Studies in Fetal Behavior:

Revisited, Renewed, and Reimagined

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Among the earliest volumes of this *Monograph* series was a report by Lester Sontag and colleagues, of the esteemed Fels Institute, on the heart rate of the human fetus as an expression of the developing nervous system. Here, some 75 years later, we commemorate this work and provide historical and contemporary context on knowledge regarding fetal development, as well as results from our own research. These are based on synchronized monitoring of maternal and fetal parameters assessed between 24 and 36 weeks gestation on 740 maternal-fetal pairs compiled from eight separate longitudinal studies, which commenced in the early 1990s. Data include maternal heart rate, respiratory sinus arrhythmia, and electrodermal activity and fetal heart rate, motor activity, and their integration. Hierarchical linear modeling of developmental trajectories reveals that the fetus develops in predictable ways consistent with advancing parasympathetic regulation. Findings also include: within-fetus stability (i.e., preservation of rank ordering over time) for heart rate, motor, and coupling measures; a transitional period of decelerating development near 30 weeks gestation; sex differences in fetal heart rate measures but not in most fetal motor activity measures; modest correspondence in fetal neurodevelopment among siblings as compared to unrelated fetuses; and deviations from normative fetal development in fetuses affected by intrauterine growth restriction and other conditions. Maternal parameters also change during this period of gestation and there is evidence that fetal sex and individual variation in fetal neurobehavior influence maternal physiological processes and the local intrauterine context. Results are discussed within the framework of neuromaturation, the emergence of individual differences, and the bidirectional nature of the maternal-fetal relationship. We pose a number of open questions for future research. Although the human fetus remains just out of reach, new technologies portend an era of accelerated discovery of the earliest period of development.

Chapter 1. Fetal development research in context: Seventy-five years of influence of the Fels Longitudinal Study

"We must regard our interest in the problem of normal fetal behavior as a direct outgrowth of the widespread tendency within the past few years to approach more nearly the beginnings of human life in the hope of obtaining a picture of behavior as it emerges." (Sontag & Richards, 1938, p 1)

So began the introduction to one of the earliest *Monographs of the Society for Research in Child Development.* The volume, titled "Studies in Fetal Behavior", reported results of a systematic study of fetal development by investigators of the Fels Institute of Yellow Springs, Ohio. The *Monograph*, focused exclusively on fetal heart rate as an indicator of behavior, was the first of a series of three reports by the group that year; the latter two were published in *Child Development*. In 1929, the Fels Institute launched one of the seminal longitudinal studies of the time that ushered in the period of rapid knowledge acquisition about early child growth and development. The research was essentially predicated on the question "What makes people different?" (Roche, 1992). Prior to the consolidation of interest in development of individuals from infancy through childhood and into later life, the early longitudinal studies, including the Fels Longitudinal Study, were necessarily multi-disciplinary investigations of maturation guided by interests and perspectives that included medicine, public health, and anthropology. The approach was descriptive and yielded measurement techniques that were quantitative and rigorous (Sontag, 1971). Its legacy is undisputed. The contribution of the Fels Longitudinal Study to the knowledge base regarding child body composition, growth, and physical maturation has been well documented (Roche, 1992) and includes a commemoration in the *American Journal of Physical Anthropology* (Sherwood & Duren, 2013). The impact of the seminal work on autonomic responsiveness by John and Beatrice Lacey (Lacey & Lacey, 1962), long-standing

members of the Fels Institute, on the role of the autonomic nervous system in developmental psychophysiology cannot be overstated. At about the same time, the 1962 publication of *Birth to Maturity* (Kagan & Moss, 1962), generated from study of a subset of Fels study participants from birth to adolescence or early adulthood, served to solidify interest in how individual differences detected in early childhood predict behavioral and psychological development within the burgeoning field of child psychology.

Here we focus on a unique feature of the Fels Longitudinal Study - the enrollment of women in late pregnancy and the investigation of the fetus as the precursor to the child. This specific interest has been attributed to the founding philanthropist, Samuel Fels (Richards & Newbery, 1938). The founding director, Lester Sontag, then resident physician at Antioch College (hence, the location in Yellow Springs), took the lead in this effort. Fetal data collection was conducted on a relatively small subsample of participants, presumably due to the time intensive and specialized methods required for prenatal assessment. As a result, most published findings are based on 30 or fewer fetuses; few enough to enumerate individual values in some reports. Despite this, the nature of the questions posed then (e.g., How does maternal smoking affect the fetus? Are there individual differences in fetal reactivity to stimulation?) (Sontag & Richards, 1938; Sontag & Wallace, 1935a) were prescient and many of the findings, necessarily reliant on rudimentary methods, have withstood the test of time.

Among the goals of this *Monograph* is to commemorate the ground-breaking prenatal work of the Fels investigators. In doing so, we provide contemporary information on the current level of understanding of the earliest period of development and, when possible, juxtapose these against the findings generated from the Fels studies. Our research team has been conducting fetal neurobehavioral research since 1991. The inaugural article describing this work was

published in 1996 in *Child Development* with a report on fetal development in 31 fetuses measured six times during the second half of gestation (DiPietro, Hodgson, Costigan, Hilton, & Johnson, 1996b). We opened that publication with the same quotation that opens this *Monograph* to underscore that the questions posed by the Fels investigators remained fresh over seven decades later. In the following pages we hope to convey the remarkable degree to which their work provided the foundation for what was to follow over the next 75 years.

Chapter 2. Why Study the Fetus?

"What, of all the multitude of responses described for and attributed to the newborn and young infant, may we describe for the organism in utero?" (Sontag & Richards, 1938, p 1)

The behavior and environment of the human fetus has been the source of fascination since antiquity. The first known images of the fetus are stone sculptures created by the Olmec civilization of Mexico between 900 and 600 B.C. (Tate & Bendersky, 1999). Somewhat fanciful and unlikely renderings of fetal behavior within the uterus are attributed to the physician Soranus of ancient Ephesus, who provided the most comprehensive description of obstetric knowledge available during the 2nd century A.D. One of his works, *Gynecology*, served as the primer for obstetrics and midwifery for centuries, and his drawings of the intrauterine environment persisted through at least the 12th century. This work revealed early appreciation for the manner in which adverse circumstances during pregnancy may affect the developing fetus and its ultimate effects on the newborn and child, likening the process to building a house with inadequate foundation (English translation, Temkin, 1991). Literary, cultural and religious works are replete with references to fetal behavior and date as far back as to the *Mahabharata* (circa 5th century B.C.). In that epic, a young warrior prince learned how to circumvent his enemy having overheard his father describe a specific battle strategy to his mother when she was pregnant with him. The bible includes acknowledgement of the vigor of fetal behavior ("But the children struggled together within her", *Genesis 25:22*) and the implication that the fetus responds to voices ("For behold, when the sound of thy greeting reached my ears, the babe in my womb leapt for joy", *Luke 1:44*). Allusions to the importance of the prenatal persist throughout literary history and include, for example, a suggestion by Shakespeare that strong maternal emotions affect the fetus (*King Henry (3), Act IV, Scene IV).*

Contemporary academic interest in the fetal period arose at the intersection of three converging influences that were crystallizing in the 1970s. The first was the emergence of infant behavior as a new area of study within the field of child development. This area transitioned fairly rapidly from documentation of infant capabilities by age (i.e., as ascertained by group means) to recognition of individual differences in these capabilities (i.e., variation around the mean). The term "neurobehavior" was used to connote aspects of basic human functioning that are phenotypic expressions of the processes that underlie the development and expression of autonomic and behavioral regulation. Assessments of the infant repertoire and related insights into neonatal and early infant neurological and neurobehavioral development by Prechtl (Prechtl & Beintema, 1968) and Brazelton (Brazelton, 1973) provided tools for research. Pre-term infants, suddenly surviving at higher rates due to refinements in technology to support neonatal intensive care provided an additional framework for considering the gestational origins of behaviors (Als, 1982). Prenatal behaviors came to be recognized as both contributory to intrauterine survival and anticipatory of postnatal life (Prechtl, 1984). For example, stepping movements assist in the fetal transition to vertex positioning; sucking movements and swallowing of amniotic fluid help regulate fluid volume and entrain the oromotor system.

Second, the growing field of developmental psychobiology, focused on experimental animal models, also supported the importance of emerging behavior to the ontogeny of the individual and adaptation to pregnancy and parturition (Hofer, 1988; Smotherman & Robinson, 1987). The manipulations afforded by the use of animal models were particularly effective in documenting fetal responses of the chemosensory systems and the role those behaviors exerted on subsequent prenatal and postnatal development. As a result, the notion that fetal behaviors were not simply epiphenomena secondary to neural maturation became well-established.

The third influence was the rise to prominence of fetal heart rate as an indicator of the well-being of the fetus in clinical obstetrics as the result of the new tools to measure it reliably. Fetal heart rate monitoring became the foundation for assessment of fetal well-being or distress in both the antepartum and intrapartum periods following the observation that acute hypoxic events or general placental under-perfusion were often associated with characteristic changes to heart rate patterns (Hon, 1958; Martin, 1978). Technologies to view and monitor the fetus, developed for clinical purposes, also provided developmentalists with new opportunities to document human fetal development. The advent and applicability of these methods will be discussed in the next chapter.

As each avenue of inquiry gained steam, the National Institute of Child Health and Development convened a series of conferences to integrate knowledge across obstetric and developmental fields with the goal of advancing research and garnering interest in fetal neurobehavioral development (Krasnegor et al., 1998a, 1998b). However, although each field benefits from insights from the other, the goals of obstetrics and developmental science are inherently different and do not necessarily intersect. Clinical fetal assessment is oriented towards identifying markers of fetal distress to detect conditions that threaten pregnancy outcome and are amenable to obstetric intervention, thereby optimizing pregnancy outcomes (Ware & Devoe, 1994). As such, it requires identification of performance criterion that can successfully distinguish between outcomes in order to inform management decisions. The focus of fetal neurobehavioral development research is to gather information on the functional development of the fetus with the putative expectation that function provides meaningful representation of nervous system development. Thus, its goals are both to characterize normative development and to document variation of individuals along a continuum, including

those within the normal range. Doing so affords evaluation of key developmental constructs, including the vulnerability of the fetal nervous system to risk factors that adversely affect development and whether individual differences in traits that are of presumed constitutional origin begin prior to birth.

Fetal neurobehavioral development, as true for virtually all aspects of development, is characterized by both change and constancy. Fetuses clearly mature over time and the manifestation of this maturation can change, but there is also constancy in the expression of underlying maturational constructs within individuals. There have been a number of efforts to linguistically parse the two in developmental theory. For example, the terms "differential continuity" (Caspi, 1998; Putnam, 2011) and "stability" (Bornstein & Suess, 2000; McCall, 1981) have both been used to describe preservation of individual differences over time as might be assessed using statistics that evaluate relative or rank order. "Absolute continuity" (Caspi, 1998; Putnam, 2011) and "continuity" (in contrast to "stability") (Bornstein & Suess, 2000; McCall, 1981) have been used to indicate preservation of mean levels of an attribute over time within a group. This distinction is further complicated by the fact that in early development, the nature of how an underlying construct is expressed changes in tandem with the expanding developmental repertoire. The terms homotypic or heterotypic have been used to describe the nature of such measurement (Putnam, 2011). Heart rate, for example, is a homotypic attribute as it can be measured in similar units over time, but activity level is a heterotypic attribute as the fetus does not locomote, so its expression is necessarily different in the fetus and 1 year old infant. A final lexical conundrum is how best to describe well-known phenomena of fairly abrupt shifts in developmental rates or trajectories. Perhaps the best known of these is the shift in multiple dimensions of biobehavioral function that occurs approximately 3 months

postpartum. The term “discontinuity” is generally used to describe such periods during which developmental maturation, as expressed by behavior, appears to undergo qualitative changes as a result of reorganization across one or more domains (Zeanah, Boris, & Larrieu, 1997).

Throughout this report, we use the term “continuity” to refer to aspects of function that develop incrementally and express the same underlying construct over time; expression may be either within-domain (homotypic) or cross-domain (heterotypic). We use “discontinuity” to indicate observation of statistically significant changes in the rate of development of these constructs over time. “Stability” is used to reflect preservation of rank order among individuals of a within-domain function. Finally, we use the term “predictive validity” to describe associations between prenatal and postnatal measures, whether homotypic or heterotypic.

Irrespective of terminology, it is clear that by the end of gestation behaviors and other developmental parameters which are measured extensively in the neonate and infant, and are integral to theories of development, originate neither at term gestation (i.e., 40 weeks) nor with birth. At parturition, the full-term fetus demonstrates virtually the same neurobehavioral repertoire as the newborn infant, with systematic developmental progress towards these mature patterns of function evident throughout gestation. Convergent evidence from a number of sources has supported the utility of fetal measures as markers for neurological development in normally progressing pregnancies (Amiel-Tison, Gosselin, & Kurjak, 2006; DiPietro, Irizarry, Hawkins, Costigan, & Pressman, 2001; Hepper, 1995; Krasnegor et al., 1998b; Nijhuis & ten Hof, 1999; Sandman, Wadhwa, Hetrick, Porto, & Peeke, 1997). Antenatal conditions or exposures with established postnatal developmental sequelae are generally accompanied by alterations to fetal neurobehavioral development. Examples include congenital anomalies related to the nervous system (Hepper & Shahidullah, 1992; Horimoto et al., 1993; Maeda et al., 2006;

Morokuma et al., 2013; Romanini & Rizzo, 1995), intrauterine growth restriction (Kurjak, Talic, Honemeyer, Stanojevic, & Zalud, 2013; Nijhuis et al., 2000), exposure to maternal use of potentially neurotoxic substances including licit (Jansson, DiPietro, & Elko, 2005; Mulder, Morssink, van der Schee, & Visser, 1998; Visser, Mulder, & Ververs, 2010) and illicit substances (Gingras & O'Donnell, 1998), and environmental contaminants (DiPietro, Davis, Costigan, & Barr, 2013). These types of studies support the position that measurement of fetal functioning can provide information on the ontogeny of neural maturation and its disruptors.

Gestation encompasses both fetal growth (i.e., an increase in cell mass or number) and fetal development (i.e., differentiation of function). The constructs that have undergirded our work spring from a developmental psychology sensibility focused on function. However, over the last decade the topic of "fetal programming" has been applied broadly to represent discoveries of prenatal influences on postnatal disease, often with adult onset (Barker, 2006; Gluckman, Hanson, Cooper, & Thornburg, 2008). Although this approach has generated an enormous literature, thereby sparking great interest in the prenatal period, it has principally relied on an epidemiologic framework and readily available data sources that generally involve weight at birth as representative of the culmination of the prenatal milieu. However, a growing literature applies the construct of programming to developmental function, as mediated by the adaptation of maternal and placental neuroendocrine and other systems, primarily in response to maternal stress (Entringer, Buss, & Wadhwa, 2010; O'Donnell, O'Connor, & Glover, 2009; Sandman, Davis, Buss, & Glynn, 2011).

Models of fetal neurobehavioral development

In the early 1980s, Als (1982) presented a model of the synactive organization of development that reflected conceptualization of early neurobehavioral functioning from the

embryo through the first 12 weeks of infancy, without indication of a functional discontinuity or other demarcation as the fetus approached and surpassed term. The hierarchical and expansive nature of development was depicted as four nested, concentric cones with autonomic regulation at the core, subsumed sequentially by motor behavior and then state control. The outer ring revealed the culmination of this process and the resultant coordinated infrastructure necessary to engage the attentional, interactive, and learning systems. Each of these domains was assessed by newly developed neurobehavioral exams designed to detect individual variation among full-term (Brazelton, 1973) and preterm (Als, Lester, Tronick, & Brazelton, 1982) infants. This model had a tremendous influence on our work, as it was not a great leap to apply these constructs to a downward extension to measurement of the fetus. This is particularly apt when one considers the preterm infant to be a fetus in the wrong place at the wrong time. The current literature on fetal development can still be distributed into these four domains; measures of each recorded longitudinally from 20 and 38 weeks were provided for the small sample that comprised our original report (DiPietro et al., 1996b).

Our research program has had three main aims: 1) documentation of normal ontogeny of fetal neurobehavioral development between and within individuals; 2) examination of maternal and exogenous influences on its expression; and 3) evaluation of the manner in which the fetus affects the pregnant woman. These aims evolved over the course of our project. We initially approached fetal neurobehavioral data collection in the same way that we had previously approach infant assessment. That is, we were focused on measuring the individual, despite the obvious challenges, and were enthusiastic about finally approaching the origins of individual differences prior to the “contamination” afforded by the postnatal environment of rearing. Somehow, we managed to overlook that the fetus was embedded within another, separate

individual and that there is no other period in development in which the proximal environment is so physiologically entangled. Over time, Aim 3 was added based on our developing appreciation of the complexity of the relationship. Individual studies often included a mix of protocols that could address more than one aim. Since the inception of our research program, all cohorts included the same core data collection protocol consisting of a baseline, unperturbed recording of fetal neurobehavioral and maternal psychophysiological measures, typically for 50 minutes. Although the number of data collection sessions and gestational age at testing varied by cohort, most involved at least 3 visits during or near the 24th , 30th to 32nd, and 36th weeks of gestation. The data analysis presented in this *Monograph* is based on that source of data. Protocols that included experimental manipulations of either mother (e.g., induction of maternal relaxation or stress to evaluate fetal responsivity) or fetus (e.g., external stimulus presentation) were administered after the 50 minute baseline period. Data from these experimental components of our work are not included in this *Monograph* as they were generally specific to only a single cohort. Results from those studies are mentioned as supportive citations when relevant to the discussion.

Figure 1 illustrates our conceptualization of fetal neurodevelopment as one of mutual and spiraling engagement between the pregnant woman and fetus. Development of function cannot be illustrated as easily as can physical growth and differentiation but its progression is as orderly and predictable. We think that the Als model (Als, 1982) accurately encapsulated the key elements of neuromaturation as involving streams of autonomic, motor, state and learning. Here we show, in a slightly different way, the scaffolding that serves to bolster each. Foundational autonomic differentiation both expresses and contributes to developing sympathetic and parasympathetic processes, thereby establishing the basis for reactivity and regulation to

endogenous and exogenous stimuli. The emergence of spontaneous motor behavior serves to innervate the developing musculature and neural systems of motor control while fostering fetal interaction with features of the intrauterine environment, resulting ultimately in volitional behavior. Neural regulation of the cardiac, somatomotor, and related systems become more tightly integrated over time and expressed through the emergence of fetal states that are the rudiments of mature sleep-wake cycles that further moderate sensory input. The culmination of this process is an organism that is able to interact with the environment and, as a result, process information in the service of memory and learning. Each progresses from the undifferentiated and uncoordinated to more refined and localized presentation. We have deliberately avoided providing gestational age specific details because the expression of each unfolds over time and in dynamic relation to the other systems; as such, each lacks clear origin. And, as is often the case with infant research, fetal capabilities may begin much earlier than documented at any point in time and individual variation can accelerate or delay the process.

Our research has indicated complex and at times unexpected interaction between pregnant woman and fetus. Although this schematic includes elements of the maternal nervous and cardiovascular systems, as those were the focus of our maternal measures, clearly all maternal organ systems are affected by pregnancy, and likely affect the developing fetus in ways that have not been entirely articulated. Not shown, for example, is that the growing fetus is accompanied by a growing uterus and placenta, both of which have implications for maternal physiology. We give prominence to the role of the fetus on the pregnant woman to draw attention to this underappreciated directionality. Understanding the nature and degree of these dynamic and bidirectional processes has only begun.

The lower part of the figure draws from the construct of canalization, which was applied more narrowly to postnatal mental development in a highly influential paper (McCall, 1981). The concept of canalization suggests that early development is more influenced by species-typical processes whereas later development is more subject to the expression of individual differences and environmental influences. McCall (1981) graphically represented this construct as the potential variation in trajectory that a ball rolling down a 3-dimensional "scoop" can exhibit from the narrow to wider ends. The application of this construct to the fetal period is perhaps most obvious in relation to physical growth. The rate of fetal growth in normal pregnancies is most invariant in the first trimester, before significant influences on the trajectory of linear growth and weight including constitutional ones, are expressed. This accounts for the greater validity of ultrasound measurements of fetal size to ascertain gestational age earlier in pregnancy than later. We propose that fetal neurobehavioral development follows a similar pathway progressing from more canalized development of the nervous system, as expressed via less variability in fetal neurobehaviors among individuals to less canalization over time. That is, as gestation progresses, individual differences, whether constitutional or mediated by maternal and other environmental influences, become increasingly prominent. Given the continuity between the prenatal and postnatal periods, one may not expect the degree of inter-individual variation between conception and term gestation to be as wide as in later postnatal life, but the emergence of variation even within this constrained gestational period should be evident.

In the following pages we will provide empirical results of our research on elements of the first two levels of function - autonomic and motor development. This will include documentation of developmental trajectories in normally developing fetuses and stability coefficients to evaluate the origins of individual differences for each parameter and determine

whether predictions arising from a canalization approach to variation among individuals are correct. We hypothesize that each stream of development will show progressive neuromaturation during the second half of gestation and that variation in development will be accompanied by variation in maternal autonomic nervous system activity. In turn, we will explore how fetal motor activity may influence maternal autonomic functioning. Documentation of the developing integration between fetal autonomic and motor processes will illustrate the degree of correspondence between systems that is requisite for attainment of the next level in the functional hierarchy, thereby setting the stage for fetal state expression. We conclude with discussion of how these data contribute to understanding of how the fetus becomes a child and suggestions for the next generation of fetal neurodevelopment research.

Chapter 3. Methods to monitor the fetus

"*If we would pursue our quest beyond the newborn period, we find ourselves suddenly in an entirely new situation, where our organism is not seen, nor scarcely felt nor heard". (Sontag & Richards, 1938, p 1)*

Unlike the infant or child, the fetus cannot be seen, touched, handled, or heard. Like the infant and young child, the fetus does not feel compelled to cooperate with investigators. This combination makes fetal research particularly challenging. Most of current understanding of neurobehavioral development in the prenatal period, and the basis for the work of Sontag and colleagues, involves assessment of cardiac patterning and motor activity. In this section we consider factors related to the measurement of each. The methods used by the Fels investigators were marked by ingenuity and meticulous work. In their earliest reports, fetal heart rate was detected by a ascultation (i.e., use of a stethoscope on the maternal abdomen to listen for the fetal heart beat) and measured by timing beats with a stopwatch which were then averaged or plotted over brief intervals (Sontag & Wallace, 1936). As shown in Figure 2, fetal movement was initially detected through a device characterized by four rubber air sacs, encased in a plaster of Paris cast molded to the maternal abdomen, with pneumatic transduction to a polygraph (Sontag & Wallace, 1933). Detection required that the fetal movement was strong enough or localized in such a way as to displace the maternal abdomen. Since the device interfered with fetal heart rate measurement, subsequent studies relied on maternal report of felt fetal movements signaled through maternal control of a switch that lit an incandescent bulb (Richards, Newbery, & Fallgatter, 1938; Sontag & Wallace, 1935b; Welford & Sontag, 1969).

The ability to view and monitor the fetus has progressed steadily over the last 75 years, but the fetus remains just out of reach. Visualization of the fetus commenced with the advent of

real-time ultrasound in the 1970's; at present ultrasound is routinely used to evaluate structural malformations of the fetus as well as characterize features of the intrauterine environment, such as sufficiency of amniotic fluid and vascularity of the umbilical cord. Most current knowledge of the ontogeny of specific fetal behaviors – thumb-sucking, stretching, startling – has been generated from formative research using 2-dimensional images initiated not long after these devices were widely available for commercial use (deVries, Visser, & Prechtl, 1982; Ianniruberto & Tajani, 1981; Roodenburg, Wladimiroff, van Es, & Prechtl, 1991). The real-time imagery afforded by 2D ultrasound was and remains pivotal to documentation of the qualitative expression and development of motor behaviors during gestation. Since then, advancements in ultrasound technology have provided 3D and 4D dimensionality; discussion of the opportunities posed by the new generation of monitors can be found in the final chapter.

At roughly the same time (i.e., 1960s), electronic fetal monitors were developed to detect and time fetal heart rate automatically, thereby supplanting the use of auscultation (Stout & Cahill, 2011). In the prenatal period, electronic fetal monitoring, or fetal cardiography, relies on Doppler to detect motions of the fetal heart (i.e., the change in frequency generated by each systole) to identify heart beats and quantify rate using autocorrelation techniques (Parer, 1999). Cardiography is routinely used in clinical practice to infer fetal well-being during the antepartum and its capacity to withstand the stress of labor in the intrapartum. Electronic fetal monitoring is also used in fetal assessment as a component of the biophysical profile, which also includes ultrasound-based observation of fetal breathing motions, fetal motor tone, and amniotic fluid volume (Walton & Peaceman, 2012). Possible scores extend from 0 to 10 although most normal fetuses score are constrained in the upper range. As a result, and not unlike Apgar scores, the

biophysical profile may be too blunt an instrument to be a useful research tool for developmentalists.

A subset of electronic fetal monitors is equipped with the capacity to extract fetal motor activity data from Doppler signals using the same transducer that detects fetal heart rate. Fetal actocardiography identifies fetal movements by preserving the remaining signal after bandpassing both the highest (i.e. fetal heart rate) and the lowest (i.e., maternal somatic activity) frequency signals. During fetal quiescence, the returned Doppler waveform retains the same frequency as the interrogating signal; during activity, the echo is returned at the frequency proportional to the velocity with which the fetal body part moves towards or away from the transducer. The resultant signal provides a continuous measure of fetal movement (Maeda, Tatsumura, & Nakajima, 1991) with output consisting of a series of spikes that are similar to that generated by actigraphy methods used in infants and children, although sampled at a higher rate. Reliability studies comparing actograph based to ultrasound visualized fetal movements have found high accuracy in detecting both fetal motor activity and quiescence (Besinger & Johnson, 1989; DiPietro, Costigan, & Pressman, 1999; Maeda, Tatsumura, & Utsu, 1999). The benefit of this method is that continuously collected data can be digitized, allowing precise quantification and analysis. However, the signal is not without sources of known and unknown signal distortion and care must be taken to control artifact produced by sharp maternal abdominal movements (e.g., coughing) and large excursions of the fetal diaphragm during fetal breathing and hiccuping (Maeda et al., 1991). In addition, fetal movement data are limited to the presence, amplitude, and duration of movements, and provides no information on movement quality, nature, or origin as is afforded by ultrasound imaging.

This brief review of existing methodology underscores the dependency of prenatal research on technology. In the work described throughout this report, we rely primarily on fetal actocardiography to quantify fetal heart rate and motor activity and use ultrasound imaging primarily to ascertain the optimal location on the maternal abdomen for transducer placement and to collect information on amniotic fluid volume.

To date, most of the preceding history of fetal monitoring relied on Doppler detected fetal heart rate as the only commercially available method for either clinical use or fetal research. This technique has been routinely shown to validly quantify fetal heart rate, but is method does not generate true interbeat (IBI) intervals as is standard in developmental psychophysiological research. Admittedly, this came as somewhat of a surprise to us upon embarking on our initial fetal research project. The potential afforded by the development of new methods to reliably detect the electrical signal generated by the fetal R-wave from electrodes applied to the surface of the maternal abdomen will be discussed in the final chapter of this *Monograph.*

Chapter 4. Description of our research program

The results described in the following sections are based on eight longitudinal cohorts of maternal-fetal pairs collected between June 1997 and February 2013, collectively known as the Johns Hopkins Fetal Neurobehavioral Project. Aggregating data across cohorts allows us to ask questions for which we were never suitably powered and to provide normative data that allows identification of fetuses with developmental trajectories that deviate from normal. Three additional, earlier cohorts (n = 151) are not included in this report because data collection and analysis were based on our initial hardware and software systems that used different artifact rejection algorithms and computational definitions for some variables. As a result, and as expected, mean values for some variables differ between the earlier and later cohorts limiting our ability to analyze them in concert. In addition, the earlier system did not include maternal psychophysiological data collection, which was introduced with the first cohort included here. Findings generated by the first three cohorts have been previously detailed (DiPietro, Costigan, Shupe, Pressman, & Johnson, 1998; DiPietro, Hodgson, Costigan, Hilton, & Johnson, 1996a; DiPietro et al., 1996b) and compatibility or incompatibility of findings with the current results will be noted in relevant chapters.

Enrollment for all cohorts was limited to healthy women with normally developing pregnancies at the time of enrollment. Even though this was a low risk sample, significant pregnancy complications emerged (e.g., pregnancy induced hypertension), medical conditions were detected either before (e.g., mild dilation of the renal pelvis) or after (e.g., atrial septal defect) birth, participants delivered prior to term, and neonates had unexpected outcomes (e.g., sepsis). Such complications and conditions may have unrecognized short or longer term consequences for the developing fetus. It becomes difficult, and somewhat arbitrary, to draw the

line as to which types of prenatal or postnatal circumstances should result in exclusion from a "normal" developmental sample. We elected to retain data in the larger sample for most participants, with the recognition that the effects of idiosyncratic conditions on the results would be diluted but also to examine the influence on development of the most common conditions in targeted analyses (Chapter 11). The most frequently encountered complications included preterm delivery [n = 49; most (n = 40) delivered at 35-36 weeks], gestational diabetes (n = 23), and intrauterine growth restriction (n = 9). Exclusions from the main analyses were limited to only those prenatal conditions that reflect major congenital malformations (e.g., cleft palate) or those with well-established and pervasive consequences for child development (e.g., trisomy 21). Table 2 provides the summary information for final sample size for each cohort, including exclusions from each cohort based on conditions, resulting in a final sample size of 740 maternal-fetal pairs. Data generated from each cohort have been previously published, and although some publications may include similar analyses on these smaller samples, others are reports based on Aim 2. Some enrollees were excluded from existing reports on those cohorts because of tighter exclusion criteria relating to issues described in the next two paragraphs.

Complicating eligibility criteria further is the circumstance that a number of women participated in our studies more than once. Women tended to enjoy participation in our research program and as a result it was not uncommon for women to re-enroll in subsequent studies with successive pregnancies. As a result, 197 fetuses were distributed among 106 of the participants. This circumstance is not unlike the original Fels study which embraced sibling recruitment within the context of the longitudinal design and retained them in subsequent developmental analyses (Kagan & Moss, 1962). Most siblings in our studies were distributed across different cohorts, but for two of the studies in which enrollment spanned several years some women with

closely spaced pregnancies who participated in the same protocol twice (n = 18). Data from those siblings were excluded from analysis of those cohorts and are not represented in existing publications. The aggregation of data across cohorts provides the opportunity to evaluate whether siblings develop more similarly than non-siblings. However, from an analysis perspective, because siblings share genes and environment, this raises the issue of non-independence of observations. Whether or not siblings develop more similarly than non-siblings is an empirical question that we can evaluate by treating siblings as observations nested within mothers (Chapter 10). Prior to that analysis, we have retained siblings in the larger dataset. For research questions in which the shared (maternal) environment of siblings may artificially inflate the results, such as stability correlations over time, secondary analyses were conducted without siblings to determine whether their inclusion affected results.

Sample

Recruitment was limited to non-smoking women, over the age of 18, with singleton pregnancies and without significant pre-existing conditions that would jeopardize normal progression of pregnancy (e.g., lupus erythematosus). Accurate dating of the pregnancy, based on early first trimester pregnancy testing or examination and generally confirmed by early ultrasound was required. Pregnancies were detected early (M = 4.9 weeks; sd = 1.6) with the first prenatal visit occuring shortly thereafter (M = 8.0 weeks, sd = 2.3); most pregnancies were planned (77%). In each cohort, data were collected on a wide array of sociodemographic indicators, antenatal and perinatal risk factors, labor and delivery outcomes, and infant characteristics. Selected maternal characteristics are presented in Table 3; infant characteristics are presented in Table 4. Women were self-referred volunteers recruited through local university and hospital publications; over time, word of mouth from current or previous participants was

also a source of recruitment. As a result, and due to the heavy participation burden (i.e., 3 to 6 mid-day visits to the laboratory every 3 to 6 weeks), participants reflected a population of predominantly well-educated, married, and mature individuals who either were employed locally or had occupations that allowed flexibility. Such relative homogeniety can be regarded as an advantage if the goal is to describe normative development of low risk fetuses, but a detriment to generalizability. Indeed, data generated from recruitment of a low-income, Medicaid-eligible sample of women yielded significant differences on most of the fetal neurobehavioral measures evaluated in the direction of less optimal development (DiPietro et al., 1998). That cohort was one of the initial three cohorts and as such data from those participants are not included in this report. In addition, we have a long-standing collaboration that has generated fetal data from several cohorts of low-income, narcotic addicted and treated women. These cohorts are not included in this report since but reveal the influences that sociodemographic and medical risk impart (Jansson et al., 2012; Jansson et al., 2005). Thus, the current analysis is based on a sample at low medical and socioeconomic risk, allowing documentation of fetal neurobehavioral development under optimal conditions. In turn, these data can provide a backdrop for subsequent research on populations at higher sociodemographic and biological risk. The issue of generalizability will be revisited in the Discussion.

Procedure

Visits were conducted in early afternoons to control for potential diurnal or postprandial effects. Precise times varied by cohort but were scheduled at between 13:00 and 15:00. Women were routinely instructed to eat lunch 1.5 hours prior to the visit and not eat thereafter. Brief ultrasound scans administered prior to the recording were used to determine fetal position, optimize transducer placement, estimate amniotic fluid volume, and provide photographs to

parents. Women were positioned in a semi-recumbent, left-lateral posture to avoid compression of the vena cava. Maternal-fetal monitoring proceeded for a period of 50 undisturbed minutes. We would like to be able to provide a scholarly rationale for selecting this recording duration, but in actuality it was determined by the amount of data that would fit on a 3.5" floppy disk in 1991. There were two exceptions to length of recordings at the midpoint visit as a result of the experimental portion of different study protocols. For Cohort I, this visit was limited to 30 minutes to allow viewing of a labor & delivery video following the baseline recording; for Cohort V, only data from the baseline (18 minute) portion of the relaxation protocol were used. Two variables based on counts were prorated accordingly.

As presented in Table 1, three data periods with the greatest commonality across protocols were selected for this report and analysis. We refer to these as gestational periods 1 (G1), 2 (G2) and 3 (G3). These span from near the end of the second trimester to just before term gestation (i.e., 37 weeks). All protocols had data collection midway between these periods, either at 30 or 32 weeks and thus data generated at either point were selected for G2. The exception is Cohort VI, which used a different gestational age sampling strategy to maximize continous data; thus data are provided from a broader gestational period for each visit of those participants. However, to accommodate participant scheduling all cohorts had some degree of variability in the actual timing of visits with respect to gestational age. This is a common feature in studies of the fetus and other studies routinely that aggregate data collected over wider gestational ages. We have taken an additional step by quantifying the actual gestational age for each participant at each visit and use these values – as opposed to the protocolized gestational age - in longitudinal modeling to more precisely characterize fetal development. However, it is worth noting that even in the presence of fairly early pregnancy dating, as the case in the current

sample, gestational age ascertainment is always an estimate due to biological variability in the timing of ovulation with respect to the last menstrual period (DiPietro & Allen, 1991). Table 5 presents sample sizes and gestational age data at the time of actual participation by cohort. Disparities in sample sizes reflect missed visits.

Maternal-fetal monitoring

Data were acquired in seven channels (six for fetal or maternal data and one event marker) via an internal analog to digital board using streaming software (HEM Data Corporation, Southfield, MI) and sampled at 1000 Hz. Channels were multiplexed together into a single file at all points for data analysis.

Fetal data collection. Fetal data were collected from the output port of a Toitu (MT320, Toitu Japan) fetal actocardiograph. As previously described, this monitor detects fetal heart rate and motor activity through a single wide array transabdominal Doppler transducer. Sample digitized fetal data during periods of rest and activity are presented in Figure 3. Data were analyzed off-line using software developed to our specifications (GESTATE; James Long Company, Caroga Lake NY). The system sampling rate was used to provide millisecond resolution for measuring maternal interbeat interval. This exceeds the resolution of the actocardiograph, so fetal data streams were resampled at 24 Hz. Doppler detection of the fetal heart can be plagued by movement artifact because when the fetus moves, the heart moves with it. This is particularly problematic earlier in gestation when the heart is small and it is more difficult to maintain within the Doppler field. Data collection at 20 weeks gestation is quite challenging and none of our studies began prior to this gestational period. Digitized data were initially processed to detect and eliminate artifact. Error rejection algorithms were established during the first year of this project through a process of comparing digitized data to the

polygraphic output of the monitor for several hundred records and ultimately validated on 7,500 minutes of collected data. In brief, this process is based on median moving averages (per 1 s) using an algorithm to determine the range of acceptable values. Determination of this range is based on expanding and contracting percentiles of prior data points that resets upon detection of expected values. A further complication in developing an accurate algorithm is our observation, consistent with Sontag & Richards (1938), that the fetal heart rate can change much more precipitously than commonly observed in the postnatal period. As a result, we routinely compare each polygraphic paper output of the actocardiograph against the processed digitized data. Details of the error rejection algorithm are available upon request. Interpolated values were used to maintain the temporal nature of the data but not used in data quantification. The original voltage output for the fetal movement signal was calibrated in arbitrary units (a.u.s.) consistent with the Toitu MT320 actograph display. Derivation of fetal variables is described in Chapters 5 (fetal heart rate) and 6 (fetal movement).

Maternal data collection. Maternal physiological signals were amplified using a multi-channel, electrically isolated bioamplifier (Model JAD-04; James Long Company, Caroga Lake, NY). The electrocardiogram was recorded from 3 carbon fiber disposable electrodes in triangulated placement (right mid sub-clavicle, left mid axillary thorax, and upper left thigh). Electrodermal activity was monitored from two silver-silver chloride electrodes with a gelled skin contact area placed on the distal phalanxes of the index and middle fingers of the non-dominant hand. Electrodes were affixed with adhesive collars to limit gel contact to a 1 cm diameter circle, and secured with velcro. Skin conductance was measured by administering a constant 0.5 volt root-mean-square 30 Hz AC excitation signal and detecting the current flow. Respiration was monitored with a bellows apparatus stretched across the ribcage below the

breasts. Data quantification proceeded off-line using the PHY General Physiology System and IBI Analysis Systems (James Long Company). Maternal variable derivation is detailed in Chapter 8.

Approach to statistical analysis

Standard techniques were used to examine distributions and evaluate outliers for each fetal and maternal variable, and provide descriptors. Levene's equality of variance test was used to evaluate whether variability among fetuses increased pairwise, from G1 to G3. The primary approach to longitudinal data analysis relied on hierarchical linear modeling (HLM) to characterize developmental trends (Raudenbush & Byrk, 2002; Singer, 1998) via the Mixed procedure in SAS (Version 9.2). HLM models account for dependency in repeated measures data (e.g., FHR nested within fetus over time) and support examination of linear change encompassing the first through the last gestational weeks with available data (i.e., 23 to 38 weeks). Gestational age, centered at 23 weeks, was specified as a random effect in models thereby allowing gestational age within each period to vary across subjects. In contrast to repeated measures analysis of variance, which excludes cases with missing data, restricted maximum likelihood estimation (REML) within the HLM framework accounts for missing data within the modeling framework. Missing data (Table 5) were due to variation in study design across cohorts, missed visits by participants, or exclusions based on signal quality or other unique situations. Based on prior work suggesting discontinuity in the growth of fetal measures at G2 (DiPietro, Caulfield, et al., 2004; DiPietro et al., 1996b), a SAS macro procedure was executed to output individual-specific growth parameters (i.e., intercept, linear slope G1 to G2, linear slope G2 to G3) for adjacent gestational periods. Paired *t*-tests were used to compare rate of change from G1 to G2 versus G2 to G3 for each fetal measure.

Statistical approaches specific to analysis question are noted within the relevant chapters. For example, stability of fetal measures over time was evaluated by Pearson correlation coefficients computed among G1, G2, and G3, with and without siblings (Chapters 5 & 6). Potential maternal sociodemographic (e.g., education), physiological (e.g., maternal heart rate; Chapter 8) and fetal (e.g., sex; Chapter 9) moderators of fetal development were entered in HLM models as predictors of developmental trajectories of fetal measures. Contrast estimates were specified to estimate moderating effects within each gestational period. Sibling similarity was evaluated by simultaneous estimation of variability in fetal measures at the individual and family level via the addition of the family unit as a predictor in HLM models (Chapter 10). Chapter 11 relied primarily on descriptive techniques to describe the development of fetuses with anomalous conditions, with the exception of the subset of fetuses with intrauterine growth restriction which were compared to normally growing fetuses using similar HLM procedures.

Chapter 5. Fetal heart rate and variability

Overview

"In the course of observation of fetal heart rate it occurred to us that a study of its minute variations, particularly during the later months when the vagal-sympathetic balance may be emerging, might yield findings of great importance…". (Sontag & Richards, 1938, p 19)

Fetal heart rate (FHR) is the most conspicuous and accessible indicator of fetal functioning. Fetal heart rate, considered as an expression of behavior, was the exclusive focus of the 1938 *Monograph.* Remarkably, the core findings regarding developmental changes in rate and variability and the sources of variation related to fetal and maternal factors were largely correct, despite their small sample (n = 30 but no more than 24 recordings at any gestational period) and reliance on a stethoscope held to the maternal abdomen to detect and time fetal heart rate. Characteristics of fetal heart rate remain the cornerstones of clinical antenatal and intrapartum assessment of fetal well-being (Bocking, 2003). Lack of heart rate variability, elevated or depressed baseline rates, the presence of episodic decreases in heart rate below baseline (i.e., decelerations) and/or the lack of episodic increases in heart rate (i.e., accelerations) can suggest a physiological adversity and connote fetal distress. The non-stress test is the primary clinical tool for evaluating prenatal heart rate, and although the name may suggest assessment along a dimension of reactivity, that is not the case. A reassuring non-stress test simply reflects the presence of two accelerations of 15 bpm or greater for at least 15 seconds within a period of 20 minutes. Interested readers are directed to (Stout & Cahill, 2011; Walton & Peaceman, 2012) for detailed discussion of clinical interpretation of fetal heart rate patterns.

The development of fetal heart rate over the course of gestation is the product of neural and non-neural influences. The former is driven by the developing parasympathetic and

sympathetic innervations with increasing parasympathetic influence as gestation progresses and changes in autonomic control from the medulla oblongata to higher cortical processes over time (Dalton, Dawes, & Patrick, 1983; David, Hirsch, Karin, Toledo, & Akselrod, 2007; Martin, 1978; Parer, 1999; Yoshizato et al., 1994; Yu, Lumbers, Gibson, & Stevens, 1998). Non-neural influences are less well articulated, but include variation in sensitivity to metabolic and other physiologic processes. As a result, patterning of fetal heart rate reveals information regarding chronic or episodic influences of oxygenation on fetal functioning as well as the status of the developing nervous system within the context of gestational stage.

Measurement of heart rate and variability has had a distinguished history in developmental science in infancy and childhood. Both have been implicated as markers of physiological regulation that correspond to individual differences in child temperament, performance, and behavior (Calkins, 1997; Doussard-Roosevelt, McClenny, & Porges, 2001; El-Sheikh & Buckhalt, 2005; Feldman, 2006; Fox & Porges, 1985; Porges, 1992; Richards & Cameron, 1989; Snidman, Kagan, Riordan, & Shannon, 1995). Measures of variability, including time dependent and time independent computational methods, are widely viewed as more utile than rate alone as they reflect more multifaceted nervous system inputs (Bernston et al., 1997; Grossman, van Beek, & Wientjes, 1990). Variability in any system connotes adaptive flexibility. Heart rate variability is a psychophysiological construct with both trait-like correspondence to an individual's capacity for behavioral and autonomic regulation and an indicator of attentional status during periods of challenge or effort. There is evidence of relatively robust stability (i.e., maintenance of ranking among individuals over time) in heart period and/or cardiac patterning from the neonatal period onward (Bar-Haim, Marshall, & Fox, 2000; Bornstein & Suess, 2000; Fox, 1989; Izard et al., 1991; Snidman et al., 1995).

Stability in heart rate or its derivatives implies that autonomic regulation of cardiac patterns is an individual difference that is retained as gestation progresses, even if the mean values of fetal heart rate or variability change for the group over time. How early in ontogeny do individual differences in these indicators emerge? Sontag and colleagues reported stability in fetal heart rate, based on Spearman's rank order coeffecient *(rho)* ranging from *rho* = .45 to .87 in successive months commencing at the 6th prenatal month (Sontag & Richards, 1938) and *rho* = .71 for variability measured in weekly intervals (Welford, Sontag, Phillips, & Phillips, 1967) in late gestation. This was perhaps the first report that, relative to their peers, fetuses with faster heart rate and greater variability earlier in gestation also had faster heart rate and greater variability later in gestation. Subsequent reports, including one based on a cohort included in this analysis, also established stability in both FHR and variability in monthly intervals and over periods of up to 18 gestational weeks(DiPietro, Bornstein, Hahn, Costigan, & Achy-Brou, 2007; DiPietro, Costigan, Pressman, & Doussard-Roosevelt, 2000; DiPietro, Hodgson, Costigan, & Johnson, 1996; Nijhuis et al., 1998).

Here we report results of our aggregated sample for fetal cardiac measures derived from Doppler-based fetal cardiography. As noted earlier, measurement of heart rate in the fetus through the use of standard fetal cardiography imposes limits on the metrics used to quantify fetal heart rate variability due to the inability to time R-waves. Thus, heart rate is computed in beats per minute, as opposed to inter-beat interval. We include two measures of variability – one based on continuous data, the other on episodic changes. Variables include: 1) mean fetal heart rate (FHR), computed in 1 minute epochs and averaged over the standard 50 minute recording period; 2) short-term fetal heart rate variability (FHRV), calculated as the standard deviation of FHR values per 1 minute epoch, and averaged over the recording; and 3) FHR accelerations,

identified as each instance in which FHR values attained 10 bpm above baseline for greater than or equal to 15 s, consistent with clinical criteria. Accelerations provide a different type of indicator of variability than the continuous FHRV measure by capturing fairly large but delimited episodic excursions above baseline. An additional metric of variability (i.e., root mean square) is also computed by our analysis system but its values correspond so highly to those derived from standard deviation (*r*s = .89, .92 and .90 at G1, G2 and G3) that we rely on the simpler metric. Finally, the number and size of decelerations of fetal heart rate, defined as reductions in baseline of 15 bpm or more for at least 15 s, was quantified. It is often difficult to distinguish true decelerations from decrements in heart rate that occur within the context of episodes of very high heart rate variability and the intervals between closely spaced accelerations can be mistakenly identified as decelerations. As a result, all instances of decelerations were confirmed by visual inspection.

Results

Detection of fetal heart rate depends on maintenance of the fetal heart within the Doppler field. Signal loss comprised 6.4%, 4.5% and 4.7% of the recording period at G1, G2 and G3, respectively. All FHR data were excluded for 2 cases with congenital septal defects. Transient expression of fetal heart rate arrhythmias and inadequate signal quality resulted in additional loss of FHR data at G1 (*n* = 5; 1 arrhythmia, 4 signal loss), G2 (*n* = 2; 1 arrhythmia, 1 signal loss) and G3 (*n* = 5; 1 arrhythmia, 4 signal loss).

Developmental trends. Mean fetal heart rate (FHR), variability (FHRV), and acceleration values are presented in Table 6. Decelerations in fetal heart rate, the ominous converse of accelerations, were also defined according to clinical criteria but after the G1 period (13.2%), were rarely observed (2.5% and 3.5% of recordings at G2 and G3 weeks). All fetal

cardiac measures changed significantly over time; results of repeated measures modeling as a function of gestational age are presented in the last column of Table 6. Fetal heart rate decreased, on average, 0.48 bpm per week over the gestational period studied, while variability in heart rate increased. The number of accelerations increased nearly seven-fold over this time period, from approximately 1 to nearly 7 per 50 minute recording. For each variable, the degree of variability within gestational period (i.e., the standard deviation) increased significantly from G1 to G3, *F*s range from 2.08 to 4.97, *p*s < .001.

Figures 4 and 5 depict individual data points for fetal heart rate and variability, respectively, and Lowess curve estimates of mean values as a function of gestational age. As shown, Lowess curves suggest steeper rates of change for both fetal heart rate measures from G1 to G2, followed by slower rates of change from G2 to G3; the same pattern is evident for accelerations (not shown). To test this empirically, a spline term was created generating two slope estimates per fetus to evaluate relative rate of change between gestational periods via a paired *t*-tests. Significantly steeper rates of change were detected between G1 and G2 relative to G2 to G3 for each measure, $t(737) = -13.05$, $p < .001$ for FHR, $t(737) = 6.69$, $p < .001$ for FHRV, and $t(737) = 10.83$, $p < .001$ for accelerations. In an effort to better determine the point of inflection for this change during the second period, comparisons between mean cardiac measures during the first gestational period and individual weeks of gestation within G2 revealed that discontinuity in each of the FHR measures was observed by 30 weeks gestation (*ps* < .001).

Within and across time associations. Correlation coefficients were computed for each pair of gestational periods to examine the degree to which heart rate parameters remain stable in individual fetuses. Data are presented in Table 7a and show fairly strong correspondence for FHR and FHRV between gestational intervals, and significant but smaller associations for

accelerations. Table 7a also includes interrelations among cardiac measures. As expected, both measures of variability are strongly associated, but despite some statistically significant associations due to sample size (e.g., $r(573) = .09$, $p < .05$ between G1 FHR and FHRV), rate and variability were relatively orthogonal.

Data presented in Table 7a include fetuses from subsequent pregnancies of the same woman, raising the possibility that the stability values may be artificially inflated due to shared genetic or intrauterine influences. Correlations were recomputed for data generated from only the initial pregnancy of those women who participated in more than one study. Those results, presented in Table 7b, reveal no biasing effects of the inclusion of siblings on the magnitude of the stability coefficients. The within-sibling contribution to fetal heart rate measures is presented in Chapter 10.

Discussion

The observed decline in fetal heart rate and corresponding increase in variability over segments within the second half of gestation was reported in the original *Monograph* (Sontag & Richards, 1938) and many times since (Nijhuis et al., 1998; Pillai & James, 1990b; Ribbert, Fidler, & Visser, 1991; Van Leeuwen, Lange, Bettermann, Gronemeyer, & Hatzmann, 1999), including individual cohorts subsumed in this analysis (DiPietro, Costigan, et al., 2006; DiPietro, Caulfield, et al., 2004; DiPietro et al., 2010). Prior quantification of variability has been based on a number of time dependent and independent methods computed over various intervals. Although we have applied complex time dependent computational techniques to fetal heart rate data, such as approximate entropy (ApEn), (Fleisher, DiPietro, Johnson, & Pincus, 1997) to characterize variability, here we used basic computation of mean standard deviation epoched into one minute intervals, averaged over the recording period. Our experience has been that for

Doppler-based methods of FHR detection, the simplest measure is equally as effective as the more complex. However, more complex quantification of variability may not have provided added value because it exceeds the measurement resolution of Doppler detected heart rate. Comparison of a range of computational techniques to quantify variability from 5 minute segments of interbeat intervals derived from fetal magnetocardiography in 11 fetuses measured repeatedly indicates that time dependent measures of complexity, such as ApEn, show somewhat stronger within-individual stability than a measure based on standard deviation (Van Leeuwen, Cysarz, Edelhauser, & Gronemeyer, 2013).

Mean fetal heart rate nears 170 bpm at 10 weeks, and slows to approximately 150 bpm seven weeks later (Gembruch, Shi, & Smrcek, 2000) reflecting a rate of change earlier in gestation that is more precipitous than observed during the 12 week gestational span studied here. The finding of further deceleration in the rate of heart rate decline during the second half of our observation period underscores the somewhat discontinuous nature of developing cardiac regulation. We have previously detected changes in slope as occurring between 28 and 32 weeks for each of these measures (DiPietro, Caulfield, et al., 2004; DiPietro et al., 1996b), although the discontinuity is less evident with more frequent gestational sampling (DiPietro et al., 2010). Consideration of this period as the point at which heart rate variability is functionally “mature” is consistent with the significant differences in variability across the power spectra between preterm infants born before and after 32 weeks gestation (Longin, Gerstner, Schaible, Lenz, & Konig, 2006). Analysis of the power spectrum in fetal heart rate generated by a fetal electrocardiogram system reveals transition for 5 of 6 components (e.g., very low, low, and high frequencies and their ratios) in directions consistent with augmented parasympathetic input at approximately 32 weeks followed by a leveling off through term (David et al., 2007). This period

of gestation coincides with both structural and functional neuromaturational changes in central and autonomic components, including transition to higher levels of cortical control in general (Kinney, Karthigasan, Borenshteyn, Flax, & Kirschner, 1994; Sachis, Armstrong, Becker, & Bryan, 1982) and of the heart rhythm in particular (Ogawa et al., 1996; Yoshizato et al., 1994).

Group differences in fetal heart rate measures have previously been reported. Fetuses drawn from samples of socioeconomically disadvantaged women in Baltimore, MD and Lima, Peru, displayed lower levels of variability and/or fewer accelerations at comparable gestational ages as compared to the more advantaged population of pregnant women that comprise the current analysis. Moreover, the inflection point at which the rate of development in fetal heart rate variability levels off is earlier in gestation and/or shows a decelerating trajectory for accelerations in the low income sample (DiPietro, Caulfield, et al., 2004). Racial differences in the incidence of accelerations during non-stress testing have also been detected (Johnson, Paine, Strobino, & Witter, 1998) although it is not clear whether this reflects a racial or socioeconomic influence. The mediating influences that underlie these differences have not been identified, but the influence of general adverse prenatal conditions on the trajectory of fetal cardiac patterns further supports that these measures reflect developing neural control during gestation.

Perhaps the most useful application of these aggregated data involves stabilization of the estimate of the degree to which individual differences in cardiac measures are preserved over time. From G2 to G3 (approximately 31 to 36 weeks gestation), 22% of the variance in both FHR and FHRV is shared within individual fetuses. Stability in FHR is initially established somewhat more strongly commencing with the first recording (R^2 = .44). Although there was significant stability in the number of fetal heart rate accelerations between each period, the shared variance was considerably smaller. This may suggest that variables based on mean values

generated from continuously sampled data may be preferable to those based on *a priori* criteria. Alternatively, the steep trajectory in the incidence of accelerations may indicate a stronger influence of maturational processes that manifest later in gestation. As predicted, and can be observed by the scatter plots in Figures 4 and 5, individuals become significantly more different from one over time, thereby confirming that even measures of autonomic function become less canalized, and more variable, with advancing gestation.

In contrast to the large and confirmatory literature on the normative development of fetal heart rate and variability, information on the predictive value of these measures for postnatal outcomes of interest to developmentalists is relatively sparse. This includes limited information on the degree to which there is within-domain or homotypic prediction. In one of the early cohorts not included in this analysis, fetal heart rate at 36 weeks was significantly associated with infant heart rate at 1 year, $r(30) = .40$ and fetal heart rate variability was significantly associated with infant heart rate variability, $r(30) = .47$ (DiPietro et al., 2000), despite differences in precision of measurement between fetal (i.e., Doppler-based detection of heart beats) and infant (traditional ECG) methods. Similar results were found between fetal and infant cardiac measures at age 2 for Cohort I (DiPietro et al., 2007).

Fetal cardiac measures also reveal cross-domain, or heterotypic, predictive validity to period, consistent with the view that variation in parasympathetic influence on heart rate variability reflects individual differences in autonomic regulation and the rate of neural maturation. Early reports identified associations between the fetal heart rate response to labor and neurobehavioral performance in neonates (Emory & Noonan, 1984; Emory, Walker, & Cruz, 1982) and with some developmental outcomes in high risk pregnancies (Todd, Trudinger, Cole, & Cooney, 1992). In the fetus, higher heart rate variation has been positively associated with

faster neural conduction in neonates (Cohort VI) (DiPietro et al., 2010) and with higher developmental assessment and language scores at age 2 (Bornstein et al., 2002), a finding subsequently replicated in a different sample (Cohort I) (DiPietro et al., 2007). These associations were detected as early as 28 weeks gestation and were similar for both FHRV level and its trajectory. Evidence linking fetal cardiac measures to infant temperament is somewhat less well-established, but includes association between higher fetal heart rate and lower threshold to novelty (Snidman et al., 1995) and lower emotional tone (DiPietro et al., 1996). Thus there remains tremendous opportunity to further investigate the manner in which the expression of autonomic regulation in the developing fetus previews both individual differences in child cardiac patterns as well as indicators of higher order developmental and maturational processes.

Summary. The development of fetal heart rate and indicators of variation progresses in predictable ways during the second half of gestation. Individual differences in autonomic regulation, as indexed by fetal heart rate and fetal heart rate variability, clearly emerge by midway through gestation. Stability coefficients for heart rate variability based on a small sample of preterm infants measured between 32 and 36 weeks post-conceptional age (DiPietro, Caughy, Cusson, & Fox, 1994) are remarkably similar to those reported in Table 7a (i.e., $r(20) = .46$ for preterm infants v $r(543) = .47$ for fetuses). Together these suggest that heart rate, and its variation, are intrinsic properties of the human fetus that reflect ontogenic expression of the developing nervous system.

Chapter 6. Fetal motor activity

Overview

"…it is the purpose of the present paper to deal with the measurement of fetal activity, and of its use as a criterion of individual differences in behavior". (Richards et al., 1938, p 69)

Motor activity is a core construct pertaining to individual differences after birth and has been among the most widely studied and validated dimensions of early temperament, with the presumption of constitutionality (Eaton & Saudino, 1992; Goldsmith et al., 1987; Rothbart, Ahadi, & Evans, 2000; Saudino & Eaton, 1991). There is a sizable literature on the development and functionality of fetal motor behaviors, initially through the use of 2D ultrasound images and subsequently using 3D/4D imaging. Spontaneous motor activity commences during the late embryonic period, and by the 16th week of gestation the fetus shows a range of motion of the limbs, fingers and head, including stretching, yawning, hand to face contact, swallowing and tongue protrusion. In general, fetal movement progresses from uncoordinated movements that involve the entire body to more integrated and fluid behavior patterns progressing from least to most well differentiated (Amiel-Tison et al., 2006; Birnholz, Stephens, & Faria, 1978; de Vries et al., 1982; Grant-Beuttler et al., 2011; Kurjak et al., 2006; Roodenburg et al., 1991). Readers are directed to a comprehensive synthesis of the emergence of human motor function by (Einspieler, Prayer, & Prechtl, 2012). Data generated from animal models confirmed that variation in fetal motor behavior both reflects ontogenic adaptation to the intrauterine environment which, in turn, fosters subsequent maturation (Hofer, 1988; Smotherman & Robinson, 1987).

Although the developmental psychobiology and neurology approaches have implicated specific fetal motor behaviors in preparatory or adaptational roles, the amount of motor activity

exhibited by fetuses has also been implicated. Greater fetal motor activity appears to mitigate the effect of maternal hyperglycemia on the development of excessively high weight for gestational age at the time of birth (Zisser et al., 2006), a potential functional relationship that was originally proposed by Sontag and colleagues (Sontag, 1944). Animal models that allow manipulation of fetal motility have indicated that the wide inter-individual variation in umbilical cord length is attributable, in part, to more persistent tensile forces exerted by more active fetuses (Moessinger, Blanc, Marone, & Polsen, 1982). Although there is limited research in this arena, there is little debate that the fetus exerts influence over its proximal environment.

Most women can feel the fetus move from approximately the 18th week of gestation (i.e., the period of "quickening"), women detect only a small proportion of fetal movements, generally relegated to those that are sustained or of larger amplitude (Fai et al., 1996; Johnson, Jordan, & Paine, 1990; Kisilevsky, Killen, Muir, & Low, 1991; Lowery, Russell, Wilson, Walls, & Murphy, 1995). As a result, although maternal report of felt fetal movement is used as a clinical indicator of well-being (Rayburn, 1990), its limitations should be acknowledged when used in research if there are no alternate methods available. Normative estimates regarding fetal motor activity based on ultrasound or actography vary due, at least in part, to differences in how investigators define the end of one movement and the beginning of the next (ten Hof et al., 1999). In general, data from our prior work confirm that of others such that in the latter half of gestation fetuses move approximately once per minute, and are active between 10% and 30% of the observation time (DiPietro, Caulfield, et al., 2004; Nasello-Paterson, Natale, & Connors, 1988; Roberts, Griffin, Mooney, Cooper, & Campbell, 1980; Roodenburg et al., 1991). Fetal activity patterns exhibit cyclic periodicities during relatively short cycles (Robertson, 1985)

during the day and there is some evidence that fetal motility peaks late in the evening (Patrick, Campbell, Carmichael, Natale, & Richardson, 1982).

Reports of developmental trends in fetal motor activity are less consistent. Some studies report that the fetus becomes less active as term approaches (Roodenburg et al., 1991; ten Hof et al., 2002), although others fail to show changes during the third trimester (Manning, Platt, & Sipos, 1979; Patrick, Campbell, Carmichael, & Probert, 1982). Differences in how fetal movement is defined across studies make comparisons difficult (ten Hof et al., 1999) as can inherent attributes of motor activity. For example, if fetuses make fewer individual movements over time, but the duration of each increases, a measure of overall fetal motor activity would be unaffected (Roberts et al., 1980). Within our own cohorts, we have reported declines in fetal motor activity during the second half of gestation (DiPietro, Costigan, et al., 2006; DiPietro et al., 1996b), no change (DiPietro et al., 1998), or an increase (DiPietro et al., 2010). Conclusions depend on whether variable definitions addressed movement frequency or signal amplitude, although these are interrelated because decisions made on whether the fetus is moving or not are based on amplitude thresholds of the actograph output.

Here we focus on quantitative aspects of fetal motor activity through the use of Doppler-based actocardiography. Analyses are based on the following three variables: 1) vigor, defined as total value of all actograph data points per minute divided by the number of these data points, averaged over the 50 minute recording period; 2) movement bouts, identified as each time the actograph signal equaled or exceeded a predetermined threshold (15 a.u.s.), and remained at or above this amplitude for at least 10 contiguous seconds (i.e., at least 10 s were required to elapse without movement before a new movement was identified); and 3) total movement, calculated as the number of bouts multiplied by the mean duration of each bout (s), yielding the total time

spent moving, in seconds, per 50 minute recording. Thus to capture the various facets of motor activity, these variables range from the unadjusted, continuous output of the actocardiograph (vigor) to a count of discrete movements (bouts) and a composite (total movement) based on discrete (bouts) and continuous (duration) measures.

Results

Excursions of the chest wall that accompany deep and continuous fetal breathing generate a characteristic signature on the actograph output and provide a source of signal artifact. At times, fetal breathing movements (FBM) persisted for significant portions of the recording interval and interfered with data quality to the extent that the fetal movement data were determined to be unusable; there were also several instances of data loss due to equipment or signal failure. These include 5 recordings at G1 (1 FBM, 4 signal failure), 4 at G2 (3 FBM, 1 signal failure), and 7 at G3 (4 FBM, 3 signal failure). Fetal motor activity is typically marked by wide inter-individual variation (Groome, Swiber, et al., 1999), so we took a conservative approach to outliers and excluded only those that were ≥ 4 SD above the mean for any fetal movement variable; 4 cases had values that met this criterion at G3 and were excluded from analyses.

Developmental trends. Mean values for fetal movement vigor, number of movement bouts, and total movement values are presented in Table 8, as are repeated measures modeling results. On average, fetuses move slightly more than once per minute during the second half of gestation and spend approximately 28% of the recording period moving. The degree of variability within gestational period increased significantly from G1 to G3 for each motor measure, *F*s range from 1.36 to 1.66, *p*s < .001.

Modeling results indicated that fetuses displayed mildly decreasing levels of motor vigor over time as shown in Figure 6; computation of the slopes between adjacent data points revealed the decrease occurred from G1 to G2, β = -.39, *SE* = .11, *t* = -3.37, *p* <.01, but not thereafter, β = .17, *SE* = .11, *t* = 1.59, *p* = .11. Also shown in Figure 7, the number of individual movement bouts decreased, on average, by approximately 1 movement per week across the gestational span studied. There was greater decline in movement bouts from G1 to G2, t(739) = -5.68, *p* <.001. Total movement (not shown) displayed a more complex pattern over time, decreasing from G1 to G2, β = -73.98, *SE* = 23.7, *t* = -3.12, *p* <.01, but increasing from G2 to G3, β = 57.70, *SE* = 25.6, *t* = 2.25, *p* <.05. As a result, the overall model (Table 8) reflected no linear change.

Within and across time associations. Correlations coefficients were used to examine stability over time and interrelations among fetal movement variables. Fetal hiccups can provide a source of signal artifact that also generate a characteristic signature on the actograph and are audibly identifiable during data collection. Fetal hiccups of at least a minute in duration were present in 16% (*n* = 90), 9% (*n* = 61), and 8% (*n* = 47) of recordings at G1, G2, and G3 with mean durations of 8.0, 7.1, and 7.7 minutes, respectively. During post-data collection processing, we implement a procedure to identify and remove their influence, but this is not always possible and can affect data validity. Since hiccups were only present in one individual fetus at all three recordings, their presence during one or more weeks can mask time to time stability values. As a result, for these analyses we excluded those instances in which hiccups were present for greater than 5.0 minutes (i.e., 10% of the recording time). These reflect 70, 39, and 32 cases at each gestational period, respectively.

As seen in Table 9a, motor vigor, bouts, and total movement show moderate but significant stability. The strongest associations are observed for motor vigor, suggesting that the

continuous nature of this variable and its lack of definitional criteria provides the most reliable indicator of fetal motor activity. Within gestation relations among fetal movement measures are also provided in Table 9a. All measures of fetal motor activity were positively related, particularly within gestational period.

Data presented in Table 9a include fetuses from subsequent pregnancies of the same woman, again raising the possibility that the stability values may be artificially inflated due to shared maternal genetic or intrauterine environment. Correlations were recomputed for data generated from only the initial pregnancy of those women who participated in more than one study, and are also exclusive of hiccups greater than 5.0 minutes. Those results, presented in Table 9b, reveal no effect of the inclusion of siblings on the magnitude of the stability coefficients. Additional information on fetal motor activity in relation to siblings is presented in Chapter 10.

Potential effects of the local intrauterine environment. Fetal motor activity may be mechanically affected by characteristics of the local intrauterine environment. During the ultrasound that preceded the data recording at each visit, fetal position (i.e., vertex, breech, transverse) was recorded and the volume of amniotic fluid was estimated by measuring the deepest pockets in each uterine quadrant and summing them. The amniotic fluid index (AFI) decreased linearly over time from 15.6 cm at G1 to 14.0 cm at G3, $\beta = -0.80$, $SE = 0.09$, $t = -9.39$, $p < .001$. At the first gestational period, 56% of fetuses were already in the vertex position; by the last gestational period this had increased to 94%. With the exception of 36 cases (4.9%), once fetuses assumed a vertex position, they remained in it until delivery based on records obtained at birth.

Analysis of variance at each gestational age revealed that fetal position had no effect on any fetal movement variable. In contrast, fetuses with greater amounts of amniotic fluid were modestly more active than those with less. Correlation coefficients were significant at each gestational period for total movement, ranging from $r = .13$ to .23, $ps < .001$. Vigor was significantly associated with AFI at G1, $r(572) = .25$, $p < .001$, and G3, $r(592) = .09$, $p < .05$. Movement bouts were unrelated to fluid level. Oligohydramnios is a diagnosed clinical condition characterized by amniotic fluid index values $\leq$ 5 cm; pregnancies that experience this condition are often subject to obstetric management intervention. Five cases met this criterion at 36 weeks. Although the sample is too small for statistical comparison, these cases exhibited slightly more than half of the total movement displayed by the full sample (M = 463 s versus 850 s).

Using an HLM framework, multivariate models of FM and AFI change over time revealed significant slope covariance between AFI and motor vigor, $\beta = .04$, $SE = .02$, $Z = 2.16$, $p < .05$, and with total movement, $\beta = .06$, $SE = .02$, $Z = 2.53$, $p < .05$. The multivariate approach also generates an empirical test of directionality of the association, comparing relative effect sizes of simultaneous standardized (i.e., Z-scored) estimates within a single model. Results indicated that each FM measure at G1 significantly predicted the change in maternal AFI over time (vigor: $\beta = -.15$, $SE = .03$, $t = -4.83$, $p < .001$; bouts: $\beta = -.09$, $SE = .03$, $t = -2.94$, $p < .01$; total movement: $\beta = -.10$, $SE = .03$, $t = -3.05$, $p < .01$), and not the converse (ps from .29 to .57).

Discussion

Fetuses were observed to make fewer individual movements as gestation advances, although the prorated value of these declines only from 1.3 to 1.1 per minute, with a slight corresponding decline in vigor. In contrast, total movement time did not change, with fetuses

exhibiting motor activity between 27% and 29% of the mid-afternoon observation period. It is possible that the decline in motor activity reflects increasing intrauterine constraint, but the fact that the greatest decline for each motor variable occurred between the first two gestational periods argues against this interpretation. Moreover, this decline parallels that observed in preterm infants (Prechtl, Fargel, Weinmann, & Bakker, 1979). Motor inhibition is the hallmark of development during early childhood and as such, is reflective of maturation of neurological processes (Eaton, McKeen, & Campbell, 2001). We consider the decline in fetal movement as term approaches as part of this continuum. For all measures, regardless of whether change was observed or not, greater variability among fetuses in motor activity evolved over time. However, examination of Figures 5 and 6 reveals that this is less pronounced for fetal motor activity than for heart rate measures, perhaps due to the large degree of between individual variability present as early as G1.

The fairly substantial stability in motor variables provides support for the position that activity level is a temperament variable reflective of relatively stable individual differences. The magnitude of these time to time correlations are consistent with, and thereby replicate, prior findings based on our earliest cohorts that are not included in this report (DiPietro, Bornstein, et al., 2002; DiPietro et al., 1996). Considering the circumstance of the fetus during this period of gestation – positioned in flexion often with knees near the ears – it seems clear that motor activity is an endogenously generated attribute. This supposition is further supported by the lack of our ability to detect motor activity differences in breech versus vertex fetuses, confirming reports of others (Kean, Suwanrath, Gargari, Sahota, & James, 1999; Van der Meulen, Davies, & Kisilevsky, 2008) despite the dramatic difference in postural constraints imposed by these presentations.

There are only a handful of studies that have evaluated the conservation of individual differences in prenatal motor activity to the postnatal period. These include significant fetal to neonatal associations for activity levels during active sleep (Groome, Swiber, et al., 1999), in the number of leg movements for girls only (Almli, Ball, & Wheeler, 2001) and, in twin pregnancies, consistency between rankings of the more active twin before and after birth (Degani, Leibovitz, Shapiro, & Ohel, 2009). We have reported that fetal motor activity is significantly associated with activity levels measured in a laboratory situation at age 1, but only for boys (DiPietro, Bornstein, et al., 2002). However, fetal activity level is significantly predictive of maternal report of activity level at 6 months (DiPietro et al., 1996) and 2 years (DiPietro, Bornstein, et al., 2002) for both sexes. The Fels investigators were the first to report a potential experiential or practice effect of fetal motor activity on development by the finding of accelerated maturation based on Gesell assessment at 6 months for infants that had expressed greater fetal motor activity (Richards & Newbery, 1938). We have also found a significant association between greater fetal motor activity and more optimal motor and/or reflex performance in the neonatal period (DiPietro, Bornstein, et al., 2002; DiPietro et al., 2010). Lastly, fetal motor activity has also been linked to a range of temperament attributes that access regulatory behavior (DiPietro, Bornstein, et al., 2002; DiPietro et al., 1996).

Although existing models posit a role of fetal behavior on the local intrauterine environment, the directionality of the positive association between fetal motor activity and amniotic fluid volume was somewhat unexpected. Although it may not be surprising to observe that fetuses with more amniotic fluid move more, we found that early fetal motor activity appears to generate the higher volume of amniotic fluid over time. This finding is conceptually consistent with the report that higher fetal motor activity yields longer umbilical cords, and not

vice versa (Moessinger et al., 1982). Unlike that study which was based on experimental manipulation, the current observational result is based on longitudinal modeling techniques. Nonetheless, because amniotic fluid levels are regulated by fetal swallowing and micturition (Einspieler et al., 2012; Levy et al., 2005; Ross & Nijland, 1998), which are more common in active states, these findings suggest that more active fetuses are more active producers of amniotic fluid.

Summary

Fetal motor activity is difficult to measure and cannot be as precisely quantified as fetal heart rate and its derivatives. Current knowledge regarding the emergence of qualitative aspects of fetal motor behaviors, observed via ultrasound, is more expansive than that related to motor activity level. A few studies have used 2D ultrasound to measure fetal motor activity, but the issues in determining the scope and boundaries of individual movements when the subject of study cannot be directly viewed remain the same as for fetal studies that rely on actography. Despite these methodological challenges, fetal motor activity emerges as an individual-level characteristic with implications for postnatal expression of variation in motor development and temperamental qualities of activity level. Moreover, developmental trajectories over time may reveal the degree of inhibitory control exerted by the developing nervous system. Support for this proposition is provided by observation of greater motor activity near term in fetuses exposed to higher levels of environmental contaminants with neurotoxic properties (DiPietro, Davis, et al., 2013). Finally, other than our original validation of the actocardiograph used in our research program (DiPietro et al., 1999), we have not used ultrasound to visualize fetal motor activity. There is no doubt that advances in ultrasound technology will play an enormous role in

elucidating qualitative aspects of fetal movements, further revealing the complexities of behavioral ontogeny of the human fetus.

Chapter 7. Integration of fetal movement and fetal heart rate

Overview

It will be seen that on the whole the heart rate is greater during minutes wherein activity occurs. (Sontag & Richards, 1938, p 33)

Fetal autonomic processes, as expressed by fetal heart rate patterns, become more linked with fetal motor activity over gestation such that movements become frequently accompanied by transient increases in heart rate. Sontag & Richards (1938) observed synchrony between elevated heart rate during periods of motor activity but lacked a method to quantify the strength of the relationship. Since then, cardiac-somatic coupling has become implicated as a function of parasympathetic control which becomes the increasingly prominent influence as gestation advances (Obrist, 1981). The association between movement and heart rate in the fetus has been most often attributed to centrally mediated coactivation of cardiac and somatomotor processes, as opposed to an increase in fetal cardiac output as a result of motor activity (Johnson, Besinger, Thomas, Strobino, & Niebyl, 1992; Timor-Tritsch, Dierker, Zador, Hertz, & Rosen, 1978; Vintzileos, Campbell, & Nochinson, 1986). This distinction was based on observations that changes in fetal heart rate can occur slightly prior to or simultaneously with a movement. Also, in animal preparations, stimulation of single central loci can rapidly increase both heart rate and blood flow to muscles (Koizumi & Kollai, 1981), and muscle paralysis does not eliminate cardiovascular responses to direct afferent stimulation (Pascoe, Bradley, & Spyer, 1989).

The development of this relationship has been previously detailed in reports on individual cohorts (DiPietro, Caulfield, et al., 2004; DiPietro et al., 1996a; DiPietro et al., 2001). The number of movements that are coupled with changes in fetal heart rate increases over gestation and the temporal relationship becomes closer. In this chapter, we provide data on the

development of the linkage between the autonomic and motor systems. We refer to this phenomenon as fetal movement-fetal heart rate (FM-FHR) coupling and measure it by identifying the onset of each individual fetal movement and applying *a priori* criteria to fetal heart rate deviations from baseline to determine whether, and when, a change in heart rate occurs. Coupling was defined as occurring each time a fetal movement was accompanied by an excursion in FHR $\geq$ 5 bpm for $\geq$ 5 sec above the FHR baseline within 5 s prior to the movement onset or 15 s after it. This definition was based on previously developed criteria (Baser, Johnson, & Paine, 1992; DiPietro et al., 1996a). Movements that occurred during a coupled segment were not additionally defined as coupled. FM-FHR coupling was computed as the number of coupled fetal movements divided by all fetal movements during the observation period. When coupling was detected, the latency between the onset of the fetal movement relative to the onset of the FHR change was calculated in seconds and the mean latency was computed over the recording. Primary analytic variables include FM-FHR coupling and latency.

Results

Exclusions noted in prior chapters for FHR and FM data individually were applied to the analyses of coupling measures. These include cases of congenital septal defects (n = 2), transient arrhythmias (ns = 1 at each G period), fetal breathing (ns = 1, 3, and 4 at G1, G2, and G3, respectively), and signal loss (ns = 4, 1, and 4 at G1, G2, and G3, respectively). There was a significant negative association between the number of times a fetus moved and the FM-FHR coupling value at each gestational period (rs = -.18 to -.30, ps < .001). Two outliers at G2 were excluded as they displayed the highest coupling scores (i.e., .70 and .63) in conjunction with low number of movements (i.e., 17 and 22). Latency values that exceeded +/- 3 SD of the mean at each gestational period were excluded (ns = 4, 9 and 5 at G1, G2 and G3, respectively).

Developmental trends. The mean coupling index, latency, and the number of coupled movements are presented in Table 10 by gestational period. Figures 8 and 9 provide individual data points for the first two measures and the Lowess curve estimates of mean values. Note that although latencies of up to -5 s were possible, this was relatively uncommon; mean latency values were negative in only 12 instances distributed across all gestational periods. Repeated measures modeling as a function of gestational age demonstrates increasing integration of somatic and cardiac systems with advancing gestation. The number of coupled movements increased, on average, by one movement every two gestational weeks from G1 to G2, β = 3.69, *SE* = .31, *t* = 12.03, *p* <.001, but not thereafter, β = .31, *SE* = .33, *t* = 0.92, *p* = .36. FM-FHR coupling increased from G1 to G2, β = 0.09, *SE* = .01, *t* = 18.43, *p* <.001, and again from G2 to G3, β = 0.03, *SE* = .01, *t* = 5.38, *p* <.001, although the rate of change from G1 to G2 was greater than that from G2 to G3, *t*(737) = -52.18, *p* <.001. The latency between movement onset and fetal heart rate change also changed significantly over time, but in the opposite direction such that latencies decreased across gestational periods (G1 to G2, β = -0.98, *SE* = .10, *t* = -10.28, *p* <.001; G2 to G3, β = -0.28, *SE* = .09, *t* = -3.00, *p* <.01). The degree of change between the first two data points was also significantly greater than between the latter two data points, *t*(737) = -33.58, *p* <.001. The degree of within gestational period variability increased from G1 to G3 for FM-FHR coupling, *F* = 1.82, p < .001, but not for latency.

Within and across time associations. Consistent with the treatment of FM associations, the examination of the degree of stability in fetal FM-FHR coupling excluded instances of hiccups longer than 5 min in duration (i.e., 10% of the recording time; *n* = 70, 39, and 32 cases at G1, G2, and G3, respectively). FM-FHR coupling showed stability across gestational periods, *r*(385 to 480) = .15 to .28, *ps* < .01; the magnitude of these associations remained unchanged

controlling for the number of movements at each gestational period. Latency was mildly stable for adjacent periods only: G1 to G2, $r(427) = .11$, $p < .05$; G2 to G3, $r(471) = .17$, $p < .001$. In general, fetuses that displayed higher levels of FM-FHR coupling also displayed shorter temporal latencies at each gestational period, and this association strengthened over time: G1, $r(501) = -.12$, $p < .01$; G2, $r(599) = -.27$, $p < .001$; and G3, $r(564) = -.30$, $p < .001$. Correlations were recomputed exclusive of sibling cases retained in the full data set. These results, similar to FHR and FM analyses, revealed minimal effects of the inclusion of siblings on the magnitude of FM-FHR coupling or latency stability coefficients. Additional information on the degree of sibling variance in coupling is presented in Chapter 10.

Discussion

Here we show the progressive integration of fetal motor activity and fetal heart rate during gestation. The developing association is expressed by increases in the absolute number and proportion of coupled movement relative to total movements and by a decrease in the temporal relationship between the two parameters. The observed decline in the rate of change from G2 to G3 is consistent with a period of discontinuity at the mid-point of the sampled period as observed with other measures. The stability in the magnitude of FM-FHR coupling and the within-individual correspondence between higher levels of FM-FHR coupling and tighter latencies suggests that this integration reflects an individual difference in neural integration. We have previously reported a relationship between higher FM-FHR coupling and steeper developmental trajectories with faster brainstem auditory evoked responses in neonates during the first week of life (DiPietro et al., 2010).

The significant negative association between the number of fetal movements and FM-FHR coupling values at each period has at least two explanations. The first may simply reflect a

measurement issue: fetuses that move infrequently have a lower bar in achieving a high coupling value. That is, to achive a coupling value of .50 when there are only 4 movements requires only two of them to be coupled, but this number would be 45 in the case of 90 movements. The alternative consideration is that this association reflects underlying neuroregulatory processes as revealed by coincidentally high levels of motor activity and low levels of integration. We are unable to distinguish between the two possibilities, and it is likely that both are contributory to the observed associations.

Measurement of the integration between fetal heart rate and motor activity reported here used *a priori* criteria to define the relationship. In prior work conducted on Cohort I, we evaluated this relationship using a purely empirical approach through the use of time series analysis of the second by second linkage of the two streams of raw data (DiPietro et al., 2001). This metric revealed a similar progression in the magnitude of the cross-correlation function over time, with coalescence around a peak latency of 5 s between fetal motor activity and heart rate by 32 weeks gestation, without appreciable change thereafter. Despite the considerable difference in quantitative approach, it is notable that latencies in that analysis declined from 6 to 5 s over the comparable gestational period here in which latencies declined from 5 to 4 s. This supports the robust nature of the developing association between these two parameters. The cross-correlation approach is labor and data intensive and was not implemented on all of the cohorts in the current analysis, so was not included in this *Monograph*. However, application of this technique to data generated by a large sample of fetuses in Lima, Peru essentially replicated the development of the cross-correlation function in both magnitude and latency at comparable gestational ages (DiPietro, Caulfield, et al., 2006). It is worth noting, however, that the earliest data point in those analyses was 20 weeks gestation, at which time the latency between fetal

movement and fetal heart rate was 16 s, nearly triple that observed four weeks later. This indicates a rapid period of neuroregulatory maturation just prior to the G1 period of 24 weeks which is the lower gestational boundary of this report.

The development of integration between fetal movement and fetal heart rate provides the foundation for expression of the third functional level in Figure 1, that of fetal states. In the early 1980s, Dutch investigators and their colleagues identified four fetal behavioral states that correspond to quiet and active (REM) sleep, and quiet and active waking states. This approach identifies fetal states using pre-defined patterns of fetal heart rate and motor activity in three minute windows, along with eye movements as observed on ultrasound (Nijhuis, Prechtl, Martin, & Bots, 1982; van Vliet, Martin, Nijhuis, & Prechtl, 1985). The linkage of eye movements with patterns of fetal heart rate and motor activity is thus the next step in the process of the consolidation of fetal state and the distinction between brain activity characteristic of REM and non-REM periods. Linkages among the three parameters are evident as early as 28 weeks gestation but become progressively strengthened over gestation (Martin, 1981); these associations continue to mature in pregnancies that persist beyond term (van de Pas, Nijhuis, & Jongsma, 1994). By 36 weeks, coincidence among all three parameters in normally developing fetuses has been described as high enough that behavioral states can be identified from heart rate patterns alone (Pillai & James, 1990b; Visser, Mulder, Stevens, & Verweij, 1993). Despite some debate as to whether a period of quiet waking in fetuses exists that is comparable to alertness in neonates (Pillai & James, 1990a), the original definitions remain widely used. There is a large literature articulating the development of state parameters around this framework (Arabin & Riedewald, 1992; Arduini et al., 1986; Mulder et al., 1994; Nijhuis et al., 1999; Nijhuis & van de Pas, 1992; Swartjes, van Geijn, Mantel, & van Woerden, 1990; Timor-Tritsch et al., 1978; van

de Pas et al., 1994; Visser, Poelmann-Weesjes, Cohen, & Bekedam, 1987), some based solely on motor activity and heart rate and some including eye movement.

Fetal state ascertainment is done by manual coding of coordinated polygraphic output of each state variable. We have not routinely coded fetal states during the 50 minutes of our baseline data collection and thus do not include state data in this *Monograph*. In part, this is due to the methodological difficulties in collecting fetal eye movement data. Prominent among these is the difficulty in preserving a view of the fetal eye using ultrasound for prolonged periods of time and the complexities of simultaneously using two transducers to visualize the fetal face and extremities. Moreover, we have observed that that periods of heart rate variation often do not fit easily into the original definitions or published exemplars. There has traditionally been a lack of attention given to establishing inter-observer reliability on the coding of fetal heart rate categories based on the original definitions which further complicates the implementation of that system. Our experience has been that whether or not ultrasound is used to collect eye movement data, there tends to be over-identification of active sleep states at the expense of indeterminate sleep states and that often higher levels of motor activity are accepted during putative periods of active sleep. Despite challenges in the operationalization of fetal state coding, the underlying construct is clearly worth efforts to measure it as an indicator of neurobehavioral consolidation and potential regulatory function. Consistent with the individual variation and stability in FM-FHR coupling, there have been reports of within individual stability in some aspects of state through the neonatal period (Groome, Singh, et al., 1997; Groome, Swiber, Atterbury, Bentz, & Holland, 1997) and we have reported that fetuses with higher degrees of state concordance, regardless of the time spent in specific states, become infants with better state regulation (DiPietro, Costigan, & Pressman, 2002). A report suggests that elements of fetal state

transitioning are associated with self regulation through adolescence (Van den Bergh & Mulder, 2012).

Summary

These data reflect the gathering correspondence between the autonomic and motor components of the developmental hierarchy. By 36 weeks gestation, one in three fetal movements is associated with a corresponding increase in fetal heart rate within, on average, 4 seconds. This integration reflects developing neuroregulatory coordination between the somatic and cardiac systems that provides the foundation for the higher order processes, including the expression of fetal behavioral states.

Chapter 8. The maternal context

Overview

"The existence of a profusion of myths and superstitions has probably somewhat inhibited until modern times scientific thought and investigation into maternal-fetal relationships from the standpoint of how fetal development may be influenced by varying maternal factors."(Sontag, 1941, p 996)

There is no other period in ontogeny when the physiological development of an individual is embedded so deeply in the physiological adaptation of another. Although there are no direct neural connections between pregnant woman and fetus, speculation on the role that the maternal experiential environment exerts on prenatal development has proliferated since antiquity. In the last decade, there has been a resurgence of academic interest in downstream effects of psychological and physiological influences that may flow from the pregnant woman to the developing fetus (Van den Bergh, Mulder, Mennes, & Glover, 2005; Entringer et al., 2010; Visser et al., 2010). This approach has been primarily observational and focused on whether baseline levels of maternal psychological characteristics, particularly those related to stress and anxiety (Van den Bergh, 1990; DiPietro, Hilton, Hawkins, Costigan, & Pressman, 2002; Groome, Swiber, Bentz, Holland, & Atterbury, 1995) and products of the hypothalamic-pituitary-adrenal axis (Class et al., 2008; DiPietro, Kivlighan, Costigan, & Laudenslager, 2009; Sandman, Wadhwa, Chica-DeMet, Dunkel-Schetter, & Porto, 1997) are associated with fetal development.

Perhaps the most compelling demonstration that alterations to maternal psychophysiological functioning have downstream effects on the fetus is provided by studies that rely on experimental models that manipulate maternal state. Our own work (Cohort I) has

indicated that inducing short-term maternal arousal, using either the Stroop Color-Word task (DiPietro, Costigan, & Gurewitsch, 2003) or a labor and delivery video (DiPietro, Ghera, & Costigan, 2008) affects fetal heart rate patterns and/or motor activity during application of the stimulus followed by return to baseline or near-baseline levels after its termination. We have also shown that the use of guided imagery to induce maternal relaxation is accompanied by fetal neurobehavioral changes during the procedure (Cohort IV) (DiPietro, Costigan, Nelson, Gurewitsch, & Laudenslager, 2008). Other investigators have also used stressful (Copher & Huber, 1967; Fink et al., 2010; Monk et al., 2004), relaxing (Fink et al., 2011), and/or emotion-provoking (Araki et al., 2010; Benson, Little, Talbert, Dewhurst, & Priest, 1987) experimental manipulations to evaluate a fetal heart rate or motor response. Despite demonstration of fetal responsivity to maternal state alteration, these reports have been unsuccessful at establishing the mechanisms through which the signal from the pregnant woman is transduced to the fetus. Moreover, our interest and use of these procedures has been to develop protocols that can be used to reliably generate a fetal response to evaluate individual differences in fetal reactivity and recovery, the constitutional components of temperament, without using external devices and within the context of individual differences in maternal reactivity and recovery. As such, we caution against extrapolating the results of these studies to assumptions regarding the effects of maternally reported mood states or clinical conditions on fetal development.

In the late 1960's, a seminal paper by Bell (1968) challenged the prevailing view of parent-child interaction as essentially a unidirectional phenomenon from parent to child. This work expanded on a perspective introduced by a classic study of parenting in which the authors expressed skepticism that "...there is any single direction of cause and effect relations in the child rearing process" (p 174) (Sears, Maccoby, & Levin, 1957). The recognition that intrinsic,

"congenital" characteristics of individual children elicit a repertoire of responses from parents aimed at maximizing socialization placed the child firmly within subsequent models of the care-giving environment. The resulting paradigm shift led to the now accepted view of the maternal-child relationship as dynamic and transactional.

The maternal-fetal relationship is emerging to be equally complex. Our most unexpected finding to date is that the contribution of offspring behavior to the environment of rearing, so to speak, begins before birth. Using second by second time series analyses of contemporaneous maternal-fetal recordings in two cohorts (i.e., Cohort I and a Peruvian cohort not included in this *Monograph*), we have shown that spontaneous fetal motor activity transiently stimulates maternal sympathetic arousal (DiPietro, Caulfield, et al., 2006; DiPietro, Irizarry, Costigan, & Gurewitsch, 2004) even though women consciously perceive only a small proportion of fetal movements. In both cohorts, spontaneous fetal movements generated an increase in maternal heart rate and electrodermal activity within 2-3 seconds at each of the six gestational periods studied. Moreover, using an experimental paradigm to evoke a fetal startle response using an acoustic stimulus generates a physiological reaction consistent with an orienting response in pregnant women blind to condition (i.e., fetal stimulation v sham) (DiPietro, Voegtline, et al., 2013).

In this section we evaluate cross-sectional and longitudinal associations between four indicators of maternal autonomic functioning: maternal heart rate (MHR), respiratory sinus arrhythmia (RSA), respiratory period (RP) and electrodermal activity (skin conductance level, SCL) in relation to fetal measures. Maternal ECG data underwent R-wave detection, manual editing for artifact, and interbeat interval (IBI) computation in ms. To maintain consistency with the fetal metric, IBI data were converted to heart rate. Respirations were measured by

quantifying inspiration to inspiration and expiration to expiration periods based on the detected peaks and troughs of the respiratory waveform(s). In addition to computation of RP, these values were included in computation of peak to valley changes in IBI from inspiration to expiration (ms) (Grossman et al., 1990). SCL was scaled from 0 to 25 μS. As for the continuous fetal measures, values were averaged over the 50-minute monitoring period. Relations with fetal motor activity excluded cases of hiccups longer than 5 min.

Results

MHR and RSA data were excluded for women with a detected cardiac arrhythmia (*n*=2) and transient irregular heart rate pattern (*n* = 1). The bellows apparatus used to monitor maternal breathing presents special challenges in use during pregnancy; as a result, respiratory artifact resulted in additional loss of RP and/or RSA data at G1 (*n* = 24 to 25), G2 (*n* = 32 to 34), and G3 (*n* = 43 to 49). SCL was excluded from analysis in instances of artificially high SCL levels (*n* = 5 at G1, *n* = 6 at G2, *n* = 5 at G3).

Change in maternal measures over gestation. Maternal values at each gestational period and results of repeated measures modeling are presented in Table 11. Maternal heart rate and skin conductance changed significantly over time. Neither change was linear in nature such that the fastest MHR and lowest SCL levels were exhibited at G2. RSA exhibited a similar pattern, and although the overall change from G1 to G3 was not significant, the changes from G1 to G2 and G2 to G3 were (*p*s = <.001). RP remained steady at 4 s, or approximately 15 breaths per minute.

Longitudinal and cross sectional associations for maternal measures. Correlations computed between gestational periods revealed high levels of stability in each maternal measure: MHR (*r*s = .74 to .77, *p* <.001), RSA (*r*s = .55 to .65, *p* <.001), RP (*r*s = .35 to .47, *p* <.001), and

SCL (*r*s = .45 to .53, p <.001). Inter-relations of mean maternal values computed across the three time periods revealed a negative association between MHR and RSA, r = -.50, p <.001; MHR was unrelated to other measures. Smaller but significant associations were also detected between RSA and RP, r = .17, p < .001, and RP and SCL, r = .12, p < .01.

The role of maternal parity. Parity had been previously shown to have an effect on maternal physiological measures in Cohort 1 (DiPietro, Costigan, & Gurewitsch, 2005) so was examined as a potential moderating or mediating variable. Fifty-eight percent of the sample was nulliparous; prior births for multiparae ranged from 1 to 4. Compared to multiparous women, women with first pregnancies had significantly slower mean MHR, $t(735) = -3.58$, $p < .001$, d = .27, lower RP (i.e., faster breathing), $t(726) = -2.54$, $p < .05$, d = .19, and higher SCL, $t(737)$ = 2.03, $p < .05$, d = .15. Fetal measures were unrelated to maternal parity and, within parous women, the number of prior pregnancies. As a result, parity is not included in subsequent models.

Associations between maternal physiology and fetal measures. Initial analyses for the relations between maternal and fetal measures relied on bivariate cross-sectional correlations between each maternal measure (i.e., MHR, RSA, RP and SCL) and the three FHR measures and three FM measures. To reduce the number of correlations, a coupling composite indicator was created by standardizing (i.e., *Z*-scoring) the FM-FHR coupling and inverse latency values and summing them. Following this, multivariate hierarchical linear models were used to examine whether maternal physiological measures at G1 corresponded to the trajectory of fetal cardiac and motor development from G1 to G3; and, conversely, whether fetal measures at G1 predicted the trajectory of maternal physiological measures.

Of all the maternal physiological measures, maternal HR yielded the most consistent relations with fetal variables. Within each gestational period, maternal heart rate and fetal heart rate were significantly and positively correlated, *r*s(573 to 647) = .17 to .18, *ps* <.001. In the HLM framework, the covariance estimate for the MHR and FHR slopes was significant, β =.03, *SE* = .01, *t* = 2.93, *p* < .01, suggesting these parameters change together over time. Comparisons of relative effect sizes of simultaneous standardized (i.e., Z-scored) estimates within a single model revealed that, in the case of MHR and FHR, FHR at G1 exerted a significant influence on MHR trajectory, β = -.06, *SE* = .02, *t* = -3.30, *p* < .01, whereas the reverse was not found, β = .02, *SE* = .02, *t* = 0.76. A comparison of effect sizes indicated that the impact of FHR at G1 on MHR trajectory was significantly larger, β = .07, *SE* = .03, *t* = 2.52, *p* < .05. MHR was unrelated to the remaining two fetal cardiac measures either cross-sectionally or over time.

Commencing at G2, MHR was also associated with each fetal movement measure such that women with faster heart rates had fetuses that were more active. Specifically, MHR was associated with both motor vigor and total movement at G2 and G3, *r*s(567 to 608) .15 to .17, *ps* <.001; the association with movement bouts was significant only at G2, *r*(608) = .10, *p* <.05. In the HLM framework, MHR and fetal motor vigor slopes covaried at a trend level, β =.02, *SE* = .01, *Z* = 1.95, *p* = .05. Estimates indicated MHR at G1 significantly predicted the trajectory of motor vigor, β = .09, *SE* = .03, *t* = 3.30, *p* < .01. The slopes of fetal movement bouts or total movement did not covary with MHR change over time. However, MHR at G1 significantly predicted the trajectory of FM bouts, β =.10, *SE* = .03, *t* = 3.44, *p* < .001, and total movement, β =.08, *SE* = .03, *t* = 2.66, *p* < .01. Conversely, FM measures at G1 did not predict MHR trajectory. A comparison of effect size magnitude confirmed that MHR at G1 was more impactful on FM trajectory than the reverse (*ps* < .05). Faster MHR was also modestly, but

negatively associated with coupling index at 31 and 36 weeks, $r(597) = -.12$ and $r(561) = -.18$, ps $< .01$ and $< .001$. The use of a standardized coupling composite precluded evaluation of trajectories.

Cross sectional associations between fetal measures and SCL were confined to an association between total movement and SCL levels at G1, $r(502) = -.09$, $p = .05$. However, multivariate HLM models showed significant slope covariance for maternal SCL with both FM vigor, $\beta = -.04$, $SE = .02$, $Z = -2.47$, $p < .05$, and total movement, $\beta = -.05$, $SE = .02$, $Z = -2.61$, $p < .01$, over time. Fetal movement bouts covaried at a trend level, $\beta = -.04$, $SE = .02$, $Z = -1.68$, $p = .09$. Further, each of the three FM measures at G1 significantly predicted the trajectory of maternal SCL such that greater motor activity early on resulted in greater subsequent rise in SCL levels with advancing gestation (vigor: $\beta = .07$, $SE = .02$, $t = 2.92$, $p < .01$; bouts: $\beta = .08$, $SE = .02$, $t = 3.28$, $p < .01$; movement: $\beta = .07$, $SE = .02$, $t = 2.67$, $p < .01$).

Significant results for RP were most consistent at G2 and included associations with FHR $r(611) = -.12$, $p < .01$, movement vigor, bouts, and total movement $r(574) = .12$, $p < .01$, $r(574) = .16$, $p < .001$, $r(574) = .09$, $p < .05$, respectively, and coupling composite, $r(563) = -.17$, $p < .001$. Several other equally modest associations were significant at G3, but not before. No other associations were detected in HLM models. Maternal RSA and fetal measures were unrelated.

Composite maternal autonomic analysis

Analysis of individual maternal physiological parameters does not fully capture the multidimensional nature of the maternal physiological environment. To this end, a composite variable was created to evaluate the broader maternal autonomic context. Maternal RSA, which is influenced predominantly by parasympathetic processes, and maternal SCL, which is of sympathetic origin, were divided at their respective medians (*medians* = 35 ms and = 6.49 μS)

and combined to create four groups (low RSA/low SCL (24.4%), low RSA/high SCL (25.7%), high RSA/low SCL (25.6%), high RSA/high SCL (24.3%). RSA and SCL were essentially orthogonal, $r(731) = -.04$. Maternal autonomic group was entered into general linear models at each gestational period to evaluate group differences in fetal measures.

Maternal autonomic groupings were associated with significant variation in FHRV, $F(3, 597) = 4.62$, $p < .01$, accelerations, $F(3, 597) = 2.93$, $p < .01$, and total movement, $F(3, 510) = 3.42$, $p < .01$. These significant associations were only detected at the final gestational period (G3) although the associations could be seen to emerge over time. Figure 10 illustrates the results for FHRV and total movement. For FHRV, posthoc contrasts indicated that fetuses of women with high SCL coupled with low maternal RSA had significantly lower heart rate variability than their high SCL/high RSA counterparts ($p < .001$). This was not the case when SCL levels were low; here, similar FHRV levels were observed irrespective of RSA. Results for accelerations (not shown) paralleled FHRV. The coupling composite measure was unrelated to the maternal autonomic profile. For fetal motor activity, fetuses of women with high SCL moved the least, but again only in the context of low RSA (relative to high RSA/high SCL, $p < .05$). Fetuses of mothers who had high SCL/high RSA presented the highest variability relative to all other groups ($p < .01$) and moved at a comparable level to those with high RSA/low SCL. Thus, fetal neurobehavioral functioning was associated with the specific mix of activation and suppression presented by the arms of the maternal autonomic nervous system.

Discussion

Pregnancy represents a period of dynamic change in psychophysiological processes that provides the backdrop for fetal neuromaturation. The modest cross sectional associations between maternal and fetal heart rate are similar in magnitude to an earlier report based on

Cohort IV (DiPietro, Irizarry, et al., 2004) but are much smaller than that reported for a more prolonged period of monitoring on a small sample (i.e., 24 hours, r (10) = .78) (Patrick, Campbell, Carmichael, & Probert, 1982). Remarkably, Fels investigators reported finding similar positive associations between fetal motor activity and maternal heart rate, skin conductance and respiratory period although details of those results are limited (Sontag, 1944).

Evaluations of directionality afforded by the longitudinal modeling approach confirm our earlier reports, based on time series analyses within each 50 minute period, that fetal motor activity provides sympathetic maternal stimulation, as indicated by electrodermal activity (DiPietro, Caulfield, et al., 2006; DiPietro, Irizarry, et al., 2004). We also found that fetal heart rate earlier in gestation appears to influence changes in maternal heart rate; the two were relatively unrelated when examined within the second by second timeframe of the earlier reports. In contrast, maternal heart rate is linked to greater fetal motor activity. Together these findings provide support for bidirectionality within the maternal-fetal relationship and underscore the role of the fetus in shaping the maternal environment. What these data cannot provide, however, are biological mechanisms through which these effects, in either direction, are mediated. We have previously speculated that the association between fetal motor activity and maternal skin conductance may be mediated through perturbations to the uterine wall, which contains adrenergic innervations (Owman, Rosengren, & Sjoberg, 1967). Similar potential mechanism through which heart patterns, either of mother or fetus, are not as easily identified and we are left with the assumption that heart rate serves as a proxy measure for other unmeasured physiological processes or, in the case of the maternal heart rate, auditory stimulation.

Although it is easiest to evaluate the relation between individual maternal and fetal measures, the intrauterine milieu is subject to a range of maternal influences including postural,

neuroendocrine, and psychophysiological. Here we focus only on selected components of the latter. Although the parasympathetic and sympathetic arms of the autonomic nervous system are often regarded as exerting push-pull tension, requiring dominance of one over the other at any point in time, these processes are orthogonal to one another. Such recognition, conceptualized as the "doctrine of autonomic space" (Bernston, Cacioppo, & Quigley, 1991), reveals that specific viscera can be subject to sympathetic and/or parasympathetic control and for those that are dually innervated, the two processes can either be coupled or independent. This principal was confirmed within this sample through the lack of association between maternal RSA (i.e., a predominantly parasympathetic process) and SCL (i.e., of sympathetic origin) and that the composite measure provided information that either alone did not. In particular, variability in fetal heart rate, which was unassociated with individual maternal parameters, was lowest in women with high sympathetic but low parasympathetic tone, and highest when both parasympathetic and sympathetic tone were elevated. This suggests that there is value in considering broader, multifaceted characterization of the maternal autonomic environment when possible.

Summary

In general, and despite significant associations, the maternal psychophysiological measures used here did not contribute much explanatory power for variation in fetal development, particularly when analyzed individually. It is clear that the fetus reacts to phasic changes in maternal arousal based on studies that employ an experimental design; however, those reports also typically fail to detect associations between baseline or delta values between maternal physiological measures and fetal responses that can adequately explain any observed downstream fetal effects (DiPietro et al., 2003; DiPietro, Ghera, et al., 2008; Fink et al., 2011;

Monk, Myers, Sloan, Ellman, & Fifer, 2003). Moreover, findings that the developing fetus influences the pregnant woman reiterate the complicated manner in which the fetus affects the maternal prenatal milieu. This is not a new concept since, in 1938, Fels' investigators speculated that the coincidence between higher maternal basal metabolic rate and fetal motor activity was the result of the former on the latter (Richards et al., 1938). Yet it remains an under-articulated phenomenon in studies that primarily try to ascertain the unidirectional influence of pregnant woman on fetus. Despite the advances in knowledge provided by our research and that of others regarding associations between aspects of maternal and fetal functioning under either baseline or perturbed conditions, the physiological processes that mediate this relationship remain largely as enigmatic as they were 75 years ago.

Chapter 9. Sex differences in fetal development

"The sex difference…favoring a slightly faster rate for females has been reported also in children, but differences here are not so clear cut". (Sontag & Richards, 1938, p 5)

Overview

Fetal sex differentiation commences late in the embryonic development. By the time gestation ends, boys are at substantially higher risk of morbidity and mortality in the neonatal and post-neonatal periods (Di Renzo, Rosati, Sarti, Cruciani, & Cutuli, 2007; Eriksson, Kajantie, Osmond, Thornburg, & Barker, 2010; Gualtieri & Hicks, 1985). Given this long-standing observation, it is now clear that at least part of this differential is the result of heightened male vulnerability to prenatal exposures and/or a greater adaptational capacity of female fetuses (Bale, 2009; Sandman, Glynn, & Davis, 2013). Although boys, on average, are heavier at birth, the higher survival and lower rates of respiratory failure of female preterm infants born at the same gestational age as male preterm infants suggests that female fetuses mature faster. There is some supportive evidence to this effect, such that girls have more mature skeletal systems at birth (Tanner, 1978) and faster conduction of brainstem auditory evoked responses (DiPietro et al., 2010), but the empirical evidence based on developmental criteria is not overwhelming.

Many fetal neurodevelopment studies fail to report analyses for fetal sex; those that do are somewhat conflicting. The commonly held perception that a faster fetal heart rate in the first trimester indicates that the fetus is female is not supported by the evidence (McKenna, Ventolini, Neiger, & Downing, 2006). Small longitudinal samples tend to yield no sex differences in fetal heart rate or a variety of variability measures during the second half of pregnancy (DiPietro et al., 1996b; Lange, Van Leeuwen, Geue, Hatzmann, & Gronemeyer, 2005; Nijhuis et al., 1998). Male fetuses showed higher heart rate variability in one of our earlier samples (n = 103)

(DiPietro et al., 1998) but not in two later cohorts (I and VI) that were somewhat larger and included in this analysis (DiPietro, Caulfield, et al., 2004; DiPietro et al., 2010). However, during labor, based on a sample of over 1800 clinically monitored deliveries, female fetuses displayed faster fetal heart rates even after controlling for potential confounding variables, but this difference was not seen in another group of fetuses monitored at term but prior to labor (Dawes, Dawes, Moulden, & Redman, 1999). There is at least one other report of faster fetal heart rates during labor for girls, although the difference is complicated by the degree to which fetuses are adversely affected by the stress of delivery (Bernardes, Goncalves, Ayres-de-Campos, & Rocha, 2009).

The cultural belief that male fetuses are more active than female fetuses has persisted since antiquity (Temkin, 1991). That boys are more physically active than girls is the most commonly observed behavioral sex difference in infancy (Campbell & Eaton, 1999) and childhood (Eaton & Enns, 1986). Yet, empirical evidence for fetal sex differences in motor activity has not been consistently empirically detected by others or in our own work. Male fetuses were more active than female fetuses in our initial small cohort (DiPietro et al., 1996b) but not in later ones (DiPietro, Caulfield, et al., 2004; DiPietro et al., 1998; DiPietro et al., 2009; DiPietro et al., 2010). Ultrasound observations of motor activity have also not generated observation of sex differences (de Vries, Visser, & Prechtl, 1988; Hepper, 2012) although there is one report that male fetuses make more leg movements (Almli et al., 2001). There is a single report that male fetuses move more often at term but not before (Robles de Medina, Visser, Huizink, Buitelaar, & Mulder, 2003).

Sex difference research during pregnancy has an additional and less examined dimension – the role of fetal sex on the pregnant woman. Much of this research involves potential antigenic

(Gualtieri & Hicks, 1985) or pro-inflammatory effects (Challis, Newnham, Petraglia, Yeganegi, & Bocking, 2013) on maternal and placental physiology as a result of carrying a male fetus. Other idiosyncratic findings include a well-established heightened incidence of hyperemesis of pregnancy in women carrying female fetuses (Veenendaal, van Abeelen, Painter, van der Post, & Roseboom, 2011) and some evidence that women carrying male fetuses consume more calories (Tamimi et al., 2003). Thus, analysis included examination of the role of fetal sex on both fetal and maternal measures.

Results

Fetal sex was equally represented in the sample (50.7% male). There were no sex differences in the infant characteristics detailed in Table 4, with the exception that boys were 137 g heavier (*M* boys = 3471 g, *M* girls = 3334 g), $t(727) = 3.66$, $p < .001$, and slightly (.5 cm) longer (*M* boys = 51.4 cm, *M* girls = 50.9 cm), $t(680) = 2.35$, $p < .05$, than girls. Boys were also more likely to be delivered via Cesarean section, 32% versus 24%, $\chi^2(1, 728) = 5.08$, $p < .05$. Twice as many Cesarean deliveries were the result of fetal distress, as indicated by worrisome patterns of fetal heart rate and variability during labor, for boys as compared to girls. When averaged over gestation, amniotic fluid levels were higher in pregnancies carrying male (14.9 cm) than female (14.4 cm) fetuses, $t(736) = 2.21$, $p < .05$, $d = .16$.

Fetal heart rate measures. Means for fetal measures for each gestational period, stratified by fetal sex, are presented in Table 12. Female fetuses had faster heart rates at G2 and G3, $\beta = 1.05$, $SE = 0.49$, $t = 2.14$, $p < .05$, $d = .16$, and $\beta = 2.24$, $SE = 0.64$, $t = 3.50$, $p < .01$, $d = .26$; no differences were detected at G1. The small but significant size differential at birth cannot explain this result, as the correlation between birth weight and FHR at G3 was essentially zero $r(601) = .01$. There was a significant sex difference in the developmental trajectory of FHR, as

noted by the *t*-value in the final column. The rate of change in FHR differed by fetal sex such that males showed greater decline from G2 to G3, β = -1.20, *SE* = 0.63, *t* = -1.89, *p* = .05. In contrast, male fetuses had significantly greater FHRV than female fetuses at G2 and G3, β = -0.28, *SE* = 0.09, *t* = -3.08, *p* < .01, *d* = .23, and β = -0.26, *SE* = 0.12, *t* = -2.13, *p* < .05, *d* = .16; again no differences were detected at G1. The overall developmental trajectory was also significantly different between males and females (see Table 12). Male fetuses showed a faster gain in FHRV compared to females from G1 to G2, β = 0.22, *SE* = 0.11, *t* = 2.14, *p* < .05. There were no sex differences in either the number of accelerations or in their trajectory. Post hoc analyses revealed no sex differences in potential confounding influences, including variation in gestational ages at G2 or G3 or the degree of signal artifact that may have contributed to these findings.

Fetal movement measures. Fetal sex was unrelated to either mean values or trajectories for vigor and total movement (Table 12). Male fetuses exhibited more individual bouts of movement relative to females at G3 only, β = -2.50, *SE* = 1.25, *t* = -2.01, *p* < .05, *d* = .18. This circumstance resulted in a significant overall trajectory difference for movement bouts between males and females. These findings were maintained after controlling for other factors that were modestly, but significantly related to fetal movement measures including amniotic fluid index and birth weight. Given that there was no sex difference in the total amount of time spent moving, an additional analysis was conducted for the other component variable of movement duration. There was a trend for female fetuses to exhibit longer movement bouts (17.1 s) than male fetuses (15.2 s), *t*(598) = -1.62, *p* = .10, *d* = .12.

Fetal movement-heart rate coupling. Coupling and latency levels or trajectories did not vary significantly by fetal sex (see Table 12) at any gestational period. At G2, there were

trends for male fetuses to exhibit higher FM-FHR coupling, β = 0.01, *SE* = 0.007, *t* = 1.84, *p* = .07, *d* = .14 and shorter latencies, β = -.25, *SE* = 0.14, *t* = -1.73, *p* = .08, *d* = .13, relative to females.

Maternal physiological measures. Women carrying female fetuses had faster heart rates at G2 (87 v 85 bpm), β = 1.65, *SE* = 0.72, *t* = 2.28, *p* < .05, *d* = .17, and G3 (85 v 83 bpm), β = 1.6, *SE* = 0.79, *t* = 2.09, *p* < .05, *d* = .15, compared to those carrying male fetuses and showed a greater increase in heart rate from G1 to G2, relative to women carrying males, β = -1.24, *SE* = 0.55, *t* = -2.26, *p* < .05. There were no differences in maternal physiology by fetal sex for other cardiac related measures, including RSA or RP. Although there was also no mean difference in SCL, its skewed distribution may obscure sex differences which may be confined to the tails. Subsequent analysis revealed that at G3 there were more male (*n* = 39) than female (*n* = 21) fetuses in the top 10% of maternal SCL values (i.e., > 12.65 μS), χ^2 (1, *N* = 599) = 5.67, *p* < .05.

Discussion

Given the existing literature, the findings that girls have faster heart rates and that boys had greater heart rate variability were unexpected. The few prior reports of faster fetal heart rates in girls were based on data generated from labor, not from the antepartum. Reports of sex differences in FHR variability are less common, although there are two reports of greater variability in male fetuses using time domain but not other methods (Bernardes, Goncalves, Ayres-de-Campos, & Rocha, 2008; Goncalves, Bernardes, & Ayres-de-Campos, 2013). Confidence in our findings is bolstered by the large sample size distributed over multiple time points and our relatively lengthy recording period of 50 minutes; few existing studies that fail to detect sex differences have both features. Conversely, in large samples small differences can

reach statistical significance; for fetal heart rate the sex differential was only 2 bpm. Nonetheless, neither finding supports the putative interpretation of accelerated maturation of parasympathetic control in female fetuses.

The *lack* of a sex difference in actograph detected fetal motor activity was equally unexpected. With respect to fetal movement, we conclude that with the exception of the observation that male fetuses initiate more new movements just prior to term, male and female fetuses do not differ in quantitative measures of motor activity, at least during baseline, undisturbed conditions. The large degree of intrafetal variation in fetal movement measures makes them particularly susceptible to vagaries of small samples and thus we are more confident in these results than in those based on individual cohorts. The single finding that boys make more individual movement bouts near the end of gestation, but do not exhibit more overall movement, is difficult to interpret. The prior report of a sex difference slightly later in gestation was based on ultrasound observed movements quantified in a manner more similar to our total time spent moving variable (Robles de Medina et al., 2003). Those authors attributed this finding to higher levels of wakefulness in male fetuses (Robles de Medina et al., 2003) which is consistent with one of our own findings of more frequent periods of activity relative to quiescence for male fetuses at 36 weeks only (DiPietro et al., 1998). Similarly, based on FHR patterns alone, male fetuses at term were observed to exhibit more episodes of wakefulness than female fetuses (Bernardes et al., 2008). We conclude that there is some element of variation in fetal movement or behavioral organization at the end of term, but that its nature remains elusive. It is worth noting, however, that if the difference of 2.6 movements per 50 minute period observed here is multiplied over the course of 24 hours, this reflects up to 75 more movement bouts by male fetuses per day. Perhaps this may fuel the general perception that male fetuses are

more active than female fetuses, particularly if movement onset, and not duration, is most salient to pregnant women.

There are several provocative reports of fetal sex differences in specific aspects of neurobehavior that extend beyond the domains measured here. Using ultrasound imaging, two reports suggest differential expression and development of oral motor behaviors. Female fetuses display more mouthing movements than male fetuses as early as 18 weeks gestation (Hepper, Shannon, & Dorman, 1998), as well as more frequent and complex lingual, laryngeal and pharyngeal movements (Miller, Macedonia, & Sonies, 2006). Several reports of sex differences related to fetal reactivity and habituation to externally applied stimuli have emerged and provide consistent findings favoring advanced development in female fetuses. Female fetuses react with greater decelerations to speech sounds near term than male fetuses (Groome, Mooney, et al., 1999). Commencing at 31 weeks gestation, male fetuses reacted with greater increases in fetal heart rate to a startling stimulus than female fetuses and, whereas the males' recovery curve following stimulation continued to lessen through term, the female regulatory response was mature by 31 weeks (Buss et al., 2009). As compared to male fetuses, female fetuses require fewer trials to habituate to vibroacoustic stimuli (Hepper, 2012; McCorry & Hepper, 2007), display the capacity to habituate earlier in gestation (Leader, Baillie, Martin, & Vermeulen, 1982a), and show faster improvement in habituation performance from 31 to 35 weeks gestation (McCorry & Hepper, 2007). The ability to habituate connotes information processing capacity and as such, this convergent evidence suggests accelerated neuromaturation in female fetuses. Our inability to discern a female advantage in neuromaturation may have been limited by the aspects of fetal functioning we measured, particularly as the most consistent sex differences have been observed for the higher order processes.

The finding that heart rates were higher in both female fetuses *and* their mothers corresponds with the correlation between maternal and fetal heart rates noted in Chapter 8. That analysis also establishes the directionality of the effect such that earlier fetal heart rate influences maternal heart rate. Variation in maternal physiological indicators, potentially as the *result* of fetal sex, may be less generally expected but aligned with the growing literature pointing to a differential role of fetal sex on the intrauterine milieu (Clifton, 2010; Ghidini & Salafia, 2005; Prior, Wild, Mullins, Bennett, & Kumar, 2013). Indeed, in the second century A.D., Soranus promoted the notion that women carrying male fetuses have better color as the result of the stimulation provided by the excessive movement of male fetuses and that pallor of women carrying female fetuses was the result of lack of such stimulation (Temkin 1991). At least two studies have reported that male fetuses have longer umbilical cords (Mills, Harley, & Moessinger, 1983; Soernes & Bakke, 1986), consistent with the role of greater fetal motor activity in generating this difference (Moessinger et al., 1982), although the sex difference was not confirmed in another report (Balkawade & Shinde, 2012). Our finding of a sex difference in estimated amniotic fluid volume also supports a role for fetal sex in the local intrauterine context. Amniotic fluid volume is significantly regulated by fetal swallowing behavior and may reflect the reduced oral motor activity displayed by male fetuses as noted above.

Despite the general lack of differences in fetal motor measures, individual differences in prenatal motor activity are more predictive of child motor activity levels for boys than they are for girls (DiPietro, Bornstein, et al., 2002) and physical maturation exerts different effects on motor activity levels in girls and boys in early childhood (Eaton & Yu, 1989). Unexpected interactions have also been reported that suggest fetal sex imparts complexity in the bridge to the postpartum (Sandman et al., 2013). For example, the timing of exposure to maternal

neurohormonal influences (i.e., corticotrophin releasing hormone and cortisol) differentially affects physical and neuromuscular maturation of male and female fetuses as evaluated by neonatal exam (Ellman et al., 2008) and the timing of fetal exposure to insufficient oxygenation has differential effects on the developmental competencies of boys and girls (Anastario, Salafia, Fitzmaurice, & Goldstein, 2012). We previously reported an unexpected observation of variation in maternal cortisol trajectories during pregnancy depending on fetal sex (DiPietro, Costigan, Kivlighan, Chen, & Laudenslager, 2011) as well as differential associations between maternal testosterone and fetal growth (Voegtline, Kivlighan, Henderson, & DiPietro, 2013).

Summary

The original Fels reports were relatively silent on the subject of fetal sex, with the exception of a passing reference to a lack of a sex difference in fetal motor activity by maternal report (Richards et al., 1938). Although we found small but consistent sex differences on the development of fetal heart rate and variability, and a single difference in fetal motor measures near term, intra-fetal variation confers far more variance on fetal development than does fetal sex. This is compatible with observations of postnatal sex differences, in which modest mean differences are accompanied by highly overlapping distributions (Campbell & Eaton, 1999; Jacklin, 1981). Somewhat unexpectedly, we have also documented fetal sex effects on aspects of maternal function. Given these findings, and the existing literature on vulnerabilities linked to fetal sex, investigation of the potential moderating role of sex on the development of both fetus and pregnant woman remains a fertile area of inquiry.

Chapter 10. Siblings

Overview

"These results suggest that what one might call "autonomic constitution" may be at least partially an inherited characteristic."(Jost & Sontag, 1944, pg 310)

The 89 children who comprised the data for the classic report of child development generated by the Fels study as described in *Birth to Maturity* (Kagan & Moss, 1962) were from 63 families with half of the children from a subset of 19 families. The inclusion of a subset of women who participated in our research with successive pregnancies affords both a potential confound within the full cohort as a result of clustering, but also an opportunity to evaluate whether fetal development of siblings is more similar than fetal development in unrelated pregnancies. Successive children born to the same woman share both genes and features of the maternal prenatal environment, although the degree to which the latter remains constant across pregnancies is unknown. Moreover, the fetal origin of the placenta further obscures the degree to which siblings experience constancy in the intrauterine environment. Developmental science has most often relied on studies comparing fraternal and identical twins to parse the genetic contribution to behavior. Although there are a few studies of fetal behavior in twins, most consider chorionicity over zygosity (Gallagher, Costigan, & Johnson, 1992; Tendais, Visser, Figueiredo, Montenegro, & Mulder, 2013). Moreover, twinning is an anomalous condition of human pregnancy and it cannot be presumed that the intrauterine milieu for both fetuses is the same. In contrast, sibling studies can provide significant information on confounding influences that may affect conclusions regarding causal relationships (Lahey & D'Onofrio, 2010). For example, comparisons of siblings who were differentially exposed to maternal smoking during pregnancy to children from unrelated pregnancies have been used to disaggregate the biological

effects on development conferred by the exposure to that of other shared influences (D'Onofrio et al., 2010).

The conclusion described in the quotation that starts this chapter was generated by the Fels investigators by contrasting postnatal autonomic functioning (e.g., heart period, skin conductance, etc) between twins, siblings, and unrelated participants. We are unable to find any reports of fetal behavior in non-twin siblings so believe this to be first analysis of data generated from successive pregnancies. The analysis focused on examining the degree to which siblings displayed similar fetal neurobehavioral development as compared to unrelated fetuses using statistical techniques that have previously been applied to evaluating sibling relatedness during postnatal development. To enable these analyses, women (as well as fetuses) were given unique identifiers in the dataset.

Results

In the full cohort, the subset of siblings (n = 197) reflects 26.6% of the dataset. The subset is generated from 106 women; of these, 91 participated twice, 14 participated three times, and 1 with each of four pregnancies. Sample characteristics of these women were similar to the larger sample that participated once with the exception of maternal education; returning participants were more educated $t(632) = 4.43$, $p < .001$. The majority of these women were nulliparous prior to enrolling in the study for the first time (n = 83, 91.2%). All available sibling data were used to compute intraclass correlations; analysis of developmental trajectories and sex composition were limited to fetuses of women who participated twice.

Infant characteristics of the sibling subset, including birth weight, gestational age, and Apgar scores were comparable to the larger sample and there were no significant differences. Sixty-one sibling pairs were of the same sex (67%; 34 pairs of boys and 27 pairs of girls); 30

were opposite sex (33%). All were full siblings. Among siblings, there were also no differences in infant characteristics, irrespective of sibling sex composition.

Within-sibling intraclass correlations. Table 13 presents intraclass correlation coefficients (ICC) between sibling pairs for each of the fetal measures by gestational period. The ICC values were generated by entering maternal ID as a random effect in unconditional mixed models. This approach yields an estimable variance component in the fetal measure that is attributable to relatedness (Donner & Koval, 1980; Raudenbush & Byrk, 2002; Robertson, 1959). Thus, intraclass correlation coefficients provide information on the degree to which fetuses from the same woman express similarity in fetal neurobehavioral development within each gestational period. For example, Table 13 indicates that at G1, the 61 pairs of fetal siblings had fetal heart rate values that were modestly (ICC = .27, $p < .05$) related. Sibling values for all other measures were not significantly associated at G1, but by G2 and through G3, all cardiac and two of the three motor measures showed significant sibling relatedness. Of all the variables, motor activity (total movement) and the coupling composite displayed the least relatedness among siblings.

Developmental trajectories. To determine if the rate of development of siblings is also similar over time, sibling pairs were examined within separate multivariate HLM models for each fetal measure. The multivariate framework simultaneously estimates the trajectory of one sibling in relation to the other. Models revealed a significant slope covariance for fetal heart rate variability among siblings, $\beta = .08$, SE = .04, $Z = 1.92$, $p = .05$, suggesting a similar pattern of variability change across gestational periods. Fetal heart rate approached a trend level slope covariance ($p = .11$), while accelerations and motor activity measures showed no evidence of familial association in developmental trajectory.

Fetal sex. As it is possible that there may be greater concordance among fetuses of the same sex, analyses were conducted separately for concordant and discordant pairs. Models were constructed to separate variance components for sibling pairs of the same sex and mixed sex; differences between the coefficients were tested using a Fisher's *r* to *Z* transformation test. Despite variation in the levels of the same and between sex correlations, only one comparison between same and mixed set pairs reached statistical significance (total movement at G2), suggesting a chance finding.

Maternal context. Paired *t*-tests were used to examine change in maternal psychophysiological measures across pregnancies. Maternal SCL was consistently higher at each gestational period for the first pregnancy relative to the second (G1: $t(60) = 2.73$, $p < .01$; G2: $t(73) = 2.12$, $p < .05$; G3: $t(64) = 3.23$, $p < .01$). There was a trend for maternal RSA to be higher during the second pregnancy at G3, $t(51) = 1.90$, $p = .06$. Maternal HR and RP were unaffected. Maternal physiological changes were unaffected by whether successive pregnancies were concordant or discordant for fetal sex.

Discussion

Although perhaps not unexpected, this is the first empirical analysis demonstrating that siblings share similarities in aspects of neurobehavioral development during the prenatal period. The effect was demonstrated for levels of both cardiac and motor activity measures. Most ICC correlations were not significant until G2 indicating that siblings became more similar as they matured. However, the magnitudes of the associations were quite modest, and familial relations accounted for far less variance than did unexplained variance. Fetal sex concordance did not amplify the relationship. Maternal sympathetic activation, as indexed by skin conductance, lessened with the subsequent pregnancy. This extends the observation of changes in maternal

skin conductance over time within the same pregnancy and is consistent with the finding that nulliparous women within the full cohort had higher SCL compared to multiparous women (Chapter 8).

Summary

Siblings shared some similarities in levels of fetal cardiac and motor behavior variables, but not in the rate of change over time. The genetic versus the maternal environmental contribution to the congruence in fetal neurobehaviors within siblings cannot be distinguished by this study. However, detected familial associations were relatively modest which corroborates the lack of impact that exclusion of siblings had on the stability coefficients for fetal cardiac and motor measures presented in Tables 7b and 9b. As such, it makes us more comfortable reporting those results with siblings included as this did not introduce a considerable degree of bias in inflating those associations. Also, with the exception of sympathetic dampening in successive pregnancies, the maternal psychophysiological context does not appear to change appreciably with successive pregnancies. As the first report of fetal neurobehaviors and maternal psychophysiological parameters in successive pregnancies, these results provide context for understanding the foundation for familial association of development observed in the postnatal period and highlight the intrauterine context as the earliest shared environment of siblings.

Chapter 11. Deviations from normal development

Overview

Sontag and colleagues did not comment on the implications of disruptions to normal prenatal development for fetal functional measures, perhaps because so few congenital conditions were identified at that time. Since then, a substantial body of evidence has shown that fetuses with genetic, chromosomal or structural defects that affect neurological development tend to exhibit differences in neurobehavioral development as compared to fetuses without these conditions. Such observations have been used, in part, to validate the construct that fetal neurobehavioral measures are expressions of neural development and maturation. Most reports include a broad range of conditions (Einspieler et al., 2012; Horimoto et al., 1993; Maeda et al., 2006; Morokuma et al., 2013; Shinozuka, Masuda, Okai, Kuwabara, & Mizuno, 1989; Vindla, Sahota, Coppens, & James, 1997). Anencephalic fetuses comprise perhaps the largest subgroup of brain malformations studied (Leader, Baillie, Martin, & Vermeulen, 1982b; Terao et al., 1984; Visser, Laurini, de Vries, Bekedam, & Prechtl, 1985; Yoshizato et al., 1994). Observation of disordered patterning of motor activity and heart rate in these fetuses has been used to draw inferences regarding the degree to which each is subject to central control ranging from the brainstem to the cortex.

Fetuses with chromosomal anomalies are sometimes included in these studies or presented in single case reports. The literature is small, but Trisomy 21 (i.e., Down Syndrome), has been associated with reduced fetal habituation (Hepper & Shahidullah, 1992) and both very fast and very slow heart rates in early pregnancy (Liao, Snijders, Geerts, Spencer, & Nicolaides, 2000; Martinez et al., 1996).

Structurally and chromosomally normal fetuses can also show altered growth and/or development. There have been a number of studies of fetuses expressing intrauterine growth restriction (IUGR). Growth restriction is the result of insufficient transport of oxygen and nutrients across the placenta, and although pregnancy complications such as preeclampsia can result in IUGR, most instances are of unknown origin. Compared to their normally growing counterparts, growth restricted fetuses display reduced heart rate variability (Graatsma et al., 2012; Nijhuis et al., 2000; Sriram et al., 2013), reduced cardiac responsiveness to external stimulation (Gagnon, Hunse, Fellows, Carmichael, & Patrick, 1988), and make fewer general movements (Bekedam, Visser, de Vries, & Prechtl, 1985; Vindla, James, Sahota, & Coppens, 1997). A report that a characteristic pattern of responsivity to external stimuli in mid-gestation is expressed by fetuses who ultimately are in the lowest third of the distribution of birth weight suggests that fetal growth associations with neurobehavior may span a broader portion of the continuum (Sandman, Cordova, Davis, Glynn, & Buss, 2011)

Here we analyze the small subset of growth restricted fetuses observed in these cohorts in relation to normally growing fetuses. In addition, we describe the fetal development of three cases that were excluded from our analysis due to conditions with potential developmental impact.

Results

The normative group included all fetuses in the original cohort, with the exclusion of growth restricted fetuses (n = 9) and those that delivered prematurely (n = 49). For space considerations, we limit figures to fetal heart rate, a measure of variability (accelerations), total movement, and FM-FHR coupling. Figure 11(a – d) include mean values for the full sample

along with shaded areas that reflect the 10^{th} to 90^{th} percentiles (*ns* at each data point range from 531 to 606).

Intrauterine growth restriction (IUGR). IUGR fetuses were all born at term, *M* GA = 38.5 weeks, range 37 to 42 weeks, but at low birth weight (i.e., < 2500 g), *M* weight = 2238 g, range 1880 to 2486 g. Mean Apgar values were 6.9 and 8.5 at 1 and 5 minutes. In this sample, with the exception of a case of suspected mid-gestation cytomegalovirus infection and the coincident congenital malformation, growth restriction was idiopathic. Mean fetal neurobehavioral values for the IUGR cases, which include the individual listed in Table 2 with both a physical malformation (i.e., ambiguous genitalia) and growth restriction, are presented in Figures 11 and 12. Compared to normally growing fetuses, there was a trend for IUGR fetuses to have faster FHR at G1, β = -4.33, SE = 2.4, t = -1.81, p = .07. IUGR fetuses displayed less gain in FHRV (not shown) between G1 and G2, β = -.93, SE = .48, t = -1.93, p =.05, and by G2 and G3 showed significantly lower FHRV (not shown) and fewer accelerations (ps < .01). Fetal motor development was less affected; IUGR fetuses showed a decline in total movement from G1 to G2, β = -444.16, SE = 217.5, t = -2.04, p < .05, not observed among normally growing fetuses, resulting in trend level lower movement at G2 (p =.06) but no differences in movement vigor or bouts. Growth-restricted fetuses displayed lower coupling by G2, β = -0.07, SE = 0.03, t = -2.01, p <.05, which continued through G3 β = -0.09, SE = 0.04, t = -2.20, p <.05. Latency was unaffected (not shown).

Congenital anomalies. Exclusion cases in Table 2 were evaluated individually. The fetal kidney malformation in Cohort IV was detected at the first study visit and resulted in discontinued eligibility. Data for both cases of fetal demise, one at 31 weeks and the other at term, were limited to one recording made more than a month prior to each, so were not evaluated

further. The cleft palates observed in two fetuses were not associated with other malformations suggestive of central effects, and, as would be expected, those fetuses did not exhibit remarkable fetal neurodevelopmental courses. Alterations to central nervous system development are implicated in the remaining three exclusions that reflect a chromosomal anomaly (i.e., Trisomy 21), a structural malformation (i.e., agenesis of corpus callosum), and a genetic condition (i.e., Williams Syndrome). All three conditions were identified after birth. These are plotted individually in Figure 11(a – d).

Trisomy 21 was associated with both FHR and motor effects such that the three cardiac measures (FHRV not shown) and FM-FHR coupling were exceptionally low. Motor activity followed a different course, commencing with exceptionally high levels of motor activity, including individual bouts (not shown) at G1 and G2 but then plummeting by G3. Agenesis of the corpus callosum was characterized by high FHR and no accelerations until the final gestational period, along with very low levels of motor activity. FM-FHR coupling exceeded the 90th percentile at G3 which is likely an artifact of the low number (23) of movements. Participants in Cohort IV were monitored twice; as such there are only two data points for the fetus subsequently diagnosed with Williams Syndrome. FHR and FHRV were unaffected, although the number of accelerations at G2 and G3 was comparable to those for IUGR fetuses. Most distinguishing, however, was an increase in movement bouts and vigor (not shown) along with a high degree of total motor activity, particularly at G2. As with Trisomy 21, FM-FHR coupling fell beneath the 10^{th} percentile on both occasions. Conversely latency values exceeded the 90^{th} percentile (higher scores indicate lesser integration).

Discussion

The information contained in this chapter is meant to be illustrative and not conclusive. Nonetheless, despite the small number of individuals with fetal growth restriction, we found a reduction in both measures of fetal heart rate variability, as well as FM-FHR coupling, without corresponding differences in fetal heart rate and a tendency towards reduced fetal motor activity. The significant cardiac differences and FM-FHR differences became more pronounced over time in tandem with the trajectory in which reduction in growth is typically observed for IUGR fetuses. These findings parallel cross-sectional and longitudinal reports of others on somewhat larger samples (Bekedam et al., 1985; Graatsma et al., 2012; Nijhuis et al., 2000; Vindla, James, et al., 1997). However, IUGR is of heterogeneous origin and those reports include cases of the most prominent etiology – cigarette smoking and hypertensive disorders (Kramer, Platt, Yang, McNamara, & Usher, 1999) – whereas ours do not. The current findings confirm that alterations to fetal neurobehavioral development in pregnancies putatively characterized by diminished placental perfusion and transport are not attributable to these confounding influences.

Although the development of IUGR fetuses differed statistically on some measures from normally growing fetuses, mean values fell within the 10^{th} to 90^{th} normative percentiles. In contrast, values for individual fetuses with conditions more clearly linked to central nervous system alterations are more consistently near or beyond normal limits. This is particularly true for FM-FHR coupling values for two of the three anomalous cases. The existing literature on fetal heart rate in fetuses with Trisomy 21 is based on recordings made in the first 18 weeks of pregnancy and yield conflicting results. Examination of data by gestational age indicates tachycardia between 10 to 14 weeks gestation (Liao et al., 2000) followed by FHR at the 5^{th} percentile between 14 and 18 weeks (Martinez et al., 1996). The low fetal heart rate observed in this fetus in the second half of gestation is consistent with the latter report. One other report of a

single Trisomy 21 case also found very low fetal motor activity within the same gestational time frame (Vindla, Sahota, et al., 1997).

Summary

Intrauterine and congenital conditions that affect neurological development are expressed by deviations from normative trajectories of fetal heart rate patterning and motor activity. Structural malformations of the brain and chromosomal or known genetic abnormalities that significantly affect development are rare enough to have generated relatively few reports in which fetal neurobehavioral development of affected individuals can be compared to normative data. We were able to find only one other report of individual trajectories of baseline fetal heart rate and motor activity in a subset of fetuses with heterogeneous CNS anomalies that was comparable to the data reported here. Fetal measures for anomalous cases also tended to cluster around, below, or above 10^{th} to 90^{th} percentiles (Vindla, Sahota, et al., 1997). Thus, deviations of fetal development in affected individuals can include both dampening and disinhibition of normative processes.

Chapter 12. General Discussion

"It would seem to us, however, that in the last analysis the most significant contribution to be made from this type of work is the clarification of developmental sequences as they may manifest themselves in fetal life." (Sontag & Richards, 1938, p 65)

Consistent with the orientation of the Fels Longitudinal study, our work has been focused on documenting the normal trajectory of fetal neurobehavioral development and establishing the fetal origins of individual differences. Then, as now, the ultimate goal in this pursuit is to understand how the fetal period provides the foundation for subsequent human development and its implications for the prediction of individual outcomes. Subsequent research conducted under the auspices of the Fels Institute, which has been administered through Wright State University since 1977, focuses predominantly on physical growth and maturation. We hope that this *Monograph* reintroduces developmentalists to the foundational research on fetal physiological and behavioral development conducted by the founding Fels investigators. Our work conducted over the last two decades, using technologies unavailable in the 1930s, essentially supports and extends the initial findings that were originally reported during this remarkable work.

Continuity and discontinuity in fetal development. Fetal heart rate, motor activity, and their interrelationship develop in predictable ways during the second half of gestation. Variation among fetuses in motor activity is greater than for cardiac rate and patterning which, as might be expected, is more canalized. On the other hand, all cardiac and motor measures as well as FM-FHR coupling showed increased levels of variability among fetuses over the 12 weeks of gestation studied here. This confirms the prediction, based on Bell's conceptualization of the construct, that development becomes less canalized over time and more subject to constitutional and environmental influences. All studied parameters showed within-individual stability,

suggesting that the precursors of individual differences in autonomic control, motor regulation and the neural integration that regulates both parameters, originate before birth. In general, and consistent with prior work, we were unable to identify covariates either extrinsic (e.g., maternal physiological measures) or intrinsic (e.g., fetal sex) that contributed much explanatory variance to these measures, although at times the associations attained significance. Sibling analyses provided suggestion of shared genetic influence, although this could not be distinguished from the influence of any shared maternal prenatal environment in successive pregnancies. Thus, although we were able to document individual differences among fetuses, no single factor provides an obvious pathway. This suggests that either unidentified constitutional factors or unmeasured features of the intrauterine milieu are paramount, or that the potential contributory factors measured here might exert stronger influence earlier in gestation than the period under study.

Developmental trajectories in cardiac and motor variables revealed a decline in slope between pairings of the three data points for most measures, suggesting a transitional period midway through the second half of gestation after which the rate of development slows. This supports our initial observation of a general developmental discontinuity between approximately the 28th to 32nd gestational weeks (DiPietro et al., 1996b), with confirmation in ensuing cohorts (DiPietro, Caulfield, et al., 2004; DiPietro et al., 2010). Transitions in other aspects of fetal functioning has also been reported during this gestational period, including characteristics of fetal breathing movements (Kozuma, Nemoto, Okai, & Mizuno, 1991; Pillai & James, 1990c; Roodenburg et al., 1991), responsiveness to vibroacoustic stimulation (Buss et al., 2009; Kisilevsky, Muir, & Low, 1992; Kuhlman, Burns, Depp, & Sabbagha, 1988), habituation performance (Groome, Gotlieb, Neely, & Waters, 1993), and the development of fetal states

(Nijhuis et al., 1999; Pillai & James, 1990b). This period coincides with rapid increase in neural development and myelination, including refinement of cortical and vagal processes (Kinney et al., 1994; Sachis et al., 1982; Yoshizato et al., 1994). Equally distinct developmental shifts in multiple domains occur at several points during the first years of life and are assumed to reflect key periods of neural reorganization (Zeanah et al., 1997).

The deceleration in neurobehavioral maturation after approximately 30 to 32 weeks gestation may suggest that antenatal neural development through term is somewhat over-determined. Ancillary support for this position is provided by the ultimate developmental and cognitive success of preterm infants who are born after this gestational period, despite immaturity in other organ systems. Studies of preterm infants also reveal a decline in the developmental trajectories of sleep-wake cyclicity (Feldman, 2006) and a reorganization in cardiac vagal tone between 30 and 33 weeks post-conceptional age (Doussard-Roosevelt, Porges, Scanlon, Alemi, & Scanlon, 1997; Feldman, 2006). This is not to imply that fetal neurodevelopment ceases after this period. For example, there is a linear increase in periods of wakefulness as the fetus progresses past term gestation (Junge, 1979; van de Pas et al., 1994), consistent with the observation that the neurobehavioral repertoires of preterm or full-term infants do not change in a manner indicative of neural reorganization around term gestation (Prechtl, 1986). Thus we suggest that the first well-known developmental transition during the 3rd postnatal month (Zeanah et al., 1997) is preceded by one during the 7th month of gestation.

Throughout our research program the most surprising findings have involved violations of expectations on the directionality of maternal-fetal influence. We happened across this somewhat serendipitously as a result of data-driven times series analysis of second by second streams of maternal and fetal variables. That analysis revealed that the lagged relationships

between maternal heart rate, skin conductance, and spontaneous fetal motor activity were instigated by fetal movement such that maternal heart rate and skin conductance demonstrated brief, phasic increases within 2 to 3 s of onset of a fetal movement (DiPietro, Irizarry, et al., 2004). This finding was replicated on another, sociodemographically distinct sample (DiPietro, Caulfield, et al., 2006). We have also documented a maternal physiological response when fetal motor activity is induced by a percussive external sound, despite maternal auditory masking (DiPietro, Voegtline, et al., 2013). In the current report, we extend this body of knowledge by showing that the trajectories of maternal heart rate and skin conductance appear to be influenced by fetal heart rate and motor activity, respectively, at preceding gestational periods and not the other way around (Chapter 8). We also show that fetal sex plays a contributory role in maternal physiological parameters (Chapter 9).

Limitations of the current analysis. The data and results described in prior chapters are not without limitations in application and interpretation. Many specific issues are not unlike those inherent to the Fels project (Roche, 1992), including variation in the number of assessments across participants and, at times, tolerance of wider gestational ages at testing than optimal as depicted in the scatter plot figures. Moreover, combining multiple cohorts into a single database has both advantages and disadvantages. The clear advantage is the power to detect associations that could not be detected in smaller individual samples while minimizing the Type I errors that can be generated by vagaries of small samples or outliers. This is perhaps best illustrated by our detection of sex differences in fetal heart rate and our confidence in confirming a general lack of sex differences in motor activity (Chapter 9). The disadvantage is that the large sample size and multiple fetal and maternal parameters may generate significant findings that are small and inconsequential, and multiple comparisons maximize this possibility. We have tried to

manage these competing influences by taking a conservative approach to our descriptions and interpretations of results and focusing on those with consistent patterns of associations and/or that replicate prior work. Many of the results discussed throughout the manuscript reflect medium to large associations, corresponding to *rs* that range from .3 to .5, respectively. Rather than applying an alpha level correction to the report of the predominantly descriptive results, we describe the size of the relations indexed by correlation coefficients in a manner consistent with Cohen's (1988) lexicon. For example, the stability analyses of fetal heart rate and variability (Table 7) are described as showing large or strong associations; the correlation between the two variables was noted to be significant due to the sample size (e.g, $r = .09$ at G1) and dismissed as not meaningful. Effect size was reported where appropriate, such as for group differences in fetal sex, and used to compare the relative influence on maternal and fetal trajectories on one another. HLM analysis has no gold standard for reporting effect size, particularly for estimates of trajectory (Feingold, 2009). However, it yields β values that reflect the magnitude of the change over time in actual units for each variable, and we have reported these. Finally, our inclusion of scatter plots for all key fetal neurobehavioral measures allows readers full understanding of the range of values within and across gestation.

Perhaps the two most important challenges to generalizability are those raised by limitations introduced by our population and gestational age range of study. As with much developmental research, our reliance on self-selected volunteers resulted in a sociodemographically skewed sample of women from more stable and advantaged environments and under-representation of minorities, particularly of Hispanic origin. This is particularly common in longitudinal protocols with high participant burden, and congruent with the sociodemographic characteristics of the 30 participants in the original *SRCD Monograph* (Sontag

& Richards, 1938). The exclusion of women with significant medical or pregnancy complications further homogenizes the sample but was done so to remove these sources of biological risk from analysis of normative data. Although the current analysis failed to find associations with our primary socioeconomic indicator – maternal education – and fetal measures, we have previously reported significant differences between participants in our standard self-referred cohorts and low income women, both in Baltimore (DiPietro et al., 1998) and Lima (DiPietro, Caulfield, et al., 2004). As a result, the current findings should be interpreted as normative data only for fetuses of women at low socioeconomic and medical risk and would not necessarily be expected to generalize to other populations. Future research that contributes to understanding the mechanisms and implications of variation in fetal development based on socioeconomic disadvantage is critical to fully interpreting the role of the fetal period in postnatal life.

The second limiting issue is the 12 week gestational span of fetal data collection, commencing slightly after the midpoint of gestation. This is consistent with other longitudinal studies (Sandman et al., 2013; ten Hof et al., 2002) and not the result of lack of interest in earlier gestation, but of the capabilities of cardiography in maintaining adequate signal detection of the fetal heart for prolonged periods of time. For example, the fetal heart rate error rejection declines precipitously between 20 and 24 weeks, from 16% to 8.5% in our original cohort (DiPietro et al., 1996b) and from 11% to 7% in Cohort I of this report (unpublished data), despite the use of ultrasound to guide transducer placement by a highly experienced clinician. Due to the difficulties in collecting Doppler-based fetal heart rate before the mid-point, studies of fetal development in the first half of gestation tend to be focused on development of specific motor behaviors visualized through ultrasound. Given the decline in the rate of change in the

developmental trajectory observed beyond 30 and 32 weeks, it would be expected that neurodevelopment would either maintain or surpass the trajectories described between G1 and G2. Also, significant stability correlations in fetal cardiac, motor and coupling measures were present by the 24th week of gestation; we are unable to ascertain the gestational period at which evidence for individual differences begins. Finally, we may have missed detection of moderating or mediating associations that may be present earlier in pregnancy with consequences for the developmental trajectory later in pregnancy. This may be particularly germane to maternal neurohormonal influences, for example, that have been observed to be predictive of neurodevelopmental measures early in the second trimester, but not later (Class et al., 2008; Ellman et al., 2008). Thus, just because we were unable to detect cross-sectional or time-lagged associations with a 12 week interval does not mean that these variables do not interact over the course of gestation and thereby influence development.

Higher order processes.

The data presented here inform the first three levels of the developmental hierarchy presented in Figure 1. This *Monograph* does not contain data directly pertinent to understanding the culmination of the autonomic, motor and state integration process as reflected in fetal responsivity to the environment and, ultimately, learning and information processing. However, fetal heart rate and motor activity are the most commonly used indicators in studies that evaluate higher order processing and it would be remiss not to provide overview of the knowledge base in this domain. The developing sensory capacity of the fetus, particularly related to auditory processing, has been fairly well-articulated based on animal and some human models (Busnel, Granier-Deferre, & Lecanuet, 1992; Lecanuet & Schaal, 1996; Querleu, Renard, Boutteville, & Crepin, 1989). There is a large literature originating in the 1920's and 1930's on fetal

responsivity to the application of intense vibroacoustic stimulation applied near or on the maternal abdomen. Sontag and colleagues provided one of the earliest empirical reports of a large fetal heart rate response using an electronic door buzzer (Sontag & Wallace, 1935b), following an earlier report using a warning horn (Peiper, 1925, as cited in Sontag & Wallace, 1935b). Much of the subsequent impetus for this research was driven by the search for a clinical tool to distinguish between inactive and unwell fetuses during fetal assessment procedures (Tan, Smyth, & Wei, 2013; Zimmer & Divon, 1993). Depending on the stimulus intensity, fetal responses range from startle and tachycardia to a more constrained, localized response. Developmentalists further elucidated fetal responsivity to stimulation by characterizing the more graded fetal heart rate and motor responses to less intense vibroacoustic or airborne sound stimuli (Kisilevsky & Muir, 1991; Lecanuet, Granier-Deferre, Cohen, Houezec, & Busnel, 1986). Prior to term, fetuses have been observed to respond with both accelerations and small decelerations in heart rate to stimulation (DiPietro et al., 1996b; Kisilevsky et al., 1992). At term, auditory stimuli can also elicit deceleratory responses suggesting the possibility of a biphasic orienting response (Lecanuet, Granier-Deferre, Jacquet, & Busnel, 1992). These and other studies provide the basis for understanding fetal signal detection and information processing.

Fetal habituation reflects a slightly higher level of information processing and has also been well-documented (Goldkrand & Litvack, 1991; Groome et al., 1993; Kuhlman et al., 1988; Leader, Baillie, Martin, Molteno, & Wynchank, 1984; Madison, Madison, & Adubato, 1986). However, with few exceptions (Hepper & Shahidullah, 1992; Sandman, Wadhwa, Hetrick, et al., 1997), dishabituation is not assessed making it difficult to distinguish true habituation from response fatigue. In addition, when intense vibroacoustic stimuli are used to evaluate

responsivity, women cannot be masked and there is evidence that at least part of the fetal response may be mediated by maternal anticipation (DiPietro et al., 1996b; Visser, Zeelenberg, de Vries, & Dawes, 1983). A subset of habituation studies combines habituation protocols with repeated testing to infer short term memory (Dirix, Nijhuis, Jongsma, & Hornstra, 2009; vanHeteren, Boekkooi, Jongsma, & Nijhuis, 2000).

The majority of studies on prenatal learning are based on postnatal testing following a naturally occurring or experimentally presented stimulus using either operant conditioning procedures that reveal preference, or other indicators of recognition such as orienting responses. A seminal study (DeCasper & Fifer, 1980) provided initial evidence that fetuses learn to recognize their mother's voice and refinements revealed neonatal preferences for the filtered maternal voice that more closely approximates the intrauterine environment (Spence & DeCasper, 1987), prefer voices spoken in their native language (Moon, Lagercrantz, & Kuhl, 2013; Moon, Panneton, & Fifer, 1993), and can discriminate between familiar and non-familiar words (Partanen, Kujala, Naatanen, et al., 2013). Studies conducted with the fetus, based on discerning fairly small heart rate or motor responses to various stimuli, provide support for the prenatal capacity to differentiate among stimulus properties, including speech sounds (DeCasper, Lecanuet, Busnel, Granier-Deferre, & Maugeais, 1994; Granier-Deferre, Ribeiro, Jacquet, & Bassereau, 2011; Hepper, Scott, & Shahidullah, 1993; Lecanuet et al., 1992). We have reported that changes in fetal heart rate and motor activity to maternal reading aloud (Cohort VIII) appear to be a response to variation in normal maternal speech patterns which were partially dependent on whether women had been previously speaking naturally (Voegtline, DiPietro, Costigan, & Pater, 2013). Sontag and colleagues noted a fetal response to music that was independent from the maternal response (Sontag, Steele, & Lewis, 1969) and there has been resurgence in fetal

response to and retention of musical passages (Granier-Deferre, Bassereau, Ribeiro, Jacquet, & Decasper, 2011; Partanen, Kujala, Tervaniemi, & Huotilainen, 2013).

Together these and other findings coalesce to indicate that the fetus has the capacity to detect, respond to, and ultimately remember stimuli experienced during the prenatal period, at least for relatively short intervals. A synthesis of the findings by gestational age suggests that rudimentary capacity for retention of information may be expressed as early as 30 weeks gestation (Granier-Deferre, Bassereau, et al., 2011); serial assessments of responsivity to a rhyme passage with prior exposure revealed the onset of a response consistent with fetal learning at 34 weeks (Krueger & Garvan, 2014). This literature is quite small and studies are extremely difficult to execute well and thus often based on small numbers of participants. Nonetheless, there is evidence that, as expected, these capacities mature over time in tandem with central nervous system development and as foundational neurodevelopmental processes mature. This brief overview cannot adequately represent this complicated and compelling area of research; interested readers are directed to several excellent reviews (Granier-Deferre, Bassereau, et al., 2011; James, 2010; Moon & Fifer, 2000).

Chapter 13. Fetal neurobehavioral research reimagined

"It has been the object of this study to demonstrate the fact that fetal heart rate, as well as activity, is definitely measurable, and that its measurement provides a relatively discriminating method of determining individual differences between fetuses and between different periods in the life of the individual fetus". (Sontag & Richards, 1938, p 64)

Thus begins the summary and concluding chapter of the inaugural Fels report. The authors went on to enumerate suggestions for future work. However, it is not a stretch to think that today's available technologies to view and monitor the fetus were unimaginable to investigators in the 1930s. No other developmental period is as dependent on technology to ask even the most basic questions as is the prenatal period, so we begin by evaluating the capabilities of newer methods that have become available to fetal investigators. We follow conclude this chapter with our own thoughts as to the future of fetal neurobehavioral research.

Technology and fetal research

Imaging the fetus. The emergence of 3- and 4-dimensional scanning has allowed striking rendering of facial expressions that have the potential to instigate a new generation of research questions. We can now clearly observe the fetus yawn, suck its thumb, and stretch. Although these behaviors have been discernable to trained observers since the inception of real time 2-dimensional imaging, the images provided by 3D techniques leave little to the imagination. The application of this technology to understanding motor development and, potentially, the development of emotions is exciting and nascent(Hata, Dai, & Marumo, 2010; Kurjak, Azumendi, Andonotopo, & Salihagic-Kadic, 2007; Reissland, Francis, Mason, & Lincoln, 2011). Ultrasound images, irrespective of dimensionality, are the only data source currently available that can provide insight to the content and qualities of specific motor

behaviors. However, ultrasound as a methodology is not without its limitations. As the fetus grows, the field of view becomes more restricted and two transducers are often required to maintain simultaneous imaging of the head and lower limbs. At the time of this writing, the images that 4D scanning puts in motion are disjointed and the technique is difficult to implement for continuous periods. In addition, quantification of behaviors, generally based on videotaped sessions, requires the same type of intensive behavioral coding techniques and training that are used in studies of postnatal behavior.

Other imaging techniques have been applied to the study of fetal development. These include the use of Doppler technology to measure blood flow in specific areas of the brain, including the middle cerebral artery, in response to maternal vocalizations (Emory, 2010; Feng, Raynor, Fiano, Graham, & Emory, 1997). Doppler flow studies have revealed a previously unknown sex difference in perfusion of the developing fetal brain (Prior et al., 2013). New tools available to map the connectivity of the developing human brain from the molecular to the functional were heretofore unimaginable (Kang et al., 2011; Thomason et al., 2013). An anatomical atlas of the human fetal brain, based on specimens ranging from 15 to 21 post-conceptional weeks, is now publicly available and includes the full transcriptome (i.e., comprehensive portrait of RNA activity) (Miller et al., 2014). Although the techniques behind the documentation of functional and anatomical fetal brain architecture remain fairly distal for developmentalists, there is no doubt that over time these technologies will benefit understanding of fetal neurodevelopment and the central nervous system foundation on which it is built. Once data are generated for gestational periods beyond the 2nd trimester, this information may be particularly useful in understanding the basis of the developmental discontinuity present near 30 weeks gestation.

Measuring fetal heart rate. Isolation of the electrical fetal electrocardiogram (fECG) from the larger maternal signal through transducers placed on the maternal abdomen has been a surprisingly difficult enterprise. This is particularly true towards the end of gestation as the rapidly accumulating vernix provides partial electrical isolation of the fetus. Individual research teams, including Fels Institute investigators in later years (Welford et al., 1967), have had some success in deriving fECG data from customized methods by a configuration of cutaneous or sub-cutaneous electrodes applied to the maternal abdomen (David et al., 2007; Ferrazzi et al., 1989; Groome, Mooney, Bentz, & Singh, 1994; Patrick, Campbell, Carmichael, & Probert, 1986). Reports using this methodology are often limited to small numbers of participants measured for short duration. As a result, most fetal heart rate data, including those presented in this *Monograph*, have been collected through detection of heart beats by Doppler ultrasound. The fetal R-wave can also be detected through the use of magnetocardiography (VanLeeuwen, Cysarz, Lange, & Gronemeyer, 2006), but access to this equipment and its technical demands also present challenges to developmental investigators.

The introduction of technology for both signal detection and post-processing software capabilities indicates that this situation is changing. At least one new commercially available device can extract the fetal R-wave from 5 electrodes applied in configuration on the maternal abdomen (AN24, Monica Healthcare, Nottingham, UK). As this technology becomes increasingly validated (Cohen et al., 2012; Stampalija et al., 2011) and adopted for use in fetal neurobehavioral studies (Graatsma et al., 2012; Graatsma, Jacod, van Egmond, Mulder, & Visser, 2009) it provides a transformational opportunity to more precisely characterize variation in fetal heart rate using time dependent and independent techniques. The lack of fECG data has precluded the use of metrics routinely applied in postnatal psychophysiological research that rely

on interbeat interval. These include measures of variability, such as vagal tone (Porges, Doussard-Roosevelt, Portales, & Suess, 1994) based on specific frequency spectra within heart period data and/or preservation of the temporal sequencing of beats. For example, the presence of fetal respiratory sinus arrhythmia (RSA), a key measure of postnatal psychophysiological functioning, during intervals of fetal breathing movements has been established (Donchin, Caton, & Porges, 1984; Groome et al., 1994; Myers, Fifer, Haiken, & Stark, 1990). However, its continuity with the intrapartum transition to true respiration and predictive validity to developmental outcomes remains uninvestigated. The capacity to implement fECG monitoring more seamlessly will make this type of pursuit more feasible. In addition, greater precision in timing of beats will afford opportunities to more accurately characterize fetal reactions to stimulation with rapid rise times as well as more subtle changes associated with fetal orienting and habituation responses.

Although we embrace the potential of fECG technology, we think it is worth noting that our own work, and that of others, has been successful to date in documenting normative trajectories, individual differences, and predictive associations despite the muted precision of Doppler methodologies. Indeed, results of the Fels research on fetal cardiac patterns, generated from listening to the fetal heart rate with a stethoscope, have withstood the test of time suggesting that measures of rate and variability are quite robust regardless of metric. We do not mean to imply that the utility of Doppler detected fetal heart rate has run its course, but rather view the precision offered by fECG as opening up the ability to pose new and more complex questions about cardiac patterning and in vivo responsiveness.

Implementing fetal neurobehavioral research

Selection of methodology (i.e., imaging versus actigraphy; 3D versus 2D ultrasound; Doppler-detected fetal heart rate versus fetal ECG) is based on feasibility and the nature of the research question. Investigators that study fetal neurobehaviors arise from two distinct worlds: the academic realm of developmental sciences and the clinical realm of maternal-fetal medicine. There are far more obstetrically oriented studies of fetal heart rate and motor activity than there are developmentally oriented ones. Results from each field can often inform the other, but the professional trappings of journals, jargon and meetings often keep the two quite segregated. We have also observed that it is not uncommon for investigators to "dabble" in fetal research but then move on to other, potentially more easily accessible, research populations. Of all the developmental periods, fetal research is probably as difficult to conduct as its discoveries are rewarding. Part of this involves access to pregnant women and the medicolegal issues raised by use of measures that were primarily devised to assess fetal well-being. A record of fetal heart rate data can provide forensic evidence of fetal distress in the event of a poor pregnancy outcome, as can an ultrasound scan that fails to detect an obvious and potentially remedial anomaly. Unfamiliarity with the technologies necessary or available to view and monitor the fetus is also contributory.

It is critical to remember that although the subject of study may be the fetus, the research participant is the pregnant woman. Impressive technologies are of little use unless women accept them and believe them to be safe. Longitudinal studies during gestation cannot be successful if pregnant women do not return for a second visit. We believe that pregnant women participate in prenatal research for reasons of altruism and interest but also a generally unarticulated desire to receive additional antenatal surveillance of the well-being of their fetus. We credit our ability to conduct this research program over a long period of time to our

collaborative arrangements with clinicians. Participants are aware that fetal data collection is conducted by an expert in antenatal testing. The physical location of our data collection site within a maternal-fetal medicine unit further ensures that we are able to solicit clinical advice expeditiously when the need arises, which it does. This multidisciplinary collaboration integrates developmental sensibility and training in measurement, study design and data analysis with content expertise in maternal-fetal medicine, obstetric technologies, and clinical insight. Although other research teams have successfully used other strategies to recruit and retain participants, we suggest that developmentally oriented fetal research may be more effectively cultivated by developing working collaborations than by any particular benefit of the latest technologies.

The future of fetal neurodevelopment research

The original *Monograph* offered ten suggestions for future research that can be consolidated into the following areas: refine measurement; link the fetal period to postnatal life; record fetal and maternal responsivity to stimulation; evaluate maternal physiological and psychological influences on fetal neurobehavior; and establish the parameters for fetal conditioning. Our primary questions and considerations about future directions for fetal research echo these and we apply them within the context of existing knowledge and modern technologies.

1. *How does the fetus behave when the pregnant woman is not at rest*? Virtually everything that is known about fetal neurobehavioral development is based on data collected when women are recumbent or reclining during the day. The degree to which this reflects normative fetal behavior when women are standing, engaged in routine activities over the course of a day, or at night, is unknown. A small number of older studies recorded fetal heart rate

during maternal exercise on stationary bicycles (Webb, Wolfe, & McGrath, 1994) and there have been several comparisons of non-stress test results in different maternal positions(Cito et al., 2005). One of the few studies to examine the effects of proprioceptive cues on the fetus by passively moving pregnant women in a rocking chair found that a rocking motion elicited fetal heart rate responsivity but lateral gliding did not (Lecaneut & Jacquet, 2002). There is one interesting report of fetal heart rate when women are driving cars (Nakajima, Yamaji, & Ohashi, 2004). Beyond these, there is little information on the effects of maternal postural changes or other aspects of moving in space on the fetus; a limitation that did not escape the attention of the Fels investigators (Richards et al., 1938). In addition, most studies occur during daytime hours when investigators are at work but those findings may not generalize to other times. For example, there is evidence that fetal heart rate patterns consistent with REM sleep occur more often in the afternoon, and that the fetus is more awake and active in the early evening (Mulder et al., 1994).

The lack of a sufficient knowledge base leaves numerous unanswered questions. For example, are fetuses more or less active when women are active? Is a fetal response potentiated by a change in maternal posture or activity, and if so, do more sedentary women present a less "enriched" prenatal environment? The emergence of the human circadian cycle is also underspecified, although there are suggestive data that maternal rhythms help entrain those of the fetus (Lunshof et al., 1998). Rapidly emerging innovations in wearable computing technologies, including refinements in data intensive quantification methods, are highly suited to addressing these questions. Impending opportunities to combine maternal actigraphy with autonomic sensors and synchronize these with fetal monitors that can collect, store and/or transmit fetal cardiac data, will revolutionize the approach to this topic.

The same lack of information regarding fetal neurobehaviors over the course of a day also applies to the night. There is almost no information on the consequences of maternal sleep on the fetus or the role of maternal sleep in fetal development. A single research project with sixteen pregnant women from the 1970's noted that maternal sleep was associated with alterations to fetal heart rate and variability, including decelerative patterns in some fetuses (Hoppenbrouwers et al., 1981). A similar observation of very low periods of fetal heart rate that would be alarming if detected during the day was reported in overnight recordings from hospitalized women, leading the authors to note that it was fortunate that both the women and their obstetricians were asleep at the time (Patrick, Campbell, Carmichael, & Probert, 1982). New non-invasive fECG methods, coupled with more automated maternal polysomnography techniques will allow investigators to evaluate these associations. These include basic developmental questions, including whether there is correspondence between maternal and fetal sleep state cycles, and those of more clinical relevance, such as the acute and chronic effects of maternal sleep apnea on fetal functioning.

2. How well does the fetus predict the child? From a developmentalist's perspective, the degree to which fetal neurobehavioral measures are associated with postnatal temperament, regulatory processes, and developmental outcomes is perhaps the ultimate question. Yet exceedingly few methodologically rigorous studies exist to directly evaluate it and those that do have not been replicated. The existing reports, including one from the Fels study (Richards & Newbery, 1938) suggest that there is indeed predictive homotypic and/or heterotypic predictive validity for fetal heart rate patterns, motor behavior, and their integration; these studies have been previously referenced in prior chapters. The most distal published findings from our own work extend only through the 3rd year of life (DiPietro et al., 2007); a 5 year follow-up of Cohort

VI has concluded. Perhaps the most long-reaching report of the predictive validity of measured fetal behaviors is that of a Dutch cohort of 25 participants followed through age 15 (Van den Bergh & Mulder, 2012). We find that the detection of any significant prenatal to postnatal associations is encouraging given the vastly different proximal environments in which the fetus and child are embedded and the nature of measurement during each period. Nonetheless, the literature is currently far too small to draw conclusions about the continuity between prenatal and postnatal life.

Despite the scarcity of data, a number of single or composite measures derived from ultrasound observations have been promoted as global developmental or neurological assessments for the fetal period with implicated predictive validity to postnatal development. These include responsiveness to vibroacoustic stimuli (Divon et al., 1985), qualitative fetal movement patterns (Birnholz et al., 1978), and scoring of enumerated behaviors observed via ultrasound (Kurjak, Stanojevic, Predojevic, Lausin, & Salihagic-Kadic, 2012; Salisbury, Fallone, & Lester, 2005). However, we concur with Einspieler et al (2012) that the field is far from establishing a fetal exam based on patterning of motor behavior or any other performance metric. Although we (Chapter 11) and others have shown differences in development of fetuses with anomalous conditions, values for even those affected fetuses that are outside the distribution can overlap with those of fetuses without known afflictions. Thus they should not be used as screening or diagnostic indicators until adequate sensitivity and specificity is established. This will not be possible until there are a sufficient number of studies that measure and track a sufficient sample of individuals within and outside of the normal range of development from the prenatal to postnatal periods.

3. *What is the best way to measure emerging fetal temperament?* Motor activity is a dimension of postnatal temperament and can be measured in the fetus through observation using ultrasound or Doppler-based actigraphy, as reported here. However, the core constructs of temperament involve individual differences in the propensity to react to new information and the ability to regulate the initial response within some period of time (Rothbart et al., 2000). Fetal researchers, including ourselves, have used both external stimuli and manipulation of maternal arousal state to elicit a fetal response and used the post-stimulation period to evaluate return to baseline. However, much of this research is aimed at evaluating normative ontogeny of the fetal capacity to react and/or between-group differences, consistent with the types of questions that were initially asked when the field of infant research was emergent. Very few studies have measured fetal reactivity and evaluated its predictive validity to infant reactivity to novel situations (DiPietro, Ghera, et al., 2008; Werner et al., 2007).

In contrast to the experimental approach, which is primarily focused on reactivity, we believe there is also an opportunity to quantify behavioral regulation by identifying patterns of fetal heart rate and motor activity recorded during baseline conditions. Our experience in reviewing thousands of fifty minute long polygraphic tracings, output by the actocardiograph, has led to the observation that fetuses exhibit characteristic patterns of behaviors. Coordination between these patterns form the basis for the identification of fetal state, but states are coded in 3-minute windows. This can obscure the larger aspects of individuality that may be expressed over time. Although fetal heart rate becomes more variable over gestation, paradoxically maturation requires the evolution of periods of very low variability coupled with periods absent of motor activity (Pillai & James, 1990b). We have observed differences among fetuses in the expression of quiescence that are not distinguished by standard state scoring. Also, we have

noted individual differences in other parameters, including the shape of fetal heart rate accelerations and periodicity of fetal movement onset and offset. These reflect qualitative differences that we have as yet been unable to capture quantitatively, but may ultimately provide additional insight into the measurement of individual differences in regulatory processes.

4. *Are there sex differences in fetal neurobehavioral development?* The documentation of some sex differences *in utero* in some domains by ourselves and others, and the lack of documentation of differences in other domains are equally intriguing. There is no doubt that males are more vulnerable to prenatal adversity and exposures but the underlying contributory processes remain unknown. Indeed, the higher level of fetal heart rate variability in male fetuses reported here would suggest that male fetuses show accelerated regulatory development. It is possible that sex differentials in vulnerability generate variation in the implications of maturational rates of male and female fetuses for developmental outcomes (Sandman et al., 2013). As such, fetal neurobehavioral expression may have different "meaning" for each sex. This interpretation is consistent with our prior report that fetal motor activity predicts child motor activity for boys but not for girls (DiPietro, Bornstein, et al., 2002). Is it possible that the pervasive cultural belief that male fetuses are more active than female fetuses the result of sex differences in as yet unmeasured fetal reactivity to intrauterine changes introduced by maternal daily activities? Thus, despite the preponderance of null results in the literature regarding sex differences in neurobehavioral development, we urge investigators to analyze data by fetal sex, including main effects and interaction terms, and to report both positive and negative findings if the sample size is appropriate.

Our current finding that fetal sex affects maternal autonomic parameters is consistent with a rapidly accumulating body of evidence that details the effects of male fetuses on the

maternal-placental interface, including differences in biomarkers related to early placentation (Brown et al., 2014) and subsequent functionality (Challis et al., 2013). Moreover, male fetuses leave behind traces of their DNA, including elements of the Y-chromosome, in the maternal circulation (Nelson, 2012) and nervous system (Chan et al., 2012) that remain throughout a woman's life. The functional consequences of this microchimerism are not yet clear as it is generated by pregnancies of both sexes, but the greater prevalence of autoimmune diseases in women who have given birth to boys suggests that male microchimerism may be particularly disruptive. Results of future research in this arena are likely to be equally revealing about the consequences of fetal sex on the pregnancy and over time.

5. *How does the maternal context affect the fetus, and how does the fetus affect the maternal context?* The complexities of the maternal-fetal relationship have long-captivated the public and scientific imagination, yet the earliest relationship remains the most enigmatic. Although there would be little debate over the recognition that the maternal-fetal relationship is bidirectional, it is very challenging to operationlize measurement to encompass a transactional system. Investigators must limit data collection to a corpus of key variables and, even in longitudinal studies, the most sophisticated statistical methods do not readily uncover the "truth" about temporal relationships. We focus on the maternal autonomic nervous system in relation to fetal behaviors during undisturbed baseline periods although there are obviously many other maternal systems activated by pregnancy that influence fetal growth and development (Murphy, Smith, Giles, & Clifton, 2006; Petraglia, Imperatore, & Challis, 2010), including those of the hypothalamic-pituitary-adrenal/gonadal systems. No single project can adequately measure either the temporal chaining of within biologic system variables nor the cross-system interactions. Nonetheless, theories regarding the maternal-fetal relationship can guide hypothesis

testing regarding complex relationships. For example, oppositional strategies to maximize well-being on the part of both fetus and pregnant woman have been proposed as regulators of the hemodynamic properties of pregnancy (Haig, 1993), and ultimate developmental success has been postulated to reflect fetal adaptation to the characteristics of the maternal milieu when they are most congruent with the postnatal caregiving environment (Sandman, Davis, & Glynn, 2012).

Most prior work on this topic has focused on how maternal experiences affect the fetus. It is clear that the fetus contributes to the intrauterine milieu and that failing to consider this possibilty may lead to incorrect assumptions about the directionality of effects. We have previously reported contemporaneous associations in which fetal motor activity activates the maternal sympathetic system within a few seconds; here we show through lagged analyses that earlier levels of fetal motor activity affect the trajectory of skin conductance in pregnant women across gestation. We have suggested that this relationship, effected through unperceived perturbations to the uterine wall, reflects a fetal signaling function that prepares women for the demands of taking care of newborn infants (DiPietro, Caulfield, et al., 2006). We take this speculation further to suggest that women carrying more active fetuses are differentially prepared to respond to a more active and perhaps more challenging infant after birth than would be women carrying more sedate fetuses. But what about the fairly robust associations between fetal and maternal heart rate? These may be mutually co-determined by other factors, but just as there is evidence that the fetus responds to the maternal heart beat (Porcaro et al., 2006), is it too far-fetched to speculate that the pregnant woman can detect the second heart beating within her body? In non-pregnant populations it has been increasingly well-recognized that the brain listens to the heart; that is, the central nervous system detects and modulates information processing in

relation to the cardiac cycle (Park, Correia, Ducorps, & Tallon-Baudry, 2014). Although there are no neural connections between the fetal heart and the maternal brain, there may be other sources of signal transmission between the pair that we have not yet imagined.

6. How well does the fetus learn? The inability to directly observe and record from the fetus makes studies of fetal learning extremely difficult to implement and interpret. Effective presentation of stimuli is as difficult as monitoring responsiveness even in understanding rudimentary processes, such as habituation. Most studies rely on small alterations to fetal heart rate or motor activity against a background of wide variation in both to infer a fetal response and note change. Studies that rely on neonatal discrimination tasks to infer recognition of exposures before birth are also notoriously challenging and subject to high rates of attrition. As a result, the literature on fetal learning is sparse or absent. For example, there are no empirical reports of associative learning in the human fetus, and only one report of this capability on a single non-human primate (Kawai, Morokuma, Tomonaga, Horimoto, & Tanaka, 2004). The emergence of commercially available technologies to more precisely quantify changes in fetal heart rate in response to stimulus presentations will certainly increase the signal to noise ratio. It is also possible that 3D/4D ultrasound visualization will reveal additional methods to quantify fetal behavioral responses.

Among the outstanding methodological and conceptual issues is the relevance of fetal behavioral state to prenatal learning. The state of a newborn infant can be ascertained at a glance, but as discussed previously, fetal state is much more difficult to ascertain remotely. Fetal responsivity to auditory and light stimulation is at least partially modulated by fetal state or levels of heart rate variability as well as characteristics of stimulus presentation e.g., (Groome, Mooney, et al., 1999; Kiuchi, Nagata, Ikeno, & Terakawa, 2000; Lecanuet et al., 1986). These

findings tend to be complex and there is a lack of agreement as to the optimal protocol to maximize fetal responsivity. The mature fetus spends most time in a sleep state, which has raised questions regarding stimulus detection. However, evidence that neonatal conditioning (i.e., pairing between a tone or voice stimulus and a puff of air to the eye) occurs during sleep (Fifer et al., 2010; Reeb-Sutherland et al., 2011) increases the plausibility that the fetus garners information over time. Investigators who pursue inquiry into fetal learning will need to grapple with these procedural issues and by doing so will ultimately inform the science of early learning.

Conclusion

Fetal research continues to remain fully dependent on technology to provide a window to the developing fetus and despite emergent technologies, the subject of study remains just out of reach. Although empirical interest in the fetal period as the foundation of ontogeny has waxed and waned since Sontag & Richards (1938) published their *Monograph*, there is greater recognition that the period before birth provides the substrate for later development and sculpts the individual. There is also much left to be learned about the earliest relationship: how the maternal context affects that neurobehavioral development of the fetus, and in turn, how the neurobehavioral development of the fetus affects the pregnant woman. In the same way that the Fels investigators likely could not imagine a time that we could observe a rendering of the fetal face, so too in the future will our ability to ask and answer increasingly sophisticated questions about the origins of human development be limited only by our ingenuity. The next 75 years of discovery awaits.

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Table 1.
Sources of Data by Cohort and Gestational Age

Cohort number	Gestational weeks	Total enrollees
I	20, **24**, 28, **32**, **36**, 38	201
II	**36**	27
III	**24, 30, 36,** 38	102
IV	**32, 36**	109
V	**24,** 27**, 30**	28
VI	**24-26**, 27-29, **30-32**, 33-35, **36-38**[a]	130
VII	**24, 30, 36**	55
VIII	**24, 30, 36**	121

Note. Bold text indicates sources of data by gestational age window selected for the current analysis.

[a] Initiation of protocol proceeded in 3 waves with staggered entry and visits every 3 weeks such that data were available for each gestational age from 24 to 38 weeks. That is, the first wave was tested at 24, 27, 30, 33 and 36 weeks, the second at 25, 28, 31, 34, etc.

(n=773)

Table 2.
Attrition and Exclusion Cases by Cohort

Cohort	Enrolled	Discontinued participation[a]	Exclusions	Final sample
I	201	8	Trisomy 21 Ambiguous genitalia Fetal demise Non-viable preterm delivery	189
II	27	0		27
III	102	3	Cleft palate	98
IV	109	0	Kidney malformation Fetal demise Williams syndrome	106
V	28	2		26
VI	130	1	Cleft palate	128
VII	55	3		52
VIII	121	6	Agenesis corpus callosum	114
Total	773	23	10	740

[a] Did not complete participation in the study, as defined by the individual cohort protocol, due to voluntary withdrawal, scheduling difficulties, or moving from area.

Table 3.
Maternal Characteristics

	N (%)	Mean (*SD*)	Range
Age (years)		31.3 (4.7)	18.0 – 45.0
Education (years)		16.7 (2.3)	9.0 – 20.0
Occupation status[a]		7.2 (1.6)	0 – 9
Weight (lbs)		145.4 (28.7)	91 – 330
Height (inches)		64.9 (2.7)	57.0 – 74.0
Body mass index (kg/m^2)		24.3 (4.5)	16.5 – 48.7
Married	664 (89.7)		
Nulliparous	430 (58.1)		
Race/ethnicity			
African-American	95 (12.9)		
Asian	60 (8.1)		
Non-Hispanic White	582 (79.0)		

(n=740) Sample size includes each instance of maternal participation.

[a]Occupational status based on Hollingshead categories.

Table 4.

Infant Characteristics

	N (%)	Mean (*SD*)	Range
Gestational age at delivery (wks)		39.2 (1.6)	28.0 – 42.7
Preterm			
35-36 weeks	40 (81.6%)		
< 35 weeks	9 (18.4%)		
Birthweight (g)		3404.37 (509.5)	1429.0 – 5315.0
Birth length (cm)		51.2 (2.6)	38.1 – 59.7
Ponderal index (100*(g/cm^3))		2.54 (0.3)	1.5 – 5.0
Apgar 1-min		8.0 (1.3)	1.0 – 10.0
Apgar 5-min		8.9 (0.5)	5.0 – 10.0
Cesarean delivery	205 (28.1)		
Sex, male	373 (50.7)		

(n=740)

Table 5.
Sample Sizes at Each Gestational Period by Cohort and Mean Gestational Age (GA) at Time of Participation

	Gestational period					
	G1		G2[a]		G3	
Cohort	*n*	GA	*n*	GA	*n*	GA
I	188	24.4	173	32.5	162	36.5
II	--	--	--	--	27	36.3
III	95	24.4	91	32.4	84	36.4
IV	--	--	97	32.4	95	36.3
V	26	24.4	26	30.3	--	--
VI	116	25.3	106	31.4	92	37.2
VII	52	24.4	51	30.4	47	36.3
VIII	106	24.4	109	30.4	106	36.3
Total	583	24.6	653	31.7	613	36.5

[a] *Note.* Reflects data collected at either 30 or 32 weeks gestation.

Table 6.
Fetal Heart Rate Measures at Each Gestational Period

	Gestational period						
	G1		G2		G3		
	n	Mean (*SD*)	*n*	Mean (*SD*)	*n*	Mean (*SD*)	*t* [a]
Fetal heart rate	576	147.6 (5.6)	649	142.9 (7.0)	606	141.7 (8.1)	-19.80***
Fetal heart rate variability	576	4.26 (0.90)	649	5.17 (1.35)	606	5.80 (1.56)	25.40***
Accelerations	576	1.4 (1.8)	649	5.2 (3.9)	606	6.7 (4.1)	29.62***

[a] Test of longitudinal change from 24 to 36 weeks gestation based on actual week of gestation at observation.

****p*<.001

Table 7a
Interrelations of Fetal Heart Rate (FHR) Measures by Gestational Period and Stability Over Time [a]

	Fetal heart rate			Fetal heart rate variability			Accelerations		
	G1	G2	G3	G1	G2	G3	G1	G2	G3
G1 fetal heart rate	--								
G2 fetal heart rate	.66***	--							
G3 fetal heart rate	.46***	.47***	--						
G1 FHR variability	**.09***	.05	.02	--					
G2 FHR variability	.03	**.10***	-.05	.43***	--				
G3 FHR variability	.02	-.06	**.05**	.37***	.47***	--			
G1 accelerations	**.04**	-.06	-.04	**.67****	.27***	.25***	--		
G2 accelerations	-.03	**.02**	-.05	.26***	**.74****	.36***	.24***	--	
G3 accelerations	.01	-.03	**-.11***	.23***	.34***	**.76****	.18***	.32***	--

*p <.05, **p<.01, *** p<.001
[a]Sample size is 738; ns for pairwise comparison ranged from 464 to 649.
Note. Bolded text used to highlight intercorrelations within each gestational period.

Table 7b
Interrelations of Fetal Heart Rate (FHR) Measures by Gestational Period and Stability Over Time Excluding Siblings [a]

	Fetal heart rate			Fetal heart rate variability			Accelerations		
	G1	G2	G3	G1	G2	G3	G1	G2	G3
G1 fetal heart rate	--								
G2 fetal heart rate	.66***	--							
G3 fetal heart rate	.47***	.48***	--						
G1 FHR variability	**.10***	.06	.01	--					
G2 FHR variability	.02	**.11***	-.07	.43***	--				
G3 FHR variability	.02	-.04	**.05**	.37***	.45***	--			
G1 accelerations	**.07**	-.06	-.03	**.66*****	.26***	.23***	--		
G2 accelerations	-.05	**.01**	-.08	.26***	**.73*****	.34***	.22***	--	
G3 accelerations	.02	-.02	**-.11***	.21***	.33***	**.76*****	.15**	.31***	--

*p <.05, **p<.01, *** p<.001
[a]Sample size is 633; ns for pairwise comparison range from 404 to 552.
Note. Bolded text used to highlight intercorrelations within GA assessment.

Table 8.
Fetal Motor Activity Measures at Each Gestational Period

	Gestational period						
	G1		G2		G3		
	n	Mean (*SD*)	*n*	Mean (*SD*)	*n*	Mean (*SD*)	*t* [a]
Vigor	578	11.02 (2.3)	647	10.62 (2.4)	601	10.79 (2.7)	-2.08*
Bouts	578	65.0 (12.2)	647	59.0 (15.8)	601	55.1 (15.4)	-12.46***
Total movement (s)	578	863.7 (443.5)	647	796.0 (535.6)	601	845.2 (566.1)	-0.67

[a] Test of longitudinal change from 24 to 36 weeks gestation based on actual week of gestation at observation.

*p<.05, ***p<.001,

Table 9a
Interrelations of Fetal Motor Activity Measures by Gestational Period and Stability Over Time [a]

	Vigor			Bouts			Total movement		
	G1	G2	G3	G1	G2	G3	G1	G2	G3
G1 vigor	--								
G2 vigor	.48***	--							
G3 vigor	.46***	.53***	--						
G1 bouts	**.39*****	.21***	.18***	--					
G2 bouts	.17***	**.42*****	.17***	.19***	--				
G3 bouts	.15**	.26***	**.32*****	.20***	.25***	--			
G1 total movement	**.74*****	.34***	.26***	**.36*****	.14**	.12*	--		
G2 total movement	.25***	**.69*****	.33***	.16***	**.31*****	.20***	.36***	--	
G3 total movement	.25***	.36***	**.71*****	.14**	.14**	**.14*****	.26***	.38***	--

*p <.05, **p<.01, *** p<.001
[a]Sample size is 739; ns for pairwise comparison range from 389 to 610.
Note. Bolded text used to highlight intercorrelations within GA assessment.

Table 9b

Interrelations of Fetal Motor Activity Measures by Gestational Period and Stability Over Time Excluding Siblings [a]

	Vigor			Bouts			Total movement		
	G1	G2	G3	G1	G2	G3	G1	G2	G3
G1 vigor	--								
G2 vigor	.44***	--							
G3 vigor	.42***	.53***	--						
G1 bouts	**.37*****	.18***	.20***	--					
G2 bouts	.17***	**.43*****	.17***	.19***	--				
G3 bouts	.11*	.24***	**.31*****	.20***	.22***	--			
G1 total movement	**.73*****	.31***	.25***	**.34*****	.16***	.11	--		
G2 total movement	.22***	**.67*****	.33***	.14******	**.29*****	.17*******	.33***	--	
G3 total movement	.22***	.32*******	**.71*****	.14******	.13******	**.14****	.26***	.36***	--

p* <.05, ***p*<.01, * *p*<.001

[a]Sample size is 634; ns for pairwise comparison range from 402 to 549.

Note. Bolded text used to highlight intercorrelations within GA assessment.

Table 10.

Fetal Movement-heart Rate Coupling Measures at Each Gestational Period

	Gestational period						
	G1		G2		G3		
	n	Mean (*SD*)	*n*	Mean (*SD*)	*n*	Mean (*SD*)	*t* [a]
FM-FHR coupling	574	0.21 (0.08)	644	0.29 (0.10)	599	0.32 (0.10)	22.75***
Latency[b]	571	5.27 (1.66)	635	4.28 (1.84)	596	4.00 (1.70)	-13.43***
Number of coupled moves	574	12.9 (5.2)	644	16.6 (6.6)	599	16.9 (6.4)	12.62***

[a] Test of longitudinal change from 24 to 36 weeks gestation based on actual week of gestation at observation.

[b] *n*s for this variable reflect exclusion of outlier cases +/- 3 *SD* from mean

****p*<.001

Table 11.

Maternal Physiology Measures at Each Gestational Period

	Gestational period						
	G1		G2		G3		
	n	Mean (*SD*)	*n*	Mean (*SD*)	*n*	Mean (*SD*)	*t* [a]
Heart rate	577	82.4 (9.2)	650	86.1 (9.8)	607	84.4 (10.20	7.24***
Respiratory sinus arrhythmia	552	43.2 (25.7)	616	38.1 (24.7)	558	42.8 (30.2)	-1.48
Respiratory period	551	4.0 (0.9)	614	4.0 (0.9)	552	4.0 (1.0)	1.32
Skin conductance	572	6.9 (3.4)	644	6.8 (3.4)	602	7.3 (3.7)	2.88**

[a] Test of longitudinal change from 24 to 36 weeks gestation based on actual week of gestation at observation.

p<.01,*p<.001

Table 12.
Sex Differences in Fetal Heart Rate (FHR, Motor Activity (FM), and FM-FHR Coupling and Latency

	Gestational period						
	G1		G2		G3		
	Female	Male	Female	Male	Female	Male	t [a]
FHR							
Fetal heart rate	148.0 (5.5)	147.3 (5.7)	143.5 (7.1)	142.3 (6.9)	142.8 (8.4)	140.6 (7.7)	2.74**
Fetal heart rate variability	4.2 (0.9)	4.3 (0.9)	5.0 (1.3)	5.3 (1.3)	5.7 (1.6)	5.9 (1.5)	-2.03*
Accelerations	1.4 (1.8)	1.4 (1.9)	5.0 (4.0)	5.3 (3.9)	6.5 (4.2)	6.9 (4.0)	-0.97
FM							
Vigor	11.1 (2.3)	11.0 (2.3)	10.6 (2.3)	10.6 (2.5)	11.0 (2.7)	10.6 (2.7)	0.77
Bouts	65.6 (12.0)	64.4 (12.5)	58.5 (15.4)	59.3 (16.2)	53.9 (14.9)	56.5 (15.8)	-2.52*
Total movement (s)	882.8 (443.7)	844.6 (443.8)	798.6 (542.7)	791.1 (528.5)	869.5 (589.1)	822.8 (543.3)	-0.11
FM-FHR							
Coupling	.20 (.08)	.21 (.08)	.28 (.10)	.30 (.11)	.32 (.11)	.32 (.10)	0.56
Latency	5.34 (1.70)	5.19 (1.56)	4.37 (1.80)	4.12 (1.86)	4.03 (1.74)	3.95 (1.66)	-0.38

[a] Test of sex difference in the trajectory of each fetal parameter.
*p<.05, **p<.01

Table 13.
Intraclass Correlations for Sibling Fetal Heart Rate, Motor Activity, and FM-FHR Integration by Gestational Period

	N (pairs)	ICC
FHR		
Fetal heart rate		
G1	61	.27*
G2	75	.35***
G3	66	.18*
Fetal heart rate variability		
G1	61	.15†
G2	75	.23*
G3	66	.30***
Accelerations		
G1	61	.03
G2	75	.18*
G3	66	.16†
FM		
Movement vigor		
G1	62	<.01
G2	75	.17*
G3	64	.31**
Movement bouts		
G1	62	<.01
G2	75	.31**
G3	64	.32**
Total movement		
G1	62	<.01
G2	75	.13
G3	64	.15†
Coupling composite		
G1	61	.06
G2	75	.11
G3	64	.05

†p<.10, *p<.05, **p<.01, ***p<.001

Figure captions

Figure 1. Conceptual model of fetal neurobehavioral development within the maternal context. The upper part of the figure presents the orderly and hierarchical progression of fetal neurobehavioral parameters from autonomic regulation through information processing, following Als (1982). The bidirectional nature of influence, initially from pregnant woman to fetus, and over time, from fetus to pregnant woman, is illustrated by progressively thickening arrows. The lower portion of the figure portrays the increasing individuality in fetal neurobehavioral expression in tandem with decreasing canalization during gestation, as described by McCall (1981) during the postnatal period.

Figure 2. The apparatus devised by the original Fels investigators to detect fetal motor activity (Sontag & Wallace, 1933). From *American Journal of Psychology* (vol. 45, no. 3, July 1933).

Figure 3. Sample digitized output of fetal actocardiography data (2 8-minute plots) from a 36 week fetus. The plot at left shows three movement bouts (lower lines), each associated with an acceleration in fetal heart rate (upper lines), with periods of relative motor quiescence and low fetal heart rate variability in between. The plot at right shows persistently high levels of variability in fetal heart rate along with long periods of continuous, high amplitude motor activity except for a brief respite between minutes 34 and 36.

Figure 4. Fetal heart rate. Lowess curve estimates depict significantly decreasing mean heart rate in the second half of gestation, with more pronounced decline from G1 to G2. Scatter points represent data from individual fetuses at each gestational age and show variation within each of the gestational periods measured (i.e, G1, G2, G3).

Figure 5. Fetal heart rate variability. Lowess curve estimates depict significantly increasing mean variability, with greater gain from G1 to G2.

Figure 6. Fetal movement vigor. Lowess curve estimates depict decreasing vigor from G1 to G2 and increasing thereafter to G3, resulting in an overall lack of change across the full gestational span.

Figure 7. Fetal movement bouts. Lowess curve estimates depict significantly decreasing number of movement bouts, with greater decline from G1 to G2.

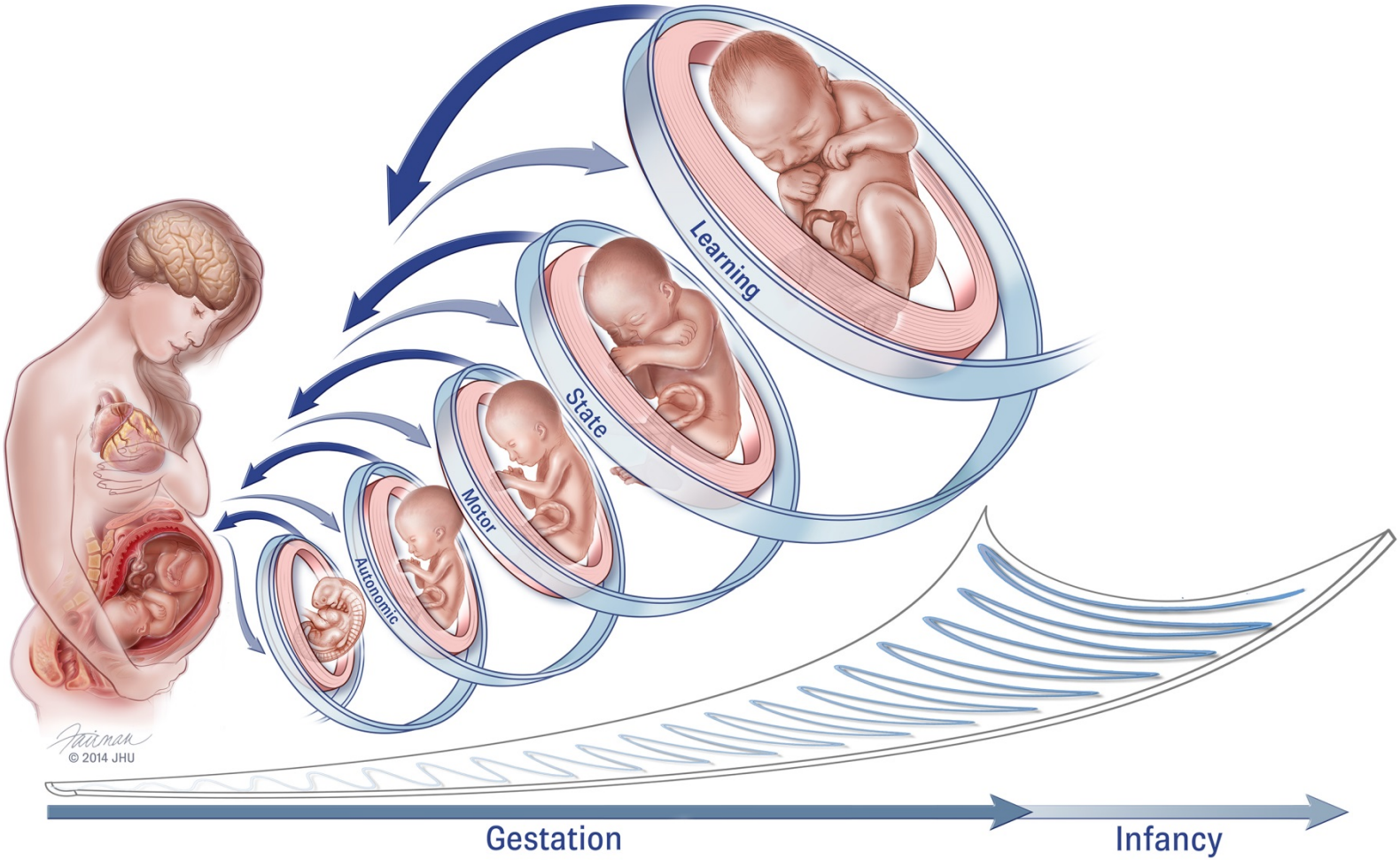
Figure 8. Fetal movement-fetal heart rate coupling. Lowess curve estimates depict significantly significantly increasing mean coupling, with greater gain from G1 to G2.

Figure 9. Coupling latency. Lowess curve estimates depict significantly decreasing mean latency, with greater decline from G1 to G2.

Figure 10. Maternal RSA/SCL autonomic profiles by fetal heart variability (a) and total movement (b) at the final gestational period (G3). High maternal sympathetic tone (i.e., SCL) is associated with lower fetal heart rate variability and motor activity only in combination with low maternal parasympathetic tone (RSA).

Figure 11. Neurobehavioral trajectories for fetuses with intrauterine growth restriction (n = 9) and three individual anomalous cases, including fetal heart rate (a), number of fetal heart rate accelerations (b), total movement (c), and FM-FHR coupling (d). The shaded areas reflect normative values (i.e. 10^{th} to 90^{th} percentiles) of the full sample.

Figure 1


Learning
State
Motor
Autonomic
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Gestation
Infancy

Figure 2.

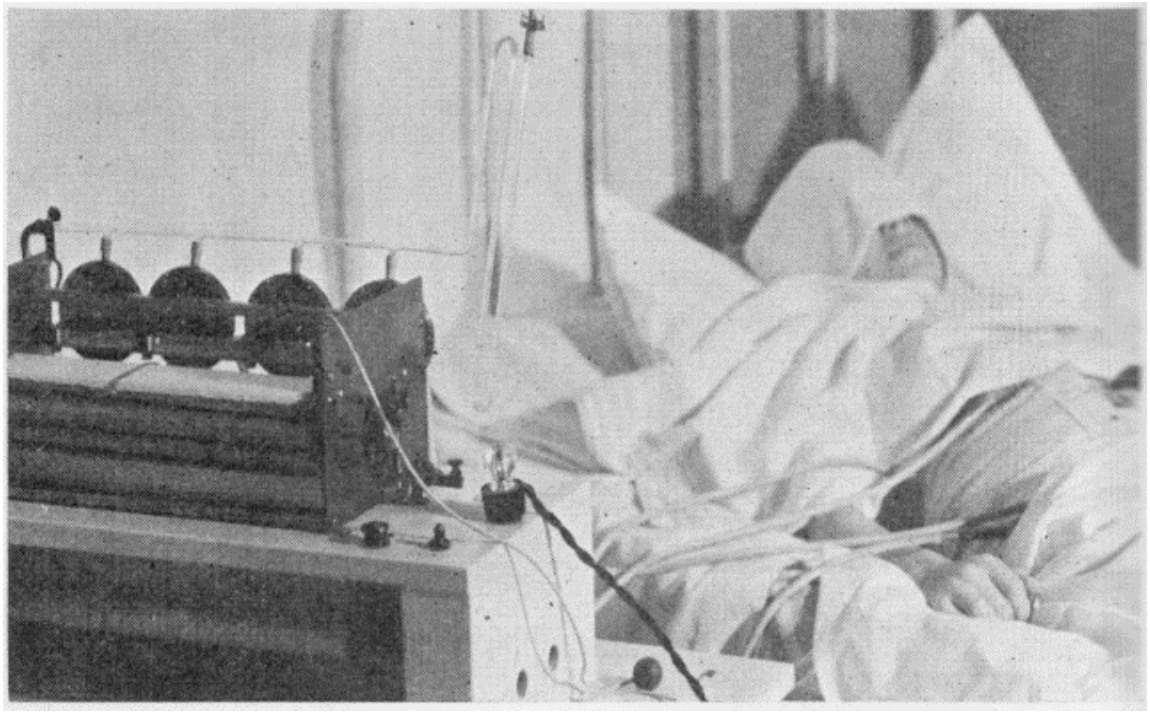
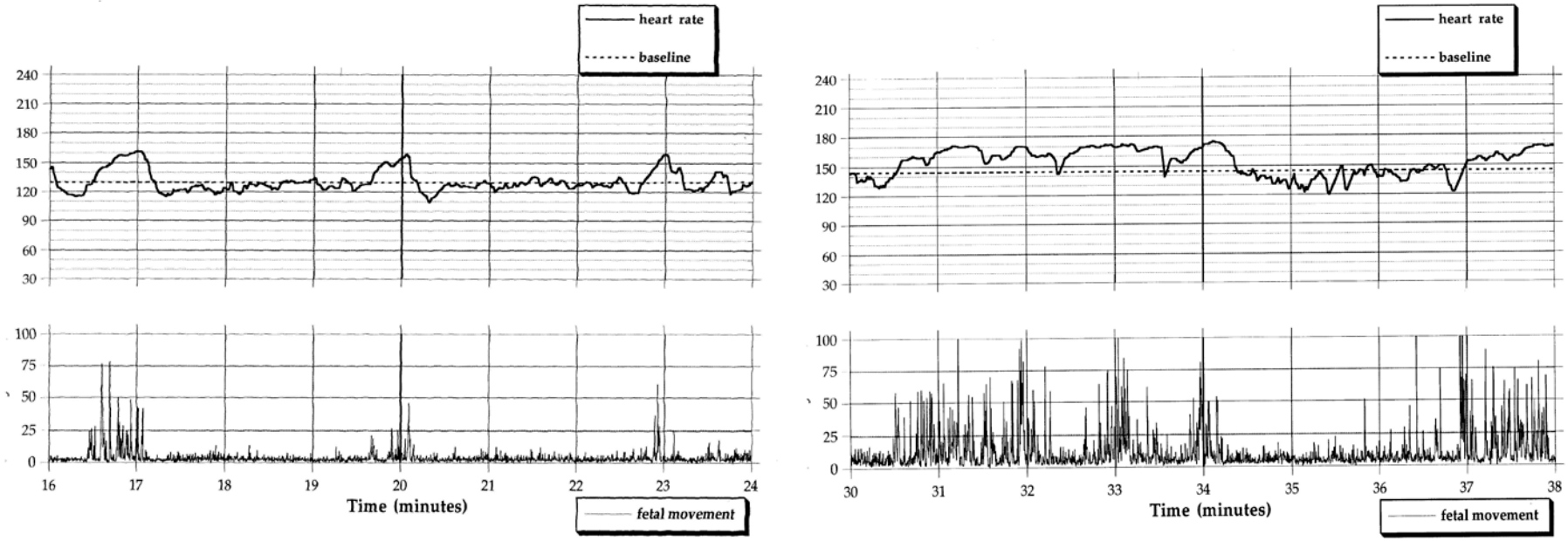


Figure 3.


heart rate
baseline
240
210
180
150
120
90
60
30
100
75
50
25
0
16
17
18
19
20
21
22
23
24
Time (minutes)
fetal movement
heart rate
baseline
240
210
180
150
120
90
60
30
100
75
50
25
0
30
31
32
33
34
35
36
37
38
Time (minutes)
fetal movement

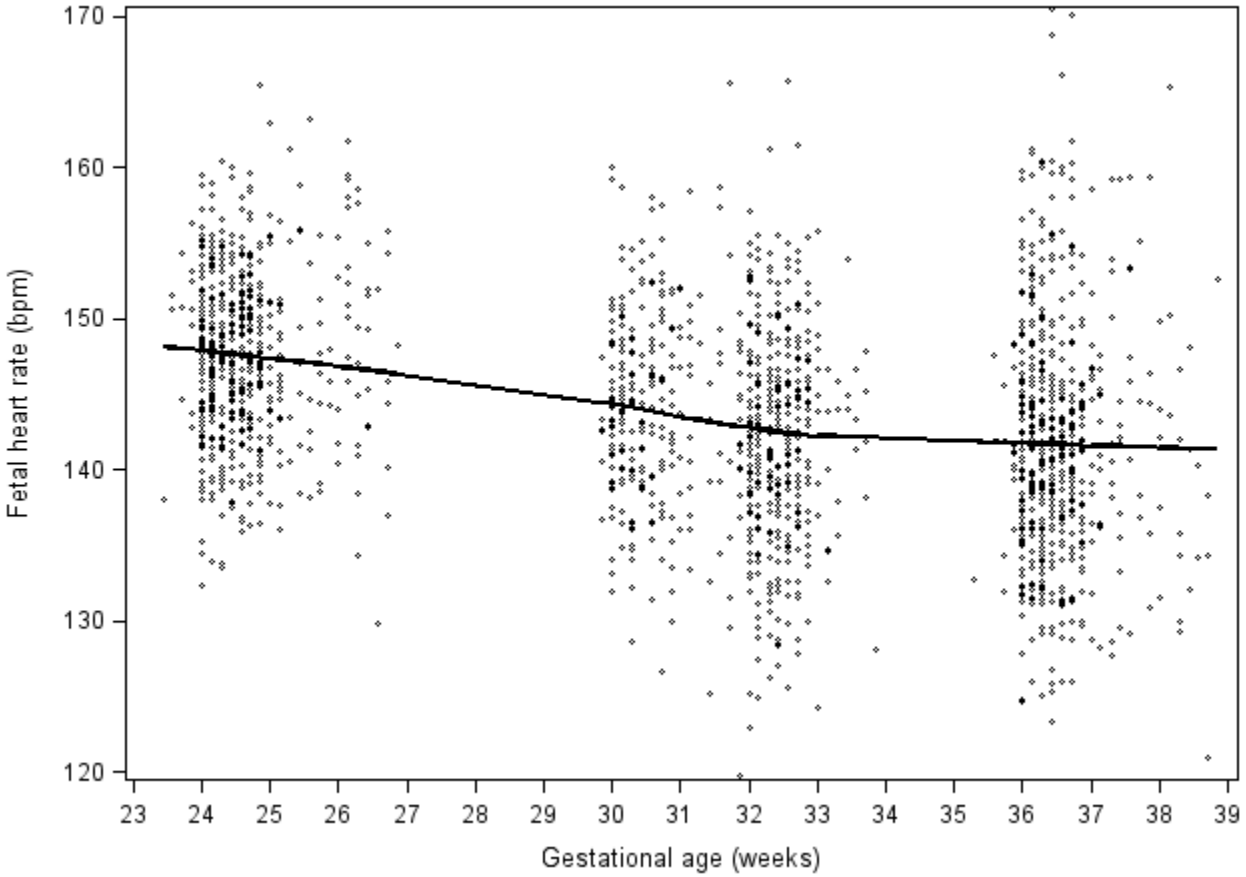

170
160
150
140
130
120
Fetal heart rate (bpm)
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
Gestational age (weeks)

Figure 4.

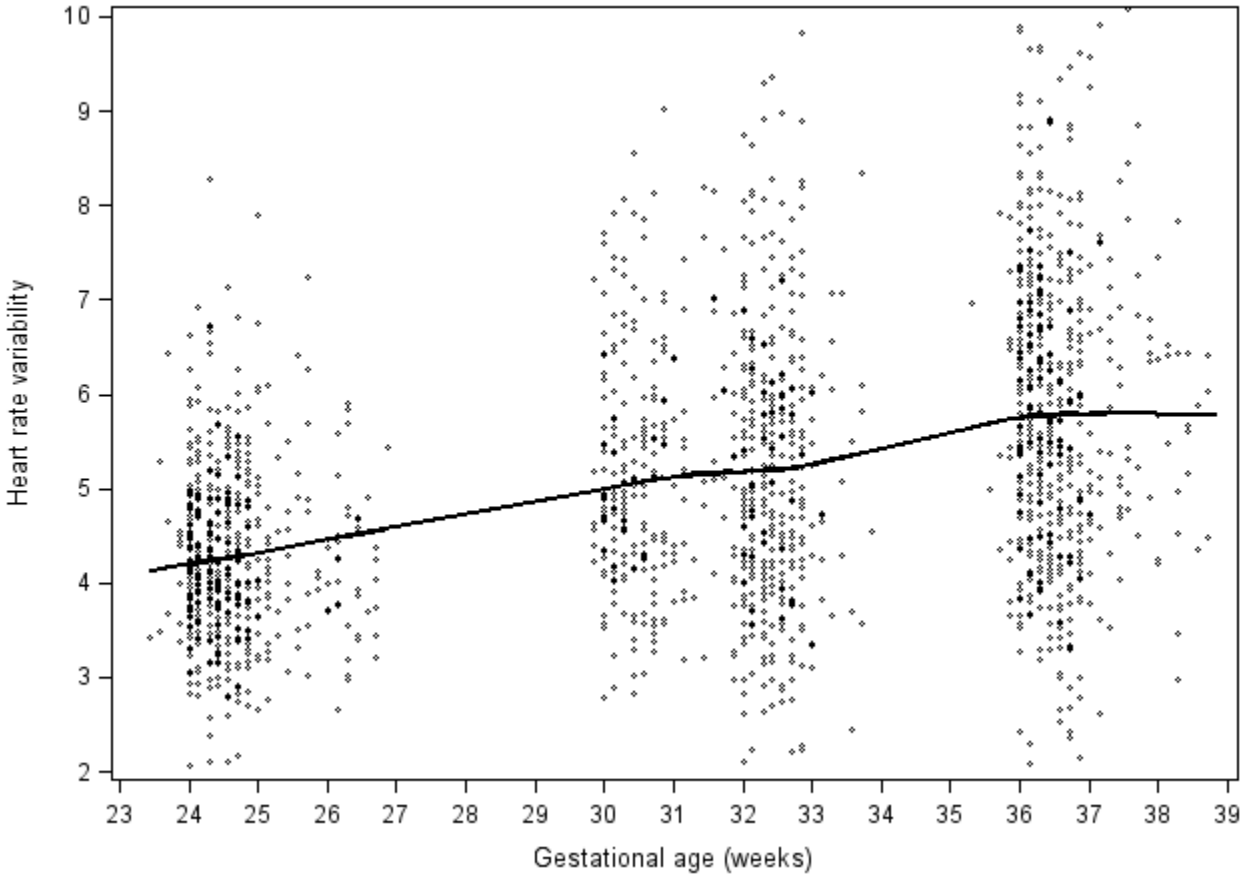

Heart rate variability
10
9
8
7
6
5
4
3
2
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
Gestational age (weeks)

Figure 5.

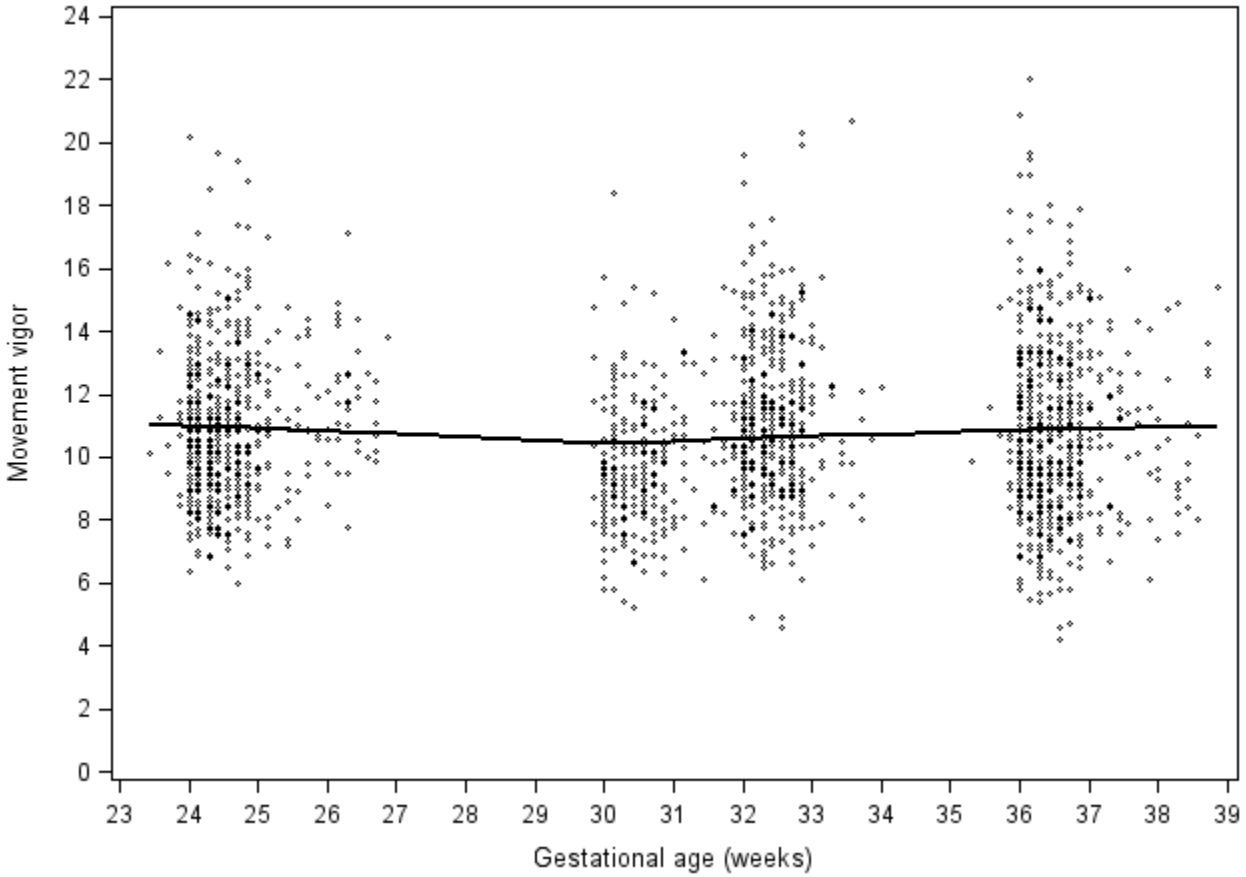

Movement vigor
0
2
4
6
8
10
12
14
16
18
20
22
24
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
Gestational age (weeks)

Figure 6.

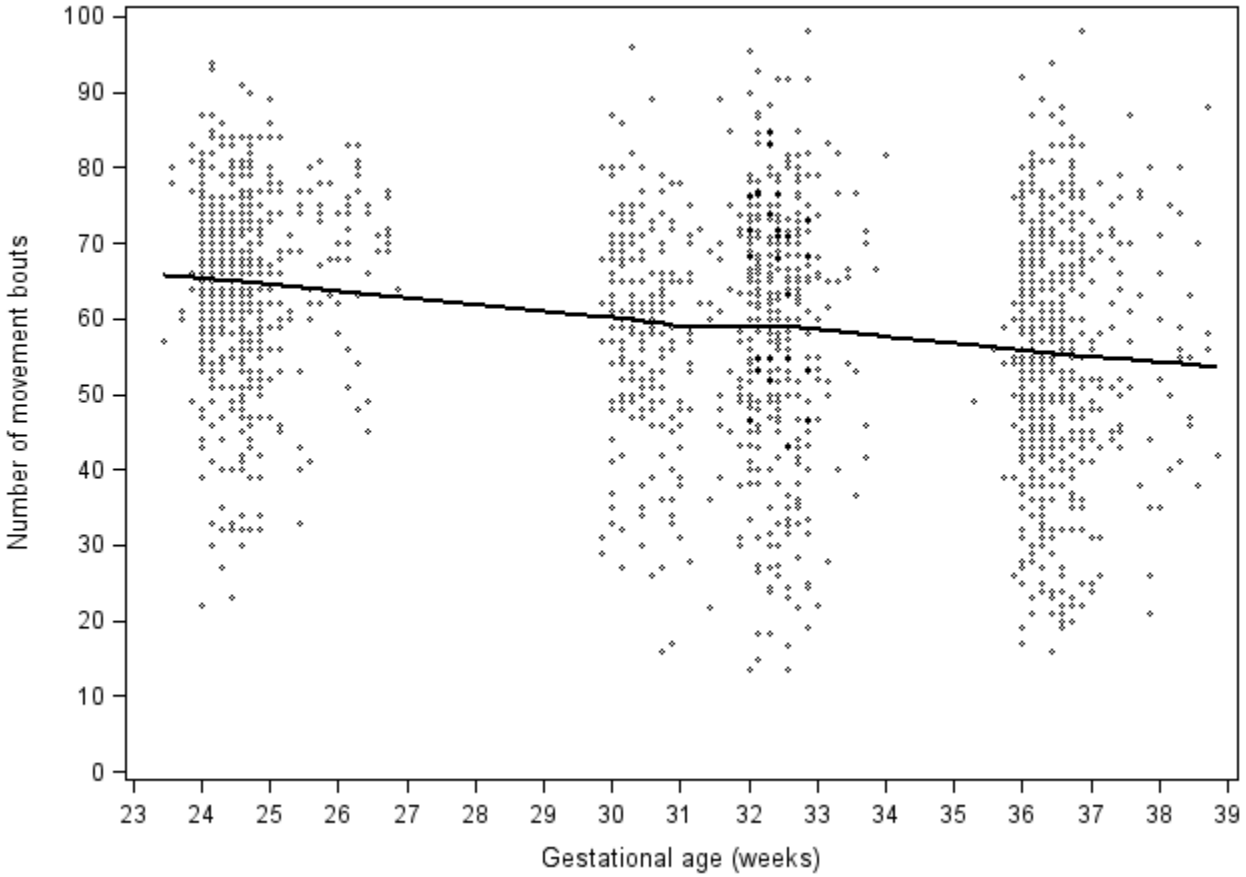

Number of movement bouts
100
90
80
70
60
50
40
30
20
10
0
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
Gestational age (weeks)

Figure 7.

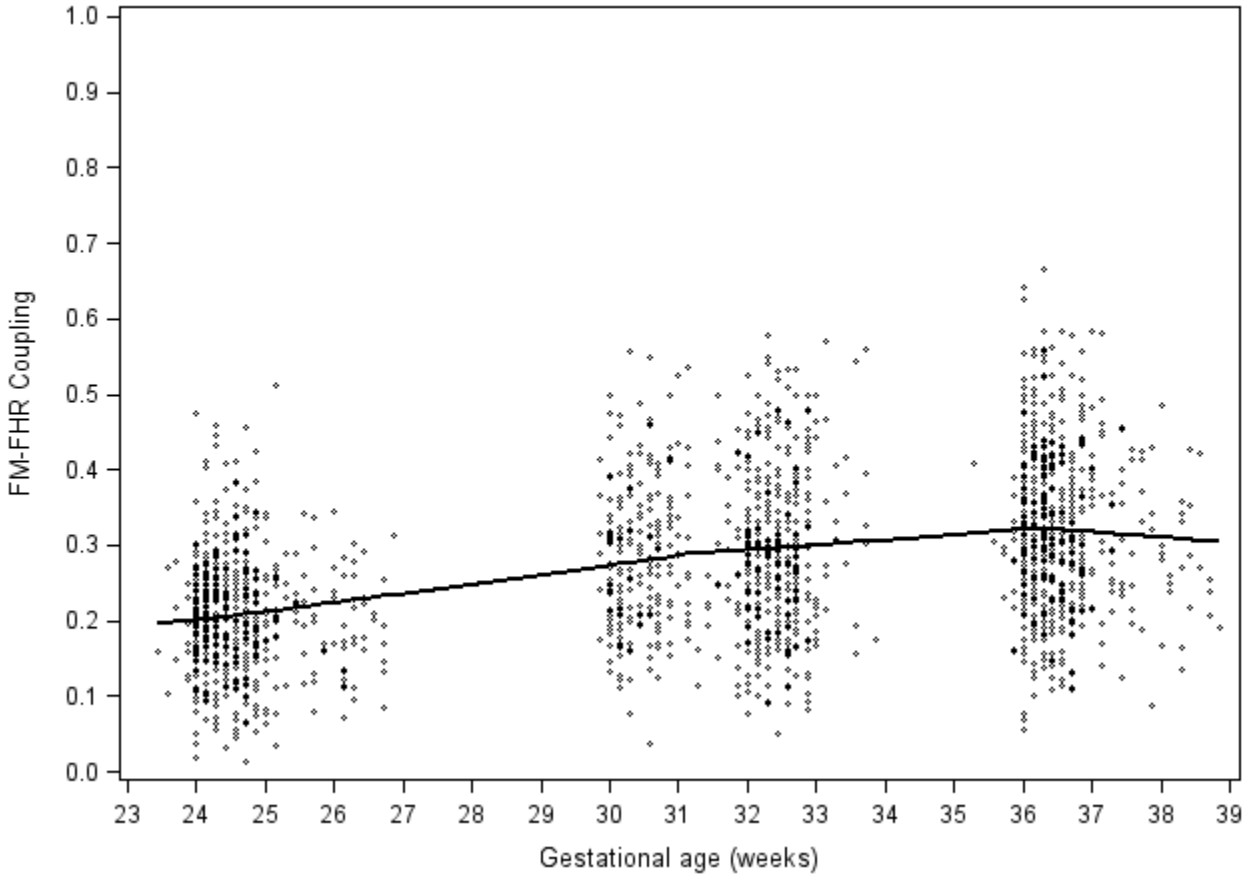
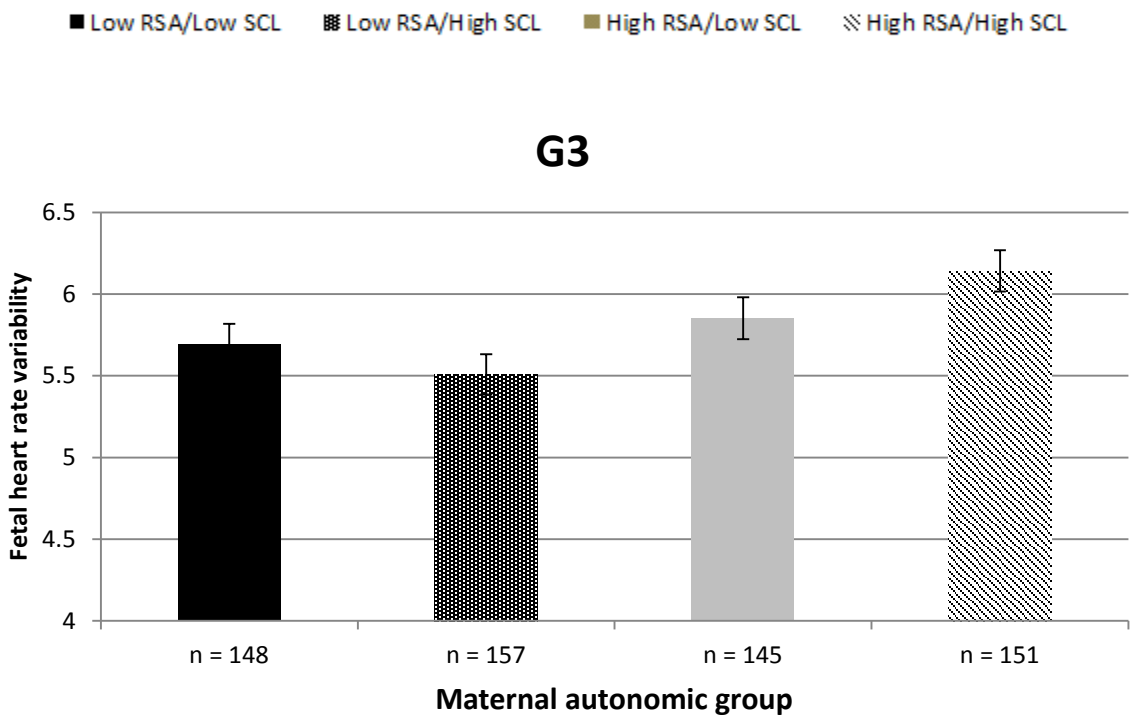
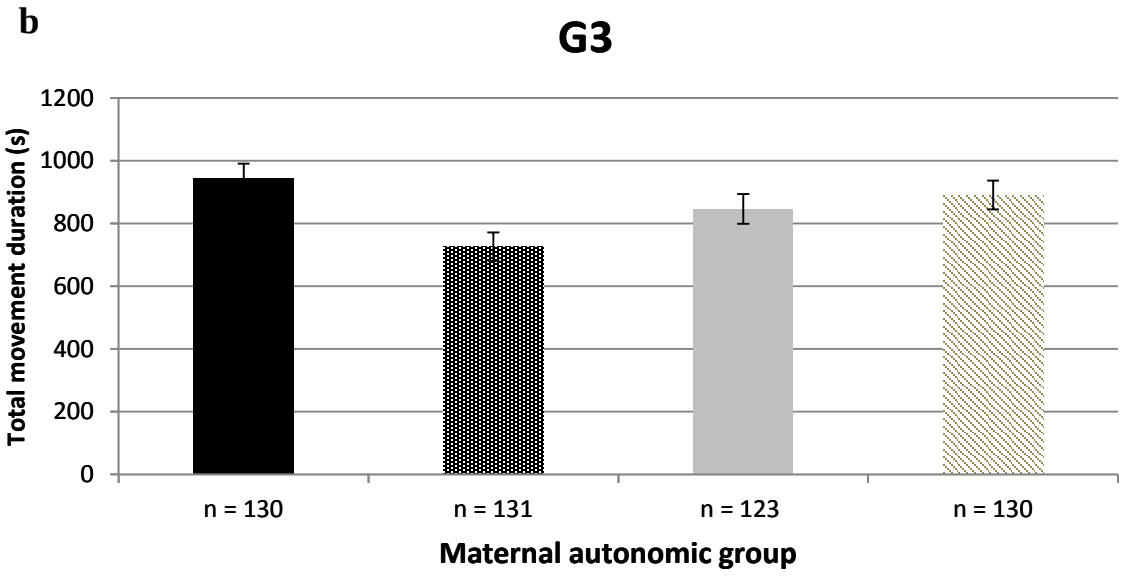
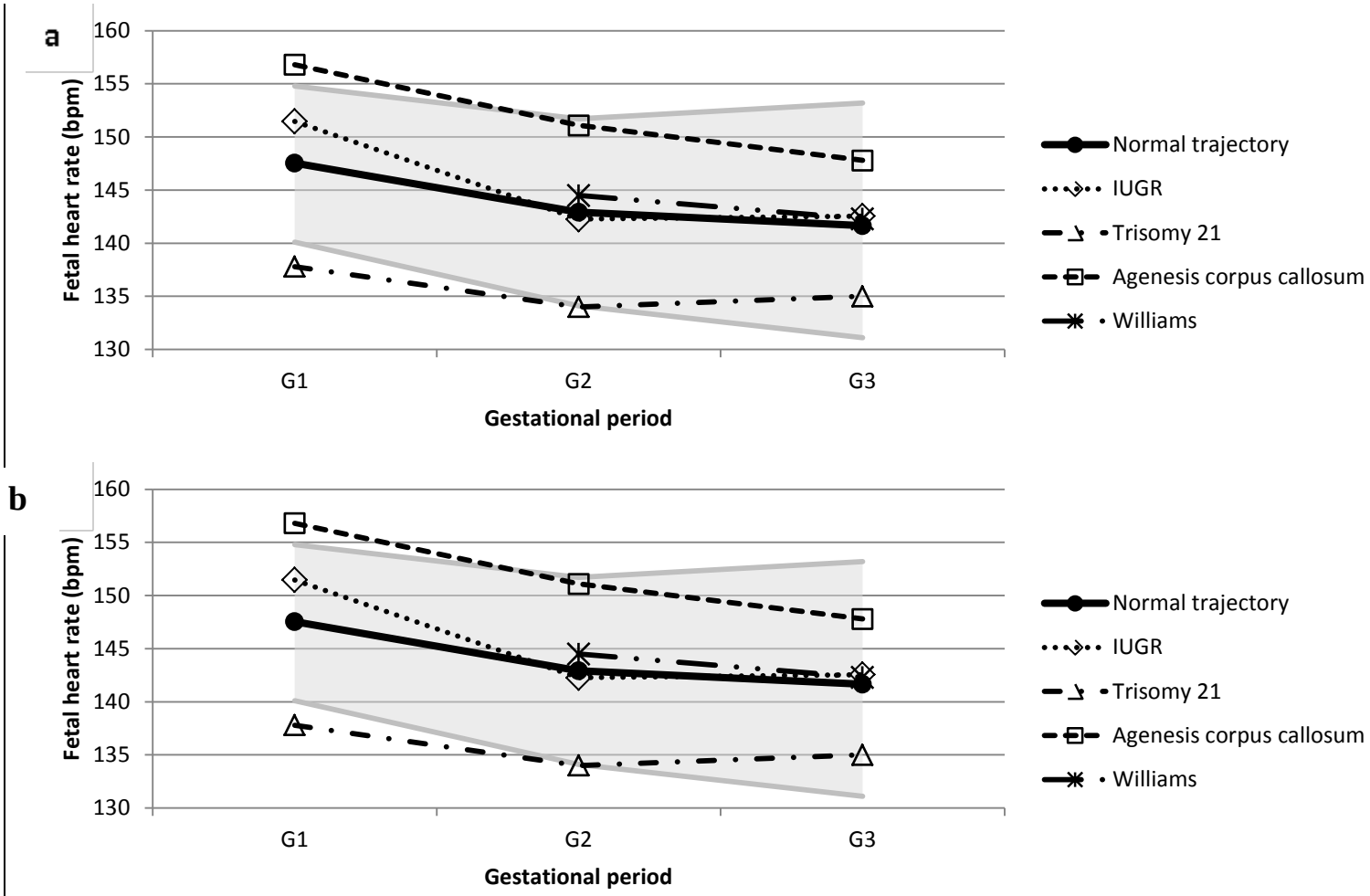

FM-FHR Coupling
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
Gestational age (weeks)

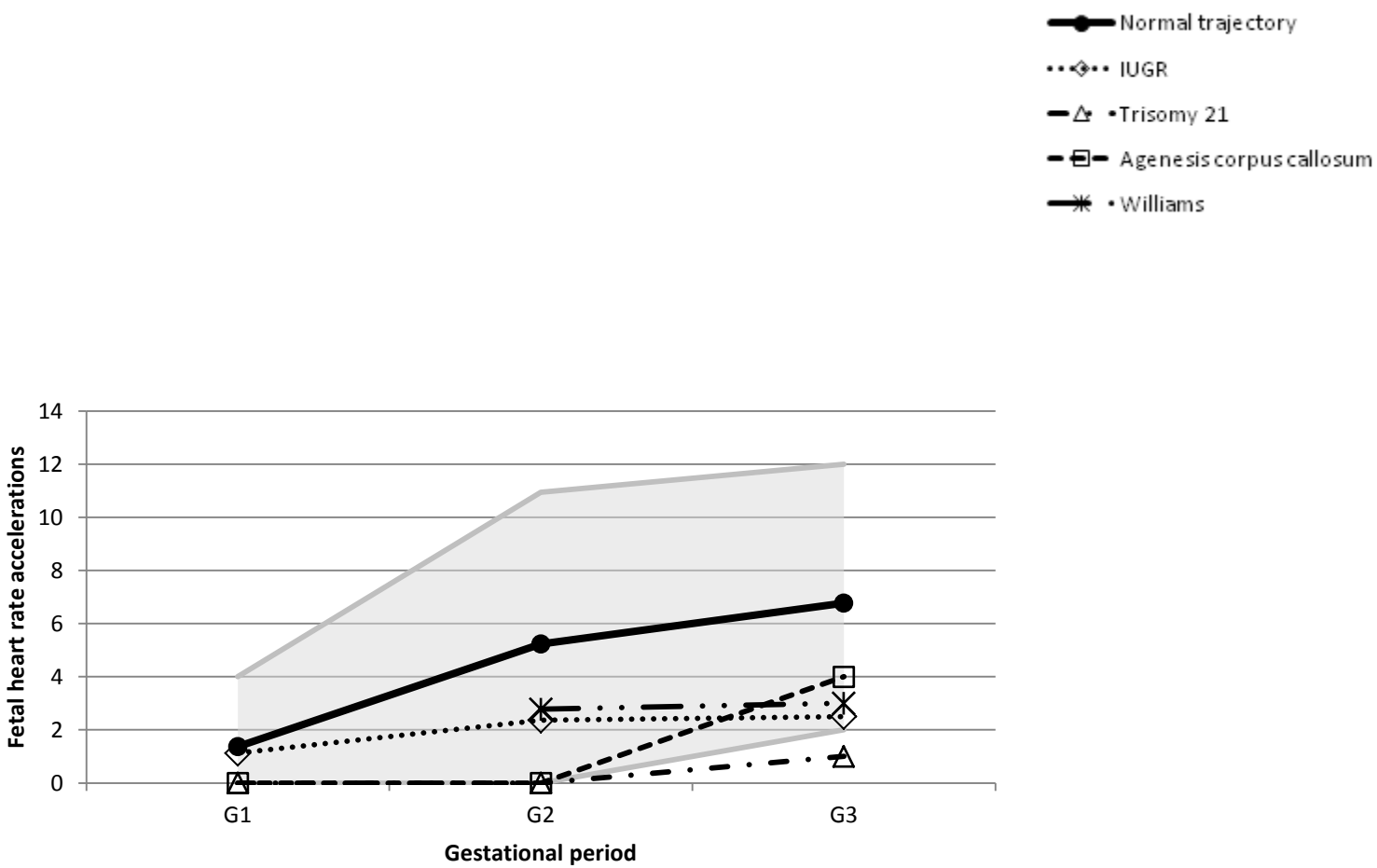
Figure 8.

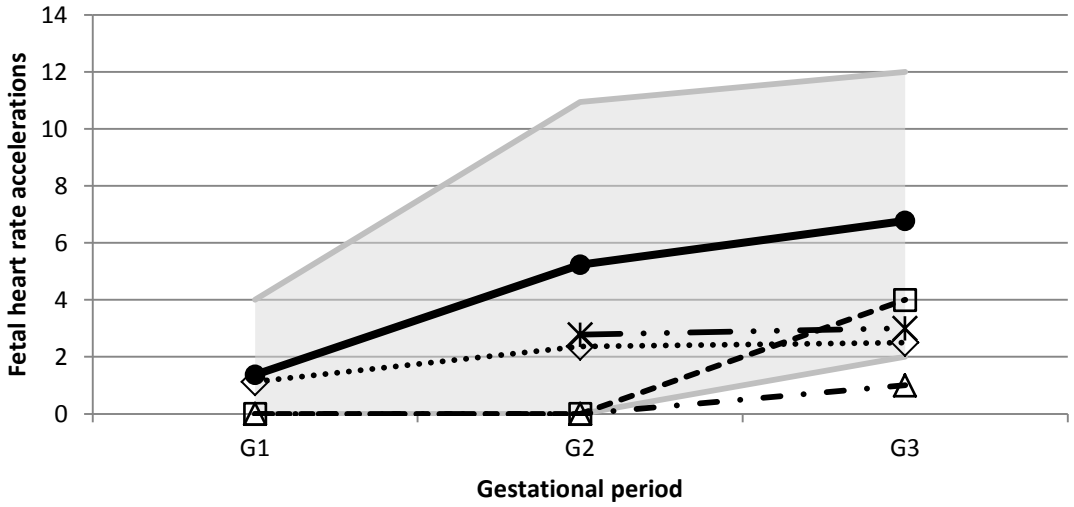
Figure 10.

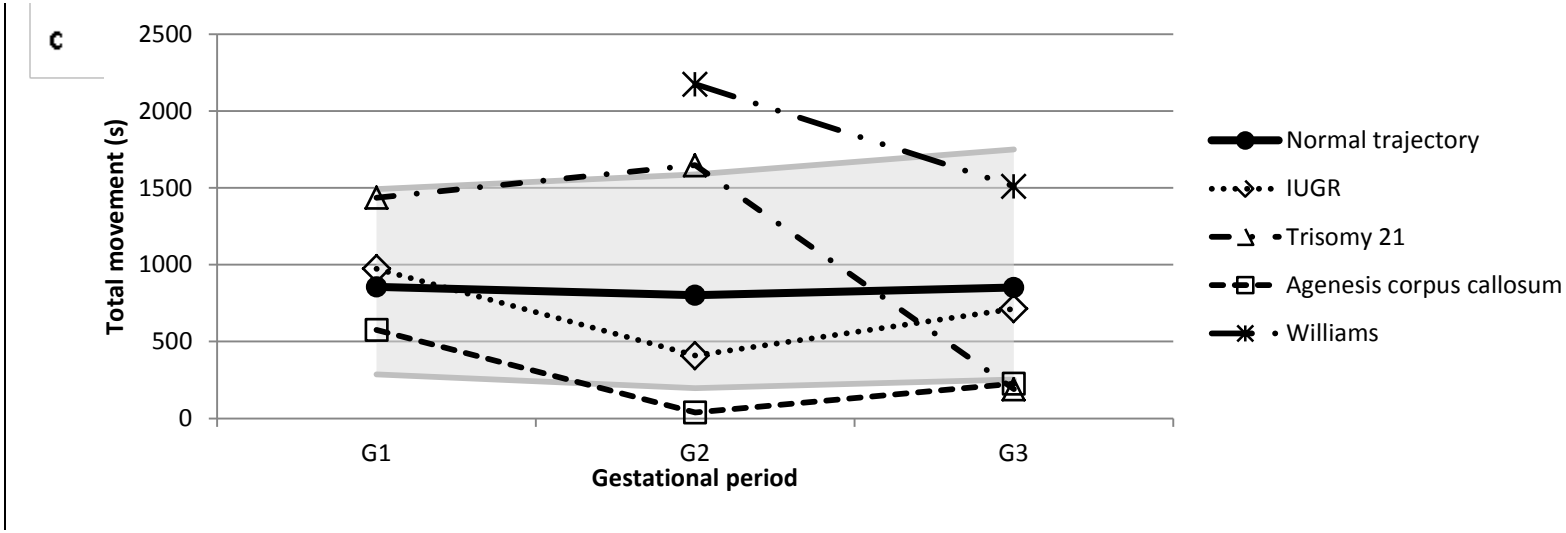

Low RSA/Low SCL
Low RSA/High SCL
High RSA/Low SCL
High RSA/High SCL
G3
Fetal heart rate variability
6.5
6
5.5
5
4.5
4
n = 148
n = 157
n = 145
n = 151
Maternal autonomic group

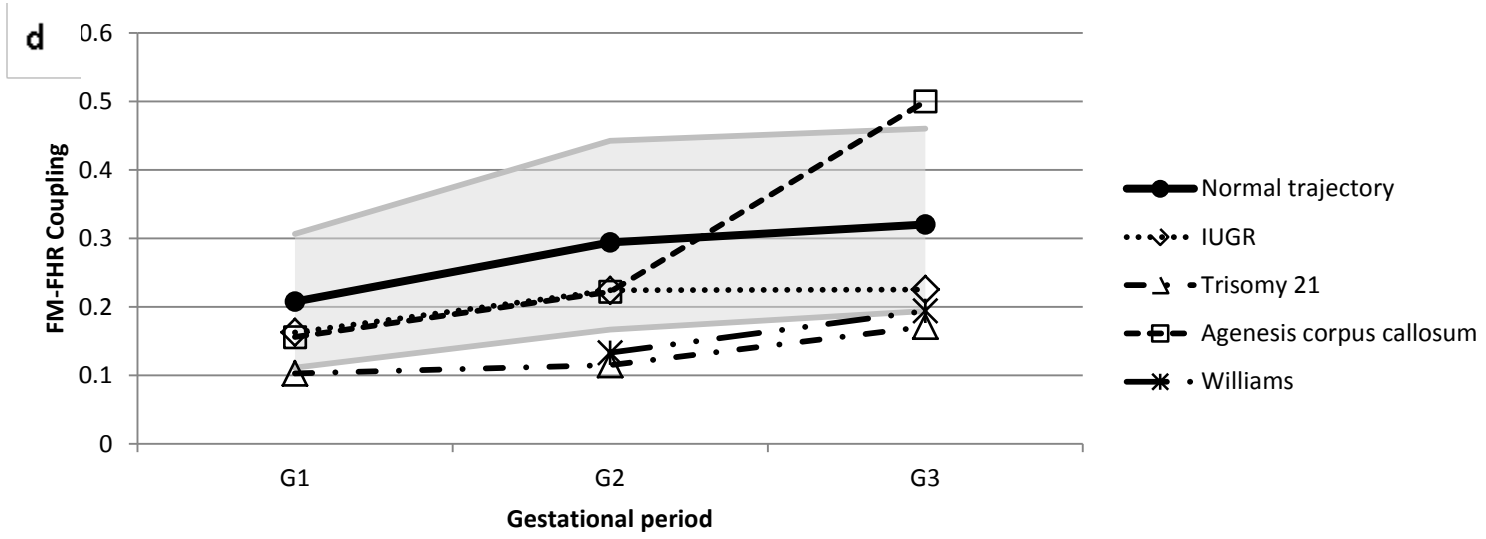

b
G3
Total movement duration (s)
1200
1000
800
600
400
200
0
n = 130
n = 131
n = 123
n = 130
Maternal autonomic group

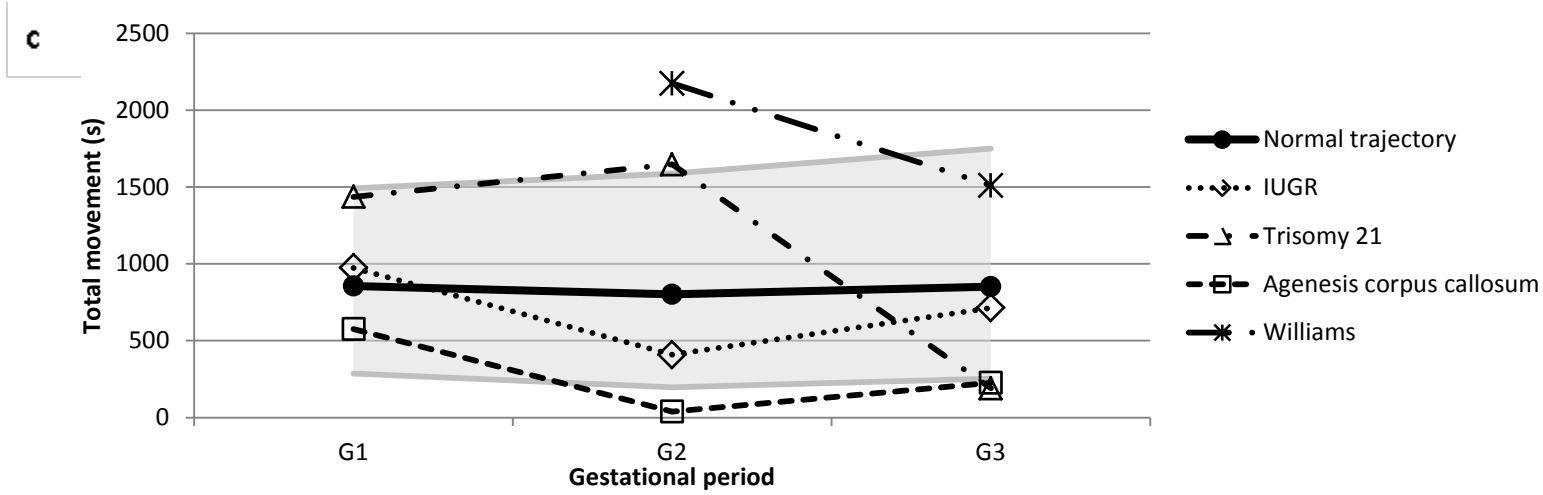

a
160
155
150
145
140
135
130
Fetal heart rate (bpm)
G1
G2
G3
Gestational period
Normal trajectory
IUGR
Trisomy 21
Agenesis corpus callosum
Williams
b
160
155
150
145
140
135
130
Fetal heart rate (bpm)
G1
G2
G3
Gestational period
Normal trajectory
IUGR
Trisomy 21
Agenesis corpus callosum
Williams

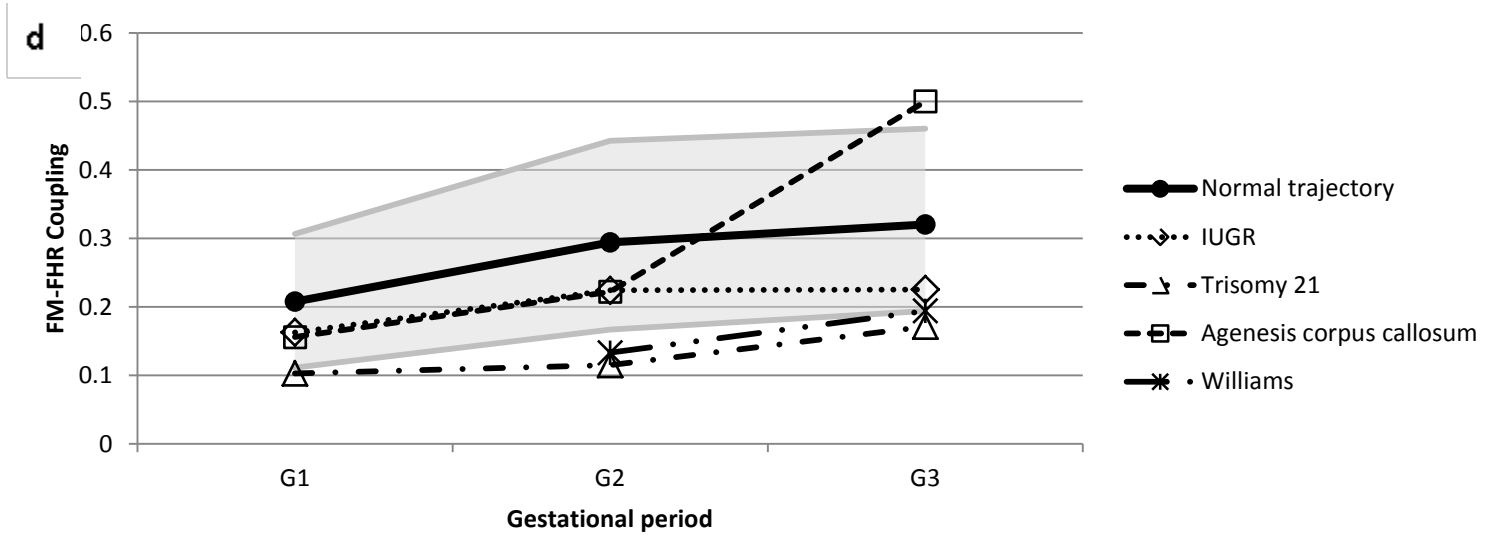

Normal trajectory
IUGR
Trisomy 21
Agenesis corpus callosum
Williams
Fetal heart rate accelerations
14
12
10
8
6
4
2
0
G1
G2
G3
Gestational period


14
12
10
8
6
4
2
0
Fetal heart rate accelerations
G1
G2
G3
Gestational period


c
2500
2000
1500
1000
500
0
Total movement (s)
G1
G2
G3
Gestational period
Normal trajectory
IUGR
Trisomy 21
Agenesis corpus callosum
Williams


d
FM-FHR Coupling
0.6
0.5
0.4
0.3
0.2
0.1
0
G1
G2
G3
Gestational period
Normal trajectory
IUGR
Trisomy 21
Agenesis corpus callosum
Williams


c
Total movement (s)
2500
2000
1500
1000
500
0
G1
G2
G3
Gestational period
Normal trajectory
IUGR
Trisomy 21
Agenesis corpus callosum
Williams


d
0.6
0.5
0.4
0.3
0.2
0.1
0
FM-FHR Coupling
G1
G2
G3
Gestational period
Normal trajectory
IUGR
Trisomy 21
Agenesis corpus callosum
Williams

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