.62	0.95
Breastfed	90	7.82 (4.16 – 13.43)	51	5.66 (2.49 – 10.35)	0.32	42	6.50 (3.22 – 9.73)	0.62	98	5.66 (3.22 – 9.73)	0.32	0.55
Not breastfed	23	4.04 (2.22 – 6.09)	21	2.78 (1.54 – 4.77)	0.03	14	2.71 (1.07 – 6.39)	0.23	42	2.46 (1.63 – 2.97)	0.032*	0.25
Male	66	6.17 (3.48 – 12.21)	45	6.27 (3.63 – 12.30)	0.77	33	6.42 (3.04 – 12.59)	0.77	80	5.70 (2.62 – 11.45)	0.57	0.87
Female	48	6.28 (3.34 – 11.01)	33	6.06 (3.03 – 10.91)	0.89	26	5.43 (3.82 – 10.20)	0.58	70	5.64 (3.08 – 9.93)	0.56	0.92
Primigravida	46	4.92 (2.55 – 9.80)	27	5.19 (3.22 – 10.13)	0.26	25	4.34 (3.04 – 11.16)	0.93	54	5.68 (2.71 – 10.27)	0.41	0.70
Multigravida	66	7.59 (4.05 – 13.14)	51	7.26 (3.49 – 12.06)	0.35	34	6.52 (3.25 – 11.53)	0.33	94	5.71 (2.68 – 10.98)	0.09	0.38
Abx exposure	62	5.16 (3.31 – 10.53)	43	7.11 (3.22 – 11.34)	0.45	33	6.12 (3.42 – 13.86)	0.43	82	5.45 (2.49 – 9.38)	0.70	0.53
No Abx exposure	50	6.95 (3.62 – 13.38)	34	5.58 (3.39 – 10.18)	0.33	24	4.64 (2.35 – 9.12)	0.12	62	5.90 (3.22 – 11.27)	0.35	0.40
SGA	8	7.66 (4.21 – 15.88)	8	8.33 (2.23 – 40.11)	1.00	2	12.62 (4.17)	0.89[a]	9	5.61 (5.17 – 13.29)	0.70	0.99
Not SGA	103	6.15 (3.35 – 11.99)	70	6.04 (3.53 – 10.87)	0.97	57	5.53 (3.11 – 10.71)	0.56	138	5.71 (2.63 – 10.78)	0.46	0.83
Pets at home	49	6.15 (3.28 – 11.31)	36	5.09 (3.09 – 11.79)	0.89	29	4.94 (2.51 – 9.94)	0.38	71	5.10 (2.48 – 9.01)	0.26	0.59
No pets at home	64	6.28 (3.58 – 12.23)	42	7.64 (3.57 – 10.91)	0.67	30	6.62 (3.93 – 12.97)	0.81	77	6.23 (3.56 – 12.94)	0.88	0.98
Other children	54	8.43 (4.16 – 13.38)	39	8.00 (3.54 – 11.34)	0.44	28	6.64 (3.13 – 11.43)	0.37	68	5.70 (2.84 – 9.13)	0.032*	0.15
No other children	59	4.97 (3.10 – 9.61)	39	5.14 (3.22 – 10.33)	0.45	31	4.24 (3.16 – 11.40)	0.94	80	5.64 (2.70 – 12.18)	0.34	0.77
Prenatal SSRI	0	-	3	3.54 (3.22)	-	0	-	-	15	4.15 (2.48 – 7.08)	-	0.37
No Prenatal SSRI	109	6.10 (3.34 – 11.70)	75	7.11 (3.49 – 11.14)	0.79	59	5.53 (3.16 – 11.40)	0.78	131	5.71 (2.71 – 10.90)	0.82	0.82
Postnatal SSRI	2	6.38 (5.27)	5	3.59 (3.38 – 7.54)	0.38[a]	2	3.82 (2.12)	0.67[a]	14	4.44 (2.46 – 8.12)	0.50[a]	0.70
No postnatal SSRI	109	6.19 (3.44 – 12.19)	72	6.69 (3.17 – 11.29)	0.97	56	6.27 (3.37 – 11.78)	0.75	133	5.69 (2.71 – 10.97)	0.55	0.85
Infant allergic eczema	10	8.27 (4.18 – 16.23)	5	9.16 (5.79 – 17.39)	0.62	9	6.62 (2.64 – 13.86)	0.62	19	4.74 (3.21 – 8.00)	0.13	0.31
No infant allergic eczema	103	6.15 (3.52 – 12.06)	72	5.98 (3.10 – 10.95)	0.98	50	5.51 (3.25 – 10.36)	0.62	128	5.84 (2.70 – 11.18)	0.73	0.96
Breastfed and other children	43	10.20 (4.67 – 14.40)	26	9.85 (7.40 – 12.42)	0.99	21	6.91 (4.83 – 16.51)	0.73	42	6.20 (4.06 – 10.87)	0.21	0.27
Not breastfed and other children	10	4.53 (2.99 – 8.84)	11	2.56 (1.54 – 3.76)	0.02***	5	4.94 (1.51 – 8.22)	0.62	21	2.71 (1.61 – 6.06)	0.18	0.19
Breastfed and no pets	51	7.18 (3.62 – 13.15)	29	9.16 (5.16 – 10.99)	0.38	23	6.77 (4.17 – 15.82)	0.52	54	7.12 (4.55 – 13.85)	0.66	0.81
Not breastfed and no pets	12	4.29 (3.33 – 6.44)	9	2.56 (1.32 – 3.43)	0.013***	6	3.89 (1.93 – 7.09)	0.71	18	4.60 (1.79 – 6.41)	0.64	0.15

*Significant at 0.05 a priori; **Significant at Bonferroni adjusted p-value 0.05/1; *** Significant at Bonferroni adjusted p-value 0.05/2; [a]Fisher's Exact test

Table B4. Logistic regression analyses of lowest quartile sIgA and perceived stress above CHILD mean (N=403)

(Ref: lower stress)	OR for prenatal	OR for postnatal	OR for both
Crude OR	1.21 (0.61 – 2.40)	1.28 (0.61 – 2.68)	1.46 (0.82 – 2.59)
Adjusted for birth characteristics (gravida, birth mode, SGA, antibiotics)	1.35 (0.67 – 2.73)	1.19 (0.55 – 2.55)	1.60 (0.88 – 2.89)
Adjusted for breastfeeding at time of stool collection	1.19 (0.57 – 2.48)	1.27 (0.57 – 2.85)	1.34 (0.72 – 2.48)
Adjusted for home environment (children and pets at home)	1.17 (0.59 – 2.33)	1.20 (0.56 – 2.57)	1.34 (0.75 – 2.40)
Adjusted for postnatal SSRI	1.19 (0.60 – 2.37)	1.15 (0.54 – 2.45)	1.40 (0.78 – 2.51)
Adjusted for maternal asthma or allergy	1.20 (0.61 – 2.40)	1.18 (0.55 – 2.51)	1.45 (0.82 – 2.58)
Adjusted for maternal depression characteristics (depression history and postnatal SSRI)	1.17 (0.58 – 2.33)	1.10 (0.51 – 2.38)	1.33 (0.74 – 2.39)
Adjusted for breastfeeding at stool collection and pets at home	1.15 (0.55 – 2.40)	1.26 (0.56 – 2.82)	1.34 (0.72 – 2.50)
Adjusted for postnatal SSRI, pets at home, breastfeeding, antibiotics	1.19 (0.56 – 2.52)	1.10 (0.47 – 2.58)	1.39 (0.73 – 2.64)

Note: Significant odds ratios were not found; therefore, a new cut-off of 17 for the highest quartile of PSS scores was used to indicate higher stress.

Appendix C: Logistic regression analyses on below median sIgA, highest quartile and lowest tertile sIgA as outcome variables (yes/no)

Table C1. Odds of below median sIgA after exposure to pre and postnatal depressive symptoms

(Ref: less depressive symptoms/below cut-off)	OR (95% CI) for prenatal	OR (95% CI) for postnatal	OR (95% CI) for both
Crude	1.70 (0.92 – 3.14)	1.39 (0.69 – 2.81)	1.92 (0.95 – 3.89)
Adjusted for antibiotics use	**1.96 (1.03 – 3.73)**	1.33 (0.65 – 2.72)	1.76 (0.86 – 3.61)
Adjusted for gravida (primiparity)	1.74 (0.94 – 3.24)	1.45 (0.71 – 2.96)	2.02 (0.98 – 4.15)
Adjusted for birth characteristics (gravida, birth mode, SGA, antibiotics)	**2.02 (1.05 – 3.88)**	1.45 (0.70 – 3.00)	1.74 (0.83 – 3.64)
Adjusted for pets at home	1.71 (0.92 – 3.19)	1.54 (0.75 – 3.16)	1.81 (0.89 – 3.68)
Adjusted for home environment (pets and children at home)	1.68 (0.90 – 3.13)	1.57 (0.76 – 3.24)	1.76 (0.86 – 3.63)
Adjusted for prenatal SSRI	1.49 (0.79 – 2.82)	1.46 (0.71 – 2.99)	1.91 (0.92 – 3.97)
Adjusted for depression history	1.50 (0.79 – 2.86)	1.27 (0.62 – 2.60)	1.89 (0.91 – 3.91)
Adjusted for maternal depression characteristics (prenatal SSRI and depression history)	1.34 (0.69 – 2.60)	1.35 (0.66 – 2.80)	1.76 (0.84 – 3.69)
Adjusted for breastfeeding	1.94 (0.94 – 4.01)	0.91 (0.42 – 1.97)	2.15 (0.96 – 4.80)
Adjusted for antibiotics, gravida, pets, breastfeeding	**2.57 (1.17 – 5.65)**	1.02 (0.45 – 2.31)	2.06 (0.88 – 4.80)
Adjusted for antibiotics, gravida, pets, depression history, breastfeeding	**2.44 (1.07 – 5.56)**	0.90 (0.39 – 2.08)	1.92 (0.80 – 4.59)

Note: No significant odd ratios were found with perceived stress.

Table C2. Odds of high sIgA in infants after exposure to pre and postnatal depressive symptoms

(Ref: less depressive symptoms/below cut-off)	OR (95% CI) for prenatal	OR (95% CI) for postnatal	OR (95% CI) for both
Crude	0.48 (0.22 – 1.07)	0.51 (0.20 – 1.27)	0.48 (0.19 – 1.18)
Adjusted for antibiotics use	0.43 (0.19 – 1.01)	0.51 (0.20 – 1.28)	0.49 (0.20 – 1.24)
Adjusted for gravida (primiparity)	0.47 (0.21 – 1.04)	0.49 (0.19 – 1.22)	0.46 (0.18 – 1.14)
Adjusted for SGA	0.47 (0.21 – 1.04)	0.50 (0.20 – 1.26)	0.51 (0.20 – 1.28)
Adjusted for birth characteristics (gravida, birth mode, SGA, antibiotics)	0.43 (0.18 – 1.00)	0.47 (0.19 – 1.20)	0.51 (0.20 – 1.29)
Adjusted for home environment (pets and children at home)	0.48 (0.22 – 1.08)	0.52 (0.21 – 1.29)	0.51 (0.21 – 1.29)
Adjusted for breastfeeding	**0.32 (0.12 – 0.88)**	0.77 (0.29 – 2.08)	0.43 (0.15 – 1.22)
Adjusted for prenatal SSRI	0.52 (0.23 – 1.18)	0.54 (0.21 – 1.35)	0.41 (0.15 – 1.12)
Adjusted for maternal depression history and prenatal SSRI	0.59 (0.25 – 1.36)	0.56 (0.22 – 1.41)	0.44 (0.16 – 1.18)

Table C3. Odds of high sIgA in infants after exposure to pre and postnatal stress

(Ref: lower stress/below cut-off)	OR (95% CI) for prenatal	OR (95% CI) for postnatal	OR (95% CI) for both
Crude OR	0.86 (0.45 – 1.67)	0.91 (0.45 – 1.87)	0.85 (0.48 – 1.48)
Adjusted for breastfeeding at time of stool collection	0.75 (0.35 – 1.59)	0.91 (0.42 – 1.99)	0.86 (0.47 – 1.59)
Adjusted for prenatal SSRI	0.95 (0.48 – 1.86)	0.99 (0.48 – 2.04)	0.95 (0.53 – 1.71)
Adjusted for maternal depression characteristics (depression history, prenatal SSRI)	0.98 (0.50 – 1.92)	1.00 (0.48 – 2.08)	1.01 (0.56 – 1.81)

Table C4. Odds of lowest tertile in infants after exposure to pre and postnatal depressive symptoms

(Ref: Less depressive symptoms)	OR for depression	OR for covariate	Decision
Crude OR	1.81 (0.97 – 3.37) 1.61 (0.78 – 3.32) **2.05 (1.02 – 4.12)**		
Adjusted for child age at stool collection (quartiles)	1.61 (0.85 – 3.04) 1.50 (0.71 – 3.16) **2.35 (1.15 – 4.82)**	1.60 (0.83 – 3.08) 2.65 (1.39 – 5.06) 3.19 (1.67 – 6.09)	In (p<0.20)
Adjusted for antibiotics exposure	**2.00 (1.06 – 3.79)** 1.54 (0.73 – 3.23) **2.08 (1.02 – 4.25)**	1.24 (0.80 – 1.91)	In (confounding)
Adjusted for gravida (primigravida)	1.85 (0.99 – 3.45) 1.66 (0.80 – 3.42) 2.01 (0.99 – 4.10)	1.14 (0.74 – 1.77)	Out
Adjusted for maternal asthma and allergy	1.74 (0.93 – 3.27) 1.57 (0.76 – 3.24) **2.21 (1.09 – 4.48)**	0.81 (0.52 – 1.26)	Out
Adjusted for breastfeeding at time of stool collection	1.83 (0.89 – 3.75) 1.21 (0.55 – 2.66) 2.14 (0.98 – 4.70)	9.18 (4.78 – 17.63) 3.75 (2.02 – 6.95)	In (always in model)
Adjusted for pets at home	1.84 (0.99 – 3.45) 1.77 (0.85 – 3.70) 1.93 (0.96 – 3.91)	1.57 (1.03 – 2.41)	In (p<0.20)
Adjusted for other children at home	1.76 (0.94 – 3.29) 1.70 (0.82 – 3.53) 1.92 (0.95 – 3.91)	0.70 (0.46 – 1.06)	Out
Adjusted for prenatal smoke exposure	1.58 (0.83 – 3.00) 1.60 (0.77 – 3.32) **2.09 (1.03 – 4.27)**	3.39 (1.43 – 8.06)	In (p<0.20)
Adjusted for postnatal smoke exposure	1.77 (0.95 – 3.31) 1.70 (0.82 – 3.53) 2.01 (1.00 – 4.04)	1.25 (0.71 – 2.19)	Out
Adjusted for prenatal SSRI	1.50 (0.78 – 2.88) 1.66 (0.80 – 3.45) 1.92 (0.94 – 3.94)	2.08 (0.77 – 5.63)	In (confounding)
Adjusted for postnatal SSRI	1.81 (0.96 – 3.42) 1.45 (0.68 – 3.10) 2.08 (1.00 – 4.04)	1.32 (0.55 – 3.18)	In (confounding)
Adjusted for antibiotics, infant age, pets, breastfeeding, prenatal SSRI	1.71 (0.77 – 3.80) 1.33 (0.57 – 3.12) 2.16 (0.91 – 5.17)		Final model

Appendix D: Sensitivity analyses for home births, age, gestational age and SSRI use

Table D1. Sensitivity analyses for home births in 415 sub-sample

(Ref: less depressive symptoms/below cut-off)	OR (95% CI)	
	Includes home births N=415	Excludes home births N=403
Crude OR	2.26 (1.19 – 4.33) 2.11 (1.01 – 4.40) 2.03 (0.98 – 4.20)	2.08 (1.08 – 4.01) 2.31 (1.10 – 4.87) 2.12 (1.02 – 4.42)
Adjusted for presence of pets at home	2.26 (1.18 – 4.34) 2.27 (1.08 – 4.77) 1.88 (0.90 – 3.93)	2.08 (1.08 – 4.03) 2.51 (1.18 – 5.35) 1.96 (0.93 – 4.12)
Adjusted for breastfeeding at time of stool collection	2.45 (1.17 – 5.14) 1.66 (0.74 – 3.69) 2.32 (1.04 – 5.20)	2.21 (1.05 – 4.65) 1.78 (0.79 – 4.01) 2.39 (1.06 – 5.39)
Adjusted for antibiotics	2.52 (1.30 – 4.88) 2.00 (0.94 – 4.25) 2.35 (1.12 – 4.96)	2.30 (1.18 – 4.49) 2.22 (1.03 – 4.78) 2.39 (1.13 – 5.05)
Adjusted for birth characteristics	2.66 (1.36 – 5.22) 2.04 (0.95 – 4.39) 2.55 (1.18 – 5.48)	2.46 (1.25 – 4.87) 2.26 (1.03 – 4.91) 2.59 (1.20 – 5.59)
Adjusted for birth characteristics and breastfeeding	3.02 (1.39 – 6.58) 1.56 (0.67 – 3.63) 2.99 (1.24 – 7.21)	2.68 (1.23 – 5.86) 1.70 (0.73 – 3.99) 3.00 (1.24 – 7.24)
Adjusted for birth characteristics, breastfeeding, pets at home	3.05 (1.40 – 6.67) 1.76 (0.75 – 4.16) 2.86 (1.19 – 6.89)	2.71 (1.23 – 5.94) 1.93 (0.81 – 4.59) 2.87 (1.19 – 6.94)

Although home births were meant to be excluded from the CHILD study, mothers that went through home delivery were still in our sample. The sensitivity analyses show that including home births in our sample change our odds ratios for low IgA in the three depressive symptom categories. Since home births seems to be a major confounding variable, we excluded the infants born from home (n=12) from this study.

Table D2. Sensitivity analyses for age at stool collection greater than 6 months in 403 sub-sample

(Ref: less depressive symptoms/below cut-off)	OR (95% CI)	
	Includes greater than 6mo N=403	Excludes greater than 6mo N=372
Crude OR	2.08 (1.08 – 4.01) 2.31 (1.10 – 4.87) 2.12 (1.02 – 4.42)	2.15 (1.07 – 4.31) 2.18 (0.99 – 4.81) 2.25 (1.07 – 4.73)
Adjusted for presence of pets at home	2.08 (1.08 – 4.03) 2.51 (1.18 – 5.35) 1.96 (0.93 – 4.12)	2.18 (1.08 – 4.39) 2.38 (1.06 – 5.32) 2.06 (0.97 – 4.37)
Adjusted for breastfeeding at time of stool collection	2.21 (1.05 – 4.65) 1.78 (0.79 – 4.01) 2.39 (1.06 – 5.39)	2.14 (0.98 – 4.69) 1.75 (0.75 – 4.09) 2.40 (1.06 – 5.44)
Adjusted for antibiotics	2.30 (1.18 – 4.49) 2.22 (1.03 – 4.78) 2.39 (1.13 – 5.05)	2.34 (1.16 – 4.75) 2.06 (0.91 – 4.67) 2.54 (1.19 – 5.40)
Adjusted for birth characteristics (antibiotics use, birth mode, gravida, SGA)	2.46 (1.25 – 4.87) 2.26 (1.03 – 4.91) 2.59 (1.20 – 5.59)	2.51 (1.22 – 5.16) 2.08 (0.91 – 4.78) 2.77 (1.27 – 6.05)
Adjusted for birth characteristics and breastfeeding	2.68 (1.23 – 5.86) 1.70 (0.73 – 3.99) 3.00 (1.24 – 7.24)	2.44 (1.08 – 5.50) 1.64 (0.67 – 3.99) 2.99 (1.23 – 7.29)
Adjusted for birth characteristics, breastfeeding, pets at home	2.71 (1.23 – 5.94) 1.93 (0.81 – 4.59) 2.87 (1.19 – 6.94)	2.50 (1.11 – 5.67) 1.90 (0.77 – 4.72) 2.85 (1.17 – 6.96)

30 (8%) infants in our sample had their stool collected at greater than six months of infant age even though the stool was meant to be collected at 3 months. Percent differences between OR's were under 10%, and so we kept this portion of infants in our sample. This portion of infants having sample collected after six months will help our analyses with postnatal stress and depressive symptoms.

Table D3. Sensitivity analyses for late preterm infants included in 403 sub-sample

(Ref: less depressive symptoms/below cut-off)	OR (95% CI)	
	Includes less than 37 weeks gestation N=403	Excludes less than 37 weeks gestation N=382
Crude OR	2.08 (1.08 – 4.01) 2.31 (1.10 – 4.87) 2.12 (1.02 – 4.42)	2.18 (1.09 – 4.35) 2.38 (1.10 – 5.21) 2.18 (0.99 – 4.79)
Adjusted for presence of pets at home	2.08 (1.08 – 4.03) 2.51 (1.18 – 5.35) 1.96 (0.93 – 4.12)	2.20 (1.09 – 4.41) 2.58 (1.18 – 5.64) 2.05 (0.93 – 4.54)
Adjusted for breastfeeding at time of stool collection	2.21 (1.05 – 4.65) 1.78 (0.79 – 4.01) 2.39 (1.06 – 5.39)	2.50 (1.16 – 5.37)* 1.81 (0.79 – 4.15) 2.69 (1.13 – 6.41)*
Adjusted for antibiotics	2.30 (1.18 – 4.49) 2.22 (1.03 – 4.78) 2.39 (1.13 – 5.05)	2.38 (1.18 – 4.82) 2.18 (0.99 – 4.79) 2.27 (1.03 – 5.02)
Adjusted for postnatal smoke exposure	1.96 (1.01 – 3.81) 2.48 (1.16 – 5.26) 2.00 (0.95 – 4.21)	2.05 (1.02 – 4.12) 2.57 (1.18 – 5.60) 2.05 (0.93 – 4.54)
Adjusted for birth characteristics (antibiotics use, birth mode, gravida, SGA)	2.46 (1.25 – 4.87) 2.26 (1.03 – 4.91) 2.59 (1.20 – 5.59)	2.51 (1.23 – 5.15) 2.25 (1.01 – 5.01) 2.29 (1.02 – 5.15)
Adjusted for birth characteristics and breastfeeding	2.68 (1.23 – 5.86) 1.70 (0.73 – 3.99) 3.00 (1.24 – 7.24)	2.96 (1.33 – 6.59) 1.69 (0.71 – 4.05) 2.80 (1.12 – 6.95)
Adjusted for antibiotics, gravida, breastfeeding, pets at home, postnatal smoke exposure	2.66 (1.21 – 5.82) 1.94 (0.82 – 4.58) 2.80 (1.19 – 6.59)	2.87 (1.28 – 6.40) 1.89 (0.78 – 4.58) 2.86 (1.17 – 7.00)

Sixteen (4%) infants in our sample were born late preterm at less than 37 weeks gestation (34-36 weeks). Percent differences between ORs were mostly under 10%, except for the OR's after adjusting for breastfeeding status. However, the multivariate logistic regression models that include breastfeeding status did not show greater than 10% difference after excluding the late preterm infants, and so we kept the preterm infants in our sample.

Table D4. Median infant sIgA levels with maternal SSRI use during pre and postnatal time period

sIgA (mg/g feces)	No SSRI use (N=374)	Prenatal use only (N=4)	Postnatal use only (N=9)	SSRI use during both pre and postnatal time period (N=14)
Median (IQR)	6.06 (3.28 – 11.36)	2.25 (1.19 – 10.17)	5.27 (1.99 – 8.74)	4.07 (3.46 – 6.72)

Note: None of the sIgA levels were significantly different when SSRIs were used than the sIgA levels with no SSRI use.

Table D5. Sensitivity analyses for SSRI use included in 403 sub-sample

(Ref: less depressive symptoms/below cut-off)	OR (95% CI)	
	Includes mothers that used SSRIs N=403	Excludes mothers that used SSRIs N=374
Crude OR	2.08 (1.08 – 4.01) 2.31 (1.10 – 4.87) 2.12 (1.02 – 4.42)	1.82 (0.88 – 3.76)* 2.34 (1.11 – 4.97) 1.55 (0.68 – 3.55)*
Adjusted for presence of pets at home	2.08 (1.08 – 4.03) 2.51 (1.18 – 5.35) 1.96 (0.93 – 4.12)	1.86 (0.90 – 3.86)* 2.53 (1.18 – 5.43) 1.40 (0.61 – 3.25)*
Adjusted for breastfeeding at time of stool collection	2.21 (1.05 – 4.65) 1.78 (0.79 – 4.01) 2.39 (1.06 – 5.39)	2.14 (0.97 – 4.71) 1.82 (0.81 – 4.12) 1.81 (0.74 – 4.46)*
Adjusted for antibiotics	2.30 (1.18 – 4.49) 2.22 (1.03 – 4.78) 2.39 (1.13 – 5.05)	1.98 (0.95 – 4.13)* 2.28 (1.05 – 4.94) 1.66 (0.72 – 3.83)*
Adjusted for postnatal smoke exposure	1.96 (1.01 – 3.81) 2.48 (1.16 – 5.26) 2.00 (0.95 – 4.21)	1.72 (0.83 – 3.58)* 2.51 (1.17 – 5.37) 1.48 (0.64 – 3.42)*
Adjusted for birth characteristics (antibiotics use, birth mode, gravida, SGA)	2.46 (1.25 – 4.87) 2.26 (1.03 – 4.91) 2.59 (1.20 – 5.59)	2.08 (0.98 – 4.42)* 2.33 (1.06 – 5.14) 1.72 (0.73 – 4.06)*
Adjusted for antibiotics, gravida, breastfeeding, pets at home, postnatal smoke exposure	2.66 (1.21 – 5.82) 1.94 (0.82 – 4.58) 2.80 (1.19 – 6.59)	2.44 (1.07 – 5.54)* 1.98 (0.83 – 4.69) 1.92 (0.75 – 4.93)*

In the sub-sample, 27 (6.7%) mothers took SSRIs during the pre or postnatal period. Although median sIgA were not significantly different with SSRI use in Table D4, the sensitivity analyses indicate excluding the mothers that took SSRIs causes significant changes to the odds ratios for low IgA. Greater than 10% change was seen for odd ratios for prenatal only depressive symptoms and both pre and postnatal groups depressive symptoms with the exclusion of SSRI use.

Appendix E: Maternal distress and sIgA median comparisons and logistic regression models

Table E1. Medians of infant fecal sIgA depending on maternal depressive symptoms after stratifying for potential covariates

sIgA (mg/g feces) Median (IQR)	Reference group										Interaction[b]
	N	Less symptoms **(3.9% SSRI use)**	N	Prenatal Only **(18.8% SSRI use)**	p-value	N	Postnatal Only **(3.2% SSRI use)**	p-value	N	Both **(16.7% SSRI use)**	p-value	
Depressive symptoms	280	6.30 (3.58 – 12.38)	49	5.19 (2.02 – 9.78)	0.057	35	5.69 (2.62 – 8.56)	0.11	37	4.43 (2.39 – 8.03)	**0.033***	-
Depression history	40	5.46 (3.58 – 11.76)	18	3.68 (1.36 – 6.59)	0.06	10	3.54 (2.21 – 5.70)	0.13	11	5.54 (2.35 – 9.01)	0.65	0.41
No depression history	236	6.37 (3.58 – 12.38)	29	5.71 (3.00 – 12.09)	0.68	25	6.39 (2.84 – 9.56)	0.47	25	3.72 (2.37 – 7.39)	**0.021***	**0.05** 0.71
Maternal asthma or allergy	175	6.55 (3.77 – 12.66)	33	4.86 (1.70 – 12.21)	0.15	18	4.81 (2.69 – 7.18)	**0.041*****	27	4.43 (2.43 – 7.96)	**0.032***	0.53
No maternal asthma or allergy	101	6.07 (3.21 – 11.73)	15	5.59 (2.78 – 8.31)	0.33	17	6.39 (2.31 –12.73)	0.93	9	3.72 (1.82 – 9.69)	0.26	**0.13** 0.92
Primigravida	112	5.32 (3.19 – 11.11)	19	5.05 (2.37 – 8.11)	0.22	12	5.00 (2.64 – 7.54)	0.30	9	5.74 (2.06 – 9.06)	0.58	0.74
Multigravida	165	7.11 (3.89 – 13.14)	30	5.66 (1.88 – 10.80)	0.11	23	6.04 (2.31 – 9.23)	0.15	27	4.43 (2.43 – 7.96)	**0.022***	
Prenatal SSRI	5	4.15 (3.76 – 9.63)	8	4.89 (2.59 – 12.30)	0.77	0	-	-	5	2.71 (2.13 – 4.17)	0.095[a]	-
No prenatal SSRI	269	6.19 (3.53 – 12.36)	40	5.27 (1.95 – 9.84)	0.12	34	5.68 (2.54 – 8.14)	0.11	31	5.54 (2..35 – 8.09)	0.12	
Prenatal smoke exposure	13	5.07 (2.39 – 13.69)	6	2.54 (1.43 – 5.32)	0.14	2	1.70 (0.68 – none)	0.11[a]	3	2.43 (1.74 – none)	0.093	1.00
No prenatal smoke exposure	263	6.27 (3.64 – 12.06)	42	5.61 (2.32 – 10.56)	0.26	33	5.71 (2.84 – 8.89)	0.23	33	4.74 (2.42 – 8.03)	0.068	
Male	159	6.62 (3.53 – 13.15)	25	5.35 (2.48 – 10.78)	0.31	20	4.56 (2.00 – 7.99)	**0.027*****	20	4.05 (2.34 – 7.82)	0.07	0.90
Female	121	6.07 (3.63 – 11.55)	24	4.98 (1.27 – 9.84)	0.11	15	6.23 (3.23 – 9.89)	0.86	17	4.74 (2.42 – 8.49)	0.26	**0.10** 0.53
Caesarean section	79	5.49 (3.53 – 10.85)	19	5.19 (2.16 – 14.04)	0.47	5	6.39 (4.18 – 11.12)	0.83[a]	12	4.05 (1.91 – 6.92)	0.057	0.83
Vaginal	198	6.62 (3.76 – 12.67)	30	5.32 (1.80 – 9.05)	0.052	30	5.46 (2.54 – 8.72)	0.058	24	5.24 (2.44 – 11.14)	0.24	**0.068** 0.53
SGA	20	7.66 (3.72 – 15.67)	4	5.60 (4.97 – 34.36)	1.00 [a]	2	15.15 (9.23)	0.36[a]	1	-	0.76[a]	-
Not SGA	256	6.23 (3.63 – 12.25)	45	5.05 (1.87 – 9.78)	0.035	33	5.66 (2.47 – 7.99)	0.052	34	4.55 (2.34 – 8.32)	**0.049***	
Breastfed	207	7.82 (4.16 – 13.43)	28	5.66 (2.49 – 10.35)	0.15	22	6.50 (3.22 – 9.73)	0.31	24	5.66 (3.22 – 9.73)	0.17	0.75
Not breastfed	64	4.04 (2.22 – 6.09)	15	2.78 (1.54 – 4.77)	0.15	11	2.71 (1.07 – 6.39)	0.61	10	2.46 (1.63 – 2.97)	**0.033***	
Antibiotics exposure	155	6.15 (3.53 – 11.41)	31	3.80 (1.54 – 10.50)	0.046	16	6.13 (2.39 – 10.42)	0.36	18	4.40 (1.77 – 7.38)	**0.027***	0.50
No Antibiotics exposure	120	6.42 (3.78 – 13.03)	15	5.71 (3.23 – 8.85)	0.23	18	5.68 (2.98 – 8.72)	0.29	17	5.74 (2.46 – 12.85)	0.51	
Other children	135	6.87 (4.05 – 12.31)	20	7.44 (1.94 – 13.28)	0.76	17	6.04 (3.15 – 8.61)	0.32	17	3.70 (2.28 – 7.69)	**0.038***	**0.17**
No other children	144	5.59 (3.37 – 12.42)	29	4.77 (2.13 – 7.15)	0.039	17	3.86 (2.31 – 8.27)	0.11	19	5.57 (2.43 – 9.01)	0.53	0.72 **0.098**
Pets at home	127	6.06 (3.31 – 12.06)	22	3.22 (1.36 – 7.73)	**0.027****	13	3.86 (1.85 – 7.99)	0.14	23	3.70 (2.35 – 5.74)	**0.022***	0.91
No pets at home	151	6.60 (3.93 – 12.70)	27	5.59 (3.49 – 10.50)	0.54	21	5.71 (2.76 – 8.89)	0.30	14	7.39 (2.56 – 12.41)	0.81	0.88 **0.04**
Postnatal smoke exposure	39	5.07 (3.54 – 9.80)	12	5.24 (1.52 – 10.92)	0.72	4	1.78 (0.54 – 11.43)	0.15[a]	9	2.43 (1.91 – 3.73)	**0.005***	0.70 0.57

No postnatal smoke exposure	241	6.42 (3.60 – 12.68)	37	5.19 (2.27 – 8.58)	0.055	30	5.70 (2.95 – 8.14)	0.19	28	5.66 (3.62 – 9.54)	0.38	**0.15**
Postnatal SSRI	10	5.19 (3.88 – 6.83)	8	4.89 (2.59 – 12.30)	0.93	1	-	0.18[a]	4	3.04 (1.78 – 4.46)	0.076[a]	-
No postnatal SSRI	266	6.42 (3.56 – 12.67)	40	5.12 (1.62 – 9.84)	0.057	31	5.69 (2.62 – 8.00)	0.13	33	5.54 (2.39 – 8.55)	0.096	
Infant allergic eczema	28	7.06 (3.89 – 11.78)	4	9.10 (3.88 – 20.41)	0.68[a]	4	2.76 (0.91 – 6.80)	0.062[a]	7	4.74 (2.30 – 8.09)	0.32	0.74
No infant allergic eczema	251	6.27 (3.57 – 12.66)	44	5.12 (1.87 – 9.46)	**0.029****	28	5.70 (2.80 – 8.91)	0.33	30	4.40 (2.41 – 8.22)	0.052	
Not breastfed and multigravida	38	4.04 (2.48 – 6.16)	8	1.70 (1.14 – 3.50)	**0.026****	6	4.18 (1.64 – 8.38)	0.76	7	2.43 (1.55 – 2.71)	**0.013***	-
Breastfed and multigravida	122	9.10 (4.87 – 14.11)	18	7.11 (3.16 – 10.80)	0.18	16	6.84 (3.21 – 10.73)	0.20	17	5.57 (4.03 – 9.74)	0.095	-
Not breastfed and not SGA	59	3.93 (2.36 – 6.07)	14	2.69 (1.46 – 4.40)	0.11	11	2.71 (1.07 – 6.39)	0.63	10	2.46 (1.63 – 2.97)	**0.03***	-
Breastfed and pets at home	89	7.82 (4.06 – 13.71)	12	5.28 (1.09 – 10.47)	0.07	6	5.92 (2.95 – 11.54)	0.43	15	4.43 (2.35 – 7.02)	**0.047***	-
Not breastfed and other children	32	4.20 (2.22 – 6.38)	6	1.70 (0.99 – 5.45)	0.07	4	5.87 (2.23 – 6.31)	0.75[a]	5	1.66 (1.53 – 2.60)	**0.028*,[a]**	-
Not breastfed and female	31	3.64 (2.08 – 6.07)	5	2.78 (0.75 – 4.00)	0.21[a]	3	6.39 (6.04 – none)	0.11[a]	5	2.48 (1.53 – 2.70)	0.078[a]	-
Not breastfed and no depression history in mother	55	4.20 (2.52 – 6.27)	7	3.22 (2.37 – 8.11)	0.60	8	4.18 (0.91 – 9.02)	0.64	9	2.48 (1.65 – 3.22)	**0.04***	-
Not breastfed and mother has asthma/allergy	43	4.20 (2.36 – 6.12)	11	2.37 (1.23 – 4.77)	0.10	5	2.71 (0.76 – 7.97)	0.47	8	2.46 (1.68 – 2.71)	**0.055***	-
Primigravida and pets at home	51	5.37 (2.71 – 12.40)	10	2.58 (0.87 – 3.97)	**0.025****	7	2.71 (1.07 – 4.34)	0.058	6	2.52 (1.77 – 6.81)	0.15	-
Multigravida and not SGA	151	6.87 (3.86 – 12.66)	29	5.71 (1.87 – 10.86)	0.13	21	5.71 (2.31 – 8.28)	0.068	25	4.37 (2.37 – 8.49)	**0.031***	-
Multigravida and has asthma/allergy	101	7.26 (4.18 – 12.25)	22	5.64 (1.50 – 13.59)	0.15	10	5.49 (2.66 – 7.49)	0.10	17	4.43 (2.46 – 7.67)	**0.030***	-
Multigravida and antibiotics exposure	85	7.23 (3.86 – 12.06)	19	3.80 (1.54 – 14.04)	0.17	12	6.22 (2.31 – 10.42)	0.20	14	4.98 (2.14 – 7.55)	**0.053***	-
Antibiotics exposure and pets at home	75	6.15 (3.52 – 10.85)	10	2.13 (1.00 – 4.02)	**0.003****	6	4.95 (2.18 – 9.58)	0.45	13	3.72 (1.76 – 5.56)	**0.017***	-
Antibiotics exposure and mother has asthma/allergy	103	6.33 (3.62 – 12.06)	22	2.80 (1.34 – 15.26)	0.10	5	2.62 (1.14 – 4.95)	**0.020***,[a]**	14	4.40 (1.77 – 7.52)	**0.047***	-
Antibiotics exposure and no other children	91	5.57 (3.35 – 13.04)	20	3.70 (1.35 – 5.55)	**0.023****	9	3.86 (2.31 – 14.28)	0.45	11	5.54 (2.30 – 7.37)	0.24	-
Antibiotics exposure and male infant	95	6.19 (3.53 – 13.56)	17	5.05 (1.87 – 17.68)	0.54	11	3.86 (2.31 – 11.23)	0.19	11	3.72 (1.74 – 5.57)	**0.02***	-
No antibiotics exposure and male infant	59	7.83 (4.01 – 13.15)	7	5.71 (3.49 – 7.79)	0.27	9	5.27 (1.49 – 6.84)	**0.037*****	8	7.56 (3.62 – 13.29)	0.92	-
Antibiotics exposure and female infant	60	5.81 (3.66 – 10.41)	14	3.19 (1.12 – 6.29)	**0.024****	5	6.23 (4.63 – 11.76)	0.86[a]	7	7.37 (2.70 – 7.96)	0.57	-

Antibiotics exposure and mother had depression history	24	5.88 (3.99 – 10.85)	11	1.87 (1.17 – 5.19)	**0.006****	4	3.54 (2.54 – 13.81)	0.43[a]	8	5.56 (2.42 – 8.75)	0.63	-
Antibiotics exposure and mother did not have depression history	130	6.24 (3.53 – 12.30)	18	6.68 (2.32 – 19.52)	0.99	12	6.31 (2.39 – 10.42)	0.51	10	3.21 (1.69 – 6.69)	**0.015***	-
Pets and no depression history	99	6.07 (3.27 – 12.06)	13	3.23 (1.68 – 8.37)	0.084	11	4.34 (1.07 – 8.00)	0.26	14	3.65 (2.40 – 6.06)	0.059	-
Pets and no other children	66	5.51 (3.01 – 11.50)	12	2.58 (0.99 – 4.45)	**0.008****	8	3.28 (1.29 – 7.07)	0.14	13	3.72 (2.33 – 8.01)	0.18	-
Pets and mother has asthma/allergy	80	5.76 (3.52 – 12.47)	16	4.04 (1.52 – 9.83)	0.12	9	3.07 (1.73 – 5.19)	**0.014*****	19	3.70 (2.35 – 7.02)	**0.042***	-
Pets and male infant	67	6.20 (3.31 – 12.66)	10	4.04 (2.25 – 6.25)	0.054	9	2.71 (0.96 – 7.99)	0.076	16	3.68 (1.91 – 5.56)	**0.043***	-

*Significant at 0.05 a priori; **Significant at Bonferroni adjusted p-value 0.05/2; *** Significant at Bonferroni adjusted p-value 0.05/1; [a]Fisher's Exact test

[b]Interactions between the covariate and depressive symptoms group variable were tested. When interaction terms with $p<0.20$ were found, the interaction term for each depressive symptoms group was reported.

Note: Stratifications with two covariates that did not have significant differences were not reported in this table.

Table E2. Logistic regression analyses for low sIgA in infants after exposure to pre and postnatal depressive symptoms

(Ref: Less depressive symptoms/below cut-off)	N for lowest quartile of sIgA	OR for depression	OR for covariate	Decision
Crude OR	100	2.08 (1.08 – 4.01) 2.31 (1.10 – 4.87) 2.12 (1.02 – 4.42)		
Adjusted for child sex	M-54 F-46	2.07 (1.07 – 3.99) 2.31 (1.10 – 4.87) 2.12 (1.01 – 4.41)	1.09 (0.69 – 1.72)	Out
Adjusted for child age at stool collection (quartiles)	1st-18 2nd-19 3rd-27 4th-36	1.88 (0.96 – 3.67) 2.12 (0.99 – 4.55) 2.35 (1.11 – 4.97)	0.99 (0.48 – 2.04) 1.65 (0.83 – 3.28) 2.51 (1.28 – 4.91)	In (p<0.20)
Adjusted for birth mode	V-70 EC-19 SC-9	2.16 (1.12 – 4.20) 2.45 (1.15 – 5.21) 2.23 (1.06 – 4.70)	1.18 (0.64 – 2.18) 0.70 (0.32 – 1.54)	Out
Adjusted for antibiotics exposure	Y-58 N-39	2.30 (1.18 – 4.49) 2.22 (1.03 – 4.78) 2.39 (1.13 – 5.05)	1.21 (0.75 – 1.95)	In (confounding)
Adjusted for gravida (primigravida)	Y-43 N-55	2.17 (1.12 – 4.20) 2.46 (1.16 – 5.21) 2.44 (1.15 – 5.16)	1.47 (0.91 – 2.36)	In (p<0.20)
Adjusted for SGA	Y-3 N-95	2.17 (1.12 – 4.20) 2.36 (1.12 – 5.00) 2.31 (1.09 – 4.87)	0.37 (0.11 – 1.26)	In (p<0.20)
Adjusted for maternal asthma and allergy	Y-61 N-38	2.18 (1.12 – 4.21) 2.28 (1.08 – 4.82) 2.27 (1.08 – 4.76)	0.85 (0.53 – 1.38)	Out
Adjusted for breastfeeding at time of stool collection	E-11 P-39 Z-47	2.21 (1.05 – 4.65) 1.78 (0.79 – 4.01) 2.39 (1.06 – 5.39)	9.40 (4.48 – 19.71) 3.87 (1.87 – 8.01)	In (always in model)
Adjusted for pets at home	Y-55 N-45	2.08 (1.08 – 4.03) 2.51 (1.18 – 5.35) 1.96 (0.93 – 4.12)	1.59 (1.00 – 2.53)	In (p<0.20)
Adjusted for other children at home	Y-43 N-56	2.04 (1.06 – 3.94) 2.42 (1.14 – 5.14) 1.95 (0.92 – 4.13)	0.81 (0.51 – 1.30)	Out
Adjusted for prenatal smoke exposure	Y-13 N-86	1.96 (1.00 – 3.96) 2.33 (1.10 – 4.95) 2.15 (1.01 – 4.55)	3.63 (1.54 – 8.52)	In (p<0.20)
Adjusted for postnatal smoke exposure	Y-23 N-77	1.96 (1.01 – 3.81) 2.48 (1.16 – 5.26) 2.00 (0.95 – 4.21)	1.79 (1.00 – 3.21)	In (p<0.20)
Adjusted for prenatal SSRI	Y-6 N-93	1.86 (0.94 – 3.70) 2.36 (1.12 – 5.01) 2.11 (1.00 – 4.48)	1.16 (0.40 – 3.34)	In (confounding)
Adjusted for postnatal SSRI	Y-6 N-92	2.15 (1.10 – 4.21) 2.01 (0.92 – 4.41) 2.11 (1.01 – 4.41)	0.85 (0.32 – 2.30)	In (confounding)
Adjusted for depression history	Y-25 N-74	2.11 (1.07 – 4.15) 2.25 (1.06 – 4.77) 2.14 (1.02 – 4.52)	1.26 (0.72 – 2.20)	Out
Adjusted for infant allergic eczema	Y-9 N-89	2.18 (1.12 – 4.21) 2.41 (1.11 – 5.23) 2.22 (1.06 – 4.65)	0.74 (0.34 – 1.63)	Out
Adjusted for birth characteristics (gravida, birth mode, SGA,	97	2.46 (1.25 – 4.87) 2.26 (1.03 – 4.91)		In (confounding)

antibiotics)		2.59 (1.20 – 5.59)		
Adjusted for SGA, gravida, antibiotics	97	2.41 (1.22 – 4.75) 2.26 (1.04 – 4.89) 2.59 (1.21 – 5.56)		Keep birth mode out
Adjusted for gravida and antibiotics	97	2.36 (1.20 – 4.62) 2.29 (1.06 – 4.94) 2.56 (1.20 – 5.45)		Keep SGA out
Adjusted for gravida and SGA	98	2.20 (1.13 – 4.28) 2.43 (1.14 – 5.16) 2.45 (1.51 – 5.23)		Keep antibiotics in
Adjusted for home environment (pets and children at home)	99	2.05 (1.06 – 3.96) 2.50 (1.17 – 5.34) 1.81 (0.85 – 3.86)		In (confounding)
Adjusted for breastfeeding and maternal asthma/allergy	96	2.46 (1.16 – 5.21) 1.74 (0.77 – 3.94) 2.61 (1.14 – 5.95)		In (confounding)
Adjusted for maternal depression characteristics (depression history, prenatal SSRI)	98	1.94 (0.95 – 3.93) 2.33 (1.10 – 4.97) 2.09 (0.98 – 4.46)		Out
Adjusted for maternal depression characteristics (depression history, postnatal SSRI)	97	2.10 (1.05 – 4.19) 1.93 (0.87 – 4.27) 2.12 (1.00 – 4.49)		In
Adjusted for prenatal SSRI and breastfeeding	96	2.05 (0.95 – 4.42) 1.81 (0.80 – 4.10) 2.39 (1.04 – 5.49)		Out
Adjusted for postnatal SSRI and breastfeeding	95	2.37 (1.10 – 5.10) 1.52 (0.64 – 3.58) 2.41 (1.06 – 5.48)		In
Adjusted for breastfeeding and prenatal smoke	96	2.26 (1.05 – 4.83) 1.81 (0.80 – 4.09) 2.42 (1.06 – 5.52)		Keep smoke in ($p<0.20$)
Adjusted for breastfeeding and postnatal smoke	97	2.17 (1.03 – 4.60) 1.94 (0.86 – 4.41) 2.37 (1.05 – 5.36)		Keep smoke out
Adjusted for age, antibiotics, gravida, depression history, postnatal SSRI, pets, prenatal smoking, breastfeeding	91	2.49 (1.06 – 5.82) 1.42 (0.56 – 3.59) 3.04 (1.23 – 7.55)		
Adjusted for age, antibiotics, gravida, postnatal SSRI, pets, prenatal smoking, breastfeeding	91	2.61 (1.13 – 6.03) 1.48 (0.59 – 3.72) 3.17 (1.28 – 7.82)		Keep depression history out
Adjusted for age, antibiotics, gravida, postnatal SSRI, pets, breastfeeding	92	2.53 (1.12 – 5.75) 1.46 (0.59 – 3.64) 3.16 (1.30 – 7.69)		Keep smoking out
Adjusted for age, antibiotics, maternal asthma and allergy, postnatal SSRI, pets, breastfeeding, gravida	91	2.82 (1.23 – 6.47) 1.43 (0.57 – 3.60) 3.33 (1.36 – 8.19)		**Final model for postnatal depressive symptoms**
Adjusted for age, antibiotics, maternal asthma and allergy, pets, breastfeeding, gravida	93	2.72 (1.21 – 6.10) 1.88 (0.78 – 4.52) 3.33 (1.37 – 8.08)		
Adjusted for age, antibiotics, maternal asthma and allergy, pets, breastfeeding, gravida, prenatal SSRI	92	2.44 (1.07 – 5.57) 1.93 (0.80 – 4.68) 3.07 (1.25 – 7.55)		**Final model for prenatal only and both**
Final model for postnatal with interaction term for antibiotics	91	0.69 (0.16 – 3.03) 2.01 (0.52 – 7.80) 5.84 (1.53 – 22.35)		
Final model for prenatal and both with interaction term for antibiotics	92	0.70 (0.16 – 3.06) 3.13 (0.83 – 11.78) 5.04 (1.32 – 19.26)		

Table E3. Interactions between depressive symptoms and covariates in final model

Variable	Interactions with depressive symptoms (p-value)
Antibiotics exposure	**0.04** 0.34 0.39
Maternal asthma and allergy	0.46
Gravida	0.79
Breastfeeding	0.77
Prenatal SSRI	1.00
Postnatal SSRI	0.91
Pets at home	0.68
Age	0.79

Note: Interactions between the covariate and depressive symptoms group variable were tested. When interaction terms with $p<0.05$ were found, the interaction term for each depressive symptoms group was reported.

Table E4. Medians of infant fecal sIgA depending on maternal perceived stress after stratifying for potential covariates

sIgA (mg/g feces) Median (IQR)	Reference group											Interaction[b]
	N	Less stress **(3.7% SSRI use)**	N	Prenatal Only **(11.9% SSRI use)**	p-value	N	Postnatal Only **(4.9% SSRI use)**	p-value	N	Both **(12% SSRI use)**	p-value	
Highest quartile of stress	218	6.12 (3.54 – 11.36)	67	5.61 (3.05 – 10.33)	0.68	41	5.66 (2.89 – 18.70)	0.97	75	5.57 (2.59 – 9.89)	0.17	-
Depression history	31	5.35 (2.79 – 10.85)	17	5.11 (1.66 – 8.32)	0.32	8	4.44 (2.10 – 17.44)	0.70	23	5.07 (2.35 – 7.96)	0.61	-
No depression history	184	6.12 (3.63 – 11.31)	49	7.82 (3.36 – 12.62)	0.62	32	6.20 (3.10 – 18.81)	0.83	50	5.72 (2.59 – 10.20)	0.26	
Maternal asthma/allergy	136	6.12 (3.55 – 11.76)	44	6.22 (3.09 – 10.09)	0.56	24	6.96 (3.23 – 21.28)	0.26	49	5.27 (2.55 – 9.45)	0.12	-
No maternal asthma/allergy	79	6.10 (3.53 – 11.03)	22	6.84 (2.66 – 12.50)	0.76	16	3.96 (1.89 – 10.12)	0.15	25	5.71 (2.45 – 11.94)	0.76	
Primigravida	85	5.14 (3.19 – 10.58)	27	5.05 (2.63 – 9.90)	0.66	16	5.64 (2.09 – 14.76)	0.84	24	5.96 (2.77 – 9.50)	0.93	-
Multigravida	131	6.67 (3.93 – 12.06)	40	7.58 (3.62 – 11.73)	0.82	24	6.84 (3.21 – 20.57)	0.89	50	5.55 (2.40 – 10.78)	0.066	
Prenatal SSRI	4	5.30 (3.65 – 11.15)	6	3.41 (2.22 – 4.88)	0.26[a]	1	-	-	7	3.60 (2.48 – 6.19)	0.41[a]	-
No prenatal SSRI	208	6.08 (3.52 – 11.29)	61	7.37 (3.27 – 10.74)	0.82	39	5.62 (2.71 – 18.48)	0.79	66	5.65 (2.61 – 9.93)	0.37	
Prenatal smoke exposure	7	2.79 (1.77 – 9.05)	7	7.83 (3.22 – 13.95)	0.28	3	2.71 (0.68 – none)	0.67[a]	7	2.43 (1.74 – 5.07)	0.48	-
No prenatal smoke exposure	208	6.14 (3.68 – 16.60)	59	5.61 (2.71 – 10.10)	0.56	37	5.69 (3.14 – 19.00)	0.67	67	5.71 (2.70 – 10.02)	0.35	
Male	125	6.42 (3.53 – 11.70)	33	7.79 (3.61 – 12.62)	0.73	22	5.66 (2.21 – 19.65)	0.73	44	5.17 (2.47 – 11.37)	0.16	-
Female	93	6.06 (3.60 – 10.99)	34	5.05 (2.38 – 9.94)	0.36	19	5.66 (3.21 – 18.48)	0.76	31	6.04 (2.70 – 9.66)	0.63	
Caesarean section	59	5.10 (3.53 – 10.35)	26	5.27 (2.56 – 10.15)	0.78	9	21.35 (2.81 – 31.40)	0.064	21	5.57 (3.02 – 6.92)	0.20	0.81
Vaginal	157	6.48 (3.81 – 12.06)	41	7.11 (3.54 – 10.66)	0.80	31	5.62 (3.07 – 11.23)	0.34	53	5.71 (2.46 – 10.82)	0.76	**0.057** 0.41
SGA	14	6.81 (3.49 – 11.88)	7	9.16 (4.77 – 43.94)	0.21	3	15.55 (9.23 –none)	0.091[a]	3	5.59 (5.57 –none)	0.77[a]	-
Not SGA	201	6.12 (3.54 – 11.40)	0	5.27 (2.79 – 10.09)	0.35	37	5.62 (2.89 – 18.70)	0.75	70	5.64 (2.47 – 9.93)	0.15	
Breastfed	164	7.36 (4.14 – 12.66)	41	7.82 (3.63 – 10.41)	0.56	26	8.12 (3.86 – 21.55)	0.24	50	5.72 (3.69 – 12.00)	0.42	-
Not breastfed	49	3.93 (2.11 – 6.11)	17	3.22 (2.37 – 4.30)	0.36	13	2.71 (1.48 – 5.49)	0.24	21	2.70 (1.70 – 6.43)	0.68	
Antibiotics exposure	123	6.12 (3.53 – 11.34)	41	4.77 (2.38 – 9.89)	0.20	16	9.00 (3.32 – 22.79)	0.16	40	5.30 (2.31 – 7.82)	0.077	-
No antibiotics exposure	92	6.11 (3.59 – 11.97)	25	8.07 (4.50 – 12.62)	0.38	22	5.64 (2.51 – 10.02)	0.34	31	5.71 (2.59 – 13.73)	0.86	
Other children	105	6.72 (4.11 – 12.06)	28	7.95 (3.86 – 11.73)	0.83	21	6.91 (3.15 – 21.21)	0.64	35	4.37 (2.08 – 6.42)	**0.004***	0.21
No other children	112	5.14 (3.28 – 10.71)	39	4.91 (2.63 – 9.98)	0.56	20	4.72 (2.41 – 11.06)	0.60	38	6.21 (2.92 – 13.64)	0.37	0.65 **0.015**
Pets at home	96	6.11 (3.52 – 11.78)	31	3.76 (2.37 – 9.98)	0.17	18	3.71 (1.79 – 14.03)	0.25	40	4.40 (2.44 – 7.59)	**0.041***	0.39
No pets at home	121	6.12 (3.63 – 11.09)	36	7.58 (4.22 – 10.46)	0.46	23	6.77 (3.23 – 19.08)	0.32	33	6.39 (2.85 – 14.14)	0.72	0.21 **0.08**
Postnatal smoke exposure	30	4.80 (3.03 – 8.68)	11	7.83 (3.22 – 13.43)	0.39	5	4.85 (1.58 – 37.98)	0.74	18	3.43 (2.03 – 9.17)	0.32	-
No postnatal smoke exposure	188	6.23 (3.68 – 11.78)	56	5.48 (2.79 – 10.09)	0.43	36	5.68 (3.10 – 17.75)	0.84	55	5.74 (3.35 – 11.52)	0.49	-
Postnatal SSRI use	7	5.27 (3.54 – 6.60)	7	4.15 (3.22 – 7.08)	0.85	2	15.08 (11.23 –none)	0.056***,[a]	7	3.60 (1.87 – 6.19)	0.41	0.99
No postnatal SSRI	208	6.17 (3.54 – 11.78)	59	7.11 (2.71 – 10.98)	0.81	38	5.64 (2.61 – 18.63)	0.77	65	5.59 (2.66 – 10.38)	0.29	

use												
Infant allergic eczema	20	8.00 (4.30 – 11.78)	9	4.77 (3.68 – 8.63)	0.28	4	2.76 (0.91 – 30.92)	0.24[a]	10	5.15 (3.15 – 14.01)	0.40	-
No infant allergic eczema	196	6.06 (3.54 – 11.31)	57	7.08 (2.67 – 10.74)	0.88	37	5.69 (3.15 – 18.70)	0.68	63	5.59 (2.59 – 9.66)	0.27	
Breastfed and other children	77	8.56 (5.22 – 13.35)	17	9.16 (7.45 – 12.24)	0.93	16	10.23 (3.63 – 21.94)	0.48	22	5.71 (3.69 – 8.39)	**0.025***	-
Breastfed and antibiotics exposure	99	7.23 (3.99 – 12.06)	24	5.48 (2.22 – 9.90)	0.21	11	18.48 (4.85 – 29.01)	0.05***	25	5.57 (3.17 – 8.13)	0.16	-
Pets and antibiotics exposure	56	6.91 (3.54 – 11.11)	19	3.05 (2.36 – 9.80)	0.061	7	4.85 (3.31 – 18.92)	0.60	22	4.26 (1.72 – 5.69)	**0.006***	-
Antibiotics exposure and no pets	66	5.24 (3.22 – 11.36)	22	5.27 (3.72 – 10.15)	0.95	9	18.48 (5.00 – 31.40)	0.024***	18	6.43 (2.82 – 13.59)	0.76	-
Primigravida and no pets	48	5.04 (3.33 – 9.63)	12	6.72 (4.94 – 12.53)	0.08	7	6.62 (5.66 – 18.48)	0.032***	9	8.11 (5.91 – 16.62)	0.054	-
Other children and pets	47	6.62 (4.05 – 12.06)	11	7.83 (3.76 – 13.50)	0.96	8	4.08 (1.89 – 20.75)	0.32	18	4.26 (2.56 – 6.14)	0.052	-
Other children and no pets	57	6.87 (4.18 – 12.15)	17	8.07 (3.96 – 11.04)	0.76	13	9.23 (4.46 – 22.17)	0.65	17	5.27 (1.71 – 7.69)	**0.037***	-
No other children and no pets	64	5.04 (3.41 – 10.26)	19	5.61 (4.77 – 10.50)	0.26	10	5.64 (2.98 – 16.28)	0.73	16	10.87 (5.75 – 16.78)	**0.015***	-
No antibiotics and no other children	35	5.08 (3.10 – 9.64)	13	5.11 (3.10 – 11.80)	0.55	13	4.07 (2.31 – 6.82)	0.51	12	9.07 (3.97 – 19.60)	0.057	-
Other children and no antibiotics	57	6.43 (4.11 – 13.14)	12	8.46 (7.26 – 13.12)	0.38	9	6.91 (2.28 – 20.08)	0.96	18	4.48 (2.38 – 9.93)	**0.05***	-
Other children and antibiotics	46	7.36 (4.20 – 11.36)	15	7.11 (2.16 – 10.03)	0.51	10	15.08 (4.46 – 25.90)	0.09	16	4.40 (1.71 – 6.42)	**0.019***	-

*Significant at 0.05 a priori; **Significant at Bonferroni adjusted p-value 0.05/2; *** Significant at Bonferroni adjusted p-value 0.05/1; [a]Fisher's Exact test

[b]Interactions between the covariate and perceived stress group variable were tested. When significant interactions were found, the interaction term for each perceived stress group was reported.

Note: Stratifications with two covariates that did not have significant differences were not reported in this table.

Table E5. Logistic regression analyses for low sIgA in infants after exposure to pre and postnatal highest quartile of perceived stress

(Ref: lower stress)	N for lowest quartile of sIgA	OR for stress	OR for covariate	Decision
Crude OR	100	1.37 (0.73 – 2.58) 1.74 (0.83 – 3.62) 1.65 (0.92 – 2.98)		
Adjusted for child sex	M-54 F-46	1.36 (0.73 – 2.56) 1.73 (0.83 – 3.61) 1.66 (0.92 – 2.99)	1.10 (0.70 – 1.74)	Out
Adjusted for child age at stool collection (quartiles)	1st-18 2nd-19 3rd-27 4th-36	1.38 (0.73 – 2.62) 1.70 (0.80 – 3.61) 1.60 (0.88 – 2.91)	0.94 (0.46 – 1.94) 1.67 (0.85 – 3.29) 2.43 (1.26 – 4.71)	In (p<0.20)
Adjusted for breastfeeding at stool collection	E-11 P-39 Z-47	1.72 (0.86 – 3.44) 1.70 (0.77 – 3.78) 1.44 (0.76 – 2.74)	9.51 (4.56 – 19.84) 3.91 (1.90 – 8.05)	Always in model
Adjusted for birth mode	SC-9 EC-19 V-70	1.36 (0.72 – 2.58) 1.63 (0.77 – 3.46) 1.73 (0.95 – 3.12)	0.71 (0.33 – 1.55) 1.17 (0.64 – 2.14)	Out
Adjusted for antibiotics exposure	Y-58 N-39	1.40 (0.74 – 2.65) 1.59 (0.73 – 3.46) 1.82 (1.00 – 3.30)	1.22 (0.76 – 1.96)	In
Adjusted for gravida (primigravida)	Y-43 N-55	1.39 (0.74 – 2.63) 1.63 (0.77 – 3.46) 1.76 (0.97 – 3.19)	1.39 (0.87 – 2.22)	In (p<0.20)
Adjusted for SGA	Y-3 N-95	1.44 (0.76 – 2.71) 1.64 (0.77 – 3.49) 1.71 (0.94 – 3.10)	0.36 (0.11 – 1.22)	In (p<0.20)
Adjusted for depression history	Y-25 N-74	1.36 (0.72 – 2.58) 1.79 (0.85 – 3.75) 1.64 (0.90 – 3.00)	1.39 (0.80 – 2.42)	Out
Adjusted for maternal asthma and allergy	Y-66 N-32	1.42 (0.75 – 2.67) 1.82 (0.87 – 3.81) 1.63 (0.90 – 2.97)	0.98 (0.60 – 1.61)	Out
Adjusted for prenatal SSRI	Y-6 N-93	1.30 (0.69 – 2.45) 1.73 (0.83 – 3.63) 1.52 (0.83 – 2.78)	1.37 (0.49 – 3.82)	Out
Adjusted for postnatal SSRI	Y-6 N-92	1.38 (0.73 – 2.61) 1.58 (0.74 – 3.34) 1.62 (0.89 – 2.96)	0.97 (0.37 – 2.55)	In
Adjusted for prenatal smoke exposure	Y-26 N-73	1.43 (0.76 – 2.71) 1.82 (0.87 – 3.81) 1.75 (0.97 – 3.17)	0.91 (0.54 – 1.53)	Out
Adjusted for postnatal smoke exposure	T-23 N-76	1.35 (0.72 – 2.55) 1.77 (0.84 – 3.69) 1.51 (0.82 – 2.76)	1.86 (1.05 – 3.32)	In (p<0.20)
Adjusted for pets at home	Y-55 N-45	1.36 (0.72 – 2.56) 1.74 (0.83 – 3.63) 1.64 (0.90 – 2.98)	1.55 (0.98 – 2.45)	In (p<0.20)
Adjusted for other children at home	Y-43 N-56	1.35 (0.72 – 2.53) 1.74 (0.83 – 3.63) 1.60 (0.88 – 2.91)	0.80 (0.51 – 1.27)	Out
Adjusted for infant allergic eczema	Y-9 N-89	1.44 (0.77 – 2.72) 1.77 (0.85 – 3.69)	0.75 (0.35 – 1.64)	Out

		1.66 (0.91 – 3.02)		
Adjusted for antibiotics and gravida	97	1.40 (0.74 – 2.65) 1.59 (0.73 – 3.48) 1.87 (1.03 – 3.41)		In
Adjusted for antibiotics and SGA	97	1.45 (0.77 – 2.75) 1.60 (0.73 – 3.49) 1.82 (1.00 – 3.32)		Keep SGA out
Adjusted for antibiotics and breastfeeding	94	1.77 (0.87 – 3.60) 1.56 (0.67 – 3.64) 1.62 (0.84 – 3.13)		In
Adjusted for postnatal SSRI and breastfeeding	95	1.70 (0.84 – 3.44) 1.46 (0.65 – 3.31) 1.45 (0.74 – 2.81)		In
Adjusted for pets and breastfeeding	97	1.65 (0.82 – 3.34) 1.73 (0.78 – 3.84) 1.47 (0.77 – 2.82)		Keep pets out
Adjusted for pets and postnatal SSRI	98	1.37 (0.72 – 2.59) 1.59 (0.75 – 3.37) 1.59 (0.87 – 2.92)		Keep pets out
Adjusted for pets and other children	99	1.33 (0.70 – 2.51) 1.74 (0.83 – 3.63) 1.57 (0.86 – 2.86)		Out
Adjusted for pets and postnatal smoking	99	1.34 (0.71 – 2.53) 1.76 (0.84 – 3.69) 1.48 (0.80 – 2.72)		In
Adjusted for breastfeeding and postnatal smoking	96	1.69 (0.84 – 3.41) 1.71 (0.77 – 3.79) 1.38 (0.71 – 2.67)		Keep smoking out
Adjusted for breastfeeding and prenatal smoking	96	1.87 (0.93 – 3.79) 1.76 (0.79 – 3.93) 1.53 (0.80 – 2.94)		Out
Adjusted for maternal asthma/allergy and gravida	96	1.44 (0.76 – 2.71) 1.72 (0.80 – 3.67) 1.74 (0.95 – 3.18)		Out
Adjusted for maternal characteristics (asthma/allergy, prenatal SSRI, gravida)	95	1.36 (0.72 – 2.59) 1.72 (0.80 – 3.69) 1.60 (0.86 – 2.97)		Out
Adjusted for depression history and prenatal SSRI	98	1.31 (0.69 – 2.49) 1.81 (0.86 – 3.82) 1.51 (0.82 – 2.80)		Out
Adjusted for depression history and postnatal SSRI	97	1.36 (0.72 – 2.60) 1.61 (0.76 – 3.45) 1.56 (0.85 – 2.89)		Out
Adjusted for breastfeeding, pets at home, antibiotics	94	1.72 (0.84 – 3.51) 1.57 (0.67 – 3.67) 1.67 (0.86 – 3.25)		Keep pets out
Adjusted for breastfeeding, antibiotics, prenatal smoking	93	1.94 (0.95 – 3.97) 1.61 (0.69 – 3.77) 1.72 (0.88 – 3.35)		Keep smoking out
Adjusted for breastfeeding, antibiotics, SGA	94	1.82 (0.89 – 3.71) 1.60 (0.68 – 3.74) 1.60 (0.82 – 3.09)		Keep SGA out
Adjusted for breastfeeding, antibiotics, gravida	94	1.77 (0.87 – 3.60) 1.54 (0.66 – 3.61) 1.68 (0.87 – 3.25)		Keep gravida out
Adjusted for breastfeeding, antibiotics, postnatal SSRI	92	1.74 (0.85 – 3.56) 1.32 (0.55 – 3.13) 1.62 (0.82 – 3.19)		In
Adjusted for breastfeeding, antibiotics, postnatal SSRI, maternal asthma/allergy	91	1.91 (0.93 – 3.95) 1.33 (0.55 – 3.18)		In

		1.65 (0.84 – 3.28)		
Adjusted for breastfeeding, antibiotics, postnatal SSRI, maternal asthma/allergy, age	91	1.86 (0.90 – 3.87) 1.38 (0.56 – 3.37) 1.72 (0.86 – 3.42)		**Final Model**
Final Model with interaction for antibiotics exposure	91	0.57 (0.14 – 2.33) 1.68 (0.53 – 5.34) 2.46 (0.85 – 7.11)		

Table E6. Interactions between depressive symptoms and covariates in final model

Variable	**Interactions with depressive symptoms (p-value)**
Antibiotics exposure	0.04 0.57 0.41
Maternal asthma and allergy	0.79
Breastfeeding	0.99
Postnatal SSRI	0.80
Age	0.55

Note: Interactions between the covariate and depressive symptoms group variable were tested. When interaction terms with $p<0.05$ were found, the interaction term for each depressive symptoms group was reported.