STATEMENT OF RESEARCH INTERESTS AND EXPERTISE

1. The overall aim of my research

a. My research contributions have been in three areas: a) gender differences theory-based approach for examining male vulnerability in very-low-birthweight (VLBW, BW < 1,500gm) infant health and developmental (cognitive/motor/language/socio-emotional) outcomes from the perinatal period to the infancy; b) generating new knowledge about the associations between biological factors, testosterone and cortisol levels, and adverse birth outcomes, infant health and development, and mental health issues; and c) developing new methods for assessing and screening infant behavioral and developmental issues such as autistic spectrum disorders regarding the male vulnerability, hormonal biomarkers, and quality of mother-infant interactions.

b. My research has been focused on examining the associations between the levels of testosterone and cortisol, infant health and growth, mother-infant interactions, and infant cognitive/motor/language/socioemotional development in VLBW infants over the first 2 years after birth. This line of research is an outgrowth of years of clinical experience with male vulnerability in infant mortality and morbidity, mother-VBLW infant interactions, and infant motor and language development that attempts to answer questions of clinical significance and relevance. My initial studies described gender differences in the interactions between mothers and their medically at-risk infants until 3 years old. Yet, more recently my research has been focused on an examination of the associations between hormonal biomarkers and their relationships to mother-infant dyads in biopsychosocial outcomes.

c. As maternal physical and mental health issues are important factors for infant health and development, my research expanded to examine whether maternal mental health issues (depressive symptoms, anxiety, perceived stress, and parenting stress) and healthy lifestyle behaviors (healthy eating, physical activity, use of cigarettes and alcohol) are associated with hormonal biomarkers (testosterone and cortisol levels) regarding maternal obstetric complications (diabetes, use of insulin, pregnancy-induced hypertension, chronic hypertension, antenatal hemorrhage, chorioamnionitis, preeclampsia, prelabor rupture of membranes, and use of glucocorticoids and antibiotics) during ante- and postpartum periods longitudinally.

d. As I have been working on the associations between hormonal biomarkers and infant health and development, I completed the critical review to suggest the most reliable biochemical measurements for testosterone in pediatric population related to the variability and determinants. The issue related to the variability including child sex and age as well as assay methods and materials while the determinants including sampling method (e.g., passive drooling vs commercially available collecting devices), multiple samplings (e.g., one vs three samples), and sampling storage (e.g., higher than room temperature and longer than a year) remains challenging. The reference values are more reliable with serum and Liquid Chromatography-Mass Spectrometry (LC/MS) as immunoassays overestimate testosterone levels, especially saliva in neonates. Testosterone measurements stratified by child sex and age were more agreeable during the first year of life. There are trends in using mass spectrometry, multi-methods, and multi-materials. It would mean that the relative comparison using LC/MS would be an option for my research as the aim of my study is not for the absolute comparison for diagnostic purposes.

e. Recently, my research is expanded to health disparities in VLBW preterm birth related to hormonal biomarkers, testosterone and cortisol. I have found that high testosterone and low cortisol levels are more common in mothers who are Black, younger, and unmarried; have less educational attainment; are in federal-assistance programs. Those biomarker levels are also

associated with adverse birth outcomes (resuscitation at birth and longer hospitalization), delayed physical growth (body weight, length, and head circumference), and adverse maternal mental health and healthy lifestyle behaviors. Mothers who are married and have higher body mass index show fewer adverse birth outcomes (low gestational age, low 1-min Apgar scores, and resuscitation at birth), whereas Black mothers show fewer healthy lifestyle behaviors. It would mean that a higher rate of VLBW preterm birth in Black women may not simply reflect racial differences. Health disparities in adverse pregnancy and birth outcomes need to be assessed from biological perspectives as demographic risk factors were related to biomarkers levels. My research project will be continued in multi-sites across the US.

2. Key aspects of my work

a. A strong assertion

i. My research studies were among the first to describe how to collect saliva samples for measuring the levels of hormonal biomarkers in VLBW neonates as early as 40 weeks' postmenstrual age (PMA) and how to collect and analyze saliva and blood samples because salivary testosterone levels are interfered with by any collection devices. Testosterone levels are also influenced by diurnal and episodic secretion patterns. Testosterone is stable in different temperatures and durations of storage however sample collection remains challenging as researchers are often faced with limited quantity and quality of samples in infants.

ii. My research studies were also among the first to describe associations of infant salivary testosterone and cortisol levels concurrently with infant health and growth outcomes during the postnatal period. I reported an inverse association between the steroid hormonal levels and infant outcomes, as well as a positive correlation between the levels of salivary testosterone and cortisol in neonates as well as in mothers during the neonatal period.

iii. My research demonstrated that gender-difference theories are applicable for infants but not adults, i.e., mothers. For example, the association of steroid hormonal levels with infant outcomes differed from those with maternal outcomes that high infant testosterone level was a risk factor for VLBW infants' health and growth outcomes, but high maternal testosterone level was a protective factor for mothers to reduce their depressive symptoms between 0 to 6 months after delivery. My research also demonstrated that maternal depressive symptoms and maternal report of infant socioemotional problems were positively associated.

iv. My research demonstrated the associations between steroid hormones and mother-VLBW infant interactions. As testosterone levels are higher in sicker and smaller infants, the elevated infant testosterone level was a biological risk factor for establishing positive mother-infant interactions. On the other hand, most VLBW infants are overexposure to pre- and postnatal glucocorticoids, elevated infant cortisol level was a protective biological factor for establishing positive mother-infant interactions. Elevated maternal testosterone level was a positive factor while elevated maternal cortisol level was a negative factor for the interactions at 3 and 6 months corrected age for prematurity (CA).

v. My research demonstrated that 6 months CA is not long enough to answer all possible developmental delays in VLBW infants. I disseminated the important finding as to motor developmental delay is the first and most observable sign of developmental delay in VLBW infants up to 6 months CA.

vi. The endpoint of my research was extended to 24 months from 6 months. I found that the effects of hormonal biomarkers differed when infants became older, e.g., a 1pg/ml increment of testosterone was related to a -0.42% decrease in body weight, a -0.18% decrease in length, and a -0.10% decrease in head circumference. Cortisol levels were not associated with any

physical growth variable. The interactions between testosterone and time on physical growth and socioemotional development also occurred that the effects of testosterone on physical growth were stronger at earlier ages before 6 months than after 6 to 24 months CA. The levels of either testosterone or cortisol were not longitudinally associated with infant cognitive, motor, language, and socioemotional development between 6 to 24 months CA.

vii. As a prevalence of adverse birth outcomes is higher in Black than White mothers and their infants, health disparities related to birth outcomes, neonatal health and physical growth, maternal mental health (depressive symptoms, anxiety, and perceived stress) and maternal healthy lifestyles behaviors (balanced eating, physical activity, smoking, and drinking) were examined based on biopsychosocial model. Maternal sociodemographic factors were associated with adverse birth outcomes, neonatal health and physical growth, and healthy lifestyle behaviors. The levels of testosterone and cortisol were associated with maternal sociodemographic factors, neonatal health and physical growth, and mental health issues. The study findings confirm that health disparities are from multiple dimensions of biopsychosocial factors. I propose using sociodemographic and biological factors concurrently to identify risk and develop and evaluate ante- and postpartum interventions.

b. Evidence about the nature of the work (references)

i. My early research examined gender differences in the quality of interactions between mother and their medically at-risk infants including infants who were born prematurely, medically fragile (including preterm and term infants), and HIV seropositive (as a reference group). Using observational and longitudinal methods, my previous study described that the quality of mother-infant interactions became less favorable after 6 months CA, esp., between mothers and sons although boys need more positive mother-infant interactions to reduce the negative health and developmental outcomes (Cho et al., 2009). As gender itself is too broad to examine the possible bioenvironmental factors that may affect the interactions, I chose testosterone as a biomarker from gender-differences theories to answer the question regarding male vulnerabilities (Cho et al., 2014). In a series of studies, I documented an inverse association of salivary testosterone and cortisol levels with infant birth outcomes (esp., birthweight, gestational age, and medical problems) (Cho et al., 2012). Also, I disseminated the associations of the steroid hormonal levels with the interactions between mothers and their VLBW infants. Elevated levels of maternal testosterone level and infant cortisol were protective factors while elevated levels of maternal cortisol and infant testosterone were risk factors for establishing positive mother-infant interactions up to 6 months CA (Cho et al., 2015).

ii. Subsequently, I explored the association of the steroid hormonal levels and maternal depressive symptoms and maternal report of infant socioemotional problems as these two variables affect the quality of mother-infant interactions regarding infant gender (Cho et al., 2016). I confirmed and documented that mothers with high levels of testosterone reported less depressive symptoms and perceived their infants had fewer socio-emotional problems up to 6 months CA after having a VLBW preterm birth (Cho et al., 2017a).

iii. Earlier, I found that testosterone levels were positively associated with language development in males, whereas cortisol levels were inversely associated with motor development both in males and females up to 6 months CA as motor development was the first observable developmental sign among the infants (Cho et al., 2017b). When I extended the research period to 24 months CA, I found that the effects of testosterone on motor and language development faded although high testosterone levels were still inversely associated with anthropometric measures (body weight, length, and head circumference). On the other hand, cortisol levels were not associated with either anthropometric measures or cognitive, motor, and language development (Cho et al., 2020).

iv. Currently, I confirmed that health disparities in VLBW preterm birth were associated with maternal sociodemographic factors, and those factors were associated with high testosterone and low cortisol levels during the postnatal period. High testosterone levels were found in mothers who were Black, younger, and unmarried, had less educational attainment, were in federal-assistance programs. Higher testosterone levels were also associated with more neonatal health problems (resuscitation at birth and longer hospitalization) and delayed physical growth (smaller body weight, length, and head circumference) as well as more maternal mental health issues and unhealthy lifestyle behaviors. On the other hand, high cortisol levels were found in mothers who were married, had higher body mass index, and had fewer adverse birth outcomes (low gestational age, low 1-min Apgar scores, and resuscitation at birth). It would mean that a higher rate of VLBW preterm birth in Black women may not simply reflect racial differences. There might be more factors in biopsychosocial model (Cho et al., in press).

c. The significance of the work in this key aspect

i. Beginning with my exploratory study in 2009, my research program has been funded continuously by NICHD (R21HD066186: 7/1/10 – 6/30/12; R01HD076871: 7/1/14 – 7/31/17). I have been addressing a similar question about male vulnerability in infant health and growth, mother-infant interactions, and infant development using biochemical measurement (blood and salivary testosterone and cortisol levels from mother-VLBW infant pairs) and observational method longitudinally. As the studies related to gender issues are too broad to examine, I have chosen testosterone as a hormonal biomarker of explaining gender effects in the outcome variables (infant health, mother-infant interactions, and infant development).

ii. The first thing I demonstrated was how to validate salivary testosterone measurement using enzyme immunoassay (EIA) procedures along with salivary cortisol in VLBW infants as saliva is physiologically meaningful, and free hormonal biomarker levels are strongly correlated with total (protein-bound) hormonal biomarker levels in the blood (r = 0.67–0.95). Saliva also has merits for infants as less invasive in sampling and simpler in assays than other samples such as blood and urine. I demonstrated to use of dual measurement to examine gender effects on the outcome variables. While others have studied either testosterone or cortisol level, pre- or postnatal period, or preterm or term infants; my work stands out by highlighting the critical importance of the use of dual measurement longitudinally from mother-VLBW infant pairs concurrently from prenatal (use of cord blood) to the infancy (use of saliva).

iii. My research serves as a foundation for the use of biochemical measurement as a completely new way of conceptualizing the impact of the steroid hormones (testosterone and cortisol) on infant health, mother-infant interactions, and infant development.

iv. As theories of gender differences guided, I validated that high testosterone level is a biological risk factor for infant health and development, mother-infant interactions, maternal mental health, and healthy lifestyle behaviors during the 2 years after VLBW preterm birth.

v. As the prevalence of VLBW preterm birth has been higher in Black women than White women, I confirmed that health disparities in VLBW preterm birth did not simply reflect racial differences as Black mothers had less favorable sociodemographic factors such as more likely to be unmarried and have less educational attainment and those sociodemographic factors were associated with different levels of hormonal biomarkers such as such as high testosterone and low cortisol levels during the postnatal period. This research project was funded from NIGMS (1U54GM104944: 7/1/19 – 6/30/22 with the no-cost extension due to COVID).

d. The impact of the work in this key area

i. After I successfully completed two series of preliminary studies using intramural funding (Dean's Scholar Award, 2009-2010) and R21 funding from the NICHD (2010-2012), my research team was able to move on to a new R01 project (2014-2019). The five-year R01 project supported by the NICHD began in June 2014. The project investigates male vulnerability in brain development and social behaviors among VLBW infants from a biophysiological perspective.

ii. The multidisciplinary impact of this work on biochemical measurement and observational method were demonstrated by publications of results of scientific studies (17 data-based papers most for which I am the first author) in top-tiered nursing and multidisciplinary journals such as *Nursing in Research and Health, Nursing Research, Biological Research for Nursing, and Early Human Development*. My publications in this area have been well cited.

iii. My previous work was summarized "Evidence-based practice for nursing" section of the March/April/2005 issue of *MCN: the American Journal of Maternal-Child Nursing*.

iv. I was invited to deliver the lecture at the Endocrine Noon Conference that organized by UAB, department of medicine, division of endocrinology, diabetes, and metabolism (2011); as well as the plenary and concurrent sessions of the symposium commemorating the 60th anniversary of the Catholic University of Korea, College of Medicine and College of Nursing in Seoul, Korean (2014).

v. A pilot study titled "Health disparities in very-low-birthweight preterm birth in Nevada" has been conducted at UNLV based on the results from my previous studies that adverse birth outcomes are related to maternal sociodemographic factors such as race and SES and those sociodemographic factors are associated with biological factors based on biopsychosocial model that embraces multiple dimensions of biological, psychological, and social factors. The study findings guided for developing the new R01 grant proposal in Fall, 2021.

e. A statement that leads to the next key area or my work

i. The results of my projects helped focus my research agenda; it was clear that smaller and sicker infants at the neonatal intensive care unit (NICU) have high testosterone levels than healthy full-term infants. I have examined the effects of prenatal glucocorticoids on infant health and development and will examine the changing point of HPA dysregulation to normative regulation throughout infancy among VLBW preterm infants.

ii. The associations between the steroid hormones and the outcome variables (infant health and growth, mother-infant interactions, and infant development) will be compared between VLBW and ELBW (extremely-low-birthweight) infants as sicker and smaller infants tend to have elevated levels of the steroid hormones.

iii. The outcomes from the critical review titled "The variability and determinants of testosterone measurements in children: A critical review" (Cho et al., 2021) will guide to use Liquid chromatography–mass spectrometry (LC/MS) rather than enzyme immunoassays (EIA) for more accurate outcomes of biological factors in my ongoing and upcoming research projects.

iv. The results of a pilot study titled "Health disparities in very-low-birthweight preterm birth in Nevada" will guide to develop multisite research across the US.

v. A pilot study titled "Hormonal biomarkers associated with internet-related disorders in young adults" will be conducted before implementing internet cognitive-behavioral therapy (ICBT) and mindfulness.

vi. A cause-and-effect study using a mouse model will be the next step to determine the effects of hormonal biomarkers in pup's physical growth and neurobehavioral development.

3. The current and next steps in my research

a. The R01 grant proposal titled "Testosterone and Cortisol as Novel Biomarkers of Health Disparities in Mothers and Their Very-Low-Birthweight Infants" was submitted in June 2021 but was not funded. The proposal was extended from the previous projects titled "Testosterone and Cortisol Levels in Infant Health and Development" and "Health Disparities in Very-Low-Birthweight Preterm Birth." The new R01 project will be submitted in Fall, 2022 after adding more biomarkers followed by the reviewers' comments on the summary statement. The main complaint from the reviews was about a huge portion of the project will be conducted under the subcontract at UAB, finding collaborations at UNLV will be unavoidable issue. As LC/MS is the most accurate assay to measure low levels of steroid hormones in women and infants, the levels of hormonal biomarkers will be measured using the LC/MS at Nevada Proteomics Center in the University of Nevada, Reno (UNR).

b. As a no-cost extension for a pilot study funded by MW CTR-IN is approved, data collection at Sunrise and Summerlin hospitals should be continued although recruitment is still restricted from both hospitals. I discussed with the PI of MW CTR-IN program if it would be acceptable to change the project but was encouraged to stick on the original study plan.

c. A potential R21 project will be likely a fundamental mechanistic study titled "Modulation of endogenous testosterone levels in mice" which aims at establishing the cause-and-effect relationship of testosterone and pup's physical and neurobehavioral development. This study will be a bona fide multidisciplinary collaboration with basic scientists at UNLV.

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