Running Head: PHYSIOLOGICAL BIOMARKERS TO PREGNANCY-SPECIFIC ANXIETY

Associations of Physiological Biomarkers to Psychosocial Measures of Pregnancy-Specific Anxiety and Depression with Support Intervention

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Keywords: Pregnancy; Prenatal Anxiety; Depression; Cytokines; Intervention; Military

Funding Source: Research reported in this publication was supported by the TriService Nursing Research Program (HU0001-11-1-TS13).

Conflict of Interest: The authors have no conflicts of interest to report.

Acknowledgements: The views expressed are those of the authors and do not reflect the official views or policy of the Department of Defense or its Components.The voluntary, fully informed consent of the subjects used in this research was obtained as required by 32 CFR 219 and DODI 3216.02_AFI 40-402. The views of Milliplex, Luminex Magpix, MyBiosource, R&D Systems, Diagnostic Automation, and BIO-TEk Synergy are not necessarily the official views of, or endorsed by, the U.S. Government, the Department of Defense, or the Department of the Air Force. No Federal endorsement of Milliplex, Luminex Magpix, MyBiosource, R&D Systems, Diagnostic Automation, and BIO-TEk Synergy is intended.

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ABSTRACT

Stress and anxiety significantly impact the hypothalamic-pituitary axis, and in pregnancy, the subsequent maternal-fetal response can lead to poor outcomes. The objective of this study was to assess the association between psychosocial measures of pregnancy-specific anxiety and physiologic inflammatory responses. Specifically, to determine the effectiveness of the Mentors Offering Maternal Support (M-O-M-S^{TM}) program to reduce psychosocial anxiety and associated inflammatory response. In conjunction with measures of pregnancy-specific anxiety and depression, serum biomarkers (IL-2, IL-6, IL-10, IL1-B, TNF-α, CRH, CRP, and cortisol) were analyzed for each trimester throughout pregnancy. Results demonstrated that women receiving the M-O-M-S^{TM} intervention had longitudinally sustained lower TNF-α/IL-10 ratios than the control group, and it was significantly associated to psychosocial measures of anxiety, specifically for *fears of labor* and *spouse/partner relationships*. Additionally, the anxiety of *spouse/partner relationships* were significantly associated to IL-6/IL-10 ratios. The findings highlight the important counter regulatory relationship between anti- and pro-inflammatory cytokines and provide insight into the distinct physiologic responses to pregnancy-specific anxiety with early prenatal intervention.

Maternal adaptive responses to psychosocial stress and anxiety, during pregnancy, are inherently complex and critical in maintaining a unique immune-privileged environment that influences birth outcomes. Specifically, the hypothalamic-pituitary-adrenal (HPA) axis stress response and the subsequent production of glucocorticoids is delicately balanced throughout the pregnancy to ensure healthy outcomes (Gangestad et al., 2012; Giesbrecht et al., 2012). As the placenta is a stress-sensitive organ, any modulation of corticotrophin releasing hormone (CRH) may augment labor (Valsamakis et al., 2019). Increased, normal, or decreased levels of CRH concentration have been associated with pre-term, term, or post-term labor, respectively. In addition to changes in stress response, the normal immune response in pregnant mothers progressively shifts from a cell-mediated, pro-inflammatory, Th1 response to a humoral, anti-inflammatory, Th2 response (Chau et al., 2016). The balance between Th1 and Th2 response and the interaction of the associated pro- and anti-inflammatory cytokines, particularly TNF-α, IL-1β, IL-6 and IL-10, are important in the development and maintenance of a normal pregnancy (Denney et al., 2011; Moreli et al., 2012).

Both prospective and retrospective pregnancy studies in humans and animals, suggest that psychosocial stress and anxiety can profoundly affect immune response, leading to complications, such as preterm birth, preeclampsia, and poor birth outcomes (Beijers et al., 2014; Glover, 2011). However, pregnancy anxiety and stress-associated physiological mechanisms contributing to poor birth and infant outcomes are not well established and inconsistent across the literature. Gelman et al., (2019) demonstrated that women with severe anxiety had higher levels of both Th1 and Th2 cytokines in the 3rd trimester than those without any reported anxiety or depression, and those with both severe depression and anxiety had the highest concentration of cytokines. Specifically, women with elevated stress scores had associated higher levels of pro-inflammatory cytokines, IL-

6 and TNF-α, and lower levels anti-inflammatory markers, IL-10 (Coussons-Read et al*.,* 2005). Conversely, studies have found positive correlations for IL-12, IL-13, and IL-10 to pregnancy-related anxiety with no associations to IL-6 or TNF-α (Karlsson et al., 2017). Additionally, an analysis of amniotic fluid obtained through transabdominal amniocentesis at 16-18 weeks, found significant differences in the levels of IL-1α, Il-1β, IL-4, IL-6, and IL-8, with no differences in TNF-α, for women who delivered preterm versus those at term (La Sala et al., 2012).

Psychosocial interventions and effective social support systems play critical roles in mitigating poor birth outcomes by affectively changing immunological status during pregnancy. Specifically, in two different studies comparing social support to inflammatory responses, women perceiving higher social support had lower serum levels of pro-inflammatory cytokines (IL-2, IL-5, and IL-6) (Giurgescu et al., 2015) and C-reactive protein (Coussons-Read et al., 2007). Also, in the Giurgescu et al. study, women with lower levels of IL-10 had an increased incidence of preterm birth. Clearly, the association between psychosocial measures and physiologic changes in pregnancy requires further consideration that includes longitudinal assessment of both measures, and adjustment for known confounders. Furthermore, there is a need to evaluate perinatal interventions and their effectiveness to modulate both psychosocial and physiologic measures, and to link effects to birth outcomes. As such, the purpose of this study was to explore the association of psychosocial measures of pregnancy-specific anxiety and depressive symptoms to serum samples of pro- and anti-inflammatory markers for women receiving the early prenatal Mentors Offering Maternal Support (M-O-M-S™) program. Specifically, we tested the hypotheses that (i) psychosocial stress of pregnancy promotes production of pro-inflammatory cytokines, and inhibits the production of anti-inflammatory cytokines, and (ii) participation in the M-O-M-S™ program

ameliorates the psychosocial stress of pregnancy evidenced by decreased changes in both psychosocial and physiologic measures of stress and anxiety.

Methods

Study Design

This study is part of the M-O-M-S™ project sponsored by the TriService Nursing Research Program as a prospective, longitudinal investigation of immune, inflammatory response to pregnancy-specific anxiety and depressive symptoms in conjunction with the M-O-M-S™ intervention with IRB approval (IRB #377034).

Participants

All pregnant military beneficiaries (both active-duty women and spouses of active duty) initiating obstetrical care from 20 June 2012 to 16 June 2015 were contacted regarding their interest in the M-O-M-S™ study and screened for eligibility. Women were considered eligible for inclusion in the study if they were, 1) ≤ 12 weeks gestation at time of recruitment and consent, 2) at least 18 years old, 3) active-duty pregnant woman, or a pregnant spouse of an active-duty member of the American Armed Services, and 4) ability to understand English. Exclusion criteria included: 1) multiple gestation, 5) diabetes mellitus requiring insulin, 3) thyroid disorders, 4) chronic renal or heart disease, and/or 5) history and treatment for asthma.

Data Collection

Once consented, women were assigned to the treatment (M-O-M-S™ program) or control (prenatal care without the M-O-M-S™) groups based on a computer-generated randomization pattern. The M-O-M-S™ intervention group attended eight, 1-hour sessions, every-other-week starting in the first trimester. Each session's content was focused on unique aspects related to pregnancy-specific anxiety previously identified (Weis, Lederman, Lilly & Schaffer, 2008; Lederman & Weis, 2009), and piloted (Weis & Ryan, 2012). Detailed explanation of the

intervention session content has been previously reported (Weis, Lederman, Walker, & Chan, 2017).

At the first trimester, and at approximate 16-week, and 28-week routine lab draws, the participants completed study questionnaire booklets containing the psychological measures and the demographic information sheet, prior to the laboratory blood draw. A maternal venous blood sample of one 10 ml serum separator tube was collected in addition to the routine obstetrical labs. Given all analytes being collected (IL-6, TNF-α, IL-1β, IL-2, IL-10, CRP, CRH, and cortisol) had some diurnal rhythm, data collection was encouraged between 10:00 and 12:00 a.m., with no requirement for fasting. The study samples were immediately centrifuged, aliquoted, and frozen at -80° for batch analysis by trimester.

Immunologic Assays

The samples for IL-6, TNF-α, IL-1β, IL-2 and IL-10 were analyzed in duplicate using a multiplex cytometric bead array (Milliplex, cat# HCYTOMAG-60K) and read on a Luminex MagPix. Singleplex ELISAs for CRH (MyBiosource, cat # MBS731545), CRP (R&D Systems, cat # SCRP00), and Cortisol (Diagnostic Automation, cat# 6101-15) were analyzed in duplicate and read on a BIO-TEk Synergy H4 plate reader. All assays were performed in accordance with the manufacturer's instructions.

Psychological Measures

Pregnancy-Specific Anxiety. *Lederman's Prenatal Self-Evaluation Questionnaire* (PSEQ-SF), a 53-item, 7 scaled instrument, measuring aspects of pregnancy anxiety related to '*Acceptance,' 'Identification with a Motherhood Role,' 'Preparation for Labor,' 'Concerns for Well-Being of Self and Baby in Labor,' 'Fear of Pain, Helplessness and Loss of Control in Labor,' 'Relationship with Mother,' and 'Relationship with Spouse/Partner,'* was given in each trimester

of pregnancy. Each item is a four-point Likert scale ranging from 1 (*Not at All*) to 4 (*Very Much So*), where higher aggregate scores indicate greater pregnancy-specific anxiety. The instrument has been used extensively both in the US and internationally with good results. The Cronbach alpha coefficients for this population ranged from $\alpha = 0.70$ to 0.94. Convergent and divergent construct validity for the items was ascertained with biochemical assessments linked to qualitative data and to other validated anxiety measures (Lederman and Weis, 2009, 2020).

Symptoms of Depression. The *Edinburgh Postnatal Depression Scale* (EPDS), a 10-item instrument measuring the presence of symptoms indicative of possible depression or possibly anxiety was given in each trimester of pregnancy. The instrument is validated for both prenatal and postnatal use (Cox et al., 1996) with each item scored 0-3, and higher scores reflecting greater symptoms of depression (Cox & Sagovsky, 1987). Aggregate scores of ≥ 13 are considered a 'positive' score requiring follow-up (Henshaw & Ericksen, 2015), and a score other than 0 for item #10 (risk of self-harm) requires immediate attention. As such, all participants with scores ≥ 13 were referred for evaluation by behavioral health, and those indicating feelings of self-harm were evaluated immediately within the obstetrical clinic and sent to the emergency department if necessary. The Cronbach alpha coefficients for this population ranged from $\alpha = 0.86$ to 0.87 across the three trimesters.

Statistical Analysis

Longitudinal mixed-effect regression models were applied to examine the slope difference of each serum biomarker between treatment groups, age groups (<= 25 vs > 25), and parity, separately as well as by subgroup. The outcome variables included IL-6, TNF-α, IL-1β, IL-2, IL-10, CRP, CRH, cortisol, the ratio of TNF-α and IL-10 and the ratio of IL-6 and IL-10. Similar mixed-effect models were used to investigate the longitudinal relationship between each biomarker and each anxiety

and stress component. These analyses were conducted and compared between the groups of aforementioned variables-treatment, age, and parity. The correlation structure of the repeated observations of each dependent variable was assumed to follow an autoregressive model of order 1. Important advantages of using these longitudinal regression models for comparing the slopes include their capability to reduce bias due to heterogeneity of the initial value and consideration of correlated structure of each dependent variable repeatedly measured over time.

Results

Three hundred and sixty-seven women were randomized to either the M-O-M-S™ intervention or control groups. Separately, women participating in the M-O-M-S™ study were recruited and consented for the biomarker component of the study. Fifty-seven women were consented of which the majority completed all serum collection points and psychosocial measures (Table 1). Analysis of the overall sample found statistically significant increases from first to third trimester for IL-6, CRP, CRH, and cortisol. No significant differences for any of the biomarkers by parity. There was a statistically significant change longitudinally in IL-6 for women older than 25 years of age. However, the same differences were not reflected in women younger than 25 years.

In terms of the M-O-M-S™ intervention, participation demonstrated a longitudinally sustained lower TNF-α/IL-10 ratio than the control $F(1, 55) = 5.01$, $p=.03$ (Figure 1). Concurrently, there was a significant negative association for IL-10 in the control group $F(1, 55) = 6.59$, $p=.01$ and for IL-6/IL-10 ratio $F(1, 55) = 11.78$, $p<.01$ (Figure 2). Comparisons to psychosocial measures of anxiety reflected a significant negative association between TNF-α/IL-10 ratio to *Preparation for Labor* ($p=.03$) (Figure 3) and for *Relationship with Spouse/Partner* ($p=.01$) (Figure 4) in pregnancy for the control group. Similarly, IL-6/IL-10 ratio reflected a negative association with anxiety related to *Relationship with Spouse/Partner* ($p<.01$) (Figure 5). The IL-6/IL-10 ratio also

reflected a higher value with a borderline significant negative effect associated with *Identification with a Motherhood Role* (p=.07)*, Relationship with Mother* (p=.06), and *Preparation for Labor* (p=.07), a finding not reflected in the treatment group, which remained relatively constant across pregnancy. Closely aligned, the treatment group had a clinically dramatic increase, albeit, borderline significant (p=.07) for IL-10 associated with *Identification with a Motherhood Role*, while the control group had a low, straight trajectory for IL-10. The control group also reflected a significant increase in IL-1β to *depressive symptoms* (p=.01) over that of the treatment group (Figure 6).

Discussion

The current study provides longitudinal data for cytokine profiles in pregnancy with and without the addition of an early pregnancy, anxiety-reducing intervention. Notably, the results highlighted the critical counter regulatory relationship between anti-inflammatory cytokines, IL-10, and pro-inflammatory cytokines, IL-6 and TNF-α. It is hypothesized that without the protective, regulatory function of IL-10, the risk of obstetric complications increases (Moreli et al., 2012). Consistent with previous literature reports (Gelman et al., 2019), our results found that IL-6 and TNF-α are significantly important in their association to pregnancy anxiety and function as drivers of inflammation. Additionally, results provided insight into the differences in distinct physiologic, inflammatory responses to pregnancy-specific anxiety based on group assignment. Women, receiving the M-O-M-S™ support intervention, had a sustained, balanced ratio of pro- versus anti-inflammatory cytokines. Conversely, the control group demonstrated a significant shift in their cytokine pattern that reflects a non-balanced inflammatory physiological state and highlighted the counter regulatory role of IL-10. Additionally, the control group had a significant increase over that of the treatment group for the pro-inflammatory marker, IL-1β associated with increased

depressive symptoms. Remarkably, our data reflects physiologic changes associated with relatively slight variation in the anxiety and depression measures, rather than severe anxiety or comorbid depression.

It is increasingly clear that maternal inflammatory responses have a strong association with poor pregnancy outcomes. Although pro-inflammatory cytokines are the most broadly understood for their significant effects on birth outcomes, the nuanced relationship with anti-inflammatory cytokines provide a mechanism as biomarkers for preterm birth and other complications (Gomes et al., 2019). Given the associations between pregnancy-specific anxiety and poor birth outcomes, it becomes important to assess the longitudinal changes in these markers both independently and in relationship to psychosocial measures of anxiety as well as association with interventions focused on maternal anxiety and depression. Within the same M-O-M-S™ patient sample, we reported elsewhere that anxiety, associated with *Preparation for Labor,* increased the odds of preterm birth by 60% (Weis et al., 2020). In parallel, the associated biomarker data reflected an increased inflammatory response within the control group for anxiety related to *Preparation for Labor,* which aligns to the reported birth outcomes.

Strengths and Limitations

While the findings are important, they must be considered in the context of the strengths and limitations. The women, participating in the biomarker element of the study, were a small fraction of the overall sample of women participating in the RCT. Due to the proximity of the clinical and research laboratories for specimen collection and processing, recruitment was limited to women receiving obstetrical care at one of two military treatment locations. Additionally, over the course of the study, participant obstetrical care transitioned almost exclusively to the non-study site location, limiting recruitment significantly. Finally, cytokine biomarkers were selected based

on published literature, and the full complement of Th1 and Th2 cytokines were not evaluated on the available serum. Instead, IL-6/TNF-α and IL-10 were utilized as representative biomarkers for their perspective pro- and anti-inflammatory function, respectively, and ratios of IL-6 and TNF-α to IL-10 were used to describe their antagonistic pro- and anti-inflammatory relationship.

In human studies, capturing and controlling all the factors that may influence cytokine variation is difficult. The sampling design attempted to control for some of the factors known to influence cytokine variation. Accordingly, pregnant women with a history of diabetes mellitus requiring insulin, thyroid disorders, chronic renal or heart disease, and/or a history of asthma were excluded from the study. Furthermore, there was no history of smoking for any of the women in the study, and none of the women in the biomarker study received steroids during their pregnancies.

The effect of BMI on the biomarkers was assessed in two ways. First, as a baseline measure (measured upon entry into prenatal care) and second, as a mean over the course of pregnancy (a weight taken at each prenatal appointment). When the baseline measure of weight was used for the BMI vs. the mean over pregnancy, there were no significant associations between BMI and any of the longitudinal biomarker values. When the time-varying nature of BMI, over the course of pregnancy was considered, there was a significant association to increases in the trend of IL-6 (p = .01). It does not appear that BMI confounded our key findings.

Conclusions

Our results suggest that the protective, balancing effect of pro- and anti-inflammatory cytokines in pregnancy is negatively affected by increased maternal pregnancy-specific anxiety. More importantly, it appears that the M-O-M-S™ early, prenatal support intervention decreased pregnancy-specific anxiety and depressive systems and promoted the unique balance of both pro-

and anti-inflammatory cytokines in pregnancy. Of particular interest is the slight variation in the psychosocial measures of pregnancy-specific anxiety that were associated with a relatively dramatic physiologic change, which highlights a more nuanced relationship between anxiety and predicted outcomes. Moreover, the theories of *chronic* stress and maternal immunity are critical in understanding the downstream effects of pregnancy-specific anxiety. Certainly, any of the psychosocial dimensions of anxiety can develop into chronic stress, if not addressed, *Preparation for Labor* and *Relationship with Spouse/Partner* were of particular interest due to their association to IL-6/IL-10 and in TNF-α/IL-10 ratios. The findings reinforce the need for early, prenatal intervention. While certain prenatal birthing classes may help alleviate some of the fears and anxiety associated with labor, these classes or programs are generally provided late in the third trimester. Additionally, currently there are generally little to no opportunities to obtain support and reflect on one's relationships relative to pregnancy and parenthood. Even more important, without intervention, anxiety associated with maternal identification will not change (Lederman & Weis, 2009 & 2020). Clearly, assessment and focused intervention must occur early in pregnancy to promote a balanced/responsive maternal immune system and improve birth and infant outcomes.

References

Bluthe, R. M., Dantzer, R., Kelley, K. W. (1997). Central mediation of the effets of IL-1 on social exploration and body weight in mice. *Psychoneurolendocrinology, 22*, 1-11.

Beijers, R., Buitelaar, J. K., & de Weerth, C. (2014). Mechanisms underlying the effects of prenatal psychosocial stress on child outcomes. Beyond the HPA axis. *European Child & Adolescent Psychiatry, 23*, 943-956. doi 10.1007/s00787-014-0566-3

Buss, C., Davis, E. P., Shahbaba, B., Pruessner, J. C., Head, K., & Sandman, C. A. (2012). Maternal cortisol over the course of pregnancy and subsequent child amygdala and hippocampus volumes and affective problems. *PNAS, Proceedings of the National Academy of Sciences of the United States of America, 109*(20), 7613-7614. doi: 10.1073/pnas

Chau, A., Markley, J. C., Juang, J., & Tsen, L. C. (2016). Cytokines in the perinatal period – Part I. *International Journal of Obstetric Anesthesia, 26,* 39-47. doi: 10.1016/j.ijoa.2015.12.005

Coussons-Read, M. E., Okun, M. L., Nettles, C. D. (2007). Psychosocial stress increased inflammatory markers and alters cytokine production across pregnancy. *Brain, Behavior, and Immunity, 21*, 343-350. doi: 10.1016/j.bbi.2006.08.006

Coussons-Read, M. E., Okun, M. L., Schmitt, M. P., & Giese, S. (2005). Prenatal stress alters cytokine levels in a manner that may endanger human pregnancy. *Psychosomatic Medicine, 67*(4), 625-631. doi: 10.1097/01.psy.0000170331.74960

Cox, J. L., Chapman, G., Murray, D., Jones, P. (1996). Validation of the Edinburgh Postnatal Depression Scale (EPDS) in non-postnatal women. *Journal of Affective Disorders, 39*(3), 185-89.

Cox, J., Holden, J., & Sagovsky, R. (1987). Detection of postnatal depression: Development of the 10-item Edinburgh postnatal depression scale. *British Journal of Psychiatry, 150*, 782–6.

Denney, J. M., Nelson, E. L., Wadhwa, P. D., Waters, T. P., Mathew, L., Chung, E. K., Goldenberg, R. L., & Culhane, J. F. (2011). Longitudinal modulation of immune system cytokine profile during pregnancy. *Cytokine, 53*, 170-177. doi:10.1016/j.cyto.2010.11.005

Gangestad, S. W., Hooper, A. E. C., & Eaton, M. A. (2012). On the function of placental corticotropin-releasing hormone: A role in maternal-fetal conflicts over blood glucose concentrations. *Biological Reviews, 87*(4), 856-873. doi: 10.111/j.1469-185X.2012.00226

Garfield, L., Mathews, H. L., & Janusek, L. W. (2016). Inflammatory and epigenetic pathways for perinatal depression. *Biological Research for Nursing, 18*(3), 331-343. doi: 10.1177/1099800415614892

Gelman, et al., (2019). The cytokine profile of women with severe anxiety and depression during pregnancy. *BMC Psychiatry, 19*(104), 1-11. doi: https://doi.org/10.1186/s12888-019-2087-6

Giesbrecht, G. F., Campbell, T., Letourneau, N., Kooistra, L., & Kaplan, B. (2012). Psychological distress and salivary cortisol covary within persons during pregnancy. *Psychoneuroendocrinology*, 37, 270-279. doi: 10.1016/j.psyneuen.2011.06.011

Giurgescu, C., Sanguanklin, N., Engeland, C. G., White-Traut, R. C., Park, C., Mathews, H. L., & Janusek, L. W. (2015). Relationships among psychosocial factors, biomarkers, preeclampsia, and preterm birth in African American women: A pilot. Applied Nursing Research, 28, 1-6. doi: https://doi.org/10.1016/j.apnr.2014.09.002

Glover, V. (2011). Annual research review: Prenatal stress and the origins of psychopathology: An evolutionary perspective. *Journal of Child Psychology and Psychiatry, 52*(4), 356-367. doi: https://doi.org/10.1111/j.1469-7610.2011.02371.x

Gomes, J., Au, F., Basak, A., Cakmak, S., Vincent, R., & Kumarathasan, P. (2019). Maternal blood biomarkers and adverse pregnancy outcomes: A systematic review and meta-analysis. *Critical Reviews in Toxicology, 49*(6), 461-478. doi: 10.1080/10408444.2019.1629873

Henshaw, C., & Ericksen, J. (2015). How to use the EPDS and maximize its usefulness in the consultation process. In J. Milgrom & A. W. Gemmill (Eds.), *Identifying perinatal depression and anxiety*. Wiley.

Hou, R., & Baldwin, D. S. (2012). A neuroimmunological perspective on anxiety disorders. *Human Psychopharmacology, 27,* 6-14. doi: 10.1002/hup.1259.

Karlsson et al., (2017). Cytokine profile and maternal depression and anxiety symptoms in mid-pregnancy – the FinnBrain Birth Cohort Study. *Archives of Women's Mental Health, 20*, 39-48. doi 10.1007/s00737-016-0672-y

La Sala, G. B., Ardizzoni, A., Capodanno, F., Manca, L., Baschieri, M. C., Soncini, E., Peppoloni, S., & Blasi, E. (2012). Protein microarrays on midtrimester amniotic fluids: A novel approach for the diagnosis of early intrauterine inflammation related to preterm delivery. *International Journal of Immunopathology and Pharmacology, 25*(4), 1029-1040.

Lederman, R., & Weis, K. (2009). Psychosocial adaptation pregnancy: Seven dimensions of maternal role development (3rd ed.). Springer.

Lederman, R., & Weis, K. (2020). Psychosocial adaptation pregnancy: Seven dimensions of maternal development (4th ed.). Springer.

Leonard, B. E., & Myint, A. (2009). The psychoneuroimmunology of depression. *Human Psychopharmacology, 24,* 165-175. doi: 10.1002/hup.1011.

Moreli, J. B., Ruocco, A. M. C., Vernini, J. M., Rudge, M. V. C., Calderon, I. M. P. (2012). Interleukin 10 and tumor necrosis factor-alpha in pregnancy: Aspects of interest in clinical obstetrics. *International Scholarly Research Network, Obstetrics and Gynecology, 2012,* 1-5. doi: 10.5402/2012/230742.

Mosmann, T. R., & Coffman, R. L. (1989). Heterogeneity of cytokine secretion patterns and functions of helper T cells. *Advances in Immunology, 46*, 111-147.

Murray, D., & Cox, J. L. (1990). Screening for depression during pregnancy with the Edinburgh depression scale (EPDS). *Journal of Reproductive Infant Psycholology, 8*, 99–107.

Seyle, H. (1936). A syndrome produced by diverse nocuous agents. *Nature,* 138, 32.

Weinstock, M. (2001). Alterations induced by gestational stress in brain morphology and behavior of the offspring. *Progress in Neurobiology, 65*, 427-451.

Thorpe, K. (1993). A study of the Edinburgh postnatal depression scale for use with parent groups outside the postpartum period. *Journal of Reproductive Infant Psychology, 11*, 119–25.

Valsamakis, G., Chrousos, G., & Mastorakos, G. (2019). Stress, female reproduction and pregnancy. *Psychoneuroendocrinology, 100*, 48-57.

doi: https://doi.org/10.1016/j.psyneuen.2018.09.031

Weis, K. L., Lederman, R. P., Lilly, A. E., & Schaffer, J. (2008). The relationship of military imposed marital separations on maternal acceptance of pregnancy. Research in Nursing & Health, 31, 196-207.

Weis, K. L., Lederman, R. P., Walker, K. C., & Chan, W. (2017). Mentors offering maternal support reduces prenatal, pregnancy-specific anxiety in a sample of military women. *Journal of Obstetric, Gynecologic & Neonatal Nursing, 46*(5), 669-685.

Weis, K. L., & Ryan, T. W. (2012). Mentors offering maternal support: A support intervention for military mothers. Journal of Obstetric, Gynecologic & Neonatal Nursing, 41, 303-313.

Weis, K. L., Walker, K. C., Chan, W., Yuan, T. T., & Lederman, R. P. (2020). Risk of preterm birth and newborn low birthweight in military women with increased pregnancy-specific anxiety. *Military Medicine, 185*, 678-685.

Table 1

Summary of Sample Characteristics

Variable Name	**Total Sample (*n*=57)**	**Intervention Group (*n*=28)**	**Control Group (*n*=29)**
Age [Years, Mean (*SD*)]	27.81 (4.50)	26.29 (4.29)	29.28 (4.26)
Race/Ethnicity (%)			
White, Non-Hispanic	32 (57.14)	18 (64.29)	14 (50)
Black, Non-Hispanic	8 (14.29)	2 (7.14)	6 (21.43)
Hispanic	10 (17.86)	5 (17.86)	5 (17.86)
Others	6 (10.71)	3 (10.71)	3 (10.71)
Prior Deliveries (%)			
0	18 (31.58)	9 (32.14)	9 (31.03)
1 to 2	35 (61.40)	18 (64.29)	17 (58.62)
3 or more	4 (7.02)	1 (3.57)	3 (10.34)
Marital Status (%)			
Married	53 (92.98)	25 (89.29)	28 (96.55)
Not Married	4 (7.02)	3 (10.71)	1 (3.45)
Military Branch[1]			
Air Force	42 (75)	21 (75)	21 (75)
Army	7 (12.50)	4 (14.29)	3 (10.71)
Other	7 (12.50)	3 (10.71)	4 (14.28)
Active Duty (%)	20 (35.09)	9 (32.14)	11 (37.93)
Active Duty Spouse (%)	51 (91.07)	24 (85.71)	27 (96.43)

[1]Military branch based on participant's branch of service unless spouse is active-duty member

Figure 1

Longitudinal Comparison of TNF-alpha/IL-10 Ratio for M-O-M-S™ Intervention and Control Groups

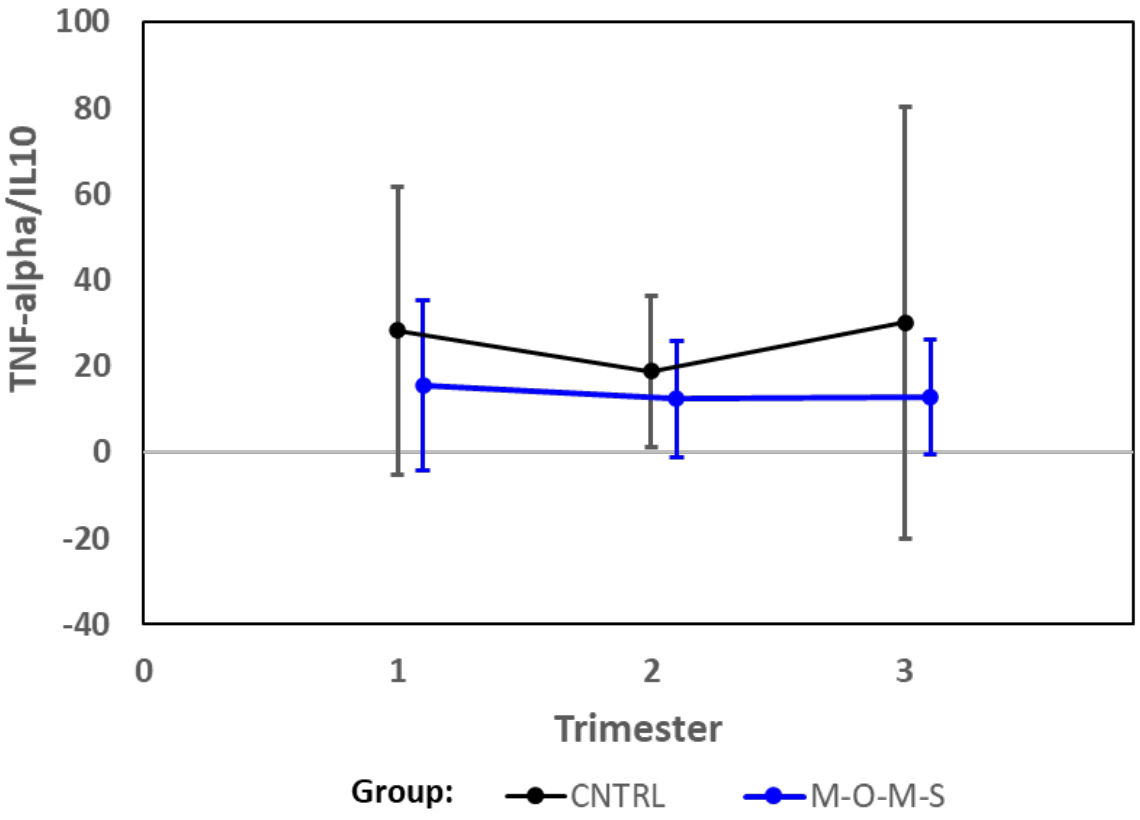

Figure 2

Longitudinal Comparison of IL-6/IL-10 Ratio for M-O-M-S™ Intervention and Control Groups

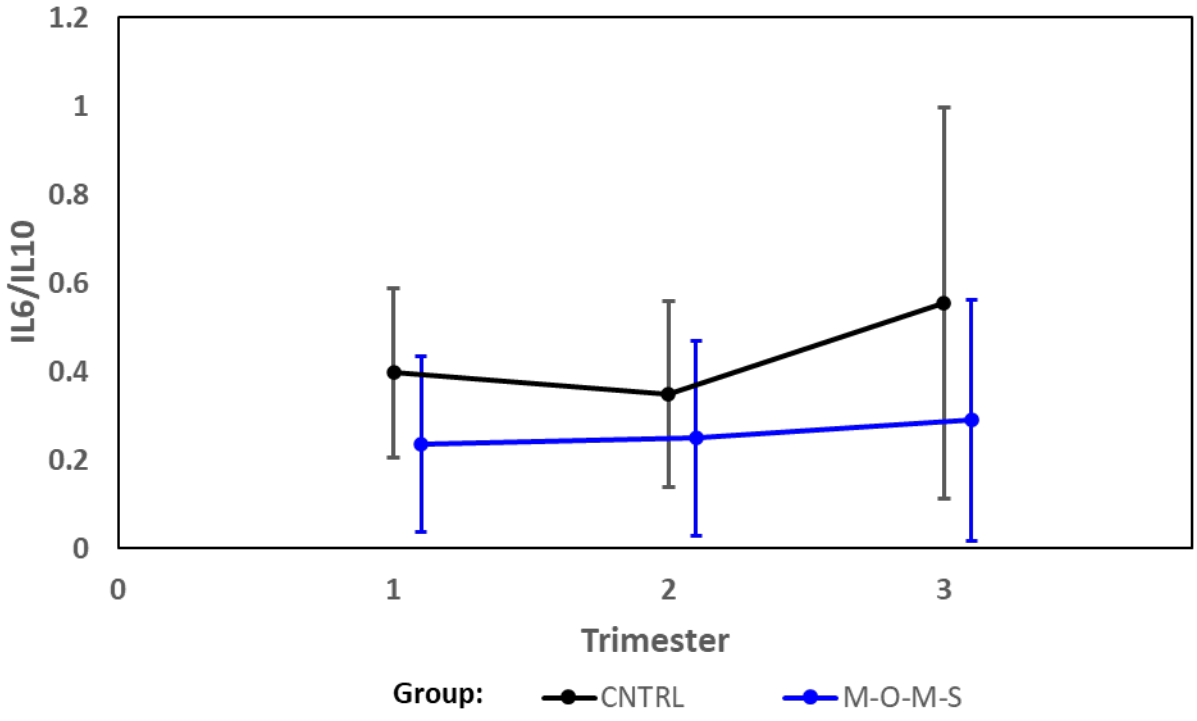

Figure 3

Longitudinal Relationship between TNF-alpha/IL-10 Ratio to Preparation for Labor anxiety for M-O-M-S™ and Control Groups

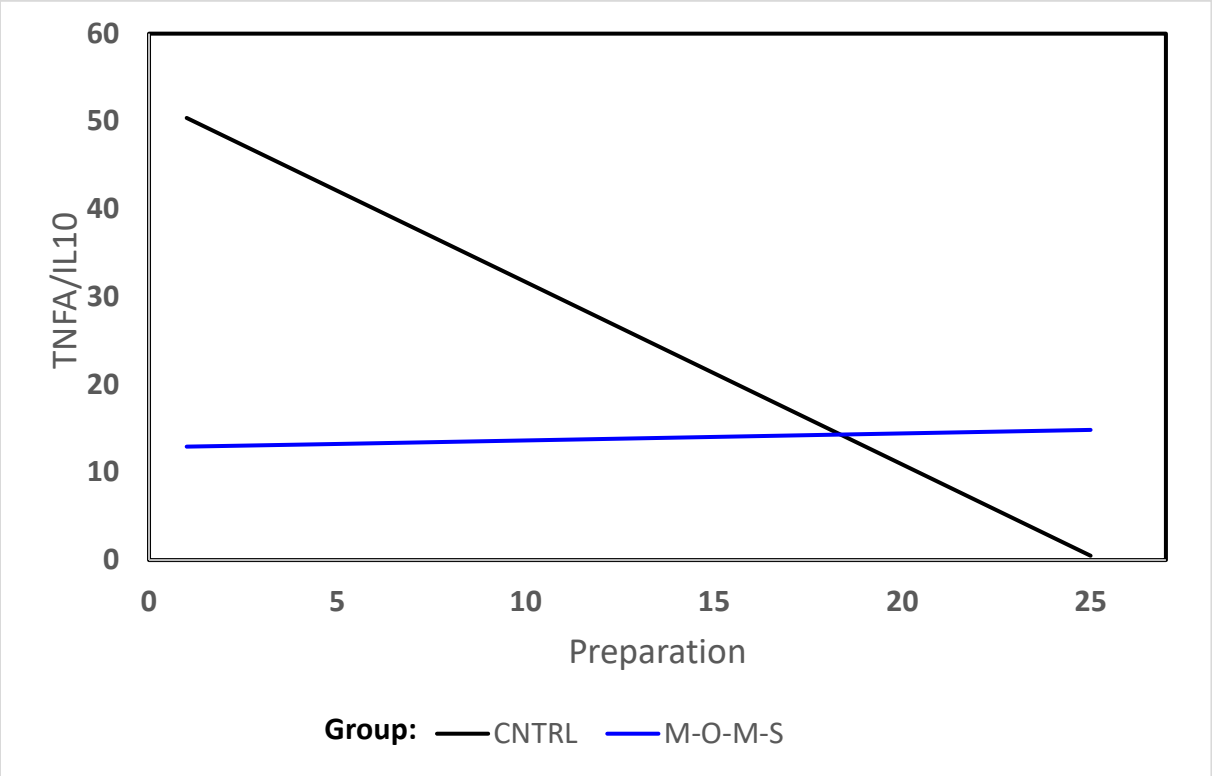

Figure 4

Longitudinal Relationship between TNF-alpha/IL-10 Ratio to Relationship with Spouse/Partner anxiety for M-O-M-S™ and Control Groups

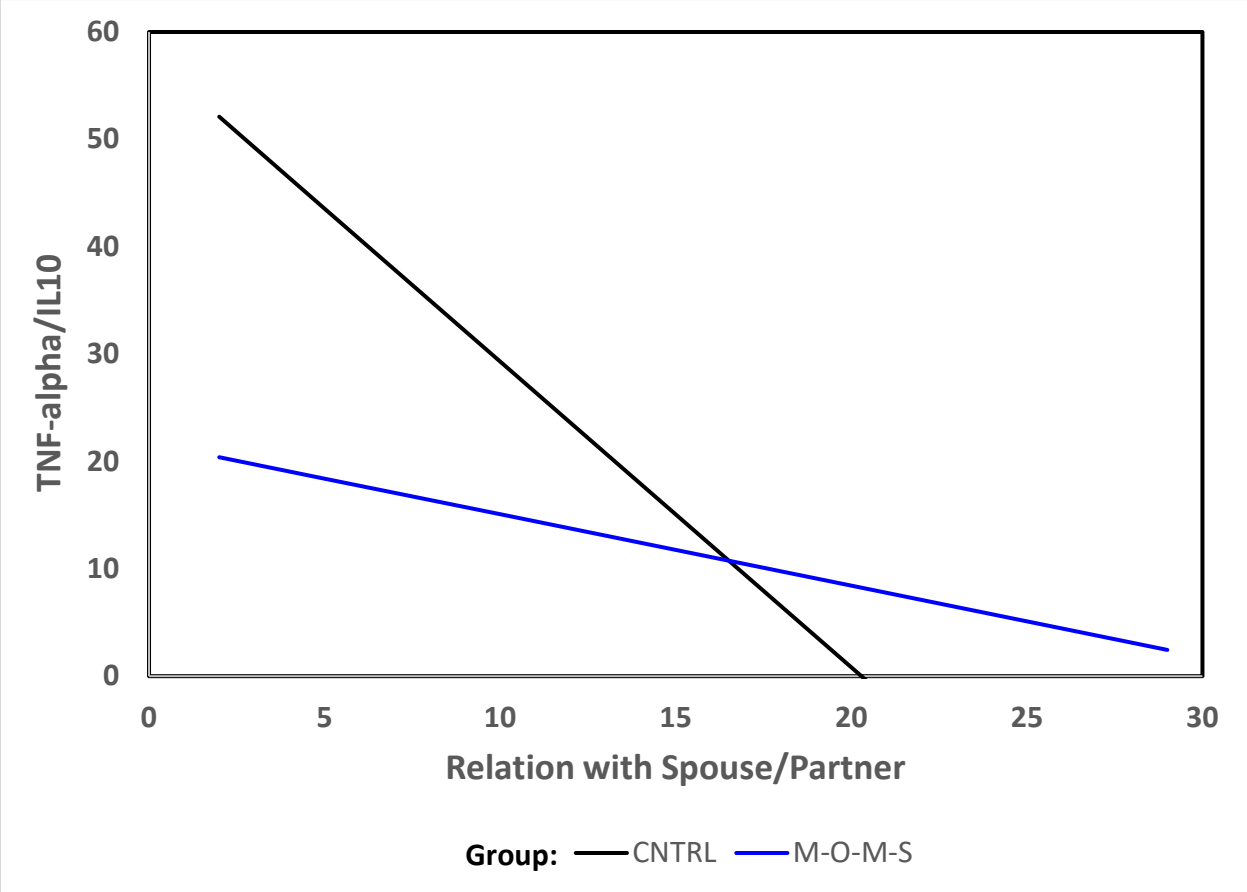

Figure 5

Longitudinal Relationship between IL-6/IL-10 Ratio to Relationship with Spouse/Partner anxiety for M-O-M-S™ and Control Groups

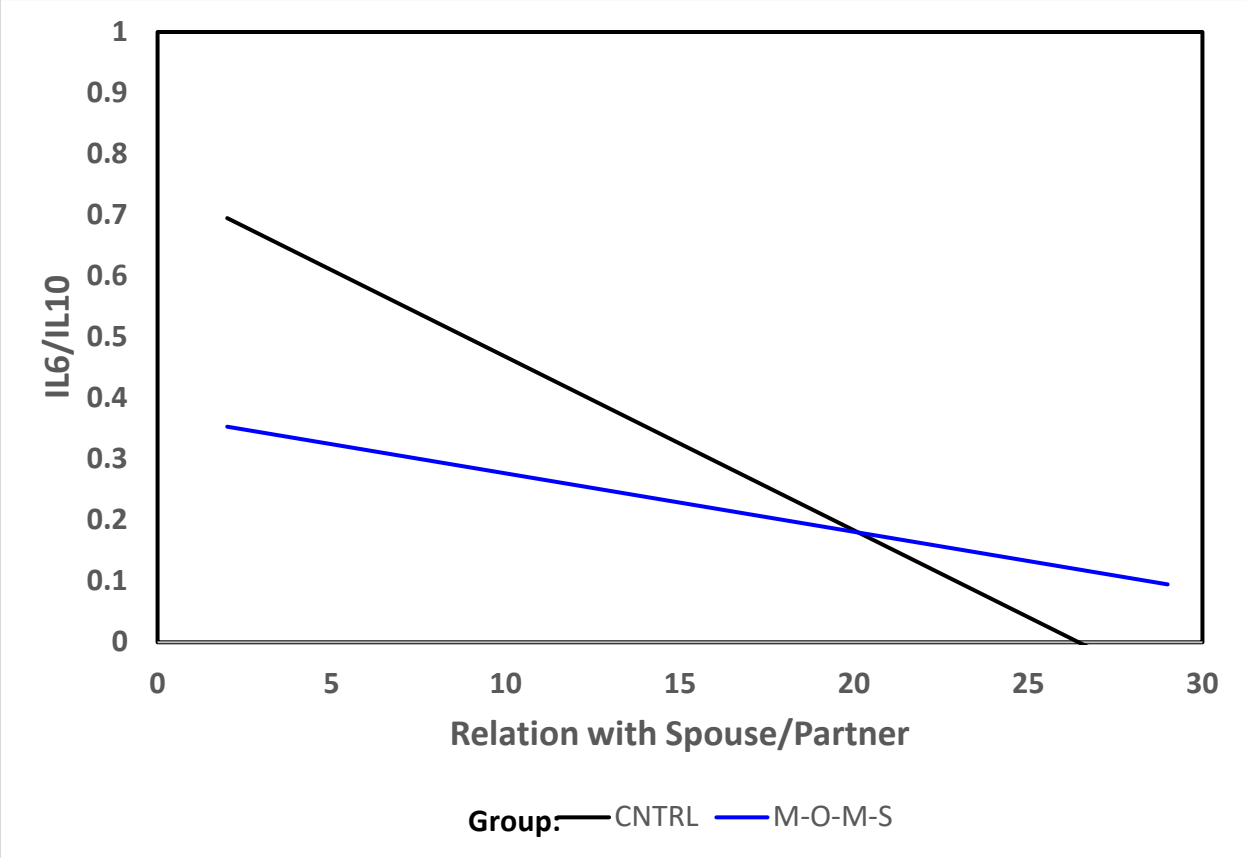

Figure 6

Longitudinal Relationship between IL1β to Depressive Symptoms for M-O-M-S™ and Control Groups