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Characterizations of Psychosocial and Biological Stress Factors and
Associations with Self-Reported Race and Bacterial Vaginosis in Pregnancy

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ABSTRACT

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Bacterial vaginosis (BV) is a reproductive tract infection syndrome whose manifestation is characterized by an overgrowth of anaerobic bacteria, and a corresponding reduction in peroxidase-producing *Lactobacillus*. Given that BV is an important risk factor associated with preterm birth, and that preterm birth is one of the most relevant contributors to infant morbidity and mortality worldwide, epidemiologic research on the causes and factors associated with BV in pregnancy is of public health importance.

A number of studies have posited the role of maternal chronic and acute stress in negatively impacting immune and hormonal functioning, thus making it biologically plausible for stress to directly affect susceptibility to infection. However, inconsistencies in the conceptualizations of the construct of stress, timing of stress exposure and BV prevalence assessment, differentiation between chronic and acute experience of stress, and the application of different instruments to measure stress further complicate the comparability of results across study populations.

The increased occurrence of BV during pregnancy among African-American women in the U.S. is well-documented. However, an excess occurrence among Afro-descendant women in other racially heterogeneous settings is unclear. Brazil provides an interesting context for testing hypothesized associations of race and BV in pregnancy, given considerable co-occurring social, economic, and racial inequality. To date, there are no published studies investigating the interrelationships of maternal psychosocial stress, race, and BV prevalence in pregnancy in a Brazilian context.

In this study, BV prevalence among black or mixed/mulatta women did not differ from that in white women. However, the excess BV prevalence among black women relative to mixed/mulatta women remained statistically significant, even after multivariate adjustment for life events, state anxiety, perceived stress, intimate partner violence, and potential confounders. These preliminary findings 1) suggest that in Brazilian pregnant women BV prevalence may differ by self-reported race, and that the associations of BV with psychosocial risk factors may differ by race, and 2) support a larger study using more complete characterization, measurement, and analysis of individual and contextual factors by racial self-identity.

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CHAPTER 1

LITERATURE REVIEW

Bacterial vaginosis in pregnancy

Bacterial vaginosis (BV) is a reproductive tract infection syndrome whose manifestation is characterized by an overgrowth of anaerobic bacteria, particularly *Gardnerella vaginalis* and *Mobiluncus* spp., and a corresponding reduction in *Lactobacillus* spp. The condition is very common, detected in about 15 to 40% of pregnant women worldwide [1], and has been cited in the U.S. literature to be most prevalent among women who have multiple sex partners, have lower income and education levels, smoke cigarettes, do not cohabit with their partners, and are African-American [2-7]. BV has been repeatedly associated with a two to three-fold increased risk of preterm delivery, usually measured as <37 weeks gestation [2, 3, 7-9].

These studies have also shown that the rate of BV prevalence is at its highest in early to mid gestation, with up to 50% of BV cases identified early in gestation spontaneously remitting by the third trimester. Research into factors associated with BV in early to mid gestation may also be of greater public health impact, as very early preterm delivery may be more frequently associated with infection status [8]. However, little is known about the factors that modulate susceptibility to developing BV during pregnancy. Results of trials in BV asymptomatic women have not shown clear benefits from antibiotic therapy, and alternative probiotic trials have either been inefficacious in interrupting the infectious process that leads to preterm delivery or have lacked the

sample size to estimate reliable intention-to-treat effects, further complicating the hypothesized causal association between BV and preterm delivery [10, 11]. Given that preterm birth is one of the most relevant contributors to infant morbidity and mortality worldwide, epidemiologic research on the causes and factors associated with BV is of public health importance.

Stress and health outcomes

The bidirectional link between stress and health is well-established in the literature [12, 13], leading to a general recognition of the important role of stress in health [14]. However, the study of “stress” is complicated by the lack of a standardized definition of the construct which results in a wide variety of definitions and operationalizations available in the literature.

A commonly-cited organizing framework for the definition of stress is the transactional, or stress and coping model, under which stress is a subjectively perceived discrepancy between environmental demands and biological, psychological, or social resources within a specific period of time [15]. An important element of this definition is stress appraisal, or the perception of environmental demands or threats and the perceived ability to meet these demands [15]. Ursin and Eriksen [16] further propose, under the cognitive activation theory of stress (CATS), that a physiological stress response is characterized by a *baseline alarm state* (consistent with chronic stress when resulting from prolonged exposure to stressful stimuli) and *acute alarm activation* (consistent with a stress response to current stimuli in one’s environment). As such, stress, beyond a

stimulus or response, is also an interaction of related experiences, pathways, responses and outcomes caused by a range of different events or circumstances [17, 18].

The study of a complex construct like stress is further complicated by temporal and spatial constraints inherent to its measurement. As Reis et al. [14] highlight, instruments to measure stress have been limited to specific contexts or aspects of stress, such as the Life Events Scale [19], thus restricting scale measurements only to specific environments and study populations. Other measurement limitations include a lack of sensitivity to chronic stress from ongoing life circumstances or from life events not listed on the scale [20]. Presumably, it is the level of appraised stress, and not only the objective occurrence of the events, that determines one's response to a stressor [16, 20, 21].

The involvement of the hypothalamic-pituitary-adrenal (HPA) and immune axes in response to psychological stress has been proposed as a central mechanism linking psychosocial factors and health outcomes [22]. It is biologically plausible for stress to directly affect susceptibility to infection, for instance, as animal models and human studies suggest that stress and depression result in impairment of the immune response. Through HPA activation during a stress response, the biological mediators released suppress some non-specific and specific parts of the immune response, including NK-cell activity, phagocytosis, production of inflammatory cytokines, and cytotoxic T-cell activity, thereby compromising the most important effectors of the response [23, 24].

Assessment of psychosocial stress and cortisol in pregnancy

A number of studies have cited the negative influence of maternal acute and chronic psychosocial stress on immune and hormonal functioning in pregnancy, which in turn may heighten susceptibility to infection, affect utero-placental function, and increase risk for preterm delivery [25-30].

Evidence for operationalizing a chronic stress latent construct with indicators of prenatal depression and trait anxiety in pregnancy has been previously reported by Ritter et al. [31], in a study where sustained (trait) anxiety and maternal prenatal depression loaded as positive indicators of chronic stress in structural equation models of pregnant, low income, inner-city women. Furthermore, evidence from animal models suggests that HPA impairment in chronically stressed rats is similar to that observed in depressed patients [23]. Social stressors may be additional sources of chronic stress and influence biological reactions to stressful stimuli in the form of either (1) larger, more sustained responses or (2) more frequent occurrence of events [32]. Variables including adverse childhood experiences, experiences of discrimination, and income and education levels have been used alone [33, 34] or in conjunction [6, 35-40] in studies of stress in pregnancy.

Regarding acute psychosocial stress during pregnancy, various studies cite selected psychosocial scale measures, including state anxiety, perceived stress, stressful life events, and social support, as well as items querying on feelings toward pregnancy and occurrence of domestic disturbance to examine the effects of stress on bacterial vaginosis status [28, 41-42]. To examine the effects of stress during pregnancy on selected outcomes, such as bacterial vaginosis (BV), various studies have focused on

acute psychosocial stress (e.g., state anxiety, perceived stress, stressful life events, and absence of social support), as well as items querying on feelings toward pregnancy, or the occurrence of childhood sexual abuse or intimate partner violence [28, 40-43].

The involvement of the HPA and immune axes in response to psychological stress has highlighted cortisol concentration as a useful measure of endocrine stress because of its roles in both modulating the stress response through the HPA axis negative feedback loop and in influencing immune function [22, 23, 41, 42]. Cortisol concentration, as an indicator of HPA function and endocrine stress, is hypothesized to covary with both chronic and acute psychosocial stress. Interestingly, lower levels of free cortisol have been reported in post-traumatic stress disorder (PTSD) patients (consistent with chronically stressed individuals) and African-Americans [23, 44], while higher levels of free cortisol have been associated with lowered immunity, inflammatory responses, and increased prevalence of BV in women reporting anxiety and acute perceived stress [23, 41].

The hypothesis that levels of maternal cortisol rise in response to stress during pregnancy has been previously studied with mixed results. Rondó et al. [45] reported an inverse relationship between the Brazilian Portuguese version of the Spielberger state anxiety inventory and cross-sectional salivary cortisol concentrations (Spearman $r = -0.39$, $p = 0.01$) in pregnant women < 20 years of age (mean gestational age = 35.97 weeks, $SD = 4.84$). In that study, conducted in health facilities in São Paulo, salivary samples were collected at participants' homes by 9:00am, followed by the anxiety inventory questionnaire. However, the analyses did not adjust for gestational age and

other maternal demographic and sociobehavioral factors, and only considered the Spielberger state-trait anxiety inventory as a psychosocial measure of maternal distress.

In North Carolina, the Pregnancy, Infection, and Nutrition study [12], which followed a protocol for saliva collection and questionnaire during clinic visits, found that morning cortisol concentrations during the 14 to 18 weeks' gestation visit and the 24 to 29 weeks' visit were not associated with any psychosocial scale measure in this study population. Furthermore, salivary cortisol was consistently associated only with body mass index and chronic hypertension at both visits.

Pluess et al. [46] found that maternal trait anxiety was consistently associated with psychological stress and the salivary cortisol awakening response only in early pregnancy (10 to 20 weeks' gestation) in a German study population. In that study, salivary samples were collected by participants at home at four different time intervals upon awakening during two consecutive days in early and late pregnancy. Trait anxiety was measured with the 16 Personality Factor Questionnaire (16-PF), and a significant inverse association between trait anxiety and cortisol concentration in early pregnancy was found after adjustment for smoking, alcohol use, and stressful experiences during pregnancy measured by the Prenatal Distress Questionnaire. Giesbrecht et al. [15] examined serial levels of salivary cortisol concurrent with level of negative mood over three days in a sample of Canadian pregnant women. Salivary sample collection was performed at home by participants, after attending an individualized training session. Results from multilevel analyses indicated a positive association between maternal negative mood and cortisol concentration, unaffected by progressing gestational age.

Comparisons across these summarized study findings are complicated by differences in instruments that defined and assessed maternal stress differently across studies, timing of stress exposures, measurements at different time windows during pregnancy, adjustment for different demographic and biomedical covariates, and cortisol measurements in single or multiple time points. An important gap that remains in the literature is more empirical support for the theoretical model in which psychosocial stressors stimulate production of biological correlates of stress [12] and consideration of race as a risk factor for BV susceptibility, rather than only as a confounder or covariate of the stress-BV association. For instance, recent findings report a race-dependent prevalence of bacterial communities in women with BV, which may directly contribute to the increased incidence of BV in Black women independent of stress mediation [103]. Also, epidemiologic research can benefit from applications of sociology paradigms such as intersectionality, where various social and biological categories interact on multiple and often simultaneous levels, to conceptualize BV as the product of intersecting dimensions of sociodemographic and stress stratification [104].

Bacterial vaginosis and psychosocial stress in pregnancy

Stressful exposures have been positively associated with susceptibility to infection (e.g., BV) in pregnancy in various settings [47-50]. In the U.S., racial disparities have been reported in both the rates of occurrence of BV [40, 43, 50] and stressful experiences during pregnancy [30, 36, 47, 48], leading to the hypothesis that racial variation in stress may explain the observed differences in infection in pregnancy

[40, 51]. Studies relating stress to the development of BV offer mixed results, however. For instance, Culhane et al. [4] evaluated current stress among pregnant women using Cohen's Perceived Stress Scale (PSS) and chronic stress measured by neighborhood proxies. The study concluded that higher levels of perceived stress were associated with modestly higher odds of BV (OR = 1.3; 95% CI 1.0 – 1.6). After adjustment for sociodemographic attributes, behavioral risk, and perceived stress, the odds of having bacterial vaginosis among women residents of communities with higher homelessness rates were significantly higher than among women residents of communities without a homelessness community level stressor (OR = 6.7; 95% CI, 1.6-27.8).

Ruiz et al. [52] found no association between the PSS and BV, but did find an elevated risk for BV among pregnant women with higher cortisol levels in a sample of 78 Medicaid-eligible women in Texas. Harville et al. [53] found that women in the highest quartile of stress, particularly state anxiety (OR=2.0, 95% CI 1.2-3.3), perceived stress (OR=2.4, 95% CI 1.5-3.9) and total stressful life events (OR=2.0, 95% CI 1.3-3.2), had the highest risk of BV. In that study, the associations with measures of stress were all nearly eliminated after adjustment for socioeconomic status and race. Paul et al. [54] found that perceived stress was not related to BV risk among either American white or African American pregnant women in multivariate models stratified by race, and Uscher-Pines et al. [55] found that, after adjusting for race, marital status, insurance, and smoking, perceived stress was not significantly associated with BV in the first trimester of pregnancy (OR = 1.01, 95% CI: 0.98, 1.05). Furthermore, Cammack et al. [43] found that early life psychosocial adversity, rather than acute perceived stress, was significantly associated with persistent BV in the second trimester of pregnancy. Treating race as a

confounder or covariate in stress-BV association analyses may over-control not only for the potential independent association of race in bacterial community differentiation driving excess prevalence of BV in Afro-descendant women, but also for the intersectionality of sociodemographic and biological processes involved in the causal chain leading to BV.

Susceptibility to BV may be a result of immune dysregulation in response to stress, or the result of an intermediate pathway of behavioral coping responses, such as use of tobacco during pregnancy, which may exert a synergistic effect with stress owing to a potential for biological interaction [54, 56]. These studies have not clearly elucidated the temporality between stress and BV incidence, as few studies systematically account for the potential modulation of the nature, duration and timing of stress before or during pregnancy and associations with BV development. Inconsistencies in the conceptualizations of the construct of stress, timing of stress exposure measurement and BV prevalence assessment, differentiation between chronic and acute experience of stress, and the application of different instruments and stratifications of exposure to measure stress further complicate the comparability of results across study populations.

Bacterial vaginosis, race, and socioeconomic status (SES)

There are significant differences in the prevalence of BV among different population subgroups in the United States [2, 51, 57]. For instance, the rate of BV is consistently higher in black women than in any other racial/ethnic group [58]. Low socioeconomic status (SES), frequently measured as lower educational attainment and household income, also have been studied as independent factors associated with BV,

either directly or as mediators of the relationship between race and adverse health outcomes in pregnancy [43, 54, 59, 60]. While the Preterm Prediction Study in the U.S. [61] found no association between low SES and BV by race, studies in Kenya and the United Kingdom reported a positive association [62, 63]. As Paul et al. [54] highlight, explanations for conflicting results include differences in the measurement of SES and stratifications by race.

Whereas estimates of the black-white difference in BV prevalence in the U.S. are thought to explain up to 30% of the national disparity in preterm births [64], the factors that influence race-specific prevalence of BV during pregnancy are not completely understood. In addition to investigating further the role of stress, a need exists to examine the pathways through which SES may mediate racial disparities in BV. Low SES may increase the likelihood that pregnant women engage in risky behaviors, such as use of cigarettes, alcohol, and illicit drugs [54, 56, 65, 66]. Furthermore, women from a disadvantaged SES may have a higher exposure, in density and intensity, to chronic stressors and a family socioeconomic context that increases exposure to domestic violence [54, 66, 67]. Exposure to such chronic stressors may in turn place women at risk of BV through hypothalamic-pituitary-adrenal axis dysfunction, impaired immune response, or altered vascular tone and reactivity [56].

Bacterial vaginosis in a Brazilian context

Of interest is the fact that the most of the studies above cited have been conducted in the U.S., while limited data exist on the association of race, stress, and BV in other

settings. Brazil provides an interesting context for testing hypothesized associations of self-reported race and health events in pregnancy since a large percentage (45% to 51%) of its population includes Afro-descendants [68], and in which there is considerable co-occurring social, economic, and racial inequality [69-71].

Poorer perinatal outcomes, as well as lower indices of health services use and quality, have been noted among Afro-descendant Brazilian women relative to their white counterparts [72-78]. Moreover, Brazilian Afro-descendant women have higher rates of low birth weight (< 2,500 g) and preterm infants (< 37 weeks) than their white counterparts [79-85]. Social and historical similarities between the racial contexts of Brazil and the United States may afford comparisons of research results [68, 70] to further our general understanding of contextual factors adversely impacting pregnancy.

Epidemiologic studies of the prevalence of BV in Brazil are limited and tend to be mostly descriptive of symptomatology within selected clinical settings in various regions throughout the country. Reports of BV prevalence assessed by Amsel clinical criteria in non-pregnant study populations range from 21.3% to 36.5% [86-89], while estimates of the prevalence of BV in pregnancy range from 20% to 26.1% [90-92]. No race-specific prevalence distributions were presented in these studies among pregnant women. A BV prevalence of up to approximately 40% has been reported in a sample of pregnant women in public maternity complexes participating in a randomized trial of probiotic treatment in Rio de Janeiro [93].

The well-documented increased occurrence of BV among self-identified African-American women in the United States is unclear among Afro-descendant women in

Brazil since few Brazilian studies report findings by race. Among non-pregnant women receiving routine gynecologic care at a university clinic in São Paulo, Tanaka et al. [87] reported an overall BV prevalence of 29%, with a higher prevalence of BV among black women (37.1%) relative to white (28.3%) and mixed women (27.9%) (n = 658, statistical significance not reported). In a study of the mannose-binding lectin (MLP) gene polymorphism and its association with bacterial vaginosis recurrence, Giraldo et al. [94] reported a BV prevalence of 18.6% in a sample (n=177) from two outpatient infectious disease clinics in the gynecology departments of two universities (São Paulo and Rio Grande do Norte, respectively). Acute and recurrent BV infections (n=33) were reported to be marginally statistically associated with non-white race (26.2%, $p = 0.05$), though no frequencies by Afro-descendant or indigenous categories within the “non-white” group were reported (perhaps due to the small number of cases), and no differences by age, education, or smoking history were found for the whole sample or between the two clinics.

Closing Remarks

Epidemiologic research into the causes and factors associated with BV as a key contributor to preterm delivery is appropriate not only in the U.S., where the preterm delivery rate has increased by 13% over the last decade [67], but also in international settings, such as that of Brazil. Silveira et al. [95] report a rising trend in preterm delivery from a review of population-based studies ranging from 3.4% to 15.0% in the Southern and Southeastern regions of Brazil between 1978 and 2004.

Historically, the fact that Brazil is the country with the highest black population

outside of Africa [71] has been used to advance the myth of a “racial democracy,” which cites the widespread racial inter-mixing of the Brazilian population as evidence for a unified and robust race that enables everyone to attain opportunities within Brazilian society in virtual absence of discrimination due to racism [96]. Nevertheless, Brazilian researchers have begun to question this myth with regards to health outcomes in light of recent data presented in the Brazilian academic literature [78, 97, 98] that racial and color-based discrimination persist in Brazil. For instance, Lopes [99] cites documentation of widespread social disadvantage of the Brazilian black population in employment, income, housing, education, and life expectancy at birth. Also, Cardoso et al. [100] present strong evidence from the Brazilian live births registry (*SINASC*) that black populations have higher infant mortality. Moreover, as articulated by Chor and Lima (2005) [78], reducing race to an economic class construct has failed to fully explain health inequalities in Brazil.

The persistent health inequalities observed in Brazil point toward the organizing hypothesis postulated by Krieger (2003) [101], in which social and economic disadvantage are mechanisms through which discrimination, or “racialization,” creates racial inequalities in health. A few suggestions of a positive association between reported experiences of discrimination and adverse health outcome in Brazil have been reported. For example, Faerstein and colleagues [97, 102] reported an elevated risk of hypertension among civil servants who self-identified as black or mulatto and who also reported experiences of discrimination. However, no studies of associations during pregnancy have yet been reported.

The present study will contribute to the literature with a characterization of psychosocial stress during pregnancy through an assessment of the internal reliability and construct validity of the Brazilian-Portuguese version of Cohen's Perceived Stress Scale. The analysis will test the applicability of the instrument in research of pregnant women within the context of a cross-sectional study in two maternity clinics in Rio de Janeiro, Brazil, and examine whether the instrument has structural population heterogeneity by self-reported race. In addition, an analysis to examine associations among selected psychosocial measures, salivary cortisol, and maternal demographic and behavioral factors in a sample of pregnant women will be presented. Finally, the present study will estimate the prevalence of bacterial vaginosis (BV) during pregnancy in the same setting, examine whether prevalence of BV differs by self-reported race, and examine whether psychosocial stress factors mediate any observed association between self-reported race and BV in pregnancy.

To the best of my knowledge, this is the first analysis considering the role of self-identified race and stress in the prevalence of BV in Brazil. A more thorough characterization of differences in such experiences and the mechanisms driving them is an important step in understanding and intervening on racial disparities observed in pregnancy and birth outcomes in Brazil.

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CHAPTER 2

STUDY QUESTIONS

The objectives of the present study are: (1) to examine whether prevalence of bacterial vaginosis (BV) differs by self-reported race, and whether psychosocial stress factors mediate any observed association between self-reported race and BV during pregnancy in a Brazilian public health center setting; (2) to assess the psychometric properties of the Brazilian-Portuguese version of Cohen's Perceived Stress Scale as applied to pregnant women; and (3) to examine associations among selected psychosocial measures, salivary cortisol, and maternal demographic and behavioral factors in pregnancy. The specific questions addressed are listed below.

Primary questions

1. Is self-reported race associated with BV prevalence? If so, does this association follow a 'color' gradient, with higher BV prevalence among Afro-Brazilian women relative to white Brazilian women?
2. Do measures of socioeconomic, chronic, and acute stress mediate the observed association between self-reported race and BV?

Secondary questions

1. Does the Brazilian Portuguese version of the Perceived Stress Scale exhibit internal reliability and construct validity when applied to a sample of pregnant women?

2. Does the Perceived Stress Scale have structural population heterogeneity by self-reported race? That is, do the latent stress factor mean scores measured by the instrument vary by self-reported race?
3. Is salivary cortisol associated with psychosocial measures of stress during pregnancy?
4. Are self-reported race and other maternal individual characteristics independently associated with salivary cortisol concentration?

A schematic of the hypothesized relationships of race, stress, and BV addressing primary questions 1 and 2 is presented in Figure 2.1. Given the cross sectional study design, implicit in the figure is the assumption that the latent factors of stress measured by variables hypothesized to be composites of socioeconomic stressors, chronic stressors, and acute stressors covary as a joint construct of latent stress. The double headed arrows between ellipses depict joint cross sectional covariance of stressors, rather than depicting a temporal causal hierarchy among them. The theoretical model posits that latent stress may mediate the relationship between color/race and BV via impaired immune response or hypothalamic-pituitary-adrenal axis dysfunction (i.e., cortisol response), which were not directly analyzed through primary questions 1 and 2 and are therefore presented with dashed lines in the figure. Additionally, latent stress may mediate the color/race-BV relationship via adoption of coping maternal behaviors, operationalized in this analysis as alcohol use and smoking during pregnancy, which in turn may be associated with alteration of immune function or other intermediate pathways associated with increased duration of BV infection.

Also, a schematic of the hypothesized relationships of psychosocial stress scales, maternal race, and cortisol concentrations addressing secondary questions 3 and 4 is presented in Figure 2.2. Note that double headed arrows between ellipses depict potential joint cross sectional covariance of stress as measured by selected psychosocial scales associated with cortisol concentration. The theoretical model further posits an independent association between maternal race and cortisol, in addition to suggesting that differences in psychosocial stress scales by race may mediate the relationship between color/race and cortisol.

Figure 2.1 Theoretical relationships between self-reported race and bacterial vaginosis, with hypothesized stress mediators.

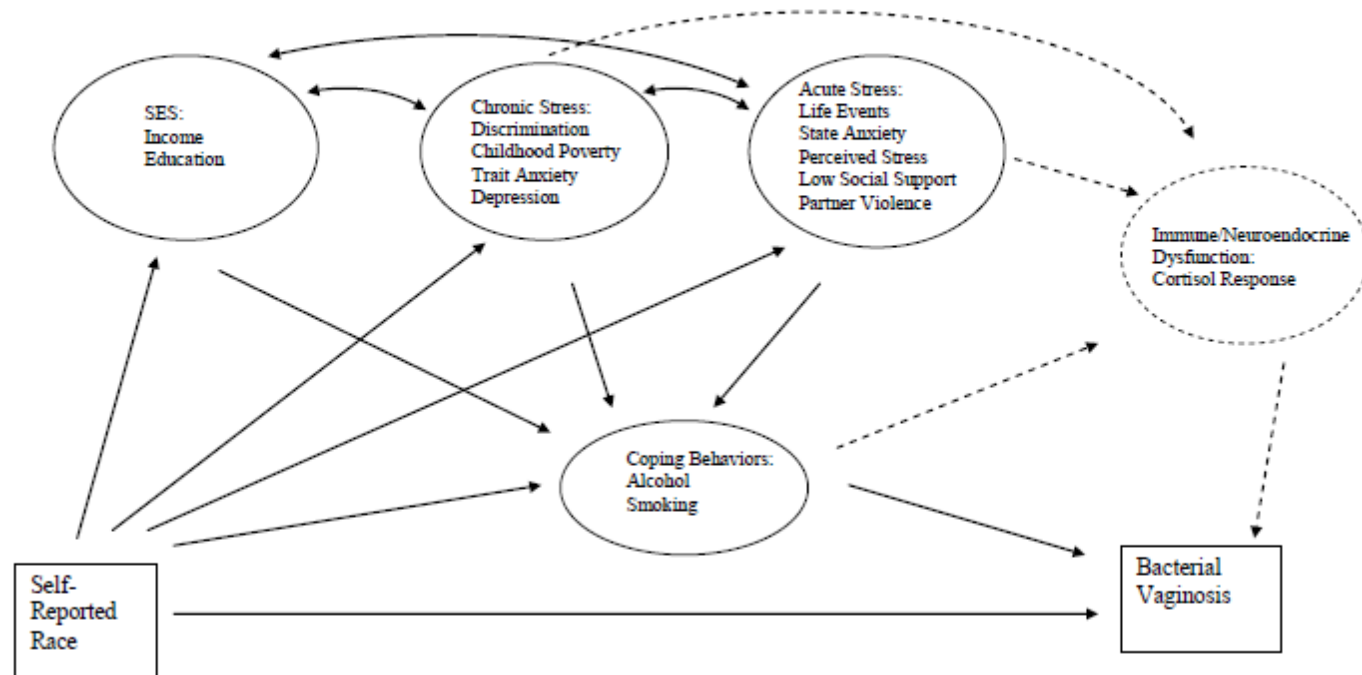
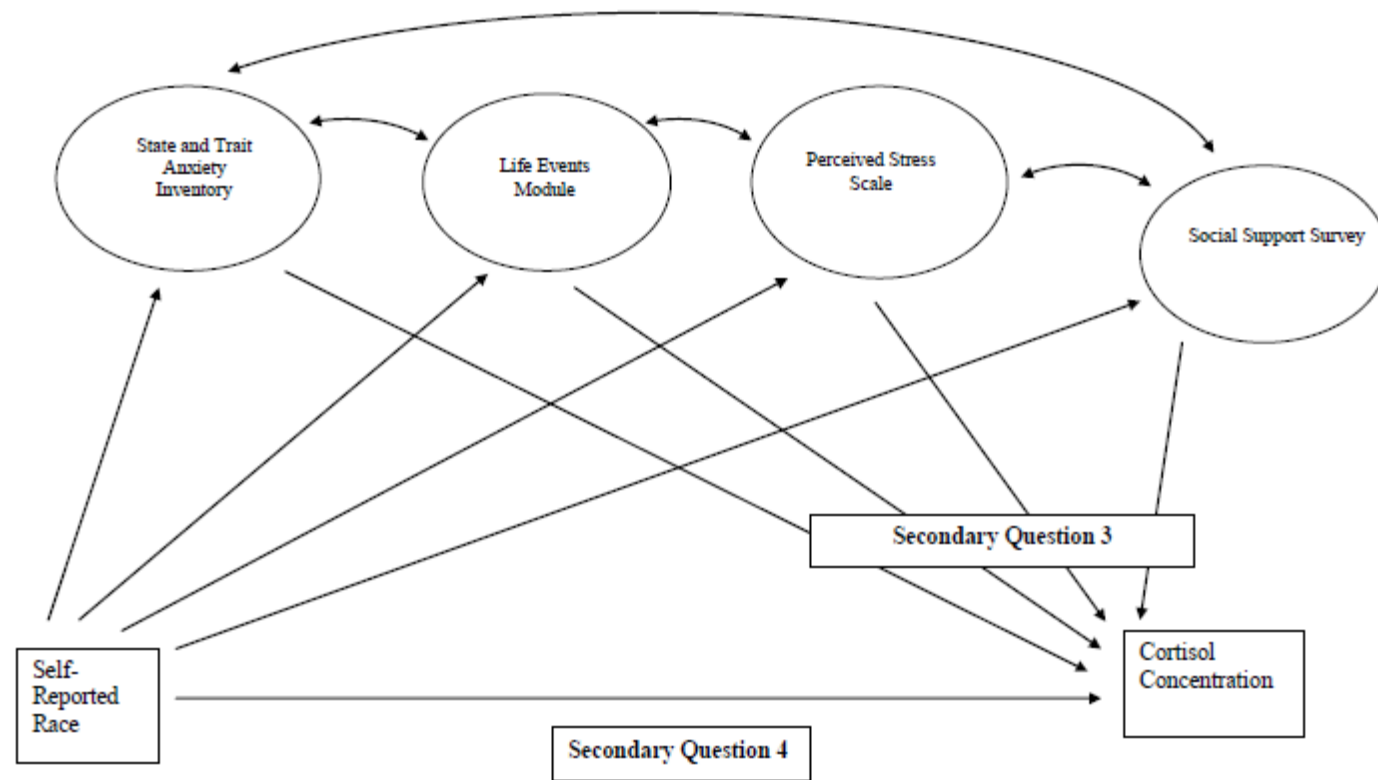


Figure 2.2 Theoretical relationships among self-reported race, psychosocial stress scales, and cortisol concentration.



CHAPTER 3

METHODOLOGY

Study Design and Study Population

The present investigation is a cross-sectional, clinical prevalence study evaluating the association of self-reported race and its mediators with bacterial vaginosis prevalence among women in their second trimester of pregnancy and enrolled in prenatal care at a maternity clinic complex in Rio de Janeiro, Brazil.

Participants were women (n=295) with singleton pregnancies, recruited from two public prenatal ambulatory clinics in the municipality of Rio de Janeiro, Brazil, between May 2008 and July 2009. One clinic (hereafter called *clinic 1*) is annexed to a maternity hospital complex located downtown. At the time of the study, clinic 1 was a private, not-for-profit institution, linked to the Brazilian Universal Health System (*Sistema Único de Saúde – SUS*), with a diverse catchment population from the entire municipality and serviced by all major routes of public transportation. The second clinic (hereafter called *clinic 2*), located in the North Zone of the municipality, is a public institution annexed to a maternity hospital complex exclusively serving patients enrolled in the Universal Health System from the immediately surrounding neighborhoods only. Both prenatal ambulatory clinics complied with the 2006 Brazilian Ministry of Health published prenatal care guidelines at the time of field activities [1].

Women were excluded if they were not residents of the Municipality of Rio de Janeiro, had a known multiple pregnancy at the time of interview, if a gestational age of

14 to 26 weeks' gestation could not be estimated by either known date of the last menstrual period (LMP) or ultrasound, or if prenatal care was initiated after 20 weeks of pregnancy. Women with prenatally-diagnosed chronic infectious diseases, metabolic disturbances, cardiopathy, mental illness, or hypertension, or vaginal bleeding, or pregnancies marked by known anomalies, as recorded in their prenatal card at the time of selection for interview, were excluded from invitation to the study.

Women in their second trimester of pregnancy were approached about participating in the study in the clinic waiting room. A list of all women eligible for recruitment was generated daily at the beginning of each nursing shift by inspecting clinical appointment cards in the prenatal weighing room. The order of appointments at each clinic was assigned by nurses in order of patient arrival. Thus, the routine in both clinics was such that most mothers would arrive at the clinic before 8:00am to ensure they would be seen by their attending obstetrician as promptly as possible once consultations began. Both clinics began consultations between 10:00am and 10:30am, allowing for the project fieldwork to take place in the waiting room well in advance of the actual timing of appointments without disrupting the clinic routine.

Written informed consent was obtained from each participant (and legal guardian for study subjects below age 18) before initiating structured face-to-face interviews. All interviews were conducted in Brazilian Portuguese by the investigator or one of four trained interviewers at a scheduled prenatal care visit between 14 and 26 weeks' gestational age. Following the interview, a trained research nurse performed speculum examinations for BV clinical assessment. All study procedures were approved by the Emory University institutional review board and the committee of ethics of the Sergio

Arouca National School of Public Health, FIOcruz, and were conducted in accordance with the ethical principles of the Helsinki Declaration and the 196/96 Resolution of the Brazilian National Committee on Health (CNS).

In total, 371 eligible women were approached about participating in the study. Of the 334 (90%) who agreed to participate, 295 women (88.3%) completed full interviews with pelvic clinical assessment. Among the 39 participants who did not complete the full study, 14 cited “fear” or “not being prepared” for the research nurse’s pelvic assessment as reasons for non-participation; 17 cited lack of time to complete the questionnaire; and 8 were lost to follow-up within the clinic routine. Non-participants (24% white, 75% black/mulatta, mean age 24) did not significantly differ from participants (23% white, 77% black/mulatta, mean age 25) by self-reported race ($p > 0.05$) or age ($p > 0.05$). Women whose self-reported race was other than white, black or mixed ($n = 28$) were excluded from analyses considering self-reported race as a covariate. One mixed participant was excluded from BV analyses for having one clinical criterion missing during data collection. All participants were given infant sock kits in gratitude for their participation.

Assessment of bacterial vaginosis (BV)

Prevalent BV was assessed using Amsel’s classic clinical criteria during the speculum examination at the time of the prenatal care visit and in accordance with the flowchart published by the Brazilian Ministry of Health [1]. An experienced research nurse at each clinic collected vaginal fluid for BV assessment, blinded to participant responses during the interviews. All samples were labeled with a study identification

number to blind all assays to questionnaire responses during microscopy, and results were linked to the patient chart number, thereafter.

Examination by microscopy was conducted by the research nurse for all participants from clinic 1 on site. For clinic 2 participants, I conducted microscopy of vaginal sample swabs at a laboratory of the National School of Public Health, FIOcruz, on the same day as sample collection. My wet mount prep and sample transportation protocol training consisted of personal communication with Roberd M. Bostick, MD, a member of my dissertation committee, and audiovisual training through the continuing medical education video titled “5 Minute Wet Mount” (Medical Education Division, Brookside Associates Ltd., 2006). The video was pre-approved as a teaching tool by Dr. Bostick, so I also reviewed the video with the two research nurses at each clinic, while simultaneously translating to Brazilian Portuguese, to standardize the collection and wet mount prep.

The research nurse at clinic 2 performed all Amsel criteria assessment with the exception of microscopy, for which she collected two vaginal swabs per patient, stored each patient’s swabs in covered vials containing saline solution and labeled by participant study number, and packed the vials in a prepared laboratory cooler bag to maintain all samples chilled. I prepared the cooler bag with frozen cooler packs the night before each data collection day and delivered it to the nurse at the start of every shift by 7:00 A.M. with a pre-packed test tube rack to keep vials upright when I transported the cooler bag to the laboratory later the same afternoon. Further microscopy and wet mount disposal protocols were reviewed with Dr. Valmir Silva on site at the laboratory of the National School of Public Health before I began working with clinic 2 vaginal swabs.

Following Amsel's criteria, results consistent with bacterial vaginosis positive status include:

- Grey-white, thin, homogenous, adherent vaginal discharge
- pH > 4.5, as measured by adhesion of graded pH paper directly onto the vaginal lateral wall
- Positive amines "Whiff" test, characterized by the release of a strong fishy odor upon addition of 10% KOH
- Clue cells present (on >20% of cells in wet mounts), characterized by bacteria-studded vaginal epithelial cells

Women with at least 3 out of 4 criteria were identified as positive for BV [2-4], and the attending obstetrician was notified for case management, as appropriate.

Assessment of self-reported race

Information on self-reported race was collected by interviewers in the face-to-face interview during the clinic visit and prior to collection of BV samples. The Brazilian census item for the self-identification of race was applied in this study, with five response categories available: *branca* (white), *preta* (black), *parda* (mixed), *indígena* (indigenous native), and *amarela* (Asian). As previously noted, women who self-identified as *indígena* (n = 6) and *amarela* (n = 22) were excluded from all analyses because of the limited number of such women.

Assessment of psychosocial measures

The standardized interview questionnaire, administered at the start of the study visit, collected information on prenatal psychosocial stress. My previous work experience as a bilingual interviewer in various projects in the U.S. and daily close

observation of the clinic routine and meetings with nurses for a preliminary period of two weeks guided my field methods for developing training protocols for my team of interviewers. My field advisor, Dr. Silvana Granada, recommended the names of the interviewers based on their good performance in previous epidemiologic field studies under her supervision. All four interviewers who worked with me in this project were university students in nursing, nutrition, social work, and law, respectively. I met with each of the interviewers to explain my project's focus, the questionnaire and study protocol, and the linkage with the research nurse following the interview, and to give them a general overview of each clinic's routine and introductions to personnel. I required that each of the interviewers run a test interview with me acting as the interviewed pregnant woman, where I created scenarios (e.g., mother was accompanied by partner, called by the nurses or her doctor in the middle of her interview, and other potential diversions that could arise in the clinic routine) to prepare the interviewers in the clinic routine. Finally, before being free to run interviews with participants on their own, I sat in on the first interview that each interviewer performed with a patient to observe the interviewer's performance and clarify any doubts and orient the interviewer with any questions she may have had. I supervised each data collection shift, and once the interviewers were fully trained, I also conducted interviews myself and organized samples as they were being collected during the entirety of every shift at each respective clinic. I numbered each questionnaire in advance for each shift, assigned a code to each interviewer, distributed all materials (i.e., clipboards, numbered questionnaires, pencils, erasers, salivary collection materials) to interviewers at the start of each shift, collected completed interviews, a log of refusals, and other materials at the end of each shift,

checked questionnaires for completion and illegible handwriting, and met with the group and the research nurse to discuss the day's shift and problems possibly encountered with the clinic routine, and to hold a group discussion regarding my solutions to standardize all decisions within the team. For example, the first few women lost their study identification number card to hand to the research nurse after interview and either the patients or nurse went back to the interviewer, encumbering both ongoing interviews and sample collection. After team discussion, I created bright patient paper slips where the interviewers had to fill out the patient's name, chart number, and study/questionnaire number at the time of signing informed consent. Thus, all team members could easily locate the patient study identification number efficiently at all steps of data collection.

The validity and/or reliability of Brazilian Portuguese versions of the scales and modules used have been previously tested and published for non-pregnant Brazilian populations [5-8]. Both published scale theoretical cutoffs and continuous score entries were considered in this analysis [5-11]. The instruments included the Spielberger State and Trait Anxiety Inventory, Cohen's Perceived Stress Scale, the CARDIA Study Life Events module, and the Medical Outcomes Study Social Support Survey:

a. The Spielberger State Trait Anxiety Inventory (SAI- TAI) [5, 11] assesses two dimensions of anxiety with 40 statements: 1) state anxiety, including feelings of nervousness, worry, and apprehension during a specific, acute moment (e.g., how the respondent feels "right now"); and 2) Trait anxiety, which assesses an "anxious" personal trait or propensity (e.g., how the respondent feels "generally"). The scale has been applied to pregnant adolescent and adult women in São Paulo by Rondó et al. (2003) [12]. The published state and trait anxiety inventory suggests dichotomizing at the 40-

point cut-off, but the scale can be analyzed as continuous scores or categorized as deemed appropriate to a given study population (e.g., quartiles) [5, 11].

b. Cohen's Perceived Stress Scale (PSS) [8, 9] measures "the degree to which situations in one's life are appraised as stressful" over the past 30 days. The scale items are designed to assess how unpredictable, uncontrollable, or overwhelming respondents evaluate their lives to be, and these three dimensions are considered central to the theoretical experience of acute stress posited by Lazarus and Folkman (1984) [13]. Given the timing assessed by the scale, the instrument is therefore more sensitive to acute, rather than chronic, stress [14]. Furthermore, the scale is not context-specific and has been validated in various cultural settings internationally. The scale scoring does not have theoretical cutoff points due to the feature of context transportability [8]. Of note is the fact that a published validated Brazilian version of the PSS has not been previously applied to pregnant populations in the literature.

c. Life Events Module [6] is an adaptation of the CARDIA Study module on stressful life events and experiences of discrimination, previously applied to a study of racial differences in preterm delivery and low birth weight among female CARDIA participants in the U.S. [15]. The module contains eight items on events such as the death of a loved one, divorce, being forced to move, etc., over the past 12 months. The items also query on the time period lapsed since the occurrence of the event from the date of interview. Two additional sections form part of the module, querying on experiences of discrimination throughout the life course in eight different environments (school, work, public spaces, etc.) and eight items on adverse childhood events, including queries on perceived childhood socioeconomic status, household violence, and availability of

food, among others. The translation and reliability of all parts of the module were tested in Rio de Janeiro by Lopes and Faerstein (2001) [6]. Since the stressful life events and experiences of discrimination items are adapted from a module comprising a segment of a larger scale, items will be treated as continuous measures with additional cutoffs possible upon examination of frequency distributions in the study population.

d. The Medical Outcomes Study Social Support Survey [10] assesses perceived social support since becoming pregnant via five dimensions of support: (1) tangible (access to practical and material resources); (2) affective (access to physical demonstrations of love and affection); (3) emotional (expression of positive affect, understanding, and feelings of trust); (4) positive social interaction (availability of a personal network with whom to have fun or relax); and (5) informational (availability of people from whom to gather counsel or advice). The reliability of the Brazilian Portuguese translation was tested and published by Griep et al., 2005 [7]. Like the anxiety scores, the published scale suggests dichotomizing at the 40-point cut-off [7], with other cutoffs possible (e.g., quartiles) upon examination of frequency distributions in the study population.

Assessment of salivary cortisol

For salivary cortisol sample collection, each participant was instructed to rinse her mouth with water before collecting two saliva samples prior to initiating the interview. Saliva was collected with *Salivette* tubes (Sarstedt Inc., Numbrecht, Germany). Briefly, the *Salivette* device consists of a small cotton roll stored in a plastic centrifugation tube. The device allows for direct sample collection, storage, centrifugation, and pipetting the supernatant from its outer chamber. After obtaining written informed consent and before

the start of the questionnaire, participants were instructed by interviewers to softly chew on the small *Salivette* cotton roll between 30 and 90 seconds to stimulate saliva secretion and saturate the cotton roll; two saliva samples were collected per subject. Saliva collection via the *Salivette* device is thus not only non-invasive to the study participant, but also easily, quickly, and neatly administered with minimal equipment in a field setting.

All saliva samples were collected and stored in the plastic *Salivette* tubes, labeled by participant study identification number to blind samples at the time of assay to interview responses. I collected all *Salivette* tubes from all interviewers at the end of each interview shift, and I kept all samples on ice in a laboratory cooler bag used only for *Salivette* transport. I transported the samples to a laboratory at the National School of Public Health the same afternoon of sample collection, and stored all *Salivettes* at -20°C at the end of each shift until analysis. I conducted all assays after being trained by Dr. Valmir Silva and performing a test run. In this preliminary run, I analyzed saliva samples belonging to me, Dr. Silva, and four volunteers (two secretaries and two doctoral students at the lab) on my own while supervised by Dr. Silva. Dr. Silva further supervised my first full plate of study participants' assays and was available at his office or his research lab for all my subsequent analyses as a resource, should I have any problems or questions regarding the equipment. Of note, my preliminary on-site training and college biology coursework served as a good platform for me to run all independent assays smoothly and without need of Dr. Silva's intervention.

After thawing, I centrifuged samples at 3000 rpm for 10 minutes before assay. Salivary cortisol concentration was assayed using a salivary cortisol competitive

enzymatic immunoassay (EIA) kit (Diagnostic Solutions Labs, DSL; Webster, TX) with reported detection limits of $0.072\mu\text{g/dl}$. Salivary cortisol values were averaged between the two samples collected for each participant. Every 10th sample was assayed twice, and the intra-assay coefficient of variation was $< 10\%$. I stored and assayed all samples at the Department of Biological Sciences, Sergio Arouca National School of Public Health, FIOcruz, Rio de Janeiro, Brazil, and all samples were destroyed after completion of duplicate assays for the entire study population sample.

The dramatic surge in maternal cortisol serum concentration as parturition approaches motivates the restriction of participants to a gestational age between 14 to 26 completed weeks. Salivary cortisol profiles exhibit decreased variability throughout the second trimester of pregnancy relative to the third trimester, reaching concentrations more than twice as high in late pregnancy than in early to mid gestation [16]. However, because of the steady rise in cortisol as gestation progresses, gestational age was controlled for in all analyses involving cortisol concentration.

I attempted a home cortisol self-collection pilot procedure collecting 6 daily *Salivette* samples, at different times of the day, among 10 women during my questionnaire pilot phase six weeks before beginning full data collection. From the experience of this pilot, I found that participant adherence was difficult to assess and that project collection of samples at participants' homes would not be feasible with my project resources at the time (e.g., I needed a larger team to trace women residents of shantytowns), and my design was therefore limited to assessment of a single salivary cortisol measurement in pregnancy at the clinic at the time of interview. I was limited not only by a cross sectional cortisol measure, but also by the fact that analyses were

morning samples without data on waking cortisol response. Average subject cortisol concentration was treated as a continuous measure in regression analyses and transformed as appropriate to meet normality criteria.

Assessment of demographic and medical characteristics

The standardized questionnaire collected information on individual and total household income, maternal literacy, and maternal educational attainment (total school years completed). Self-reported total household income was scaled to the number of household residents for a measure of per capita household income, and then categorized by Brazilian minimum salary units, where one salary unit is R\$465, or approximately U.S.\$260 [17].

Additional potential sociobehavioral covariates of the association between race or stress and BV collected via interview included self-reported use of alcohol, cigarettes, and the occurrence of physical intimate partner violence since becoming pregnant. Given that the timing of occurrence queried was since the time of conception of the index pregnancy only, physical intimate partner violence was considered ‘acute’ in timing, likely underestimating the effect of its possible preconceptional occurrence. Clinical chart extraction was conducted using a standardized form, the *Cartão da Gestante* (or Pregnant Woman’s Card) issued by all institutions participating with the Brazilian Ministry of Health. The card included data on last menstrual period, date(s) of ultrasound(s), gestational age, anthropometrics, diagnosis of pre-existing/current conditions, and obstetric history, including previous preterm, low birthweight, and caesarean section deliveries. Additional covariates collected through interview and card

extraction included in analyses were maternal age, cohabitation, parity, contraceptive use at time of conception, urinary tract infection (UTI) and antibiotic use during pregnancy, and reproductive and obstetric history.

Statistical analysis

Primary Questions: (1) Is self-reported race associated with BV prevalence? If so, does this association follow a ‘color’ gradient, with higher BV prevalence among Afro-Brazilian women relative to white Brazilian women? (2) Do measures of socioeconomic, chronic, and acute stress mediate the observed association between self-reported race and BV?

Prevalence proportion estimates for BV status were calculated for the whole sample and by self-reported race. Bivariate associations between categorical and continuous covariates and BV were investigated overall and stratified by race using the Pearson χ^2 test, Fisher’s exact test, and Student’s t test, as appropriate. Non-parametric (Mann-Whitney) tests were applied for comparisons of variables with skewed distributions, household income, and scale responses. Scale quartiles were examined for psychosocial measures to explore different dichotomous points in addition to the theoretical cut points for each scale.

Selection for inclusion in multivariate models followed a two-fold approach due to the large number of variables considered. For variables hypothesized to be stress exposures, those associated either with BV overall or by race-specific stratum at $p \leq 0.20$ were considered for inclusion in multivariate models. In addition, covariates identified

from the literature as potential confounders were included in the models to observe whether prevalence point estimates changed by more than 10%.

Given the relatively high estimated prevalence of BV during pregnancy cited in the literature, it was expected that bacterial vaginosis would be a frequent outcome in this population and that prevalence proportions of BV within racial groups would be at least 20% across racial categories under the null hypothesis of no association between self-reported race and BV. The theoretical approximation of the prevalence odds ratio (POR) to the prevalence ratio (PR) allows for the use of a generalized linear model fitting a Poisson regression model with robust standard error estimation. One could extend the odds ratio interpretation to an approximation of an incidence density ratio, assuming that duration of disease is independent of exposure. As the hypotheses considered in this analysis posit that race and stress exposures are associated with prevalence of BV (ranging from incident infection, to recurring infection, to prolonged infection given immune impairment associated with a heightened stress state), only the more conservative approximation of the odds ratio to the prevalence ratio was considered relevant in this analysis. The point estimates obtained from generalized linear models (GLMs) further conferred the advantage of having robust standard errors for more precise (e.g. less inflated) confidence interval estimates [18-20]. The estimates from a Poisson model with robust standard errors are comparable to results obtained from more frequently used log binomial GLMs, but avoid convergence problems encountered with the latter distribution [14, 20].

A hierarchical forward selection multivariate modeling procedure [21] was applied, guided by the conceptual framework presented in Chapter 2, Figure 1. Because

the analysis considers three race categories, the modeling strategy considered two distinct reference categories: The first set of models considered BV prevalence ratios among black and mixed race, respectively, relative to white race, while the second set of models considered BV prevalence ratios among black race relative to mixed race. The starting point for the model building procedure was the crude association between self-identified race and BV with no mediators. The general modeling procedure consisted of sequential forward selection of variables in different blocks. The criterion for retention of sets of variables for each sequential block was statistical significance of $p \leq 0.20$ in Type III score tests, both for parsimony and sufficient sample size relative to the number of model terms (at least seven observations per model term), following the flow hierarchy shown in Figure 2.1.

Maternal behaviors hypothesized to be coping strategies in response to stress (i.e., smoking and alcohol use in pregnancy) and potential confounders identified from the literature – including age, cohabitation, gestational age at interview, urinary tract infection during pregnancy, and contraceptive use at conception – were entered in the last steps of forward model specification. Inspection of the working correlation matrices for multicollinearity was conducted at each step. Model goodness-of-fit was assessed by examination of the Quasi-likelihood under the Independence model Criterion (QIC) statistic, where a smaller QIC statistic was indicative of better model fit. Analyses were conducted using SAS v.9.2 for Windows [22] and SPSS 17.0 [23].

Hypothesized mediation of the race-BV association during pregnancy was defined *a priori* as at least a 50% reduction in the prevalence ratio point estimate associated with race. While we were unable to fully assess statistical interaction of race and stress

variables due to sparse data and lack of model convergence, procedures for effect measure modification assessment would have considered all retained stress variables at each model step as potential effect modifiers of the race-BV association and entered as such in the model. The potential effect modifier with the highest p-value would be dropped and the model reassessed, repeating the procedure until the model contained only those effect modifier terms found to have a p-value < 0.20 . I attempted at a minimum to explore basic three-way tabulations of stress associations with BV by race. Stratified sub-analyses suggested that indicators of acute stress were associated with BV by race. For instance, among black women, state anxiety was statistically significantly associated with BV prevalence in pregnancy, suggesting that there was a different set of experiences and perceptions according to self-reported racial identity and accompanied social context. Final evidence of a possible difference in experiences and perceptions based on a woman's racial self-identity is the observation that intimate partner physical violence in pregnancy was statistically significantly associated with BV prevalence in pregnancy only among mixed women.

Confounding was assessed by dropping each potential confounder one at a time. If the percent change in the prevalence ratio was less than 10% but resulted in a more precise confidence interval, dropping the given variable from the model was considered. The adjusted prevalence point estimates for race did not change beyond 10%, but all the covariates were left in the model because they conferred a slight gain in precision as observed through comparison of confidence interval widths. Last, inspection of QIC fit criteria confirmed that the sequential addition of each block of variables improved the model fit, as given by a decreasing QIC value throughout model runs.

Women ($n = 28$) whose self-reported race was other than white, black or mixed were excluded from all analyses. One mixed participant was excluded from BV analyses for having one clinical criterion missing during data collection. The total number of women excluded from BV analyses was 29, and the total sample analyzed consisted of 266 subjects (62 white, 148 mixed, and 56 black).

Secondary questions 1 and 2: (1) Does the Brazilian Portuguese version of the Perceived Stress Scale exhibit internal reliability and construct validity when applied to a sample of pregnant women? (2) Does the Perceived Stress Scale have structural population heterogeneity by self-reported race? That is, do the latent stress factor mean scores measured by the instrument vary by self-reported race?

The internal consistency of the PSS was measured using Cronbach's alpha of the items in the total score of the best-fitting form of the Brazilian-Portuguese version of the scale [8]. Given measures of reliability published in the literature for the PSS, we expected Cronbach's alpha coefficients above 0.50, reflective of instruments with acceptable internal consistency [24]. Convergent construct validity was analyzed through comparison with the following alternative measures of maternal stress previously used in studies of pregnant women: the Spielberger State and Trait Anxiety Inventory [5], an adapted module of stressful life events [7], cross-sectional salivary cortisol concentrations [25, 26], and six items measuring depression previously used in studies of pregnant women in this setting [27]. These measures were hypothesized to be positively correlated with the PSS total score, following literature reports that are consistent with anxiety, number of stressful life events, and cortisol measurements as assessing perceived acute stress dimensions measured by the PSS [26, 28, 29]. There is evidence of a

positive linear association between acute timing and number of negative events and probability of depressive episodes [30], so it was expected to observe a moderate positive correlation between the PSS total score and depression items. No external scale data were available to assess divergent validity. Weak correlations were defined as those with a correlation coefficient below 0.30, even if statistically significant; moderate correlations were defined as those between 0.30 and 0.50; and correlations above 0.50 were considered to be strong [31].

Estimation Choice

All procedures for the analysis of structural models with ordinal categorical data were conducted using MPlus 5.21 software [32], and the robust mean- and variance-adjusted weighted least squares estimator (WLSMV) was used to estimate model parameters.

Exploratory Factor Analyses

In preliminary analyses, an exploratory factor analysis (EFA) was performed in a random split sample of 153 study participants to identify an applicable factor structure for the categorical nature of the scale items. This sub-sample was selected using the randomization function in SPSS Release 17.0.0 [23]. EFAs with oblique and orthogonal rotations were compared with the Geomin rotation [32] to determine the best underlying factor structure of the 14 items of the PSS. Items with primary factor loadings ≥ 0.40 and secondary factor loadings < 0.30 , and those that did not load on more than one factor, were retained. Items not meeting these criteria were removed sequentially, with the worst performing item removed first, then the next worst, and so on until no item had a loading < 0.30 .

The preliminary EFA was followed by an exploratory factor analysis within the confirmatory factor analysis framework (E/CFA) [32]. The E/CFA approach is an intermediate step between EFA and CFA that provides standard errors to assess the statistical significance of factor loadings, as well as modification indices to determine whether residual covariances are needed to represent method effects [32]. For all analyses, a p-value of less than 0.05 was used as the criterion for denoting statistical significance. The examination of modification indices guided any empirical path modifications to the model. Based on my review of the literature, I expected to observe items loading on two general factors of stress, corresponding to positive and negative perceived stress, respectively.

Confirmatory Factor Analyses

A CFA was then performed on the second subsample of 142 participants to determine whether the factor structure identified with the intermediate E/CFA stage required modification. The following goodness-of-fit indices and respective cut-points were used to assess the degree of fit between the model and the sample: Chi-square, the Comparative Fit Index (CFI: $>.90$ acceptable, $\geq .95$ excellent), Tucker Lewis Index (TLI: $>.90$ acceptable, $\geq .95$ excellent), the Root Means Square Error of Approximation (RMSEA: $<.08$ acceptable, $\leq .05$ excellent), and the Weighted Root-Mean-Square Residual (WRMR: $<.90$ excellent) [33, 34].

Analysis of Invariance

I conducted analysis of invariance to determine whether the measurement model for perceived stress differed either by self-reported race or by neighborhood context (i.e.,

clinic membership). This investigation is equivalent to testing for structural invariance. A method for examining invariance in multiple groups involves regressing the latent factors and indicators onto covariates that represent group membership. This approach has been referred to as CFA with covariates or MIMIC (multiple indicators, multiple causes) models [32, 33]. Note that while invariance evaluation in MIMIC modeling addresses the mean structure component of multiple-groups CFA, factor means are not estimated explicitly in the MIMIC analysis. Rather, group mean deviations, or differences, are given by parameter estimates representing the degree of group-specific deviation when the factors are assumed to be zero under the null hypothesis [33].

A primary advantage of MIMIC models is that they usually have smaller sample-size requirements than a conventional multiple-group CFA analysis for structural invariance. This advantage arises because MIMIC involves a single measurement model and input matrix, whereas multiple groups CFA involves the simultaneous analysis of two or more measurement models [33, 35]. Theoretically, the covariate is observed and assumed to be free of measurement error (i.e., its error variance is fixed to zero for model convergence), which is a reasonable assumption when the covariate represents known groups (e.g., bivariate self-reported race).

Two separate MIMIC models were examined, where the covariates included self-reported color/race (white vs. black/mulatta) and clinic membership, a proxy for potential differences in neighborhood context within the study sample. Based on preliminary evidence from independent clinic sample t-tests showing a significant difference in mean PSS scores between clinics (data not tabulated), it was of interest to test whether population heterogeneity (e.g., mean latent factor means) in the measurement model was

also present across clinics. Therefore, the latent factor was first regressed on the covariate (i.e., self-reported race and clinic membership, respectively). Also, all indicator means were fixed to zero and were regressed on the respective covariate to examine modification indices and determine the presence of differential item functioning (that is, whether any item behaved differently as an indicator of perceived stress depending on the level of the covariate).

Owing to the small number of *indígena* and *amarela* respondents, self-reported race was treated as a binary variable in this analysis: black and mixed/mulatta categories were collapsed into one group and were compared to the white category. In all analyses considering self-reported race, 28 women whose self-reported color/race was other than white, black or mixed/mulatta were excluded, and the sample for invariance analyses consisted of 267 observations.

Secondary questions 3 and 4: Is salivary cortisol associated with psychosocial measures of stress during pregnancy? Are self-reported race and other maternal individual characteristics independently associated with salivary cortisol concentration?

Exploratory analyses examined variables for missing data, normality violations, and outliers. Differences in salivary cortisol concentrations by demographic characteristics were tested using t-tests for dichotomous variables and analysis of variance (ANOVA) for categorical variables. Demographic variables included age, race, parity, years of education, cohabitation, and household income *per capita*, while behavioral and reproductive history variables included smoking and alcohol use in

pregnancy, pre-pregnancy body weight (kg), previous spontaneous abortion, and preterm delivery.

Two-tailed Spearman correlations examined unadjusted bivariate associations between continuous psychosocial measures and salivary cortisol. Given the steady rise in cortisol as gestation progresses, multiple linear regression analyses further examined associations between continuous psychosocial measures and cortisol concentration adjusted for gestational age. Salivary cortisol was log transformed for analyses because it was found to not meet normality criteria. Data were analyzed using SPSS 17.0 [23].

Statistical Power Considerations

The sampling frame includes the sequential selection of pregnant women who reside in the Municipality of Rio de Janeiro, who meet study inclusion/exclusion criteria, and who provide written consent to participate in the study the day of their prenatal care visit at the selected research site. Power calculations for the type of analyses undertaken considered the number of observations per predictor in regression analyses (ideally, no fewer than seven observations per independent variable) and an estimate of the BV prevalence in the study setting. Using a recent estimate of BV prevalence of up to 40% reported in a sample of pregnant women in public maternity complexes participating in a randomized trial of probiotic treatment in Rio de Janeiro [36], power estimations were for BV prevalence from 20% to 40%. To assess power for comparisons of BV prevalence between mixed/mulatta (referent) and black women (exposed), we calculated a normal approximation with continuity correction power estimate. Assuming BV prevalence among mixed/mulatta women of 20% compared to BV prevalence among black women of 40%, the smallest prevalence ratio that the study can detect given our sample size and

alpha at 0.05 is 2.0 with 80% power.

Organization of Results

The results for the above mentioned studies are presented in Chapters 4-6. Chapter 4, a manuscript to be submitted to *Cadernos de Saúde Pública*, addresses Primary Questions 1 and 2. Chapter 5, a manuscript submitted to the *Journal of Health Psychology*, addresses Secondary Questions 1 and 2. Finally, Chapter 6 addresses Secondary Questions 3 and 4 in a draft commentary to be submitted to *Cadernos de Saúde Pública*.

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CHAPTER 4

The association of bacterial vaginosis with self-identified race, socioeconomic, and psychosocial stress in a sample of Brazilian pregnant women in a public care setting

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ABSTRACT

OBJECTIVE: The purposes of this study were to estimate the prevalence of bacterial vaginosis (BV) during pregnancy in a Brazilian clinical setting and investigate whether BV prevalence was associated with self-reported race and whether the race-BV association differed according to psychosocial factors.

STUDY DESIGN: A cross sectional sample of 266 pregnant women with 14 to 26 weeks' gestation singleton pregnancies was recruited in two public maternity clinics in Rio de Janeiro, Brazil, from 2008 to 2009. Using univariate analyses and multivariate Poisson regression with robust standard error estimation, sociodemographic factors, state anxiety, intimate partner violence, potential confounders, and prevalence of BV were assessed.

RESULTS: The overall prevalence of BV was 26.3% (95% confidence interval [CI] 21.0%, 31.6%). Race-specific BV prevalence ranged from 22.3% (95% CI = 15.6%, 29.0%) among mixed women, to 25.8% (95% CI = 14.9%, 36.7%) among white women, to 37.5% (95% CI = 24.8%, 50.2%) among black women (Pearson χ^2 $p = 0.09$). In bivariate analyses, prevalent BV infection was associated with elevated state anxiety among black pregnant women and with intimate partner violence among mixed/mulatta pregnant women only. In multivariate models, BV prevalence among black or mixed/mulatta women did not differ from that in white women. However, the excess of BV prevalence among black women relative to mixed/mulatta women remained statistically significant (prevalence ratio [PR] = 1.73, 95% CI = 1.11, 2.70) even after multivariate adjustment for life events, state anxiety, perceived stress, intimate partner violence, and other potential confounders.

CONCLUSION: These preliminary findings 1) suggest that in Brazilian pregnant women BV prevalence may differ by self-reported race, and that the associations of BV with psychosocial risk factors may differ by race, and 2) support a larger study using more complete characterization, measurement, and analysis of individual and contextual factors by racial self-identity.

INTRODUCTION

Bacterial vaginosis (BV) is a reproductive tract infection syndrome characterized by an overgrowth of anaerobic bacteria, particularly *Gardnerella vaginalis* and *Mobiluncus* spp., and a corresponding diminished or absent flora of *Lactobacillus* spp. [1, 2]. The condition is common, detected in about 15% to 40% of pregnant women worldwide [3, 4], and has been cited in the literature to be associated with factors such as lower income and educational levels, young age, cigarette and substance abuse, not living with a partner, Afro-descendant race, and risky sexual behavior [5-11]. BV often has been linked with a two to three-fold increased risk of preterm delivery, usually measured as < 37 weeks' gestation [5-7, 12, 13]. In turn, early preterm delivery is associated with higher risk of neonatal complications, including early sepsis and bronchopulmonary dysplasia, which may result in long-term disabilities [2]. Results of trials in BV asymptomatic women have not shown clear benefits from antibiotic therapy, and alternative probiotic trials have either been inefficacious in interrupting the infectious process that leads to preterm delivery or have lacked the sample size to estimate reliable intention-to-treat effects [2, 14]. Given that BV is an important risk factor associated with preterm birth, and that preterm birth is one of the most relevant contributors to infant morbidity and mortality worldwide, epidemiologic research on the causes and factors associated with BV is of public health importance.

The increased occurrence of BV during pregnancy among African-American women compared to other racial groups in the U.S. is well-documented [6, 7, 9, 10, 15-19]. However, an excess occurrence among Afro-descendant women in other racially heterogeneous settings, such as Brazil, is unclear. Given the gap in the Brazilian BV

literature, we summarize evidence for the inter-relationships between maternal race, stress, and BV and factors measured and occurring during pregnancy that may be associated with BV prevalence in a Brazilian study population, and we propose a theoretical framework of hypothesized pathways mediating the race-BV association in light of empirical associations of race with BV.

BACKGROUND

In both pregnant and non-pregnant women, BV prevalence reports are consistently higher among African Americans, even after adjustment for identified confounders [9, 10]. The black-white difference in BV prevalence in the U.S. is thought by some to explain up to 30% of the national racial disparity in preterm birth [20], but the factors that influence race-specific prevalence of BV during pregnancy are incompletely understood.

Hypotheses for the more frequent occurrence of BV in African-American compared to white populations have included disparities in maternal stress [21]. Harville et al. (2007) found that women in the highest quartile of stress, particularly state anxiety (OR=2.0, 95% CI 1.2-3.3), perceived stress (OR=2.4, 95% CI 1.5-3.9) and total stressful life events (OR=2.0, 95% CI 1.3-3.2), had the highest risk of BV [17]. However, associations with measures of stress were all nearly eliminated after adjustment for socioeconomic status and race. Paul et al. found that perceived stress was not related to BV risk among either white or African American pregnant women in multivariate models stratified by race [16], and Uscher-Pines et al. found that after adjusting for race, marital status, insurance, and smoking, perceived stress was not significantly associated with BV in the first trimester of pregnancy (OR = 1.01, 95% CI: 0.98, 1.05) [22]. A potential

design flaw that remains in the literature is consideration of race as a confounder of the stress-BV association, rather than an independent risk factor for BV susceptibility. For instance, recent findings report a race-dependent prevalence of bacterial communities in women with BV, which may directly contribute to the increased incidence of BV in Black women independent of stress mediation [81]. Also, epidemiologic research can benefit from applications of sociology paradigms such as intersectionality, where various social and biological categories interact on multiple and often simultaneous levels, to conceptualize BV as the product of intersecting dimensions of sociodemographic and stress stratification [82]. Treating race as a confounder or covariate in stress-BV association analyses may over-control not only for the potential independent association of race in bacterial community differentiation driving excess prevalence of BV in Afro-descendant women, but also for the intersectionality of sociodemographic and biological processes involved in the causal chain leading to BV.

Social stressors may be additional sources of chronic stress and influence biological reactions to stressful stimuli in the form of either (1) larger, more sustained responses or (2) more frequent occurrence of events [23]. Evidence from animal models suggests that chronic stress exposure is associated with HPA impairment and long-term, persistent behavioral and neuroendocrine alterations [24, 25]. Hypothesized markers of chronic stress in pregnancy previously studied include depression, adverse childhood experiences, and experiences of discrimination [10, 26-31]. Inconsistencies in the conceptualizations of the construct of stress, potential differential item functioning of stress scales by race [32], timing of stress exposure measurement and BV prevalence assessment, differentiation between chronic and acute experience of stress, and the

application of different instruments and stratifications of exposure to measure stress further complicate the comparability of results across study populations.

In addition to investigating further the role of stress, a need exists to examine the pathways through which socioeconomic status (SES) may mediate racial disparities in BV. Low SES, frequently measured as lower educational attainment and household income, has also been studied as a factor associated with BV, either directly or as a mediator of the relationship between race and adverse health outcomes in pregnancy [16, 31, 33, 34]. Furthermore, women from a disadvantaged SES may have a higher exposure, in frequency and prolonged timing, during pregnancy to domestic violence [16, 35, 36] and behavioral coping responses, such as use of tobacco and alcohol [16, 18, 36, 37]. Exposure to such stressors may in turn place women at risk of BV through HPA dysfunction, impaired immune response, or altered vascular tone and reactivity, which may also be associated with markers for fetal stress and adverse perinatal outcomes [18, 36].

Brazil provides an interesting context for testing hypothesized associations of self-reported race and health events (i.e., BV) in pregnancy. Brazil is marked by considerable co-occurring social, economic, and racial inequality [38-40], and a large percentage (45% to 51%) of the Brazilian population is Afro-descendant [41]. Poorer perinatal outcomes have been noted among Afro-descendant Brazilian women [42-48], yet an excess occurrence of BV in pregnancy among Afro-descendant women, as that observed in the U.S., is unclear given that few Brazilian studies report findings by race. Brazilian reports of BV prevalence assessed by Amsel clinical criteria in pregnancy range from 20% to 40% [49-51], but no race-specific prevalence estimates or associations with

prenatal stress were presented in these studies among pregnant women.

[Figure 4.1 about here]

Our investigation was a cross sectional study conducted in two public maternity clinics in the Municipality of Rio de Janeiro, Brazil. Antenatal care coverage in this setting is high [52], with estimates for the Municipality of Rio de Janeiro that at least 60% of women enroll in prenatal care by the first trimester in the public network or in clinics linked to the Universal Health System [53].

A schematic of the hypothesized relationships of race, stress, and BV is presented in Figure 4.1. Given the cross sectional study design, implicit in the figure is the assumption that the latent factors of stress measured by variables hypothesized to be composites of socioeconomic stressors, chronic stressors, and acute stressors covary as a joint construct of latent stress. The double headed arrows between ellipses depict joint cross sectional covariance of stressors, rather than depicting a temporal causal hierarchy among them. The theoretical model posits that latent stress may mediate the relationship between color/race and BV via impaired immune response or hypothalamic-pituitary-adrenal axis dysfunction (i.e., cortisol response), which were not directly analyzed and are therefore presented with dashed lines in the figure. Additionally, latent stress may mediate the color/race-BV relationship via adoption of coping maternal behaviors, operationalized in this analysis as alcohol use and smoking during pregnancy, which in turn may be associated with alteration of immune function or other intermediate pathways associated with increased duration of BV infection.

Thus, the purposes of this study were to estimate the prevalence of BV during pregnancy in a Brazilian clinical setting, to investigate whether BV prevalence was associated with self-reported race, and to test whether the race-BV association differed according to psychosocial factors.

METHODS

Participants

Participants were women (n=295) with singleton pregnancies, recruited from two public prenatal ambulatory clinics in the municipality of Rio de Janeiro, Brazil, between May 2008 and July 2009. One clinic (hereafter called *clinic 1*) is annexed to a maternity hospital complex located downtown. At the time of the study, clinic 1 was a private, not-for-profit institution, linked to the Brazilian Universal Health System (*Sistema Único de Saúde – SUS.*), with a diverse catchment population from the entire municipality and serviced by all major routes of public transportation. The second clinic (hereafter called *clinic 2*), located in the North Zone of the municipality, is a public institution annexed to a maternity hospital complex exclusively serving patients enrolled in the Universal Health System from the immediately surrounding neighborhoods only. Both prenatal ambulatory clinics complied with the 2006 Brazilian Ministry of Health published prenatal care guidelines at the time of field activities [54].

Women were excluded if they were not residents of the Municipality of Rio de Janeiro, had a known multiple pregnancy at the time of interview, if a gestational age of 14 to 26 weeks' gestation could not be estimated by either known date of the last menstrual period (LMP) or ultrasound, or if prenatal care was initiated after 20 weeks of

pregnancy. Women were excluded from invitation to the study if they had prenatally-diagnosed chronic infectious diseases, metabolic disturbances, cardiopathy, mental illness, or hypertension, or vaginal bleeding, or pregnancies marked by known anomalies, as recorded in their prenatal card at the time of selection for interview.

Procedures

A list of all women eligible for recruitment was generated daily at the beginning of each nursing shift by inspecting clinical appointment cards in the prenatal weighing room. Potentially eligible women were approached in the clinic waiting room. Consenting patients signed a written informed consent (signed by a legal guardian for study subjects below age 18) and were interviewed by one of four trained interviewers. Interviews were conducted in Brazilian Portuguese. Following the interview, a trained research nurse performed speculum examinations for BV clinical assessment. All study procedures were approved by the Emory University institutional review board and the committee of ethics of the Sérgio Arouca National School of Public Health, FIOcruz, and were conducted in accordance with the ethical principles of the Helsinki Declaration and the 196/96 Resolution of the Brazilian National Committee on Health (CNS).

In total, 371 eligible women were approached about participating in the study. Of the 334 (90%) who agreed to participate, 295 women (88.3%) completed full interviews with pelvic clinical assessment. Among the 39 participants who did not complete the full study, 14 cited “fear” or “not being prepared” for the research nurse’s pelvic assessment as reasons for non-participation; 17 cited lack of time to complete the questionnaire; and 8 were lost to follow-up within the clinic routine. Non-participants did not significantly

differ from participants by self-reported race ($p > 0.05$) or age ($p > 0.05$). All participants were given infant sock kits in gratitude for their participation.

Measures

Bacterial Vaginosis

Prevalent BV was assessed using Amsel's classic clinical criteria during the speculum examination at the time of the prenatal care visit and in accordance with the flowchart published by the Brazilian Ministry of Health [54]. An experienced research nurse at each clinic collected vaginal fluid for BV assessment, blinded to participant responses during the interviews. Examination by microscopy was conducted by the research nurse for all participants from clinic 1 on site; for clinic 2 participants, microscopy of sample swabs was carried out at a laboratory of the National School of Public Health, FIOcruz, on the same day as sample collection by the first author. All samples were labeled with a study identification number to blind all assays to questionnaire responses during microscopy, and results were linked to the patient chart number, thereafter. Women with at least 3 out of 4 criteria – abnormal vaginal discharge; vaginal pH > 4.5 ; presence of amines (whiff test); and presence of clue cells in wet mounts examined by microscopy [55, 56] – were identified as positive for BV, and the attending obstetrician was notified for case management, as appropriate.

Self-identified race

Participants self-identified their race during the interview and prior to collection of BV samples, according to Brazilian census categories: *branca* (white), *preta* (black), *parda* (mixed), *indígena* (indigenous native), and *amarela* (Asian). Women whose self-

reported race was other than white, black or mixed ($n = 28$) were excluded from analyses considering self-reported race as a covariate. One mixed participant was excluded from BV analyses for having one clinical criterion missing during data collection. The total sample analyzed consisted of 266 subjects (62 white, 148 mixed, and 56 black).

Socioeconomic status

Interviewers asked about individual and total household income, maternal literacy, and maternal educational attainment (total school years completed). Self-reported total household income was scaled to the number of household residents for a measure of per capita household income, and then categorized by Brazilian minimum salary units, where one salary unit is R\$465, or approximately U.S.\$260 [57].

Psychosocial measures

Participants responded to standardized measures of perceived stress, maternal state and trait anxiety, adverse life events, (ever) experiences of discrimination, and social support since becoming pregnant. The validity and/or reliability of Brazilian Portuguese versions of the perceived stress scale [32, 58], the state and trait anxiety inventory [59, 60], the adverse life events module [61], the experiences of discrimination module [61], and the social support survey [62, 63] have been previously tested and published for non-pregnant Brazilian populations. Two dichotomized (yes/no) indicators of poverty in childhood – perceived poor/very poor SES in childhood and at least one instance of going without food because of lack of money in the household during childhood, from the adverse life events and experiences of discrimination modules [61, 64] were also included in analyses. Preliminary factor analyses (correlated Geomin

rotation in MPlus 5.21) confirmed *a priori* hypotheses of factors measured by psychosocial scales. Both published scale theoretical cutoffs and continuous score entries were considered in this analysis [58, 59, 61, 62].

Sociobehavioral factors and covariates

Potential sociobehavioral covariates of the association between race or stress and BV identified from the literature included self-reported use of alcohol, cigarettes, and the occurrence of physical intimate partner violence since becoming pregnant. Given that the timing of occurrence queried was since the time of conception of the index pregnancy only, physical intimate partner violence was considered ‘acute’ in timing, likely underestimating the effect of its possible preconceptional occurrence.

Clinical chart extraction was conducted using a standardized form, the *Cartão da Gestante* (or Pregnant Woman’s Card) issued by all institutions participating with the Brazilian Ministry of Health. The card includes data on last menstrual period, date(s) of ultrasound(s), gestational age, anthropometrics, diagnosis of pre-existing /current conditions, and obstetric history, including previous preterm, low birthweight, and caesarean section deliveries. Additional covariates collected through interview and card extraction include maternal age, cohabitation, parity, contraceptive use at time of conception, urinary tract infection (UTI) and antibiotic use during pregnancy, and reproductive and obstetric history.

Statistical Analyses

Prevalence proportion estimates for BV status were calculated for the whole sample and by self-reported race. Bivariate associations between categorical and continuous covariates and BV were investigated overall and stratified by race using the Pearson χ^2 test, Fisher's exact test, and Student's t test, as appropriate. Non-parametric (Mann-Whitney) tests were applied for comparisons of variables with skewed distributions, household income, and scale responses. Scale quartiles were examined for psychosocial measures to explore different dichotomous points in addition to the theoretical cut points for each scale.

Given the relatively high estimated prevalence of BV during pregnancy cited in the literature, it was expected that bacterial vaginosis would be a frequent outcome in this population and that prevalence proportions of BV within racial groups would be at least 20% across racial categories under the null hypothesis of no association between self-reported race and BV. The theoretical approximation of the prevalence odds ratio (POR) to the prevalence ratio (PR) allows for the use of a generalized linear model fitting a Poisson regression model with robust standard error estimation, and the point estimates are obtained with the advantage of having robust standard errors for confidence interval estimates [65-67]. The estimates from a Poisson model with robust standard errors are comparable to results obtained from more frequently used log binomial GLMs, but avoid convergence problems encountered with the latter distribution [16, 67].

A hierarchical forward selection multivariate modeling procedure [68] was applied, guided by the conceptual framework presented in Figure 4.1. Given that the

analysis considers three race categories, the modeling strategy considered two distinct reference categories: the first set of models considered BV prevalence ratios among black and mixed race, respectively, relative to white race, while the second set of models considered BV prevalence ratios among black race relative to mixed race. The starting point for the model building procedure was the crude association between self-identified race and BV with no mediators. The general modeling procedure consisted of sequential forward selection of variables in groups as depicted in the figure. The criterion for retention of sets of variables for each sequential group, or block, was statistical significance of $p \leq 0.20$ in Type III score tests, both for parsimony and sufficient sample size relative to the number of model terms (at least seven observations per model term), following the flow hierarchy shown in Figure 4.1. Last, maternal behaviors hypothesized to be coping strategies in response to stress (i.e., smoking and alcohol use in pregnancy) and potential confounders identified from the literature, including age, cohabitation, gestational age at interview, urinary tract infection during pregnancy, and contraceptive use at conception were entered in the forward model specification. Inspection of the working correlation matrices for multicollinearity was conducted at each step, and model goodness-of-fit was assessed by examination of the Quasi-likelihood under the Independence model Criterion (QIC) statistic. Analyses were conducted using SAS v.9.2 for Windows [69] and SPSS 17.0 [70].

RESULTS

Characteristics of the study population

[Table 4.1 about here]

Table 4.1 describes the overall characteristics of the sample. The overall BV prevalence was 26.3% (95% CI = 21.0%, 31.6%) and was highest among black women (37.5%). However, race-specific differences in BV prevalence were not statistically significant ($p = 0.09$). Most women were of mixed/mulatta race, parous, 25 years of age and under, lived with their partner, and felt supported by their partner during the index pregnancy. Also, over half of the women reported the index pregnancy as unplanned, despite use of contraception being common in the sample. No significant crude associations of individual characteristics with BV were observed.

[Tables 4.2 and 4.3 about here]

The distributions of hypothesized stress factors by BV status and race are presented in Tables 4.2 and 4.3. While no hypothesized stressors were associated with BV (Table 4.2), race-specific differences in stress exposures were observed in household per capita income, maternal education, experiences of discrimination, and state anxiety (Table 4.3). Mixed/mulatta women had lower per capita household income ($p < 0.05$), fewer years of education ($p < 0.05$), and were significantly more likely to be in the highest quartile of state anxiety ($p < 0.05$), while most women reporting at least one experience of racial discrimination were of self-identified black race ($p < 0.01$). Sources of hypothesized chronic stress were not frequent in the sample, with only few women (10.2%) reporting ever experiencing racial discrimination overall. Smoking and reported experiences of physical domestic violence during pregnancy were not frequent among participants, and most women reported high social support during pregnancy.

[Tables 4.4 and 4.5 about here]

Hierarchical regression models

Tables 4.4 and 4.5 present the results of hierarchical robust Poisson regression of socioeconomic and psychosocial stress associations with bacterial vaginosis, adjusted for covariates. There was no significant association of race and BV either in crude or adjusted models where white race was the referent category (Table 4.4). While not statistically significant, black race was associated with a 45% crude excess prevalence of BV relative to white race (PR = 1.45, 95% CI = 0.85, 2.50) (Table 4.4, Model 1). The final adjusted prevalence point estimate for black race relative to white race was similar in magnitude (PR = 1.46, 95% CI = 0.84, 2.55) in Table 4.4, Model 6, likely due to small numbers, and suggesting that neither chronic nor acute stressors, as measured, mediate the potential observed excess in BV prevalence among black women relative to white. There was no significant excess BV prevalence by mixed race relative to white throughout any of the model procedure steps (Models 1 -6, Table 4.4).

In turn, black women had a significant 68% crude excess prevalence of BV (PR = 1.68, 95% CI = 1.07, 2.64) in models where mixed race was the referent category (Table 4.5, Model 1). Note that this analysis considered only women of black and mixed race (n = 205), and excluded white women. This statistically significant finding was not attenuated by the inclusion and retention of acute stressors and covariates in the modeling procedure (Table 4.5, Models 4, 5, 6). Hypothesized mediation by acute stress, operationalized as life events, state anxiety, perceived stress, and occurrence of intimate partner physical violence during pregnancy, was not observed according to an *a priori* criterion of at least a 50% reduction in the prevalence ratio point estimate associated with race. While we were unable to fully assess effect measure modification of the race-BV

association by levels of acute stressors due to sparse data and model convergence, data-based confounding was not observed, and the excess BV prevalence among black women relative to mixed persisted after adjusting for life events, state anxiety, perceived stress, intimate partner physical violence, and covariates in pregnancy (PR = 1.73, 95% CI = 1.11, 2.70) (Table 4.5, Model 6).

In sub-analyses stratified by race (data not shown), factors hypothesized to be indicators of acute stress were associated with BV by race. For instance, among black women, state anxiety was statistically significantly associated with BV prevalence in pregnancy, suggesting that there was a different set of experiences and perceptions according to self-reported racial identity and accompanied social context. Final evidence of a possible difference in experiences and perceptions based on a woman's racial self-identity is the observation that intimate partner physical violence in pregnancy was statistically significantly associated with BV prevalence in pregnancy only among mixed women (data not shown).

Due to the inherent subjectivity of the clinical criteria, particularly that of the amines "whiff" test [80], a sensitivity analysis was conducted in which the presence of amines as a criterion for diagnosis of BV was excluded. As expected, the remaining three criteria jointly yielded lower prevalence estimates across race strata; however, associations of stress exposures with the outcome stratified by race were not meaningfully different from the estimates obtained using the *a priori* classic clinical criteria for diagnosis (results available upon request). The *a priori* clinical criteria for BV diagnosis were therefore retained for comparability with other studies of BV among pregnant women employing Amsel's full clinical criteria.

DISCUSSION

This is the first report of BV prevalence in pregnancy with respect to race in an urban Brazilian public healthcare setting. Self-identified black women had a higher prevalence of bacterial vaginosis by Amsel's criteria than white and mixed women, even after adjusting for potential confounding variables and stressors experienced in pregnancy.

The crude overall prevalence of BV among pregnant women in this study (26.3%), as assayed by Amsel's clinical criteria, was very similar to that reported by Tanaka et al. [71] in a clinical sample of non-pregnant women in São Paulo that also used classic Amsel's criteria. The BV prevalence findings of the two studies were also similar by race. Tanaka et al. [71] reported a BV prevalence of 28.3% in white women, 37.1% in black women, and 27.9% in mulatta women. In the present study, race-specific BV prevalence ranged from 22.3% (95% CI = 15.6%, 29.0%) among mixed women, to 25.8% (95% CI = 14.9%, 36.7%) among white women, to 37.5% (95% CI = 24.8%, 50.2%) among black women (Pearson χ^2 $p = 0.09$). The comparability of the findings of the two studies supports the validity of our reported associations between self-reported race and bacterial vaginosis prevalence in Brazilian women and of our clinical method for assessing BV prevalent status in a similar low-resource, public women's health care setting in a large metropolitan area in Brazil.

As hypothesized, stressors, including perceived stress, anxiety, social support and stressful life events, differed by self-reported race, particularly in the comparisons of women of black and mixed race (Table 4.5). However, the hypothesis that different

stress levels would mediate a racial difference in BV prevalence was not supported. Rather, the significant excess in BV prevalence among black women relative to mixed women was not attenuated in multivariate regression. Perhaps different characterizations of stress observed by racial identity may be influenced by intersectionality, where health risk is the product of intersecting dimensions of socioeconomic stratification [72, 73]. Future inquiry into factors associated with BV in Brazilian women should consider evaluating intersecting dimensions of stress within racial identity category. We note the need for a larger sample size in such studies to have adequate power to investigate potential effect modification by stress.

Factors hypothesized to be indicators of largely fixed socioeconomic and chronic stress were not predictive of BV prevalence in this study population. Regarding measures of socioeconomic stress, the study participants may have been too homogenous to display SES-associated differences in BV prevalence. Brazilian public maternity clinics in Rio de Janeiro serve populations of women from proximal neighborhoods and urban areas within the municipality who belong to low or middle socioeconomic classes [74]. Another explanation for the lack of association of socioeconomic factors and BV may be incomplete measurement of SES and potentially compromised ecological validity of the self-reported 'mixed' racial self-classification. While self-classification of black and white race within the Brazilian census scheme is considered reliable [75], 'mixed/mulatta' racial self-classification includes a fluid skin color gradient that may obscure potentially mixed differences across the spectrum of 'lighter' to 'darker' mixed race in Brazil and, therefore, render misclassified results by racial stratifications [16, 75, 76]. Future studies of racial disparities with respect to bacterial vaginosis and pregnancy

outcome would benefit from a multi-dimensional measure of socioeconomic status that considers individual and contextual metrics appropriate to the study population.

There is a clear advantage for analyzing race as a binary variable (i.e., white vs. non-white) in the Brazilian epidemiologic literature, both for the sake of sample size and ease of comparisons in studies in racially heterogeneous societies, such as the United States. However, grouping non-white women into a single group may obscure very distinct experiences and perceptions associated with skin tone and discrimination. The heightened state of acute anxiety reported by black women during pregnancy in this sample raises the possibility of an altered immune and/or inflammatory vaginal milieu that increases the susceptibility to developing BV in pregnancy or difficulty clearing the infection. This could lead to an increased duration of the outcome at the time of our observation of prevalent status. Further research is needed to establish whether there is a temporal sequence between “exposure” to anxiety in pregnancy and incidence and/or duration of clinical criteria consistent with a BV diagnosis and how, if at all, a history of altered vaginal flora may affect a pregnancy outcome among women experiencing state anxiety during the second trimester of pregnancy.

Furthermore, information on domestic violence was based on responses from a small number of women; however, that mixed women BV cases more frequently reported physical violence during pregnancy, either because they experienced it more frequently or because they were more willing to report its occurrence, indicates that the role of intimate partner violence in pregnancy with respect to pregnancy outcomes needs to be studied further. While there was a suggestion that intimate partner physical violence during pregnancy was associated with an excess in bacterial vaginosis prevalence in our

study, we lacked data on violence occurrence prior to pregnancy and its frequency during pregnancy, therefore substantially underestimating any potential associations with BV by race. Also, the sample size was inadequate to further investigate intimate partner physical violence by intensity, associations with other stressors, and potential effect measure modifications. In recent literature, violence in pregnancy is reported as frequent and associated with inadequate maternal weight gain during pregnancy and prenatal care in disadvantaged settings in Brazil [79], supporting the future study and characterization of violence in pregnancy and its interrelationships with other measures of psychosocial stress.

The pathways through which self-reported race and stress may impact health to produce a clinical profile consistent with bacterial vaginosis in pregnancy are clearly much more complex than the organizing diagram presented in Figure 4.1. The problem of racial disparities in pathways leading to adverse health outcome in pregnancy may perhaps be the end result of multiple interacting factors at the social, individual, and molecular levels [18]. The findings of this study provide evidence of the need to study racial “non-white” self-categorizations in Brazilian contexts separately, particularly as they pertain to Afro-descendant populations. Also, the present findings are not consistent with a uniform hypothesized mediating effect of latent stress in the race – BV relationship; rather, this study suggested the potential effect measure modification of the race – BV association by level of acute stress. One explanation is that differential item functioning of current psychosocial scales may measure stress dimensions differently by race. For instance, the Brazilian version of the standardized scale of perceived stress is not invariant by race [32], given by a statistically significant difference in the latent stress

mean for black and mixed women combined relative to white Brazilian women in pregnancy. While a difference in stress state by race may account for the race-specific BV differences suggested in our findings, consideration of culturally-appropriate scales and their psychometric properties in a Brazilian context warrants further investigation.

Limitations to this study include the inability to establish temporality of exposures with respect to the occurrence of the outcome because of the cross-sectional design. The use of robust Poisson models to directly estimate the prevalence ratio is, however, an analytical strength given that prevalence odds ratios from logistic regression models would have overestimated point estimates because the prevalence of BV was relatively high. A more complete characterization of bacterial vaginosis occurrence, duration, and recurrence is needed in future studies using a prospective design. Additional limitations include the use of Amsel's clinical criteria to identify bacterial vaginosis in this low-resource, public clinical setting instead of Nugent's scoring of gram stain (the gold standard method for BV diagnosis in research settings). Though clinical criteria are acceptable, the use of the gold standard method should be contemplated where time and study resources allow.

Our findings justify more complete characterization, measurement, and analysis of individual and social factors that may differ by racial self-identity and choice of "color" self-report in Brazilian study populations. The implication is that important differences exist between the perceptions and experiences lived by black and racially-mixed women, relative to each other and to white women, and beyond the context of "poverty" measured by low household income and maternal education. To our knowledge, this is the first analysis considering the role of self-identified race and stress

in the prevalence of BV in this setting. A more thorough characterization of differences in such experiences and the mechanisms driving them is an important step in understanding and intervening on racial disparities observed in pregnancy and birth outcomes in Brazil.

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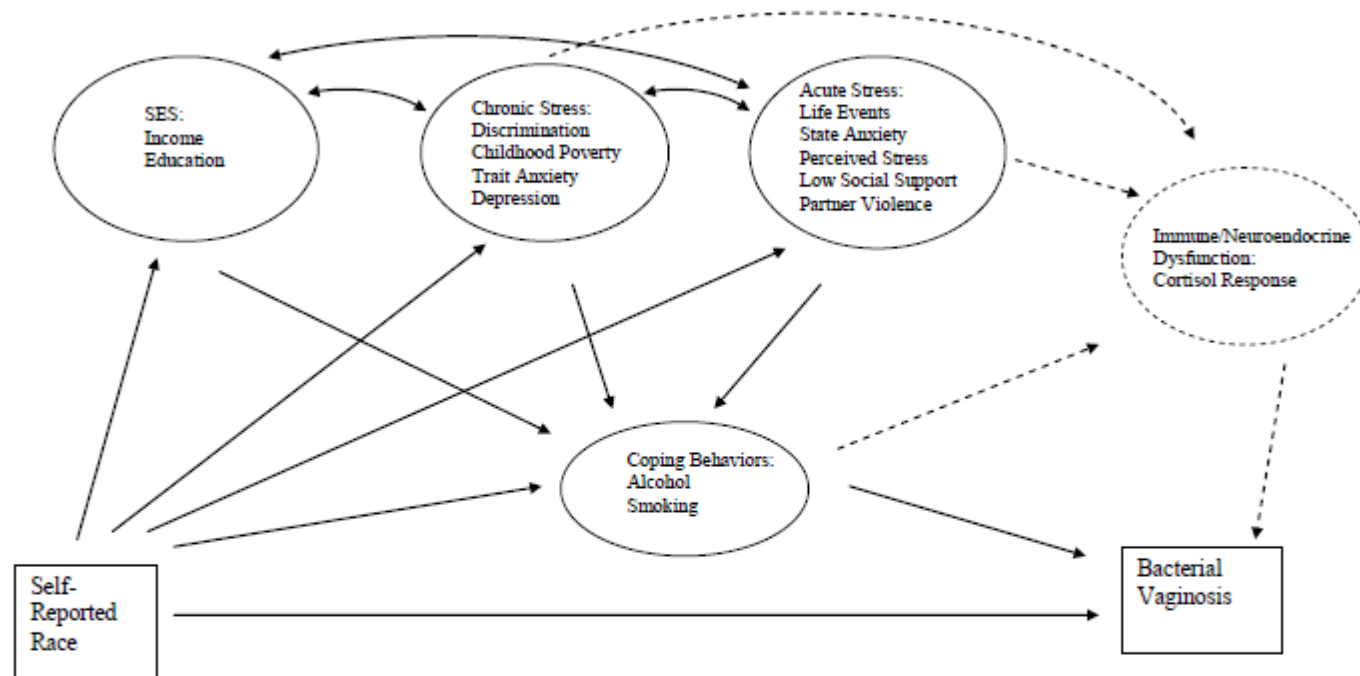
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Figure 4.1 Theoretical relationships between self-reported race and bacterial vaginosis, with hypothesized stress mediators.



<i>Model</i>	<i>Explanatory Variables</i>	<i>Interpretation</i>
1	Race	Overall effect of race; not adjusted for mediating variables.
2	Race + socioeconomic stressors	Effect of race not mediated through socioeconomic stressors
3	Race + socioeconomic stressors + chronic stressors	Effect of race not mediated through socioeconomic stressors nor chronic stressors
4	Race + socioeconomic stressors + chronic stressors + acute stressors	Effect of race not mediated through socioeconomic stressors, chronic stressors, or acute stressors
5	Race + socioeconomic stressors + chronic stressors + acute stressors + maternal behaviors	Effect of race not mediated through socioeconomic stressors, chronic stressors, acute stressors, or maternal behaviors

Table 4.1. Descriptive characteristics of a sample of pregnant women between 14-26 weeks' gestation and unadjusted associations with BV prevalence, Rio de Janeiro, 2008-2009.

	Number of women ^a n (%)	BV positive ^b n (%)	p-value ^c
Total sample	266	70 (26.3)	
Race			
White	62 (23.3)	16 (25.8)	0.09
Black	56 (21.1)	21 (37.5)	
Mixed	149 (55.6)	33 (22.3)	
Age			
< 20	60 (22.6)	16 (26.7)	0.72
21-25	97 (36.5)	28 (28.9)	
26-30	68 (25.6)	18 (26.5)	
31-35	29 (10.9)	7 (24.1)	
≥ 35	12 (4.5)	1 (8.3)	
Cohabiting Status			
Yes	204 (77.0)	52 (25.5)	0.74
No	61 (23.0)	17 (27.9)	
Contraception use at time of conception			
No	140 (52.6)	34 (24.3)	0.49
Yes	126 (47.4)	36 (28.6)	
Hormonal contraception use at time of conception^d			
(Pill, shots, IUD)	95 (75.4)	27 (28.4)	0.48
Pregnancy Wanted?			
Yes	115 (43.2)	30 (26.1)	0.83
Yes, but mistimed	93 (35.0)	23 (24.7)	
No	58 (21.8)	17 (29.3)	
Partner support during pregnancy			
Yes	246 (92.5)	66 (26.8)	0.61
No	20 (7.5)	4(20.0)	
Parity			
0	120 (45.1)	35 (29.2)	0.56
1	81 (30.5)	18 (22.2)	
≥ 2	65 (24.4)	17 (26.2)	
Continued on next page			

Table 4.1 (cont.)			
UTI in pregnancy			
No	196 (73.7)	47 (24.0)	0.16
Yes	70 (26.3)	23 (32.9)	
Antibiotic use since beginning of pregnancy			
No	121 (69.9)	28 (23.1)	0.51
Yes	50 (28.9)	13 (26.0)	
Previous: Preterm birth^a			
No	120 (82.2)	28 (23.3)	0.80
Yes	26 (17.8)	7 (26.9)	
Previous: spontaneous abortion^f			
0	11 (20.4)	1 (9.1)	0.61
1	39 (72.2)	10 (25.6)	
2+	3 (5.6)	1 (33.3)	

BV, bacterial vaginosis; *UTI*, urinary tract infection

^a Distribution for the overall study population; sample sizes may not total 266 (total number of participants with BV data) because of missing data patterns. ^b BV status as ascertained by the classic Amsel clinical criteria, where a case is defined as having at least three out of the four following criteria present upon clinical pelvic examination: a thin, ashen, homogeneous vaginal discharge, vaginal pH > 4.5, positive whiff test, and presence of clue cells in a wet mount preparation. Percentage of participants with BV by Amsel criteria, given the specified variable category. ^c χ^2 p-values; Fisher's exact test p-values where cell sizes had 5 or fewer observations. ^d Hormonal contraceptive use among 126 women reporting using a contraception method at the time of conception. ^e Among 146 parous women. ^f Among 54 women reporting a previous pregnancy loss.

Table 4.2. Distribution of stress exposures by bacterial vaginosis (BV) status in a sample of pregnant women between 14-26 weeks' gestation. Rio de Janeiro, Brazil, 2008-2009.

	Number of women n (%)	BV positive (n=70) n (%)	p-value^b
Low per capita income^c	61 (22.8)	20 (32.8)	0.25
Education			
≤ 8 years	61 (22.8)	20 (32.8)	0.19
> 8 years	205 (77.2)	50 (24.4)	
Perceived SES in childhood			
Poor/Very Poor	115 (43.1)	29 (25.2)	0.78
Average	150 (56.2)	41 (27.3)	
Food insecurity in childhood			
Yes	40 (15.0)	10 (25.03)	0.50
No	225 (84.3)	60 (26.7)	
Ever experiences of racial discrimination			
At least one	27 (10.1)	8 (29.6)	0.68
None	239 (89.5)	62 (25.9)	
Trait anxiety			
Highest quartile (Q4)	53 (19.9)	13 (24.5)	0.74
Quartiles 1-3	213 (79.8)	57 (26.7)	
Depression			
Highest quartile (Q4)	41 (15.4)	11 (26.8)	0.94
Quartiles 1-3	225 (84.6)	59 (26.2)	
Stressful life events in last 12 months			
At least one event	88 (33.0)	29 (33.0)	0.08
None	178 (66.7)	41 (23.0)	
State anxiety			
Highest quartile (Q4)	61 (22.8)	12 (19.7)	0.18
Quartiles 1-3	205 (76.8)	58 (28.3)	

Table 4.2 (cont.)			
Perceived stress in past 30 days			
Highest quartile (Q4)	57 (21.3)	11 (51.6)	0.09
Quartiles 1-3	208 (77.9)	59 (28.4)	
Social support during pregnancy			
Lowest quartile (Q1)	59 (22.1)	17 (28.8)	0.84
Quartiles 2-4	206 (77.1)	53 (25.7)	
Partner physical violence during pregnancy			
Yes	11 (4.1)	5 (45.5)	0.13
No	255 (95.5)	65 (25.5)	
Alcohol use during pregnancy			
Yes	75 (28.1)	24 (32.0)	0.19
No	191 (71.9)	46 (24.1)	
Smoking during pregnancy			
Yes	20 (7.5)	4 (20.0)	0.61
No	246 (92.5)	66 (26.8)	

^aBV status as ascertained by the classic Amsel clinical criteria, where a case is defined as having at least three out of the four following criteria present upon clinical pelvic examination: a thin, ashen, homogeneous vaginal discharge, vaginal pH > 4.5, positive whiff test, and presence of clue cells in a wet mount preparation. Data shown as n (row %). Sample sizes may not total 266 (total analyzed number of subjects with BV data) due to missing observations. ^b χ^2 p-values; Fisher's exact test p-values where cell sizes had 5 or fewer observations.

^cHousehold income per capita < ¼ minimum monthly salary (minimum monthly wage R\$465.00).

Table 4.3. Distribution of stress exposures in a sample of pregnant women between 14-26 weeks' gestation and unadjusted associations with BV prevalence, stratified by self-reported race. Rio de Janeiro, Brazil, 2008-2009.

	White (N = 62)	Black (N = 50)	Mixed (N=148)		Overall (N = 266)*
	n (%)	n (%)	n (%)	p-value ^b	n (%)
Household income ^c (median, Br. Reais)	R\$1283.00	R\$940.00	R\$1130.00	0.18	R\$1070.00
Low per capita income ^d	15 (24.6)	19 (31.1)	27 (44.3)	< 0.05	61 (23.4)
Education					
< 8 years	8 (13.1)	18 (29.5)	35 (57.4)	< 0.05	61 (22.8)
> 8 years	54 (26.2)	38 (18.4)	114 (55.3)		206 (77.2)
Perceived SES in childhood					
Poor/Very Poor	19 (16.5)	26 (22.6)	70 (60.9)	0.07	115 (43.4)
Average	43 (28.7)	30 (20.0)	77 (51.3)		150 (56.6)
Food insecurity in childhood					
Yes	5 (12.5)	11 (27.5)	24 (60.0)	0.17	40 (15.1)
No	57 (25.3)	45 (20.0)	123 (54.7)		225 (84.9)
Ever experiences of racial discrimination					
At least one	5 (18.5)	13 (48.1)	9 (33.3)	< 0.01	27 (10.2)
None	57 (23.8)	43 (18.0)	139 (58.2)		239 (89.8)
Trait anxiety					
Highest Quartile (Q4)	10 (18.9)	7 (13.2)	36 (67.9)	0.11	53 (19.9)
Quartiles 1-3	52 (24.4)	49 (23.0)	112 (52.6)		213 (80.1)

Table 4.3 (cont.)					
Depression					
Highest Quartile (Q4)	8 (19.5)	9 (22.0)	24 (58.5)	0.82	41 (15.4)
Quartiles 1-3	54 (24.0)	47 (20.9)	124 (55.1)		225 (84.6)
Stressful life events in last 12 months					
At least one event	16 (18.2)	20 (22.7)	52 (59.1)	0.38	88 (33.1)
None	46 (25.8)	36 (20.2)	96 (53.9)		178 (66.9)
State anxiety					
Highest Quartile (Q4)	10 (16.4)	8 (13.1)	43 (70.5)	< 0.05	61 (22.9)
Quartiles 1-3	52 (25.4)	48 (23.4)	105 (51.2)		205 (77.1)
Perceived stress in past 30 days					
Highest quartile (Q4)	8 (14.0)	11 (19.3)	38 (66.7)	0.12	57 (21.5)
Quartiles 1-3	53 (25.5)	45 (21.6)	110 (52.9)		208 (78.5)
Social support during pregnancy					
Lowest quartile (Q1)	10 (16.1)	13 (19.4)	40 (64.5)	0.20	21 (7.9)
Quartiles 2-4	52 (25.6)	44 (21.7)	107 (52.7)		244 (92.1)
Partner physical violence during pregnancy					
Yes	60 (23.5)	54 (21.2)	141 (55.3)	0.17	11 (4.1)
No	2 (18.2)	2 (18.2)	7 (63.6)		255 (95.9)
Alcohol use during pregnancy					
Yes	19 (25.3)	16 (21.3)	40 (53.3)	0.85	75 (28.1)
No	43 (22.4)	40 (20.8)	109 (56.8)		192 (71.9)
Smoking during pregnancy					
Yes	1 (5.0)	7 (35.0)	12 (60.0)	0.05	20 (7.5)
No	61 (24.5)	29 (19.8)	137 (55.5)		247 (92.5)

* Sample sizes may not total 266 because of missing data patterns; n (row %) for all categorical variables. ^b χ^2 p-values; Fisher's exact test p-values where cell sizes had 5 or fewer observations. Kruskal-Wallis test for non-normal continuous income data. ^c Data presentation is shown as median for total household income. ^d Household income per capita = % minimum monthly salary (minimum monthly wage R\$465.00)

Table 4.4. Results of hierarchical robust Poisson regression of socioeconomic and psychosocial stress associations with bacterial vaginosis, adjusted for covariates and considering white race as referent (n = 266). Rio de Janeiro, Brazil, 2008-2009.

Variable	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6*
	PR (95%CI)	PR (95%CI)	PR (95%CI)	PR (95%CI)	PR (95%CI)	PR (95%CI)
Color/race						
Black	1.45 (0.85, 2.50)	1.41 (0.81, 2.45)	1.48 (0.84, 2.58)	1.44 (0.84, 2.47)	1.51(0.89,2.58)	1.46 (0.84, 2.55)
Mixed	0.86 (0.51, 1.45)	0.84 (0.50, 1.43)	0.89 (0.52, 1.50)	0.79 (0.47, 1.34)	0.82 (0.49, 1.38)	0.80 (0.47, 1.37)
White	1	1	1	1	1	1
Education						
≤ 8 years		1.27 (0.80, 2.01)	Dropped	Dropped	Dropped	Dropped
> 8 years		1
Low income per capita						
< 1/2 minimum wage		1.17 (0.73, 1.88)	Dropped	Dropped	Dropped	Dropped
≥ 1/2 minimum wage		1
Experiences of discrimination (ever)						
At least 1			N/A	N/A	N/A	N/A
None			1
Childhood poverty						
Low perceived SES			N/A	N/A	N/A	N/A
Avg perceived SES			1	1
Not enough money for food						
Not enough money for food			N/A	N/A	N/A	N/A
Enough money for food			1	1	1	...
Trait Anxiety						
Highest quartile (Q4)			N/A	N/A	N/A	N/A
Quartiles 1 - 3			1	1

Table 4.4 (cont.)	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6*
Depression						
Highest quartile (Q4)			N/A	N/A	N/A	N/A
Quartiles 1 -3			1	1
Stressful life events						
At least 1				1.39 (0.92, 2.10)	1.43 (0.95, 2.15)	1.41 (0.93, 2.15)
None				1	1	1
State anxiety						
Highest quartile (Q4)				1.56 (0.93, 2.63)	1.53 (0.91, 2.56)	1.43 (0.84, 2.44)
Quartiles 1 -3				1	1	1
Perceived stress (continuous)						
				1.03 (1.00, 1.05)	1.03 (1.00, 1.05)	1.03 (1.00, 1.04)
Physical intimate partner violence						
Yes				2.00 (1.00, 4.01)	1.97 (1.01, 3.82)	1.94 (0.94, 4.0)
No				1	1	1
Social Support						
Lowest quartile (Q1)				1.76 (0.90, 3.46)	1.70 (0.89, 3.26)	1.97 (0.98, 4.0)
Quartiles 2-4				1	1	1
Alcohol use						
Yes					1.32 (0.88, 1.99)	Dropped
No					1	...
Smoking						
Yes					1.55 (0.61, 3.94)	Dropped
No					1	...

(1) Model 1: unadjusted estimates of the association between color/race and BV; Model 2: Model 1, plus SES group variables; Model 3: Model 2, plus chronic stress group variables; Model 4: Model 3, plus acute stress variables; Model 5: Model 4, plus coping behaviors; Model 6* (final model): Model 5, adjusted for maternal age, gestational age at interview, cohabitation, contraception use at conception, and UTI infection during pregnancy. (2) PR prevalence ratio. (3) 'N/A' indicates that the variable was not entered into the model because it had no association with BV in bivariate analyses. (4) 'Dropped' indicates that the variable was entered at an earlier stage of the model procedure but was dropped from the final block step because it did not meet retention p-value criterion.

Table 4.5. Results of hierarchical robust Poisson regression of socioeconomic and psychosocial stress associations with bacterial vaginosis, adjusted for covariates and considering mixed race as referent (n = 205). Rio de Janeiro, Brazil, 2008-2009.

Variable	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6*
	PR (95%CI)	PR (95%CI)	PR (95%CI)	PR (95%CI)	PR (95%CI)	PR (95%CI)
Color/race						
Black	1.68 (1.07, 2.64)	1.67 (1.03, 2.68)	1.70 (1.07, 2.70)	1.79 (1.15, 2.77)	1.78 (1.16, 2.75)	1.73 (1.11, 2.70)
Mixed	1	1	1	1	1	1
Education						
≤ 8 years		1.09 (0.65, 1.84)	Dropped	Dropped	Dropped	Dropped
>8 years		1	---	---	---	---
Low income per capita						
< 1/2 minimum wage		1.26 (0.73, 2.18)	Dropped	Dropped	Dropped	Dropped
≥ 1/2 minimum wage		1	---	---	---	---
Experiences of discrimination (ever)						
At least 1			N/A	N/A	N/A	N/A
None			1	---	---	---
Childhood poverty						
Low perceived SES			N/A	N/A	N/A	N/A
Avg perceived SES			1	1	---	---
Not enough money for food						
Not enough money for food			N/A	N/A	N/A	N/A
Enough money for food			1	1	1	---
Trait anxiety						
Highest quartile (Q4)			N/A	N/A	N/A	N/A
Quartiles 1-3			1	1	---	---

Table 4.5 (cont.)	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6*
Depression						
Highest quartile (Q4)			N/A	N/A	N/A	N/A
Quartiles 1 -3			1	1
Stressful life events						
At least 1				1.43 (0.91, 2.24)	1.46 (0.94, 2.29)	1.35 (0.86, 2.13)
None				1	1	1
State anxiety						
Highest quartile (Q4)				1.88 (1.00, 3.54)	1.84 (0.99, 3.44)	1.76 (0.93, 3.34)
Quartiles 1 -3				1	1	1
Perceived stress (continuous)						
				1.03 (1.00, 1.05)	1.02 (0.99, 1.05)	1.02 (1.00, 1.05)
Physical intimate partner violence						
Yes				2.33 (1.23, 4.41)	2.21 (1.24, 3.93)	1.96 (0.97, 3.96)
No				1	1	1
Social Support						
Lowest quartile (Q1)				1.32 (1.39, 2.97)	Dropped	Dropped
Quartiles 2 -4				1	1	1
Alcohol use						
Yes					1.40 (0.88, 2.22)	Dropped
No					1	...
Smoking						
Yes					0.63 (0.24, 1.63)	Dropped
No					1	...

(1) Model 1: unadjusted estimates of the association between color/race and BV; Model 2: Model 1, plus SES group variables; Model 3: Model 2, plus chronic stress variables; Model 4: Model 3, plus acute stress group variables; Model 5: Model 4, plus coping behaviors; Model 6* (final model): Model 5, adjusted for maternal age, gestational age at interview, cohabitation, contraception use at conception, and UTI infection during pregnancy. (2) *PR* prevalence ratio. (3) 'N/A' indicates that the variable was not entered into the model because it had no association with BV in bivariate analyses. (4) 'Dropped' indicates that the variable was entered at an earlier stage of the model procedure but was dropped from the final block step because it did not meet retention p-value criterion.

CHAPTER 5

Assessment of the reliability and validity of the Perceived Stress Scale in a sample of Brazilian pregnant women

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Abstract

The Brazilian-Portuguese Perceived Stress Scale (PSS) has been validated, but its internal reliability and construct validity have not yet been examined among Brazilian pregnant women. The PSS was applied in face-to-face interviews in a total of 295 pregnant women aged 16 – 43 years in public prenatal clinics in Rio de Janeiro, Brazil. Exploratory factor analysis (EFA) yielded an 11-item two factor model. Confirmatory factor analysis (CFA) yielded a 10-item single factor model, where χ^2 (df = 27) = 35.790, CFI = 0.975, TLI=0.981, RMSEA=0.048, and WRMR=0.610. The final instrument has internal consistency ($r=0.807$). Analysis of population invariance showed that the latent stress factor mean varies by self-reported color/race in the overall study population ($Z = 3.199$, $p = 0.001$). The PSS showed adequate reliability and validity supporting its use in this population. Further analysis of the instrument's properties across race/color categories is warranted.

The bidirectional link between stress and health is well-established in the literature [[1, 2], leading to a general recognition of the important role of stress in health [3]. While no standardized definition for “stress” exists in the literature, the construct is generally regarded as a subjectively perceived discrepancy between environmental demands and biological, psychological, or social resources within a specific period of time [4]. As such, stress is an interaction of related experiences, pathways, responses and outcomes caused by a range of different circumstances [5, 6].

Investigation of stress effects on health is complicated by temporal and spatial constraints inherent to its measurement. Instruments to measure stress have been limited to specific contexts or aspects of stress, such as the Life Events Scale [3, 7], restricting assessment only to specific environments and study populations. Other limitations include lack of sensitivity in differentiating chronic stress from ongoing life circumstances [8] and the level of appraised stress to an event [8-11].

Stressful exposures during pregnancy have been positively associated with adverse events, such as susceptibility to bacterial vaginosis (BV) in various study settings [12-16]. In the U.S., racial disparities have been reported in both the rates of occurrence of BV [17, 18] and stressful experiences during pregnancy [12-14, 19], leading to the hypothesis that racial variation in stress may explain the observed racial differences in infection during pregnancy [18, 20].

The Perceived Stress Scale (PSS) is a tool for examining issues about the association of appraised levels of acute stress with health and behavioral outcomes [8] often used in U.S. studies of BV and stress in pregnancy [15-17]. The PSS is brief and

easy to administer, with good internal reliability (Cronbach alpha ranging from 0.79 to 0.87) and validity in various study populations [3, 21-23].

While a Brazilian Portuguese validated PSS version exists [21], to date, the psychometric properties of the recently validated translation have not been examined in Brazilian pregnant women. Therefore, our objectives were: (1) to assess the internal reliability and construct validity of the Brazilian-Portuguese version of the full PSS instrument to test the applicability of the instrument in research of pregnant women within the context of a cross-sectional study in two maternity clinics in Rio de Janeiro, Brazil; and (2) to examine whether the instrument had structural population heterogeneity by self-reported race and clinic membership.

Methods

Participants

The present study uses data from a larger cross-sectional study characterizing maternal stress in pregnancy and its associations with BV. A sample of 295 pregnant women was recruited between May 2008 and July 2009, in two public prenatal ambulatory clinics in the municipality of Rio de Janeiro, Brazil. All women residents of the municipality and in their second trimester of pregnancy were invited to participate. Women were excluded if they had a multiple pregnancy, if a gestational age of 14 to 26 weeks' gestation could not be estimated by either a known date of the last menstrual period (LMP) or ultrasound, or if prenatal care was initiated after 20 weeks of pregnancy. Women were excluded from invitation to the study if they had prenatally-diagnosed chronic infectious diseases, metabolic disturbances, cardiopathy, mental illness,

hypertension/pre-eclampsia, vaginal bleeding, and pregnancies marked by fetal anomalies.

Procedures

Written informed consent was obtained from each participant (and legal guardian for study subjects below age 18), and all study procedures had Emory University and National School of Public Health/FIOCruc ethics committee approval. Interviews were administered by trained interviewers in each clinic.

In total, 371 eligible women were approached about participating in the study. Of the 334 (90%) who agreed to participate, 295 women (88.3%) completed interviews. Non-participants did not differ by self-reported race or age relative to participants. Women whose self-reported color/race was other than white, black or mulatta (n=28) were excluded from analyses considering self-reported race as a covariate.

Measures

The standardized questionnaire contained queries on sociodemographics, maternal alcohol, cigarette and drug use, contraceptive use, occurrence of intimate partner violence during pregnancy, reproductive and obstetric history, as well as other scales and module items of perceived stress, state and trait anxiety, adverse life events, and depression.

The PSS is a 14-item scale measuring the level to which situations occurring over the past 30 days are appraised as stressful [8, 21]. Construct validation was performed through comparison with alternative measures of maternal stress in pregnancy previously published, including the Spielberger State and Trait Anxiety Inventory [24], an adapted module of stressful life events [25], cross-sectional salivary cortisol concentrations [26, 27], and six items measuring depression previously used in studies of pregnant women in

this setting [28, 29]. These measures were hypothesized to be positively correlated with the PSS total score, following literature reports that are consistent with anxiety, number of stressful life events, and cortisol measurements as assessing perceived acute stress dimensions measured by the PSS [27, 30-32]. There is evidence of a positive linear association between acute timing and number of negative events and probability of depressive episodes [33], and a moderate positive correlation between the PSS total score and depression items was expected.

Other variables collected through interview as covariates for consideration in population heterogeneity analyses included: maternal age (continuous years); cohabitation (yes/no); years of education completed; gestational age at interview; and whether the pregnancy was mistimed or unwanted. Other variables collected to examine whether their distribution differed between clinics included: maternal income (in Brazilian *reais*); total household income (in Brazilian *reais*); number of previous pregnancies; smoking and alcohol use during pregnancy; contraception use at the time of conception; and emotional/verbal or physical partner violence during pregnancy. Clinic membership was also examined as a proxy for neighborhood context.

Analyses

Characteristics of the study population were assessed first, overall and by race, reporting percentages within categories and Chi-square p-values indicating the significance of any differences noted across groups. The internal consistency of the PSS was measured using Cronbach's alpha of the items in the total score of the best-fitting form of the Brazilian PSS [21]. Convergent construct validity was analyzed by examining the bivariate Pearson correlation of the total PSS score with the total scores of

the Spielberger State and Trait Anxiety Inventory, depression items, the adapted stressful life events inventory, and salivary cortisol concentrations, respectively, to examine the overall construct validity of the full 14-item scale before proceeding to factor analyses for the best-fitting measurement model. No external scale data were available to assess divergent validity.

Estimation Choice

All procedures for the analysis of structural models with ordinal categorical data were conducted using MPlus 5.21 software [34], and the robust mean- and variance-adjusted weighted least squares estimator (WLSMV) was used to estimate model parameters.

Exploratory Factor Analyses (EFA)

A preliminary EFA was performed in a random split sample of 153 study participants to identify a factor structure for the categorical scale items. This sub-sample was selected using the randomization function in SPSS Release 17.0.0 [35]. EFAs with oblique and orthogonal rotations were compared with the Geomin (correlated) rotation [34] to determine the best underlying factor structure. Items with primary factor loadings ≥ 0.40 and secondary factor loadings < 0.30 , and those that did not load on more than one factor, were retained. Items not meeting these criteria were removed sequentially, until no item had a loading < 0.30 .

Next, an EFA within the confirmatory factor analysis (E/CFA) framework [34] was run. The E/CFA approach is an intermediate step between EFA and CFA that provides standard errors to assess the statistical significance of factor loadings and

modification indices to assess for item residual covariances [34]. Based on our review of the literature, we expected to observe items loading on two general factors of stress, corresponding to positive and negative perceived stress, respectively.

Confirmatory Factor Analysis (CFA)

A CFA was then performed on the second subsample of 142 participants to determine whether the factor structure identified with the intermediate E/CFA stage required modification. The following goodness-of-fit indices cut-points guided the assessment of the degree of model fit: Chi-square; the Comparative Fit Index (CFI: $>.90$ acceptable, $\geq .95$ excellent); Tucker Lewis Index (TLI: $>.90$ acceptable, $\geq .95$ excellent); the Root Means Square Error of Approximation (RMSEA: $<.08$ acceptable, $\leq .05$ excellent); and the Weighted Root-Mean-Square Residual (WRMR: $<.90$ excellent) [36, 37].

Analysis of Invariance

To test whether the best-fitting perceived stress measurement model differed either by self-reported race or by neighborhood context (i.e., clinic membership), a CFA with covariates, or MIMIC (multiple indicators, multiple causes) model was fit. MIMIC is a method for testing structural invariance and involves regressing the latent factors and indicators onto covariates that represent group membership. Factor means are not estimated explicitly in the MIMIC analysis; rather, group mean deviations, or differences, are given by parameter estimates representing the degree of group-specific deviation [36].

Based on preliminary evidence from independent clinic sample t-tests showing a significant difference in mean PSS scores between clinics (data not tabulated), two

separate MIMIC models were examined. The respective model covariates included self-reported color/race (white vs. black/mulatta) and clinic membership, a proxy for potential differences in neighborhood context. Also, all indicator means were fixed to zero to examine modification indices and determine the presence of differential item functioning.

Results

Participants from two clinics were pooled in an overall sample (Table 5.1). Over half (58.3%) of participants ($n_1 = 172$) were recruited in clinic 1. The ages of participants ranged from 16 to 43 years (mean = 25 years, SD = 5.3). The racial distribution was: 27.1% white (*branca*), 17.6% black (*preta*), and 47.5% mulatta (*parda*). No statistically significant differences were noted in age, cohabiting status, maternal education, feelings toward pregnancy, and mean salivary cortisol by self-reported race.

[Table 5.1]

The results in the overall study population confirmed our hypothesized positive correlations between the Brazilian PSS and state ($r = 0.67$, $p < 0.01$) and trait ($r = 0.74$, $p < 0.01$) anxiety, depression ($r = 0.64$, $p < 0.01$), stressful life events ($r = 0.30$, $p < 0.01$), but not with cortisol concentrations ($r = -0.11$, $p > 0.05$) measured cross-sectionally. The same analyses were run by self-reported race, and the magnitude and significance of correlations with the PSS did not differ by racial group (data not shown).

Exploratory Factor Analysis

The EFA resulted in a 12-item scale with a two-factor solution: 6 items measuring a factor consistent with positive perceived stress and 4 items consistent with negative perceived stress, and two cross-loading items. Items 9 and 11 cross-loaded on

both factors under both orthogonal and oblique rotations and were retained for statistical analysis in the E/CFA framework. Items 8 and 12 were removed from the original 14-item measure because of loadings < 0.30 . In contrast to our results, Cohen [38] found that items 4, 5, 12, and 13 had loadings < 0.48 and could therefore be excluded from the full version of the scale.

Table 5.2 presents the items retained and factor loadings obtained with the Geomin rotation. Items loading on factor 1 (positive perceived stress) included items whose content included successful coping and control mechanisms, while items loading on factor 2 (negative perceived stress) included items consistent with adaptation mechanisms in response to hassles and unexpected events.

[Table 5.2]

Modification indices showed model fit strain suggesting that the residual error of items 6 and 10 be allowed to covary. Introducing a path allowing both item residuals to covary was empirically meaningful since the modification index likely represented a method effect from reverse-coding [36]. Thus, the final E/CFA model had 11 items (1, 2, 3, 4, 5, 6, 7, 9, 10, 13, 14), a cross-loading for item 9, a residual covariance between the residuals for items 6 and 10, and acceptable model fit indices ($\chi^2 = 45.431$, $df = 21$, $p = 0.0015$; CFI = 0.940; TLI = 0.954; RMSEA = 0.087; WRMR = 0.589).

Confirmatory Factor Analysis

The two-factor solution derived from the E/CFA framework was cross-validated on the remaining 142 subjects. In the first model run (i.e., the final E/CFA), item 9 no longer loaded significantly on factor 2 (i.e., negative perceived stress), and the residual covariance between items 6 and 10 was no longer statistically significant. No additional

modification indices were suggested by the model, so the second run freed the residual covariance between residual errors 6 and 10 and dropped the cross-loading of item 9 on factor 2. This model yielded excellent model fit indices ($\chi^2 = 31.903$, $df = 27$, $p = 0.2537$; CFI = 0.986; TLI = 0.989; RMSEA = 0.036; WRMR = 0.567). The internal consistency coefficient for the full scale in this sample was $\alpha = 0.83$. However, this model presented evidence of violation to assumptions of discriminant validity between the two latent factors: the freely estimated factor correlation was 0.84, $p < 0.001$, which is at the recommended cutoff (< 0.85) for determination of discriminant validity of the measurement model.

Given that the above model suggested a single latent stress factor, a third CFA model was run constraining the latent factor correlation to be equal to 1, and fit statistics were acceptable ($\chi^2 = 37.516$, $df = 27$, $p = 0.0858$; CFI = 0.970; TLI = 0.977; RMSEA = 0.052; WRMR = 0.636). A χ^2 difference test ($\chi^2_{DIFFTEST}$) was conducted to test the improvement between the constrained model nested within the freely estimated model ($p = 0.033$), and we failed to reject the null hypothesis that the constrained model presented no worse model fit at the $\alpha = 0.01$ level.

Therefore, a parsimonious model with a single latent perceived stress factor was run as the final model for our sample of pregnant women. Figure 5.1 shows the final CFA for the sample. The final CFA model is congeneric and has all statistically significant loadings, no suggestion of model strain in modification indices, and excellent goodness-of-fit statistics ($\chi^2 = 35.790$, $df = 27$, $p = 0.1200$; CFI = 0.975; TLI = 0.981; RMSEA = 0.048; WRMR = 0.610). The modified instrument displays good reliability

and construct validity, given by the internal consistency coefficient ($\alpha=0.807$) and factor loadings.

[Figure 5.1]

Analysis of Invariance

Two separate MIMIC models were run using the modified PSS to test invariance in the study population, entering clinic membership (clinic 1 vs. 2) and self-reported race (white vs. black/mulatta), respectively, as exogenous covariates. There was no evidence of population heterogeneity in perceived stress latent means by clinic membership ($Z = 0.075$, $p = 0.940$, $\chi^2 = 69.801$, $df = 25$, $p < 0.001$; CFI = 0.937; TLI = 0.947; RMSEA = 0.078; WRMR = 0.915).

However, the MIMIC model considering self-reported race ($n = 267$) was statistically significant ($Z = 3.199$, $p = 0.001$), indicating that latent perceived stress factor means differed by race in this study population. The final model fit indices were within acceptable ranges ($\chi^2 = 84.462$, $df = 35$, $p < 0.001$; CFI = 0.925; TLI = 0.947; RMSEA = 0.073; WRMR = 0.872). More directly, in this sample, black/mulatta pregnant women had a latent perceived stress mean that was 0.32 standard deviations higher than white pregnant women.

Discussion

In general, the performance of the Brazilian PSS as applied to pregnant women shows satisfactory results after modification and empirical testing between groups within the study sample. The final model has internal reliability, construct validity, and an expected latent stress factor structure. Our final model showed a similar internal

consistency ($r = 0.807$) to the final reduced version of the validated Brazilian-Portuguese scale ($r = 0.82$) reported by Luft et al [21].

The 14-item scale EFA corresponded to two underlying latent factors, consistent with previous studies evaluating the construct validity of the PSS [3, 21-23]. The factor loadings reported in this study were similar in magnitude and loading pattern to those reported by Luft et al. [21] in a geriatric ambulatory population in Southeast Brazil, and confirms the validity of the scale for use in our study population.

Item 12 had the least salient loadings on either factor, and the item was removed in the first stages of EFA. This result is consistent with findings of Cohen and Williamson [38], and verified by Mimura and Griffiths [22] among students in Japan and Luft et al. [21] in a geriatric population in Brazil. The final model in this analysis resulting in a single latent factor for perceived stress also is consistent with the findings by Luft et al. [21]. Moreover, the single dimensionality of the latent factor structure of a reduced scale version is verified by Cohen and Williamson [38] and Mimura and Griffiths [22]. The comparability of the present study's salient factor loadings with previous studies lends strength to the applicability of the PSS to diverse study populations, specifically, for the study of acute perceived stress in pregnancy.

Strengths of this study include the analysis of categorical data using robust estimation and an intermediate technique (E/CFA) to better guide factor analyses. We also used an efficient technique (i.e., MIMIC) for the examination of population heterogeneity between groups with adequate sample size to conduct the group analyses [36, 39].

The result of population heterogeneity by race is interpreted to be a reflection of Afro-Brazilian pregnant women reporting a higher generalized level of perceived stress. The higher standardized scores of generalized perceived stress among black/mulatta pregnant women may be reflective of the social disadvantage among Brazilian Afro-descendants frequently cited in the Brazilian literature [40-42]. Further research analyzing the specific scale items that drive the observed difference between racial self-classification in our study can further elucidate whether the PSS tends to inflate perceived stress levels among Afro-Brazilian pregnant women or whether there is a specific pattern related to “coping” and “adapting” that differs by self-reported race in this Brazilian setting.

Our results have limited generalizability, given that the study population was drawn from two purposefully-chosen public maternity clinics in Rio de Janeiro. Both clinics served a diverse catchment area, however, and were representative of public outpatient maternity facilities for low-risk pregnancies throughout the municipality. Data from the Brazilian Ministry of Health show that in the period between 2005 and 2007, 69% of live births in public maternity institutions had more than 6 prenatal visits within the public network [43]. We assume that a similar figure applies to women who initiated antenatal care before 20 weeks gestation in the two clinic populations studied, and that our study criteria did not result in substantial selection bias by differentially excluding a large proportion of women who enroll in public facilities for antenatal care after the 20th week of pregnancy.

In this analysis, race was treated as a binary variable (white vs. black/mulatta). This is an analytical limitation; however, collapsing black and mulatta race/color

categories into one is common in the Brazilian epidemiologic literature, particularly for the interpretation and comparability of results with findings from other countries [40, 42, 44]. We explored nested models within each individual racial category in addition to the binary categorization presented; however, small sample sizes constrained our ability to achieve model convergence using the MIMIC framework. Future analyses with additional methods for testing population and structural invariance should be explored to identify the scale items driving the observed difference in stress perception between racial groups.

Another limitation to MIMIC analyses is the issue of fixing the covariate variance to 0 for model convergence. Inherent in this constraint is the assumption that the observed covariate is free of measurement error. For variables that indicate clear group membership, such as clinic membership, this assumption is met. However, the variable of self-reported race may be subject to potential information bias. Bastos and colleagues [45] found differences in respondents' self-classification of race/color based on the perceived race/color of the interviewer. It is unclear to what extent such differential self-reporting of race occurred in our study population and in which direction the self-classification of race would have varied. Race matching between interviewers and respondents in future studies may be a fruitful avenue for further inquiry. Lastly, studies with larger sample sizes can allow for further control of other potential confounders, such as neighborhood level variables, cohabiting status, and correlates of intimate partner violence.

In conclusion, the psychometric characteristics of the Brazilian Portuguese PSS meet the criteria of internal consistency and construct validity, and they exhibit similar

results to the original scale and subsequent validations in diverse international settings. Also, within our study population, the PSS shows no population heterogeneity by clinic population, further strengthening its adequacy in application to various pregnant populations and settings. Future analyses of the application of the PSS should consider the heterogeneity of stressful events and the appraisal of stress level by self-reported race to determine whether Afro-Brazilian pregnant women respond differently to similar stressful events. Longitudinal studies are warranted in order to examine whether this heightened acute stress state from our cross-sectional findings may, in turn, place Afro-Brazilian women at risk for adverse health and pregnancy outcomes associated with increased reported stress in pregnancy.

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Figure 5.1. Final Confirmatory Factor Analysis (CFA) Model of the Cohen Perceived Stress Scale (PSS) in Pregnant Women (n = 142)

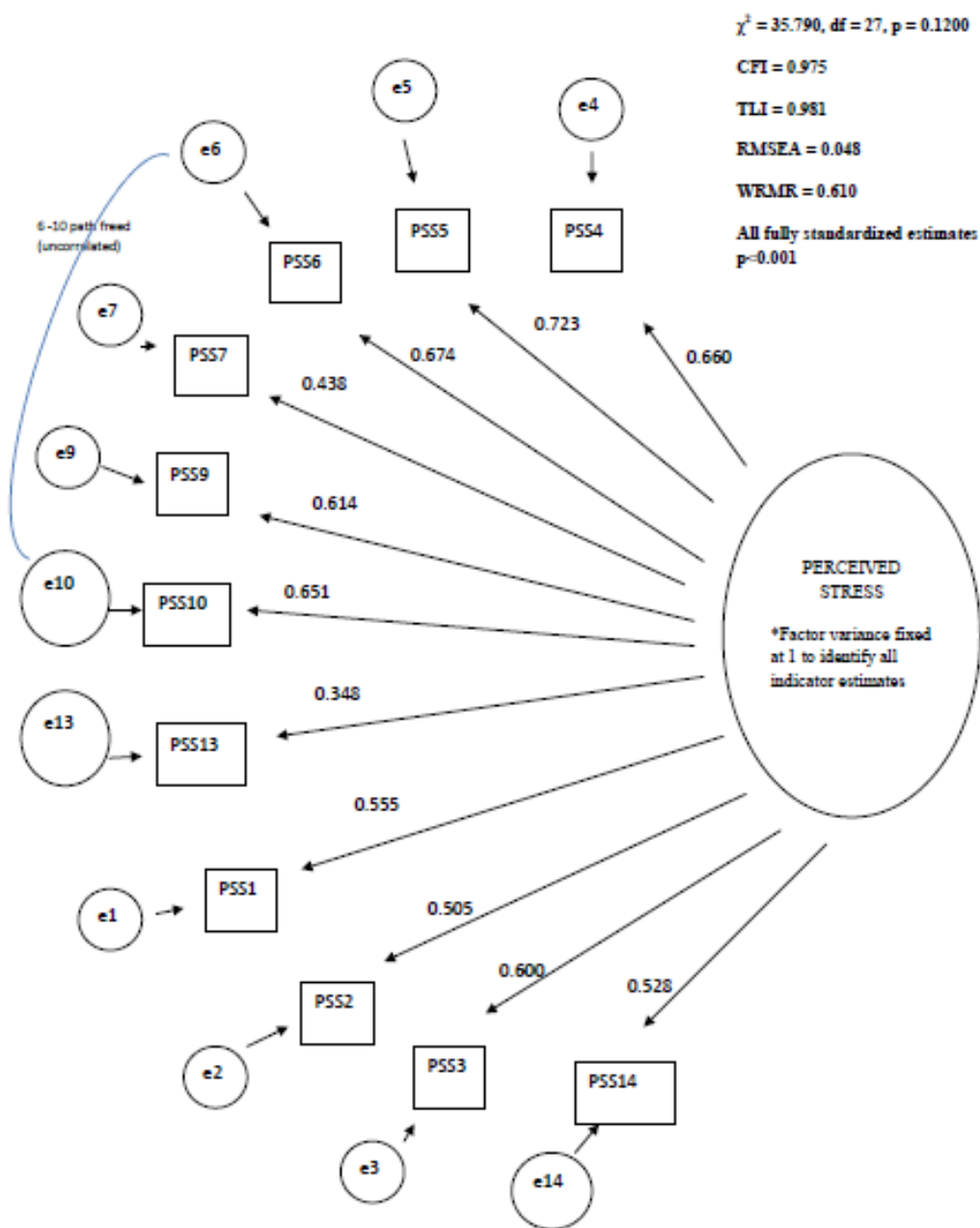


Table 5.1. Characteristics of the study sample (n=295)

Variable	N (%)	White N (%)	Black/Mulatta N (%)	χ^2 p-value
Age				
< 20	39 (13.2)	8 (10.0)	27 (14.1)	0.052
20-29	201 (68.1)	50 (62.5)	136 (70.8)	
30-43	55 (18.6)	22 (27.5)	29 (15.1)	
Clinic				
1	80 (29.4)	47(29.7)	33(28.9)	0.32
2	192 (70.6)	111(70.3)	81(71.1)	
Cohabiting Status				
Yes	224 (76.2)	60 (29.1)	146 (29.2)	0.55
No	70 (23.8)	19 (70.9)	46 (70.8)	
Education				
Elementary/Middle School	70 (23.7)	13 (16.3)	50 (26.0)	0.22
High School	189 (64.1)	57 (71.3)	120 (62.5)	
(Some) College/University	36 (12.2)	10 (12.5)	22 (11.5)	
Gestational Age at Interview				
<=17	80 (27.1)	22 (27.5)	53 (27.6)	0.14
18-21	83 (28.1)	25 (31.3)	47 (24.5)	
22-24	74 (25.1)	24 (30.0)	48 (25.0)	
25+	58 (19.7)	9 (11.3)	44 (22.9)	
Pregnancy Wanted?				
Yes	128 (43.4)	37 (46.3)	83 (43.2)	0.63
Yes, but mistimed	101 (34.2)	24 (30.0)	69 (35.9)	
No	66 (22.4)	19 (23.8)	40 (20.8)	
BV Status				
Negative	169 (57.5)	43 (53.8)	115 (60.2)	0.20
Positive	125 (42.5)	37 (46.3)	76 (39.8)	
Mean Salivary Cortisol	0.42 μ g/dl	0.46 μ g/dl	0.44 μ g /dl	0.064

Table 5.2⁶. Items Retained in the Perceived Stress Scale (PSS) after Exploratory Factor Analyses (EFA) and Respective Factor Loadings (N=153)

Item	Factor 1 ^a	Factor 2 ^b
5. How often have you felt that you were effectively coping with important changes that were occurring in your life? ^c	0.806	
7. How often have you felt that things were going your way? ^c	0.762	
10. How often have you felt that you were on top of things? ^c	0.662	
4. How often have you dealt successfully with irritating life hassles? ^c	0.637	
6. How often have you felt confident about your ability to handle your personal problems? ^c	0.511	
13. How often have you been able to control the way you spend your time? ^c	0.497	
9. How often have you been able to control irritations in your life? ^{c, d}	0.338	0.415
1. How often have you been upset because of something that happened unexpectedly?		0.724
2. How often have you felt that you were unable to control the important things in your life?		0.712
14. How often have you felt difficulties were piling up so high that you could not overcome them?		0.623
3. How often have you felt nervous and "stressed"?		0.529

Loadings on factors <.300 were suppressed and not presented in the table.

a=factor consistent with "positive" perceived stress; "coping" mechanism

b=factor consistent with "negative" perceived stress; "adaptational" mechanism

c = scored in reverse direction

d=cross-loading item retained for further analysis in CFA due to significant cross-loading result in E/CFA ($p < 0.01$)

€=Items 8 and 12 deleted for non-salient loadings (<.3) on either factor; item 11 deleted for non-significant cross-loading (E/CFA). All items theoretically load on factor 2 (negative perception of stress) in original instrument:

8. How often have you found that you could not cope with all the things that you had to do?

11. How often have you been angered because of things that happened that were outside of your control?

12. How often have you found yourself thinking about things that you have to accomplish?

CHAPTER 6

Commentary: Racial differences in salivary cortisol concentration and psychosocial stress scales among pregnant women in Rio de Janeiro, Brazil

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To the Editor:

A number of epidemiologic studies posit the negative influence of maternal psychosocial stress on immune and hormonal functioning in pregnancy, which in turn may heighten susceptibility to infection, affect utero-placental function, impact fetal development, and increase risk for preterm delivery [1-7]. In the U.S., racial disparities have been reported in stressful experiences during pregnancy [6, 8-10], leading to the hypothesis that racial variation in stress may explain disparities of adverse events in pregnancy and birth outcomes by race [11, 12]. Hypothalamic-pituitary-adrenal (HPA) axis dysregulation as a result of lifetime exposure to stress may be marked by blunted cortisol secretion, particularly among Afro-descendants [13]. However, investigations of a direct association between cortisol and psychosocial stress during pregnancy have

reported mixed findings [14-18]. Direct comparisons across study findings are difficult given the differences in instruments that defined and assessed maternal psychosocial stress across studies, different time windows in gestation for stress exposure assessment, adjustment for different demographic and biomedical covariates, and cortisol measurements in single or multiple time points. Empirical support for the theoretical model in which psychosocial stressors stimulate production of biological correlates of stress during pregnancy, which may in turn impair neuroendocrine response and heighten immune susceptibility, is therefore still limited [15].

An important question that arises, therefore, is whether psychosocial instruments commonly used in the perinatal epidemiologic literature to measure theoretical dimensions of stress are able to capture, or are associated with, biomarkers of stress during pregnancy, and whether biomarkers and psychosocial measures of stress vary by race. To examine whether an association exists among selected psychosocial measures and maternal cortisol and whether cortisol levels vary by race, we conducted an analysis in a sample of pregnant women enrolled in prenatal care in two public maternity health centers in Rio de Janeiro, Brazil.

Participants ($n = 234$) were pregnant women with singleton pregnancies in their second trimester of pregnancy recruited from a cross sectional study between May 2008 and July 2009, who provided salivary samples before 10:00 A.M. A standardized interview questionnaire was administered at maternity clinics and collected information on maternal perceived stress, state and trait anxiety, stressful life events, and social support, in addition to maternal sociodemographic factors, reproductive history, current use of alcohol and cigarettes, and the occurrence of physical intimate partner violence

during pregnancy. Clinical chart extraction was conducted using a standardized form to collect obstetric history. Saliva samples were collected with the *Salivette* device (Sarstedt Inc., Numbrecht, Germany) and assayed using a salivary cortisol enzyme immunoassay kit (Diagnostic Solutions Labs, DSL; Webster, TX). All study procedures were approved by the Emory University institutional review board and the committee of ethics of the Sergio Arouca National School of Public Health, FIOCrúz.

Differences in salivary cortisol concentrations by demographic characteristics were tested using t-tests for dichotomous variables and analysis of variance (ANOVA) for categorical variables. Demographic variables included age, race, parity, years of education, cohabitation, and household income *per capita*, while behavioral and reproductive history variables included smoking and alcohol use in pregnancy, pre-pregnancy body weight (kg), previous spontaneous abortion, and preterm delivery. Two-tailed Spearman correlations examined unadjusted bivariate associations between continuous psychosocial measures and salivary cortisol. Given the steady rise in cortisol as gestation progresses, multiple linear regression analyses further examined associations between continuous psychosocial measures and cortisol concentration adjusted for gestational age and race. Salivary cortisol was log transformed for analyses because it was found to not meet normality criteria.

Most women were under 25 years of age, of mixed/mulatta color/race, and lived with their partner. Only 20% of women had fewer than 8 years of education and less than half a monthly minimum wage *per capita*. Smoking during pregnancy was infrequent in this sample, but about one-third of women reported using alcohol during pregnancy. At

interview, median gestational age was 18 weeks, and median salivary concentration was $0.42\mu\text{g/dl} \pm 0.16$ (range 0.08 – 0.87).

Psychosocial measures were uncorrelated with salivary cortisol. Full, published scales, for consistency with external published analyses, of perceived stress, life events, and social support measures had correlation coefficients less than $r = 0.15$. Only state anxiety ($r = -0.21$, $p < 0.01$) and trait anxiety ($r = -0.15$, $p < 0.01$) were both weakly inversely correlated with salivary cortisol. In multiple linear regression modeling cortisol as a function of psychosocial stress measures adjusted for gestational age and color/race, the total model was significant ($F(8, 216) = 2.61$, $p = 0.01$) but explained only 6% of the variance of the cross-sectional salivary cortisol concentration (adjusted $R^2 = 0.06$). State anxiety (continuous score) and black color/race were inversely associated with cortisol ($\beta_{\text{state anxiety}} = -0.20$, $p = 0.04$; $\beta_{\text{black race}} = -0.16$, $p = 0.04$). Including maternal age, years of education, smoking, alcohol use, and cohabiting status as additional covariates in this model did not substantially change regression coefficient results. For parsimony, the final regression model included only state anxiety adjusted for gestational age and color/race as predictors of cortisol concentration. The final total model was significant ($F(4, 218) = 4.52$, $p = 0.002$), explaining 8% of the variance of salivary cortisol concentration (adjusted $R^2 = 0.08$). Accounting for gestational age, state anxiety and black color/race were inversely associated with cortisol concentration, respectively ($\beta_{\text{state anxiety}} = -0.16$, $p = 0.02$ and $\beta_{\text{black race}} = -0.18$, $p = 0.03$). The partial correlation coefficients for state anxiety and black race were -0.16 and -0.15 , respectively, indicating only weak unique associations of these variables with cortisol concentration after accounting for shared variance with gestational age.

Our analyses found few associations between psychosocial stress scale measures and salivary cortisol when measured cross-sectionally during the second trimester of pregnancy, consistent with findings from other studies [14-17, 19-22]. Also, our finding of an inverse relationship between black color/race and cortisol concentration in pregnancy is consistent with previous findings of lower cortisol levels during pregnancy among African American women relative to Hispanic and non-Hispanic white women in two medical research sites in California [13]. At 18 to 20 weeks' and 30 to 32 weeks' gestation, African American women had statistically lower cortisol plasma levels than their counterparts. The findings reported by Glynn et al. [13] imply that cortisol levels during pregnancy in Afro-descendant women differ from white women in the absence of a specific experimental stimulation or stressor. The relatively low cortisol levels observed in African American women is consistent with a possible lifetime exposure to continuous and augmented stress, experiences of discrimination, and socioeconomic disadvantage giving rise to HPA axis dysregulation marked by blunted cortisol secretion among Afro-descendant women in pregnancy [13]. It is possible that such mechanism may operate in Afro-Brazilian women who self-identify with black color/race rather than mixed/mulatta race. Similarly as in the U.S., poorer pregnancy outcomes, as well as lower indices of health services use and quality, have been noted among Afro-descendant Brazilian women relative to their white counterparts [23-27]. Brazilian society is marked by considerable social inequality and variation in socioeconomic indicators across the skin color spectrum [28, 29], and comparisons with U.S. research results may be plausible given the social and historical similarities between the racial contexts of both countries [28]. Further studies employing prospective serial cortisol collection

throughout pregnancy are warranted in Brazilian pregnant populations to better clarify differences in stress endocrine profile by color/race and how, if at all, such patterns are in turn associated with poor pregnancy outcomes.

Our results should be interpreted in light of a number of limitations. Salivary cortisol was assayed only at a single time point in pregnancy, and analyses were limited to morning samples. Cortisol sample collection at another time in the day may result in refined differentiation between groups because of reduced morning baseline variability [15], and not all factors known to affect baseline variation in cortisol (sleeping patterns) were controlled in our analyses. Given that state anxiety and black color/race, though significant model terms, accounted for only 8% of the variance of salivary cortisol concentration in the present analysis, other dimensions of stress and additional factors associated with cortisol concentrations were clearly uncontrolled in our analyses.

Also, with the exception of trait anxiety, which is more closely tied to a heritable construct [16], dimensions of stress measured by validated Brazilian Portuguese psychosocial scales in our analyses may more closely reflect acute, rather than chronic, stress [14, 15, 30] and likely account for the large unexplained variation in salivary cortisol observed. The finding of unclear patterns of association between acute stress and a hypothesized marker of endocrine stress (i.e., cortisol) collected cross sectionally is not unexpected, but contributes a platform for future research perspectives in Brazilian settings. To more completely characterize dimensions of stress, inclusion of culturally-appropriate chronic stress measures resulting from social disadvantage is a fruitful research avenue. That is, given that the use of racial terminology and how it may influence self-reported experiences of discrimination has not been thoroughly examined

in Brazilian study populations, discrimination instruments adapted mostly from the American literature may not necessarily apply in a Brazilian setting [31]. Finally, the cross sectional assessment of stress characterization in our study does not allow for causal interpretations of our data. Future studies with larger sample size and prospective in design are warranted to corroborate the significant associations observed in the present study.

To the best of our knowledge, this is among the first studies considering associations of multiple measures of psychosocial stress, maternal cortisol, and race during pregnancy in a public outpatient Brazilian setting. Further research into field methodology that allows for the reliable collection of serial measurements in this setting is justified, as well as further examination of instruments measuring chronic stress, particularly experiences of discrimination, in a Brazilian context. Future research inquiry confirming the apparent heightened stress state and possible impaired stress reactivity during pregnancy suggested by our cross-sectional findings is warranted to further understand and intervene on Afro-Brazilian women's excess risk of adverse health and pregnancy outcomes.

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CHAPTER 7

DISCUSSION

This study fills a gap in the epidemiologic literature with respect to BV prevalence in pregnancy in an urban Brazilian public healthcare setting. Study findings generate further hypotheses for future studies of BV incorporating sources of maternal stress by race in Brazil. In this Brazilian sample of women in their second trimester of gestation, self-identified black women had a higher prevalence of BV by Amsel's clinical criteria compared to white and mixed women, even after adjusting for potential confounding variables and stressors experienced in pregnancy. The validity of the present associations between self-reported race and BV prevalence in Brazilian women are strengthened by comparable findings from studies in a similar low-resource, public healthcare setting in a large metropolitan area in Brazil [1].

It was hypothesized that prenatal perceived stress, anxiety, life events, and social support would differ by self-reported race and exert a mediating role between the race – BV association in this study sample, following the growing evidence for racial health disparities in Brazil. The present results further confirm the presence of health outcome differences by self-identified race, particularly in the comparisons of women of black and mixed color/race. With regards to my primary study questions, my study presents evidence against the common practice of collapsing black and mixed color categories as a single Afro-descendant category in epidemiological research in Brazilian settings. A statistically significant excess in BV prevalence among black women relative to mixed women was not attenuated by the inclusion of acute stressors and covariates (Table 4.5,

Models 4, 5, 6), which could suggest that different characterizations of stress observed by racial identity may be the products of intersecting dimensions of social stratification. BV, a known risk factor for preterm birth, was a frequent outcome, and considering black and mixed women as members of a single group may obscure the distinct experiences and perceptions associated with race and a health outcome, as well as limit the effectiveness of potential strategies to intervene upon racial disparities in pregnancy outcomes in Brazil. Future inquiry of factors associated with BV in Brazilian women should consider evaluating intersecting dimensions of stress within separate Afro-descendant category. In addition, we note the need for a larger sample size in such studies to have adequate power to study measure effect modification and/or mediating effects of stress by levels of race stratification.

Factors hypothesized to be indicators of largely fixed socioeconomic stressors and chronic stress were not predictive of BV prevalence in this study population. With respect to measures of socioeconomic stressors, the study setting may explain the mostly homogenous social class group in our study, given that Brazilian public maternity clinics in Rio de Janeiro serve women from proximal neighborhoods and urban areas within the municipality belonging to low or middle socioeconomic classes [2].

We note that incomplete measurement of SES and potentially compromised ecological validity of the self-reported 'mixed' racial self-classification as additional important explanations for the lack of association of socioeconomic factors and BV in our sample. While self-classification of black and white race within the Brazilian census scheme is considered reliable [5], 'mixed/mulatta' racial self-classification includes a fluid skin color gradient that may obscure within-group differences across the spectrum

of 'lighter' to 'darker' mixed color/race in Brazil and therefore render misclassified results by racial stratifications [3-7].

A relevant gap in valid, cross-cultural adaptations of instruments for the measurement of racial discrimination in Brazilian contexts was identified in this study. That is, given that the use of racial terminology and how it may influence self-reported experiences of discrimination has not been thoroughly examined in Brazilian study populations, discrimination instruments adapted mostly from the American literature may not necessarily apply in a Brazilian setting [Bastos]. Future studies of racial disparities with respect to bacterial vaginosis and pregnancy outcome would benefit from a multi-dimensional measure of experiences of discrimination that considers individual and contextual metrics appropriate to the study population.

A salient finding consistent in all analyses in this study was the association of acute stress factors with race. As seen in the primary study, among black women, state anxiety was significantly associated with BV prevalence in pregnancy, suggesting that black Brazilian women may have a different set of experiences and perceptions conferred by self-report of racial identity and accompanied social context. The heightened state anxiety reported by black women during pregnancy in this sample raises the possibility of an altered immune and/or inflammatory vaginal milieu that increases the susceptibility to developing BV in pregnancy or difficulty clearing the infection. This could lead to an increased duration of the outcome at the time of our observation of prevalent status. Further research in this setting is needed to assess whether there is a temporal sequence between "exposure" to anxiety in pregnancy and incidence and/or duration of clinical criteria consistent with a BV diagnosis and how, if at all, a history of altered vaginal flora

may affect a pregnancy outcome among women experiencing state anxiety during the second trimester of pregnancy.

The results answering secondary questions 1 and 2 further strengthen the primary study question findings of an association between self-identified black race and BV. The Brazilian Portuguese version of the Perceived Stress Scale was positively correlated with state and trait anxiety and exhibited good internal reliability and construct validity when applied to pregnant women. Not only did the instrument exhibit good internal validity, but my analysis suggests that the interviewer administration of the instrument is valid in a resource-poor setting. Administration of the instrument by trained interviewers in poor populations, where low educational level and literacy may be likely, is deemed valid given the consistency of the instrument's psychometric properties in this population compared to previous reports. The finding of the instrument's population heterogeneity by self-reported race is a reflection of Afro-Brazilian pregnant women reporting a higher mean generalized level of stress during pregnancy.

The higher mean stress among Afro-Brazilian women may be extended to higher prenatal anxiety profile, given that the perceived stress and state and trait anxiety inventory were correlated with the same underlying factor of stress in our study population. The implication is, therefore, that Afro-Brazilian women in this public care setting are at a heightened level of acute stress during pregnancy, in terms of ability to cope with the unexpected and adapt to uncontrollable events as measured by the PSS. Another implication of our results could be that the PSS instrument performs differently by self-reported race in pregnant women. Further research analyzing the specific scale items that drive the observed difference between racial self-classification in our study can

further elucidate whether the PSS tends to inflate perceived stress levels among Afro-Brazilian pregnant women or whether there is a specific pattern related to “coping” and “adapting” (labeled as positive and negative perceptions of stress under the PSS schema) that differs by self-reported race in this Brazilian setting.

Furthermore, regarding secondary study questions 3 and 4, only black race and state anxiety were independently associated with cortisol concentration after adjustment for gestational age. The finding of an inverse relationship between black color/race and salivary cortisol concentration in pregnancy is consistent with previous findings of lower cortisol levels during pregnancy among African American women. Such a cortisol level profile is hypothesized to be consistent with a possible lifetime exposure to continuous and augmented stress, experiences of discrimination, and socioeconomic disadvantage giving rise to HPA axis dysregulation marked by blunted cortisol secretion among Afro-descendant women in pregnancy [8]. A key implication of our study results is that such blunted cortisol expression may also operate in Afro-Brazilian women who self-identify with black color/race rather than mixed/mulatta race. Given the implication of HPA axis dysregulation, black Brazilians may also have a different profile of stress reactivity when challenged with acute sources of psychosocial stress during pregnancy. Gaining a better understanding of this dynamic calls for prospective serial cortisol collection throughout pregnancy to better clarify differences in stress endocrine profile by color/race and how, if at all, such patterns are in turn associated with poor pregnancy outcomes.

The pathways through which self-reported race and stress impact health to promote a clinical profile consistent with bacterial vaginosis in pregnancy are clearly much more complex than the organizing diagram presented in Figure 1, Chapters 2 and 4.

Racial disparities in pathways leading to adverse health outcome in pregnancy may be the end result of multiple interacting factors at the social, individual, and molecular levels [8]. Overall, the findings in this study support hypotheses posited in the U.S. perinatal epidemiologic literature with regards to the associations between markers of maternal stress and BV. Of note is the need for studying racial “non-white” self-categorizations in Brazilian contexts separately, particularly as they pertain to Afro-descendant populations. Also, the present findings are not consistent with a hypothesized mediating effect of latent stress in the color/race – BV relationship; rather, this study suggested the potential effect measure modification of the color/race – BV association by level of maternal stress, supported by a significant difference in the latent stress mean for Afro-Brazilian women relative to white Brazilian women in pregnancy.

Results should be interpreted in light of a number of limitations, the most salient being the inability to establish temporality of exposures with respect to the occurrence of the outcome because of the cross sectional study design. The use of robust Poisson models to directly estimate the prevalence ratio in our primary study analyses is, however, an analytical strength given that prevalence odds ratios from logistic regression models would have overestimated point estimates because the prevalence of BV was relatively high.

Additional limitations to the primary study include the use of Amsel’s clinical criteria to identify bacterial vaginosis in this low-resource, public clinical setting instead of Nugent’s scoring of gram stain (the gold standard method for BV diagnosis in research settings). Though clinical criteria are acceptable, the use of the gold standard method should be contemplated where time and study resources allow. Limitations to the

secondary study analyses include salivary cortisol assay only at a single time point in pregnancy and the fact that not all factors known to affect baseline variation in cortisol (e.g., sleeping patterns) were controlled in our analyses.

Also, study results have limited generalizability, given that the study population comes from patients selected at two purposefully-chosen public maternity clinics in Rio de Janeiro. Both clinics served a diverse catchment area, however, and were representative of public outpatient maternity facilities for low-risk pregnancies throughout the municipality.

In conclusion, my research findings justify more complex characterization, measurement, and analysis of individual and social factors that may differ by racial self-identity and choice of “color” self-report in Brazilian study populations. A salient study implication for future epidemiologic research is that important differences may exist between the perceptions and experiences lived by black and mixed Brazilian women, relative to each other and to white women, and beyond the context of “poverty” measured by low household income and maternal education. Field methodology research that allows for the reliable collection of serial measurements not only of cortisol, but of additional, alternative, and more stable biomarkers consistent with inflammation and oxidative stress, in this setting is warranted, particularly in this urban setting where at-home sample data collection may not always be practical. A salient direction for future stress biomarker research is also more complete endocrine profiling to help elucidate the biological pathways through which cortisol concentration may be consistently lower in individuals with high stress profiles. Future inquiry confirming the apparent heightened stress state and possible impaired stress reactivity during pregnancy suggested by our

cross-sectional findings is warranted to further understand and intervene on Afro-Brazilian women's excess risk of adverse health and pregnancy outcomes. Finally, further examination of culturally-appropriate instruments measuring chronic stress, particularly experiences of discrimination, in a Brazilian context is a fruitful research venue for the more refined study of my proposed relationships between race, stress, and BV in pregnancy.

This is among the first analyses considering the role of self-identified race and stress in the prevalence of BV in this Brazilian setting. Longitudinal study designs examining the hypotheses generated by the present study are warranted in order to examine whether heightened stress state from our cross-sectional findings may, in turn, place Afro-Brazilian women at risk for adverse health and pregnancy outcomes associated with increased reported stress in pregnancy.

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APPENDIX A

ADDITIONAL RESULTS

The following is a more detailed description of the statistical analysis and results addressing secondary questions 3 and 4, which were summarized in a draft commentary in Chapter 6.

Statistical Analysis

Exploratory analyses examined variables for missing data, normality violations, and outliers. Differences in salivary cortisol concentrations by demographic characteristics were tested using t-tests for dichotomous variables and analysis of variance (ANOVA) for categorical variables. Demographic variables included age, race, parity, years of education, cohabitation, and household income *per capita*, while behavioral and reproductive history variables included smoking and alcohol use in pregnancy, pre-pregnancy body weight (kg), previous spontaneous abortion, and preterm delivery. Two-tailed Spearman correlations examined unadjusted bivariate associations between continuous psychosocial measures and salivary cortisol. Given the steady rise in cortisol as gestation progresses, multiple linear regression analyses further examined associations between continuous psychosocial measures and cortisol concentration adjusted for gestational age. Salivary cortisol was log transformed for analyses because it was found to not meet normality criteria. Data were analyzed using SPSS 17.0 [22].

Results

Characteristics of the sample

The characteristics of study participants are detailed in Table A.1. Most women were under 25 years of age, of mixed/mulatta color/race, and lived with their partner. Only one fifth women had fewer than 8 years of education and less than half a monthly minimum wage *per capita*. Smoking during pregnancy was infrequent in this sample, but about one-third of women reported using alcohol during pregnancy. At interview, median gestational age was 18 weeks, and median salivary concentration was $0.42\mu\text{g/dl} \pm 0.16$.

[Table A.1 about here]

Associations between salivary cortisol and demographic and behavioral variables

Table A.2 presents preliminary analyses examining bivariate associations between salivary cortisol concentration ($\mu\text{g/dl}$) and all variables presented in Table A.1. No clear pattern of association between cortisol and demographic, behavioral, and reproductive history variables was observed. Mean cortisol concentrations tended to be higher among women with more than 8 years of education, among non-black women, among women who reported an income of less than half the minimum monthly wage *per capita*, and among women who did use cigarettes or alcohol in pregnancy, though none of these associations were statistically significant at the $\alpha = 0.05$ level. When all maternal demographic, behavioral, and reproductive history variables were entered in a linear

regression model adjusted for gestational age (continuous weeks), color/race (entered as two dummy variables) was the only statistically significant factor associated with cortisol concentration ($\beta = -0.17$, $p = 0.03$ for black color/race). Stepwise removal of combinations of demographic variables did not result in a change greater than 10% in the color/race regression coefficient estimate. Consequently, color/race was retained as a covariate in further analyses in addition to gestational age.

[Table A.2 about here]

Associations between salivary cortisol and psychosocial stress measures

Similarly, bivariate associations between psychosocial stress measures and demographic, behavioral, and reproductive history variables were examined (Table A.3). Mean perceived stress scores were higher among women reporting use of cigarettes and alcohol during pregnancy ($p < 0.05$, respectively), and among women who lived with their partners ($p < 0.05$). In addition, mean state anxiety scores were higher among women who reported use of alcohol in pregnancy ($p < 0.01$) and among women who lived with their partners ($p < 0.05$). Mean trait anxiety scores followed a j-shaped distribution across age categories, with women under 20 and women over 35 years of age having the highest mean trait anxiety scores ($p < 0.05$). Trait anxiety mean scores were also higher among women reporting use of cigarettes and alcohol during pregnancy ($p < 0.05$, respectively), and among women who had less than 8 years of education ($p < 0.05$). No additional patterns of association between psychosocial measures of stress and demographic, behavioral, and reproductive history variables were observed.

[Table A.3 about here]

Cross-sectional continuous psychosocial measures of perceived stress, state anxiety, trait anxiety, and life events were significantly positively correlated (Table A.4), as hypothesized, and Spearman correlation coefficients ranged from 0.21 to 0.73. As expected, social support was inversely correlated with the perceived stress, anxiety, and life events measures. In addition, exploratory factor analyses [23] have previously confirmed the *a priori* hypothesis of stress factors measured by the psychosocial scales in the same population.

[Table A.4 about here]

However, psychosocial measures were largely uncorrelated with salivary cortisol. Perceived stress, life events, and social support measures had correlation coefficients less than $r = 0.15$ (Table A.4). Only state anxiety ($r = -0.21$, $p < 0.01$) and trait anxiety ($r = -0.15$, $p < 0.01$) were both inversely correlated, albeit weakly, with salivary cortisol. In multiple linear regression modeling cortisol as a function of psychosocial stress measures adjusted for gestational age and color/race, the total model was significant ($F(8, 216) = 2.61$, $p = 0.01$) but explained only 6% of the variance of the cross-sectional salivary cortisol concentration (adjusted $R^2 = 0.06$). Given the strong correlation noted between perceived stress with anxiety measures and potential collinearity problems, a second model was run without perceived stress as a predictor. Results were largely unchanged (data not shown). State anxiety (continuous score) and black color/race were inversely associated with cortisol ($\beta_{\text{state anxiety}} = -0.20$, $p = 0.04$; $\beta_{\text{black race}} = -0.16$, $p = 0.04$). Including maternal age, years of education, smoking, alcohol use, and cohabiting status as additional covariates in this model did not substantially change regression coefficient results. For parsimony, the final regression model included only state anxiety adjusted for

gestational age and color/race as predictors of cortisol concentration. The final total model was significant ($F(4, 218) = 4.52, p = 0.002$), explaining 8% of the variance of salivary cortisol concentration (adjusted $R^2 = 0.08$). Accounting for gestational age, state anxiety and black color/race were inversely associated with cortisol concentration, respectively ($\beta_{\text{state anxiety}} = -0.16, p = 0.02$ and $\beta_{\text{black race}} = -0.18, p = 0.03$). The partial correlation coefficients for state anxiety and black race were -0.16 and -0.15 , respectively, indicating only weak unique associations of these variables with cortisol concentration after accounting for shared variance with gestational age.

In a sub-analysis (data not shown) for black respondents only ($n=46$), unadjusted for gestational age, state anxiety was dichotomized at the theoretical cut point of 40 (i.e., low/medium low vs. medium/high state anxiety) to examine the relationship with cortisol concentrations through independent samples t-tests. Among black women with low/medium low state anxiety ($n = 14$), mean cortisol concentration was $0.49 \mu\text{g/dl}$ ($SD = 0.19$), while among black women with medium high/high state anxiety ($n = 32$), mean cortisol concentration was $0.39 \mu\text{g/dl}$ ($SD = 0.11$). In this unadjusted analysis, black women with medium high/high state anxiety had a significantly mean lower cortisol concentration relative to black women with low/medium low state anxiety ($t_{(44)} = 2.49, p = 0.02$).

Table A.1. Characteristics of a sample of pregnant women between 14-26 weeks' gestation (n = 234) in Rio de Janeiro, Brazil, 2008-2009

Variables	N (%)
Maternal age (years)	
< 20	55 (23.5)
20-24	84 (35.9)
25-29	57 (24.4)
30-34	28 (12.0)
> 35	10 (4.3)
Color/race	
White	56 (23.9)
Black	46 (19.7)
Mixed/Mulatta	132 (56.4)
Education	
≤ 8 years	54 (23.1)
> 8 years	180 (76.1)
Household income per capita¹	
< ½ minimum monthly salary	51 (21.8)
≥ ½ minimum monthly salary	179 (76.5)
Cohabitation²	
No	54 (23.1)
Yes	179 (76.5)
Smoking in pregnancy	
No	216 (92.3)
Yes	18 (7.7)
Alcohol in pregnancy	
No	166 (70.9)
Yes	68 (29.1)
Pre-pregnancy weight (kg)	
51 – 59	119 (50.9)
60 – 75	58 (24.8)
> 75	57 (24.4)
Parity	
0	106 (45.3)
1	70 (29.9)
> 2	58 (24.8)
Previous spontaneous abortion³	
0	10 (4.3)
1	34 (14.5)
> 2	4 (1.7)
Previous preterm delivery⁴	
No	107 (45.7)
Yes	21 (9.0)

¹Household income per capita < ½ minimum monthly wage (minimum wage R\$465, approx. U.S.\$260; 2007 Legislative Assembly of Rio de Janeiro, Rio de Janeiro). Missing observations = 4

²Missing observation = 1

³Among 48 women who reported a pregnancy loss out of 128 women who reported a pregnancy prior to index pregnancy

⁴Among 128 women who reported a pregnancy prior to index; missing observation = 1

Table A.2. Cortisol concentration by demographic characteristics in a sample of pregnant women between 14-26 weeks' gestation (n=234) in Rio de Janeiro, Brazil, 2008-2009

Variables	Salivary cortisol (µg/dL) [*]		
	Mean	SD ^{**}	p-value
Maternal age (years)			
< 20	0.45	0.16	0.61
20-24	0.46	0.17	
25-29	0.49	0.22	
30-34	0.50	0.18	
≥ 35	0.42	0.19	
Color/race			
White	0.48	0.17	0.28
Black	0.43	0.16	
Mixed/Mulatta	0.47	0.19	
Education			
≤ 8 years	0.43	0.16	0.06
> 8 years	0.48	0.19	
Household income per capita[†]			
< ½ minimum monthly salary	0.48	0.18	0.11
≥ ½ minimum monthly salary	0.43	0.18	
Cohabitation[‡]			
No	0.47	0.22	0.97
Yes	0.47	0.17	
Smoking in pregnancy			
No	0.47	0.18	0.41
Yes	0.43	0.13	
Alcohol in pregnancy			
No	0.48	0.18	0.15
Yes	0.44	0.17	
Pre-pregnancy weight (kg)			
51 – 59	0.45	0.17	0.53
60 – 75	0.48	0.18	
> 75	0.48	0.20	
Parity			
0	0.46	0.17	0.36
1	0.48	0.18	
> 2	0.44	0.02	
Previous spontaneous abortion[§]			
0	0.43	0.13	0.53
1	0.44	0.19	
> 2	0.48	0.19	
Previous preterm delivery[¶]			
No	0.47	0.18	0.14
Yes	0.41	0.14	

^{*} Limited to samples collected between 7:45am and 10am.

^{**} SD = Standard deviation.

[†] Household income per capita < ½ minimum monthly wage (minimum wage R\$465, approx. U.S.\$260; 2007 *Legislative Assembly of Rio de Janeiro, Rio de Janeiro*). Missing observations = 4

[‡] Missing observation = 1

[§] Among 48 women who reported a pregnancy loss out of 128 women who reported a pregnancy prior to index pregnancy

[¶] Among 128 women who reported a pregnancy prior to index; missing observation = 1

Table A.3. Measures of psychosocial stress by demographic and medical factors in a sample of pregnant women between 14-26 weeks' gestation (n = 234) in Rio de Janeiro, Brazil, 2008-2009

	Perceived Stress			State Anxiety			Trait Anxiety			Life Events			Social Support		
	Mean	SD**	P-value	Mean	SD	P-value	Mean	SD	p-value	Mean	SD	P-value	Mean	SD	p-value
Maternal age (years)															
< 20	26.61	12.12	0.25	46.89	8.25	0.17	48.61	8.30	0.02	1.39	1.41	0.82	63.66	12.84	0.17
20-24	23.67	9.48		45.70	8.54		44.45	9.41		1.57	1.60		61.09	14.20	
25-29	22.86	9.10		43.28	8.33		44.54	7.90		1.65	1.69		62.55	13.66	
30-34	23.86	9.45		43.75	8.46		45.14	8.26		1.29	1.18		60.00	12.78	
≥ 35	24.70	7.99		46.20	11.56		50.60	12.55		1.60	1.78		52.50	16.52	
Color/race															
White	23.41	8.34	0.67	43.98	8.28	0.47	44.66	8.70	0.54	1.63	1.35	0.75	63.72	10.56	0.26
Black	25.04	7.81		45.19	8.22		45.83	7.87		1.40	1.60		32.55	12.66	
Mixed/Mulatta	24.28	9.97		45.65	8.86		46.25	9.46		1.51	1.60		60.33	15.20	
Education															
≤ 8 years	26.21	8.66	0.06	46.27	7.41	0.27	48.57	7.87	0.01	1.45	1.72	0.70	59.38	15.40	0.17
> 8 years	23.62	9.29		44.83	8.91		44.94	9.13		1.54	1.48		62.25	13.20	
Household income per capita¹															
< ½ minimum monthly salary	25.42	9.01	0.26	46.38	7.63	0.23	47.12	8.48	0.22	1.68	1.66	0.41	58.23	16.04	0.05
≥ ½ minimum monthly salary	23.79	9.18		44.78	8.81		45.36	9.11		1.48	1.51		62.54	13.00	
Cohabitation²															
No	23.41	8.63	0.01	44.50	8.03	0.04	47.80	10.00	0.06	1.51	1.56	0.89	61.72	13.57	0.76
Yes	26.93	10.52		47.23	10.06		45.15	8.59		1.48	1.45		61.07	14.62	
Smoking in pregnancy															
No	23.86	9.12	0.03	44.98	8.61	0.24	45.39	8.84	0.02	1.48	1.51	0.22	61.96	13.66	0.13
Yes	28.67	9.17		47.44	8.27		50.50	9.51		1.94	1.83		56.83	14.54	
Alcohol in pregnancy															
No	23.45	9.13	0.04	43.86	7.82	< 0.01	44.64	8.98	< 0.01	1.42	1.51	1.44	62.16	13.83	0.31
Yes	26.10	9.13		48.34	9.56		48.57	8.39		1.74	1.59		60.16	13.60	
Pre-pregnancy weight (kg)															
51 – 59	24.05	8.72	0.82	45.46	8.44	0.58	45.84	8.69	0.63	1.35	1.37	0.20	61.32	12.72	0.73
60 – 75	23.95	8.86		44.18	7.74		44.93	8.87		1.62	1.42		62.74	12.53	
> 75	24.88	10.55		45.59	9.75		46.54	9.71		1.76	1.92		60.86	16.98	

Table A.3 continued.

	Perceived Stress			State Anxiety			State Anxiety			Life Events			Social Support		
	Mean	SD	p-value	Mean	SD	p-value	Mean	SD	p-value	Mean	SD	p-value	Mean	SD	p-value
Parity															
0	23.53	8.97	0.23	44.82	8.29	0.70	45.20	8.90	0.58	1.37	1.47	0.23	63.59	12.39	0.11
1	25.77	9.41		45.88	9.12		46.66	8.56		1.52	1.30		60.36	14.62	
> 2	23.59	9.25		44.93	8.57		45.81	9.65		1.79	1.88		59.29	14.80	
Previous spontaneous abortion ³															
0	24.50	9.25	0.20	47.70	9.60	0.47	50.10	9.69	0.20	2.40	2.59	0.61	56.90	15.76	0.35
1	23.83	8.91		45.71	9.40		45.85	9.33		1.94	1.49		62.77	10.01	
> 2	28	13.00		41.67	6.11		12.34	7.13		2.00	1.00		66.00	3.61	
Previous preterm delivery ⁴															
No	24.93	9.61	0.73	45.59	8.86	0.70	46.31	8.74	0.93	1.56	1.52	0.20	59.32	14.36	0.32
Yes	24.14	8.15		44.76	9.03		46.10	10.76		2.05	1.86		62.81	16.18	

** SD = Standard deviation

¹Household income per capita < ½ minimum monthly wage (minimum wage R\$465, approx. U.S.\$260; 2007 Legislative Assembly of Rio de Janeiro, Rio de Janeiro). Missing observations = 4

²Missing observation = 1

³Among 48 women who reported a pregnancy loss out of 128 women who reported a pregnancy prior to index pregnancy

⁴Among 128 women who reported a pregnancy prior to index; missing observation = 1

Table A.4. Spearman correlations among stress measures in a sample of pregnant women between 14-26 weeks' gestation (n = 234) in Rio de Janeiro, Brazil, 2008-2009

	Perceived Stress	State Anxiety	Trait Anxiety	Life Events	Social Support	Salivary Cortisol
Perceived Stress	1.00					
State Anxiety	0.66 *	1.00				
Trait Anxiety	0.73 *	0.66 *	1.00			
Life Events	0.26 *	0.25 *	0.21 *	1.00		
Social Support	-0.45 *	-0.44 *	-0.52 *	-0.19 *	1.00	
Salivary Cortisol	-0.11	-0.21 *	-0.15 **	0.05	0.03	1.00

* Correlation is significant at the 0.01 level (2-tailed)

** Correlation is significant at the 0.05 level (2-tailed)

APPENDIX B



TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Prezada _____

Você está sendo convidada a participar do projeto de pesquisa: “Caracterização de fatores psicológicos e biológicos de estresse materno e associação com vaginose bacteriana na gravidez”, da Fundação Oswaldo Cruz. Você foi selecionada por ter mais de 16 anos e estar com menos de sete meses de gestação. Você tem o direito de pedir outros esclarecimentos sobre a pesquisa e pode se recusar a participar ou interromper a sua participação a qualquer momento, sem que isto lhe traga qualquer prejuízo.

O estudo pretende avaliar algum tipo de estresse possivelmente presente na gestação, bem como fatores que contribuem para o bem-estar de você e seu bebê durante a gravidez. A sua participação irá contribuir para acrescentar ao nosso conhecimento de saúde da gestante, sem qualquer risco envolvido.

Gostaríamos de pedir o seu consentimento para fazer uma entrevista. Sua participação nesta pesquisa consistirá em responder a um questionário sobre suas condições de moradia, nível de instrução, sua história reprodutiva e comportamentos que influenciam na sua saúde. Além disso, o questionário inclui perguntas sobre seu nível socioeconômico, estresse, ansiedade e outras características do ambiente em que você vive. Não existe nenhum risco relacionado com a sua participação nesta pesquisa. O tempo de duração da entrevista será de 30 min., aproximadamente. Também gostaríamos de fazer um exame especular como parte de sua rotina de pré-natal na clínica sem custo nenhum. Os resultados dos exames serão acrescentados ao cartão de gestante.

Finalmente, gostaríamos de realizar uma pequena coleta de saliva. Você receberá 2 tubos plásticos e algodões para fazer a coleta de saliva na clínica. A coleta de saliva consiste só em mastigar levemente o algodão por um ou dois minutos. O algodão só contém material para preservar sua saliva e não apresenta nenhum risco para você ou seu bebê. Depois de mastigado, o algodão só precisa ser guardado no tubo plástico e entregue à entrevistadora.

As informações que você nos der serão mantidas em segredo e não serão divulgadas em qualquer hipótese. Os resultados do estudo serão apresentados em conjunto, não sendo possível identificar os indivíduos que dele participaram.

Eu,

_____, declaro ter sido informada e concordo em participar, como voluntária, desta pesquisa.
[Representante legal, em caso de mãe adolescente (menor de 18 anos) não emancipada]

Assinatura da paciente ou seu responsável legal:-

Rio de Janeiro, ____ / ____ / _____

Para maiores esclarecimentos, entrar em contato com: Dra. Silvana Granado Nogueira da Gama.
Endereço: Rua Leopoldo Bulhões 1480, sala 808, Manguinhos. Tel.: 2598-2620.

Comitê de Ética e Pesquisa da Escola Nacional de Saúde Pública Sergio Arouca

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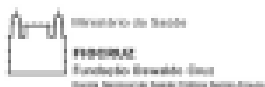
O horário de atendimento ao público do CEP/ENSP é de 14:00 às 17:00

Informações adicionais no caso de recusa da mãe em participar da pesquisa:

Motivo da Recusa:

Idade Materna: _____

Raça/Cor Materna:



"Caracterização de fatores psicológicos e biológicos de estresse materno e associação com vaginose bacteriana na gravidez"

Questionário nº

2008

Entrevista com a Gestante

QUESTIONÁRIO

INSTRUÇÕES PARA PREENCHIMENTO:

Para todo questionário, preencher 00 para não se aplica e 99 para não informado.

BLOCO A

I IDENTIFICAÇÃO DO QUESTIONÁRIO

1. Nome da Unidade: <i>Maternidade PRO MATRI</i>	
2. Nº do Frontalário <input style="width: 80%; border: none; border-bottom: 1px solid black;" type="text"/>	
3. Data da entrevista <input style="width: 40%; border: none; border-bottom: 1px solid black;" type="text"/> / <input style="width: 10%; border: none; border-bottom: 1px solid black;" type="text"/> / <input style="width: 10%; border: none; border-bottom: 1px solid black;" type="text"/>	4. Entrevistador <input style="width: 30%; border: none; border-bottom: 1px solid black;" type="text"/>
5. Trabalho em	6. Data de início <input style="width: 40%; border: none; border-bottom: 1px solid black;" type="text"/> / <input style="width: 10%; border: none; border-bottom: 1px solid black;" type="text"/> / <input style="width: 10%; border: none; border-bottom: 1px solid black;" type="text"/>
7. Capital em	8. Capital <input style="width: 20%; border: none; border-bottom: 1px solid black;" type="text"/>

II IDENTIFICAÇÃO DA MULHER E DADOS SOCIO-DEMOGRÁFICOS

"Não vamos fazer algumas perguntas sobre você, sua família e sua residência"

9. Qual o seu nome?	
10. Qual a data de seu nascimento? <input style="width: 40%; border: none; border-bottom: 1px solid black;" type="text"/> / <input style="width: 10%; border: none; border-bottom: 1px solid black;" type="text"/> / <input style="width: 10%; border: none; border-bottom: 1px solid black;" type="text"/>	
11. Qual a sua idade? <input style="width: 20%; border: none; border-bottom: 1px solid black;" type="text"/> anos	
12. Qual o seu endereço completo?	
13. Bairro	14. Município/UF
15. Ponto de referência	
16. Telefone(s) para contato <input style="width: 70%; border: none; border-bottom: 1px solid black;" type="text"/> <input style="width: 70%; border: none; border-bottom: 1px solid black;" type="text"/> (residência)	

17. Qual o seu estado civil? 1. Casada/Com Companheiro 2. Separada/Divorciada 3. Viúva 4. Solteira						__
18. Em sua opinião, qual é a sua cor/raça?						
1. Branca 2. Preta 3. Parda(morena/mulata) 4. Amarela 5. Indígena						__
19. Quem mora na sua casa?						
Nome	Idade	Grau de Parentesco	Ocupação	Renda mensal(R\$)	Assinale quem é o chefe da família	
<small>Grau de parentesco: 1- o próprio 2- cônjuge/companheiro 3- Filho(a) 4- Pai/mãe 5- Sogra(a) 6- Outro parente 7- Não é parente Ocupação: 1- Servidor público 2- Empregado não servidor público 3- Trabalha por conta própria 4- Aposentado 5- Desempregado 6- Dona-de-casa 7- Estudante 8- Pensionista</small>						
20. Em que município e estado você nasceu?						
Município: _____ Estado: _____ País: 1. Brasil 2. Outro						__
21. Há quanto tempo você mora, sem interrupção, em seu município atual de residência?						__ anos __ meses
22. Há quanto tempo você mora em seu endereço atual?						__ anos __ meses
23. Você sabe ler e escrever? 0- Não 1- Sim 2- Mais ou menos						__
24. Qual foi a última série que você completou na escola?						
__ Série Superior		__ 1 - Fundamental		2 - Médio		3 -
(0 - nunca estudou)		(1º grau)		(2º grau)		(3º grau)
III. ANTECEDENTES OBSTÉTRICOS <i>"Agora vamos fazer algumas perguntas sobre suas gestações anteriores"</i>						
25. Quantas vezes você já esteve grávida, antes dessa gravidez? (Atenção, incluir também os abortos) se nenhuma vá para a pergunta 35.						__ __

26. Qual a sua idade quando engravidou pela primeira vez?	____
27. Você realizou pré-natal em todas as gestações? 0- Não, em nenhuma 1- Não, apenas em algumas 2- Sim, em todas	____
28. Alguma gravidez resultou em aborto? (considerar aborto a perda fetal com menos de 500g ou idade gestacional menor que 22 semanas) 0- Não (Vá à questão 31) 1- Sim	____
29. Quantos foram espontâneos?	____
30. Quantos foram provocados?	____
31. Algum dos filhos nasceu morto? Quantos?	____
32. Você já teve algum bebê que tenha nascido com peso menor que 2500g? 0- Não (Vá à questão 34) 1- Sim	____
33. Quantos bebês nasceram com peso menor que 2500g?	____
34. Quando foi seu último parto/aborto, antes desta gravidez? (mês/ano)	____/____ ____
35. Você já teve algum bebê que nasceu antes do 9º mês? 0- Não 1- Sim	____

IV. HISTÓRIA DA GRAVIDEZ ATUAL

"Agora vamos fazer algumas perguntas sobre o seu pré-natal nesta gravidez:"

36. Qual foi a data da sua última menstruação? (usar 00/00/00 quando não souber)	____/____/____
37. No momento, você está com quanto tempo de gravidez? ____ meses ____ semanas	____/____ ____
38. Com quanto tempo de gravidez você começou o pré-natal? ____ meses ____ semanas	____/____ ____
39. Quantas consultas você já teve desde que começou o pré-natal?	____
40. Quantas ultra-sonografias você fez até o momento?	____
41. Qual era o seu peso antes de ficar grávida? 999 - Não sabe	____ ____

42. Você já teve pressão alta nesta gestação?	0-Não 1-Sim 2-Não sabe	<input type="text"/>
43. Você já teve diabetes gestacional?	0-Não 1-Sim 2-Não sabe	<input type="text"/>
44. Você teve alguma infecção na urina durante a gravidez?	0-Não 1-Sim 2-Não sabe Se não, vá para a questão 46.	<input type="text"/>
45. Foi prescrito algum antibiótico para você?	0-Não 1-Sim 2-Não lembra	<input type="text"/>
46. Você fez xarapá antes de engravidar?	0-Não 1-Sim	<input type="text"/>
47. Você fumou durante a gravidez?	0-Não (vá à questão 48) 1-Sim	<input type="text"/>
48. Quantos cigarros você fumou, por dia?		<input type="text"/> <input type="text"/> <input type="text"/> cigarros
49. Você usou algum tipo de droga durante a gravidez?	0-Não (vá à questão 51) 1-Sim	<input type="text"/>
50. Que tipo? _____		
51. Você ingeriu bebidas alcoólicas durante a gravidez?	0-Não (vá à questão 54) 1-Sim	<input type="text"/>
52. Caso tenha ingerido, qual a frequência? 1-Raras vezes 2-Final de semana 3-Freqüentemente 4-Diariamente		<input type="text"/>
53. Qual o tipo de bebida que você costumava beber? 1-Whisky/cachaça 2-Vinho 3-Cerveja 4-Outras		<input type="text"/>
54. Quando ficou grávida, você: 1- Estava querendo engravidar 2- Quería esperar mais um tempo 3- Não queria mais engravidar		<input type="text"/>
55. Você estava utilizando algum método para evitar gravidez?	0-Não(vá à questão 57) 1-Sim	<input type="text"/>
56. Caso tenha utilizado, qual o método? 1- Pílulas Anticoncepcionais 2- Injeção Hormonal 3- Dispositivo Intrauterino (DIU) 4- Diafragma 5- Camisinha (masculina ou feminina) 6- Coito interrompido 7- Tabela 8- Cremes Espermicidas 9- Outro: _____		<input type="text"/>

57. Você se sente apalada pelo pai do bebê durante esta gestação?	0-Não	1-Sim	<input type="text"/>
58. Durante esta gravidez, você já foi alguma vez maltratada emocionalmente (com palavras) pelo seu companheiro ou alguém importante para você?	0-Não	1-Sim	<input type="text"/>
59. Durante esta gravidez, você já foi alguma vez maltratada fisicamente pelo seu companheiro ou alguém importante para você?	0-Não	1-Sim	<input type="text"/>
60. Desde que engravidou esta vez, alguém lhe bateu, arbofeteou, chutou ou machucou fisicamente?	0-Não	1-Sim	<input type="text"/>
61. Caso afirmativo, quem foi?			

BLOCO B

"As próximas perguntas referem-se a sentimentos e pensamentos durante o ÚLTIMO MÊS. Em cada caso, será pedido você indicar o quão frequentemente você tem se sentido de uma determinada maneira. Embora algumas das perguntas sejam similares, há diferenças entre elas e você deve analisar cada uma como uma pergunta separada. A melhor abordagem é responder a cada pergunta razoavelmente rápido. Isto é, não tente contar o número de vezes que você se sente de uma maneira particular, mas indique a alternativa que lhe pareça como uma alternativa razoável."

Neste último mês, com que frequência...	Preencha a coluna com:	
	0-Nunca	1-Às vezes
	1-Raramente	4-Sempre
	2-Quase Nunca	
62. Você tem ficado triste por causa de algo que aconteceu inesperadamente?		<input type="text"/>
63. Você tem se sentido incapaz de controlar as coisas importantes em sua vida?		<input type="text"/>
64. Você tem se sentido nervosa ou "estressada"?		<input type="text"/>
65. Você tem tratado com sucesso os problemas difíceis da vida?		<input type="text"/>
66. Você tem sentido que está lidando bem com as mudanças importantes que estão ocorrendo na sua vida?		<input type="text"/>
67. Você tem se sentido confiante na sua habilidade de resolver problemas pessoais?		<input type="text"/>
68. Você tem sentido que as coisas estão acontecendo de acordo com a sua vontade?		<input type="text"/>
69. Você tem achado que não conseguiria lidar com todas as coisas que você tem que fazer?		<input type="text"/>

70. Você tem conseguido controlar as irritações em sua vida?	[...]
71. Você tem sentido que as coisas estão sob o seu controle?	[...]
72. Você tem ficado limitada porque as coisas que acontecem estão fora do seu controle?	[...]
73. Você tem se encontrado pensando sobre as coisas que deve fazer?	[...]
74. Você tem conseguido controlar a maneira como gasta seu tempo?	[...]
75. Você tem sentido que as dificuldades se acumulam ao ponto de você acreditar que não pode superá-las?	[...]

BLOCO C

"Agora vou fazer perguntas sobre como você SE SENTE HOJE. Não pense por muito tempo numa única pergunta. Tente dar a resposta que mais se aproxima de como você SE SENTE HOJE."

Preencha a coluna com:	1-Abolutamente não 2-Um pouco	3-Muito 4-Muito pouco
76. Sinto-me calma		[...]
77. Sinto-me segura		[...]
78. Estou tensa		[...]
79. Estou arrependida		[...]
80. Sinto-me à vontade		[...]
81. Sinto-me perturbada		[...]
82. Estou preocupada com possíveis infortúnios		[...]
83. Sinto-me descansada		[...]
84. Sinto-me ansiosa		[...]
85. Sinto-me "em casa"		[...]
86. Sinto-me confiante		[...]
87. Sinto-me nervosa		[...]
88. Estou agitada		[...]
89. Sinto-me uma pilha de nervos		[...]

90. Estou descontrolada	<input type="text"/>
91. Sinto-me satisfeita	<input type="text"/>
92. Estou preocupada	<input type="text"/>
93. Sinto-me super excitada e confusa	<input type="text"/>
94. Sinto-me alegre	<input type="text"/>
95. Sinto-me bem	<input type="text"/>

"*Agora vai fazer perguntas sobre como você GERALMENTE SE SENTE. Não pense por muito tempo sobre cada pergunta. Tente dar a resposta que mais se aproxima de como você SE SENTE GERALMENTE."*

Preencha a coluna com:	1-Quase nunca 2-Ar vezes	3-Freqüentemente 4-Quase sempre
96. Sinto-me bem	<input type="text"/>	<input type="text"/>
97. Canso-me facilmente	<input type="text"/>	<input type="text"/>
98. Tenho vontade de chorar	<input type="text"/>	<input type="text"/>
99. Gostaria de poder sentir-se feliz quanto os outros parecem ser	<input type="text"/>	<input type="text"/>
100. Perco oportunidades porque não consigo tomar decisões rapidamente	<input type="text"/>	<input type="text"/>
101. Sinto-me descontrolada	<input type="text"/>	<input type="text"/>
102. Sinto-me calma, ponderada e segura de mim mesma	<input type="text"/>	<input type="text"/>
103. Sinto que as dificuldades estão se acumulando de tal forma que não as consigo resolver	<input type="text"/>	<input type="text"/>
104. Preocupo-me demais com coisas sem importância	<input type="text"/>	<input type="text"/>
105. Sou feliz	<input type="text"/>	<input type="text"/>
106. Deixo-me afetar muito pelas coisas	<input type="text"/>	<input type="text"/>
107. Não tenho muita confiança em mim mesma	<input type="text"/>	<input type="text"/>
108. Sinto-me segura	<input type="text"/>	<input type="text"/>
109. Evito ter que enfrentar crises ou problemas	<input type="text"/>	<input type="text"/>
110. Sinto-me deprimida	<input type="text"/>	<input type="text"/>

111. Estou satisfeito	_____
112. Às vezes ideias sem importância me entram na cabeça e ficam me preocupando	_____
113. Levo os desapontamentos tão a sério que não consigo tirá-los da cabeça	_____
114. Sou uma pessoa ativa	_____
115. Fico tenso e perturbado quando penso em meus problemas do momento	_____

BLOCO D

"Responda a frequência com que as condições descritas abaixo estiveram presentes em sua vida durante a quarentena."

Preenchida a coluna com:	1-Quase nunca	2-Às vezes	3-Com frequência
116. Eu me senti triste	_____	_____	_____
117. Senti solidão	_____	_____	_____
118. Senti que estava deprimido	_____	_____	_____
119. Senti que não poderia sair da tristeza mesmo com a ajuda de familiares e amigos	_____	_____	_____
120. Pensei em cometer suicídio	_____	_____	_____
121. Fiz alguma tentativa de cometer suicídio	_____	_____	_____

BLOCO E

"As próximas perguntas referem-se a alguns acontecimentos ou situações desagradáveis que podem ter ocorrido com você"

	a) 0-Não (se não, vá à seguinte pergunta) 1-Sim, uma vez 2-Sim, mais de uma vez	b)(Se Sim), quando foi o ÚLTIMO VÍZ que isso aconteceu? 1-Há menos de 1 mês 2-Entre 1 e 6 meses atrás 3-Entre 7 e 12 meses atrás
122. Nos últimos 12 meses, você teve algum problema de saúde que o impediu de realizar alguma de suas atividades habituais (trabalho, estudo, ou lazer) por mais de um mês?	_____	_____

123. Nos últimos 12 meses, você esteve internada em hospital por uma noite, ou mais, em razão de doença ou acidente?	_____	_____
124. Nos últimos 12 meses, faleceu algum parente próximo seu (pai, mãe, cônjuge, companheiro, filho ou irmão)?	_____	_____
125. Nos últimos 12 meses, você enfrentou dificuldades financeiras mais severas do que as habituais?	_____	_____
126. Nos últimos 12 meses, você foi forçada a mudar de casa contra sua vontade (por exemplo, por aumento de aluguel)?	_____	_____
127. Nos últimos 12 meses, você foi ferida com uma arma de fogo (revolver, escopeta, pistola, etc) ou arma branca (faca, navalha, etc)?	_____	_____
128. Nos últimos 12 meses, você foi assaltada ou roubada, isto é, teve dinheiro ou algum bem tomado, mediante uso ou ameaça de violência?	_____	_____
129. Nos últimos 12 meses, você passou por algum rompimento de relação amorosa, incluindo divórcio ou separação?	_____	_____
130. Alguma vez na vida experimentou discriminação, não lhe foi permitido fazer algo, a incomodaram ou fizeram com que se sentisse inferior em alguma das seguintes situações devido à sua raça ou cor?		
a. Em seu colégio ou faculdade?	0. Não 1. Sim	a
b. Em seu local de trabalho?	0. Não 1. Sim	b
c. Ao obter ou alugar casa?	0. Não 1. Sim	c
d. Ao obter assistência médica?	0. Não 1. Sim	d
e. Em locais públicos, como comércio, banco, etc.?	0. Não 1. Sim	e
f. Ao obter crédito, empréstimos bancários ou hipotecários?	0. Não 1. Sim	f
g. Na rua, em um lugar público?	0. Não 1. Sim	g
h. Com a polícia?	0. Não 1. Sim	h
<i>"Agora, pense na época em que você tinha 12 anos de idade"</i>		
131. Quando você tinha 12 anos de idade, em que tipo de lugar você morava? 1. Capital de estado 2. Cidade pequena ou vila 3. Cidade grande, mas não capital 4. Zona rural		_____
132. Quando você tinha 12 anos de idade, seus pais eram vivos? 0. Não 1. Sim 2. Apenas pai era vivo 3. Apenas mãe era viva		_____

133. Quando você tinha 12 anos de idade, com quem você morava a maior parte do tempo? 1. Com pai e mãe 2. Com mãe 3. Com pai 4. Com outros parentes 5. Em instituição (orfanato ou outra) 6. Não sei	__
134. Como você classifica a situação econômica da sua família, quando você tinha 12 anos de idade, isto é, o padrão de vida de sua família naquela época? 1. Rica 2. Média 3. Pobre 4. Muito pobre	__
135. Quando você tinha 12 anos de idade, em sua casa, algum adulto batia com força nas crianças? 0. Não 1. Sim, com frequência 2. Sim, às vezes 3. Sim, raramente	__
136. Quando você tinha 12 anos de idade, em sua casa, houve ocasiões em que se deixou de comer por falta de dinheiro? 0. Não 1. Sim, com frequência 2. Sim, às vezes 3. Sim, raramente	__
137. Comparado ao padrão de vida que você tem agora, como era o padrão de vida da sua família quando você tinha 12 anos? 1. Melhor 2. Igual 3. Pior	__
138. Com que idade você começou a trabalhar?	_____ anos

BLOCO F

"A seguir, apresentamos situações em que as pessoas podem vir a procurar por outras em busca de companhia, apoio ou ajuda."

Preencha a coluna com:	0-Nunca	1-Quase sempre
	1-Frequentemente	4-Sempre
	2-Às vezes	
<i>"Se você preferir, com que frequência..."</i>		
139. Você conta com alguém que o ajude, se ficar doente?		__
140. Você conta com alguém para lhe ouvir, quando você precisa falar?		__
141. Você conta com alguém para lhe dar bons conselhos em uma situação de crise?		__
142. Você conta com alguém para levá-lo ao médico?		__
<i>"Se você preferir, com que frequência..."</i>		
143. Você conta com alguém que demonstre amor e afeto por você?		__
144. Você conta com alguém para se divertir junto?		__

145.	Você conta com alguém para lhe dar informação que a ajude a compreender uma determinada situação?	<input type="text"/>	
146.	Você conta com alguém em quem confiar ou para falar de você ou sobre seus problemas?	<input type="text"/>	
<i>"Se você precisar, com que freqüência..."</i>			
147.	Você conta com alguém que lhe dê um abraço?	<input type="text"/>	
148.	Você conta com alguém com quem relaxar?	<input type="text"/>	
149.	Você conta com alguém para preparar suas refeições, se você não puder prepará-las?	<input type="text"/>	
<i>"Se você precisar, com que freqüência..."</i>			
150.	Você conta com alguém de quem você realmente quer conselhos?	<input type="text"/>	
151.	Você conta com alguém com quem distrair a cabeça?	<input type="text"/>	
152.	Você conta com alguém para ajudá-la nas tarefas diárias, se você ficar doente?	<input type="text"/>	
153.	Você conta com alguém para compartilhar suas preocupações e medos mais íntimos?	<input type="text"/>	
<i>"Se você precisar, com que freqüência..."</i>			
154.	Você conta com alguém para dar sugestões sobre como lidar com um problema pessoal?	<input type="text"/>	
155.	Você conta com alguém com quem fazer coisas agradáveis?	<input type="text"/>	
156.	Você conta com alguém que compreenda seus problemas?	<input type="text"/>	
157.	Você conta com alguém que você ame e que faça você se sentir querida?	<input type="text"/>	
<i>"As próximas perguntas referem-se à religiosidade"</i>			
158.	Atualmente, qual é a sua religião? (aquela com que você mais se identifica)	<input type="text"/> <input type="text"/>	
01.	Adventista		11. Judaica
02.	Assembleia de Deus		12. Luterana
03.	Batista		13. Metodista
04.	Budista		14. Metodista
05.	Candomblé		15. Presbiteriana
06.	Casa de Benção		16. Testemunha de Jeová
07.	Católica		17. Umbanda
08.	Congregação Cristã do Brasil		18. Universal do Reino de Deus
09.	Espirita Kardecista		19. Outra (_____)
10.	Evangelho Quadrangular	20. Não tenho religião	

<p>159. Nos ÚLTIMOS 12 MESES (sem contar com situações como casamento, batizado, ou enterro), com que frequência você compareceu cultos, missas ou atividades da sua religião ou de outra religião?</p> <p>0. Nenhuma vez 1. Uma vez por semana 2. Mais de 1 vez por semana 3. 2 a 3 vezes por mês 4. Algumas vezes no ano 5. Uma vez no ano</p>	[__]
<p>160. Sentiu que o seu comparecimento nas atividades da sua religião foi uma fonte de apoio?</p> <p>0. Não 1. Sim</p>	[__]

Muito obrigado pela sua participação!

OBSERVAÇÕES DO ENTREVISTADOR:

Entrevistador(a):

161. Em sua opinião, qual é a cor/raça da gestante participante?

1. Branca 2. Preta 3. Parda(morena/mulata) 4. Amarela 5. Indígena [__]

Notas adicionais
