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Developmental trajectories of parents and infants from conception to 3 months postpartum: The singularity of twins

Iva Alexandra Barbosa Tendais

UMinho | 2016

Universidade do Minho
Escola de Psicologia

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September, 2016

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Doctoral thesis in Applied Psychology

Supervised by
Professora Doutora Bárbara Figueiredo

September, 2016

STATEMENT OF INTEGRITY

I hereby declare having conducted my thesis with integrity. I confirm that I have not used plagiarism or any form of falsification of results in the process of the thesis elaboration.
I further declare that I have fully acknowledged the Code of Ethical Conduct of the University of Minho.

University of Minho, 30 de Setembro de 2016

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AGRADECIMENTOS/ ACKOWLEDGEMENTS

À Professora Doutora Bárbara Figueiredo pelo desafio que me lançou para realizar este projeto. Foi a descoberta de um mundo novo que nos levou até ao Novo Mundo. Pelo exemplo de perseverança e motivação para fazer sempre mais e melhor.

Ao Professor Doutor Rui Carrapato, Professor Doutor João Bernardes, Drª Fátima Menezes, Professora Doutora Hercília Guimarães, Professor Doutor Almeida Santos pelo apoio institucional.

Ao Professor Doutor Nuno Montenegro pelo apoio institucional e instrumental ao longo deste processo. A sua colaboração foi determinante na execução deste trabalho. Agradeço pelas oportunidades de enriquecimento profissional proporcionadas, especialmente a facilitação do contato com a equipa coordenada pelo Professor Doutor Gerard Visser com grande experiência de investigação do comportamento fetal.

À Drª Dulcina Ramada pelo desafio lançado à nossa equipa de investigação de fazer um estudo sobre os gémeos, por ter facilitado o acolhimento deste projeto no Hospital de S. Sebastião e no Hospital Pedro Hispano e por ter colaborado na implementação deste projeto.

Aos médicos que realizaram as ecografias - Professora Doutora Alexandra Matias, Drª. Eduarda Marques, Drª. Teresa Loureiro e Drª. Augusta Pinto. À Professora Doutora Carla Ramalho pelo apoio e colaboração. Ao Professor Doutor João Bernardes, Professor Doutor Diogo Ayres de Campos e ao Hernâni Gonçalves pela experiência de colaboração na análise e publicação dos dados da frequência cardíaca fetal. Ao Milton Severo pela ajuda na análise estatística do comportamento fetal. Às equipas de enfermagem do Hospital de S. Sebastião, Hospital de S. João e Hospital Pedro Hispano, especialmente à Enfª. Fernanda e à Enfª Cristina pela realização da cardiotocografia. Às administrativas e auxiliares dos serviços de Ginecologia e Obstetrícia dos 3 hospitais, especialmente à D. Ana Maria, Márcia, Carina e Anabela.

To Amy Salisbury for showing her lab at Brown University and for sharing her knowledge and expertise on fetal behavior analysis.

To Professor Gerard Visser for the opportunity to spend more than a month at the the University Medical Center at Utrecht for training and analysis of our fetal behavior data.

To Eduard Mulder for giving a positive new meaning to the expression "the Dutch way". Thank you for sharing your knowledge and for helping me analyze the fetal behavior of the twins. It was an honor to work with you.

To David Kenny (University of Connecticut) for sharing his knowledge and providing continuous support on dyadic data analysis.

Aos antigos e atuais membros da nossa equipa de investigação, em particular à Raquel Costa, Alexandra Pacheco, Mariana Bianchi de Aguiar, Catarina Samorinha e Tiago Pinto por terem contribuído direta (treino e administração do NBAS, avaliação das propriedades psicométricas dos instrumentos) ou indiretamente para este trabalho. O trabalho desta equipa é um exemplo de excelência e um motivo de grande orgulho.

Ao Eng. Pedro Ribeiro pelo apoio técnico e emocional nas alturas de maior stress com os equipamentos de ecografia e gravação de vídeo.

Às famílias que participaram neste projeto, pela generosidade da partilha da vossa vivência nesta fase. Foi um enorme privilégio. É a vocês que dedico este trabalho.

À Professora Doutora Paula Cristina Martins pelo apoio na longa reta final desta tese. A sua generosidade e visão positiva tornou o fardo mais leve. Ao Vítor pelo companheirismo, apoio e conselhos sensatos.

À Drª Ana Barbosa pela amizade e partilha de conhecimentos sobre o comportamento fetal. É um exemplo inspirador de rigor e dedicação.

À Catarina Canário pela partilha generosa dos seus conhecimentos avançados sobre análise diádica.

À Ana Conde pela amizade à prova de bala, pelo apoio emocional constante e pela partilha de reflexões sempre pertinentes.

À minha mãe por estar sempre presente, disponível para ajudar e pelo espírito combativo.

Ao Germano pelo apoio incondicional, todos os dias.

À Fundação para a Ciência e Tecnologia pelo financiamento deste projeto.

This work was supported by a grant (SFRH/BD/40146/2007) from the Foundation for Science and Technology (Portuguese Ministry of Education and Science), by the Operational Program Science and Innovation 2010 (POCI 2010) of the Community Support Board III, by FEDER Funds through the Operational Competitiveness Programme, COMPETE.

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DEVELOPMENTAL TRAJECTORIES OF PARENTS AND INFANTS FROM CONCEPTION TO 3 MONTHS POSTPARTUM: THE SINGULARITY OF TWINS

ABSTRACT

Background: Twin birth rates have increased worldwide in the last decades. Compared with the well-known health risks associated with twin pregnancy, relatively little is known about the psychological adjustment of couples during the transition to twin parenthood and twins' early development. **Aims**: 1) To describe the trajectories of parents' psychological adjustment during the transition to twin parenthood; 2) to determine whether parents of twins and parents of singletons differ on psychological adjustment trajectories; 3) to investigate whether psychological adjustment trajectories vary as a function of mode of conception and parent gender; 4) to examine twins' fetal development (fetal movements and heart rate) and to investigate factors associated with it. **Method:** This prospective longitudinal study assessed 41 parent-twi-n pairs conceived spontaneously (*n* = 25) and after infertility treatment (*n* = 16) at multiple time points from the first trimester of pregnancy up to 3 months postpartum. A control sample of parents of singletons was derived from a larger longitudinal study. Parents' psychological adjustment was assessed with self-report measures. Fetal movements were assessed from ultrasound recordings and fetal heart rate variability was assessed by computerized cardiotocography. **Results:** Using dyadic growth curve analyses, we found that parents of twins reported increasingly positive attitudes to pregnancy and the baby over time, a decrease in marital relationship and increasingly positive attitudes to sex from pregnancy to the postpartum period and over the postpartum period. Anxiety and depression trajectories varied as a function of type of pregnancy and mode of conception. While parents of singletons exhibited a significant decrease in anxiety levels during the postpartum period, parents of twins showed no significant decrease in anxiety during this period. Parents of twins conceived after infertility treatment showed no significant decrease in depression during pregnancy and a significant increase in anxiety from pregnancy to the postpartum period, whereas parents of twins conceived spontaneously showed a decline in depression during pregnancy and no significant increase in anxiety from pregnancy to the postpartum period. In addition, parents of twins conceived after infertility treatment showed a higher risk for clinically significant depression symptoms than parents of twins conceived spontaneously and parents of singletons conceived after infertility treatment during the postpartum period. No systematic gender differences were found in anxiety and depression trajectories over time. Dyadic growth curve analyses also demonstrated age-dependent changes in fetal general movements and breathing movements. While the developmental course of fetal movement patterns was found to be largely independent of a number of factors (co-twin, fetal sex, gestational age at delivery and birthweight), fetal sex differences were found in one fetal movement pattern at mid-pregnancy and heart rate parameters at late pregnancy. **Conclusion:** This study provides new data on the psychological adjustment to twin parenthood and on twins' fetal development.

TRAJETÓRIAS DESENVOLVIMENTAIS DE PAIS E BEBÉS DA CONCEPÇÃO AOS 3 MESES PÓS-PARTO: A SINGULARIDADE DOS GÉMEOS

RESUMO

Enquadramento: As taxas de nascimentos de gémeos têm aumentado em todo o mundo nas últimas décadas. Comparado com os bem conhecidos riscos para a saúde associados a uma gravidez gemelar, relativamente pouco se conhece sobre o ajustamento dos casais na transição para a parentalidade de gémeos e sobre o desenvolvimento precoce dos gémeos. **Objetivos**: 1) Descrever as trajetórias de ajustamento psicológico dos pais de gémeos durante a transição para a parentalidade; 2) determinar se existem diferenças entre pais de gémeos e de únicos nas trajetórias de ajustamento psicológico; 3) investigar se as trajetórias de ajustamento psicológico variam em função do modo de conceção e do género dos pais; 4) examinar o desenvolvimento fetal (movimentos fetais e frequência cardíaca) e investigar os seus fatores associados. **Método:** Este estudo longitudinal prospetivo avaliou 41 pares de pais-gémeos concebidos espontaneamente (n = 25) e após tratamento de infertilidade (n = 16) em múltiplos momentos desde o primeiro trimestre de gravidez até aos 3 meses pós-parto. Uma amostra de controlos composta por pais de únicos foi selecionada de um estudo longitudinal mais alargado. O ajustamento psicológico dos pais foi avaliado com medidas de auto-relato. Os movimentos fetais foram avaliados a partir de gravações de ecografias e a variabilidade da frequência cardíaca fetal por cardiotocografia computorizada. **Resultados:** Usando curvas de crescimento diádicas, verificou-se que os pais de gémeos reportaram ter atitudes progressivamente mais positivas sobre a gravidez e o bebé ao longo do tempo, uma diminuição na qualidade da relação conjugal e atitudes perante o sexo progressivamente mais positivas da gravidez para o pós-parto e durante o pós-parto. As trajetórias da ansiedade e da depressão variaram em função do tipo de gravidez e modo de conceção. Enquanto os pais de únicos exibiram uma diminuição significativa nos níveis de ansiedade durante o pós-parto, os pais de gémeos não mostraram uma diminuição significativa durante esse período. Os pais de gémeos concebidos após tratamento de infertilidade não apresentaram uma diminuição significativa da depressão durante a gravidez e um aumento significativo na ansiedade da gravidez para o pós-parto, enquanto que os pais de gémeos concebidos espontaneamente apresentaram uma diminuição da depressão durante a gravidez e um aumento não significativo da ansiedade da gravidez para o pós-parto. Além disso, os pais de gémeos concebidos após tratamento de infertilidade apresentaram um risco mais elevado de sintomatologia depressiva clinicamente significativa do que os pais de gémeos concebidos espontaneamente e pais de únicos concebidos após tratamento de infertilidade. Não foram encontradas diferenças de género nas trajetórias de ansiedade e depressão ao longo do tempo. Curvas de crescimento diádico também demonstraram alterações nos movimentos fetais globais e respiratórios em função da idade gestacional. Enquanto que o curso desenvolvimental dos movimentos fetais se mostrou independente de vários fatores (co-gémeo, sexo, idade gestacional ao nascimento e peso ao nascimento), foram encontradas diferenças em função do sexo num movimento fetal a meio da gravidez e na variabilidade da frequência cardíaca fetal no final da gravidez.

Conclusão: Este estudo apresenta novos dados sobre o ajustamento psicológico à parentalidade de gémeos e sobre o desenvolvimento fetal dos gémeos.

This thesis is based on the following papers:

I. Tendais, I., Visser, G. H., Figueiredo, B., Montenegro, N., & Mulder, E. J. (2013). Fetal behavior and heart rate in twin pregnancy: A review. *Twin Research and Human Genetics, 16*, 619–628. doi:10.1017/thg.2012.149

II. Tendais, I., Figueiredo, B., Canário, C., Kenny, D. A. (2016). *Couples' psychological adjustment to twin parenthood: Mode of conception and gender differences*. Manuscript under review

III. Tendais, I., & Figueiredo, B. (2016). Parents anxiety and depression symptoms after successful infertility treatment and spontaneous conception: does singleton/ twin pregnancy matter? *Human Reproduction*. *31*(10), 2303-2312. doi: 10.1093/humrep/dew212

IV. Tendais, I., & Figueiredo, B., Mulder, E.J.H., Ramada, D., & Montenegro, N. (2016). *Fetal general movements and breathing movements' developmental trajectories in twins and associated factors*. Manuscript submitted for publication.

V. Tendais, I., Figueiredo, B., Gonçalves, H., Bernardes, J., Ayres-de-Campos, D., & Montenegro, N. (2015). Sex differences in the fetal heart rate variability indices of twins. *Journal of Perinatal Medicine*, *43*(2), 221-225. doi:10.1515/jpm-2014-0031

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ABBREVIATIONS

ART	Assisted Reproductive Technology
CNS	Central nervous system
EPDS	Edinburgh Postnatal Depression Scale
FHR	Fetal Heart Rate
IBQ-R	Infant Behavior Questionnaire-Revised
ICSI	Intracytoplasmic Sperm Injection
IT	Infertility treatment
IVF	In Vitro Fertilization
SC	Spontaneous Conception
NBAS	Neonatal Behavioral Assessment Scale
STAI	State Trait Anxiety Inventory

INTRODUCTION

The transition to parenthood is an important part of the life cycle for both parents and children. During this period, parents and children experience major developmental changes.

For parents, this stage of the life cycle has been portrayed as a developmental transition, a long term process that results "in a qualitative reorganization of both inner life and external behavior, … involving a qualitative shift from the inside looking out (how the individual understands and feels about the self and the world) and from the outside looking in (reorganization of the individual's or family's level of personal competence, role, and relationships with significant others)" (Cowan, 1991, p. 5). From a developmental point of view, the transition to parenthood involves an experience of crisis that is associated with facing new and difficult developmental tasks (Erikson, 1950, 1959). This normative family developmental transititions is often stressfull (Cowan & Cowan, 2000). Although it is inevitable and temporarily incapacitating, this crisis is pointed as necessary for normal developmental growth. However, this transition, like any other developmental transition, can contribute to alterations in the course of mental health and psychopathology (Schulenberg, Sameroff, & Cicchetti, 2004). While the birth of a child is considered a normative family developmental transitition, the birth of twins might be viewed as a nonnormative stressor (unexpected) and, therefore, can be more disruptive (Walsh & McGoldrick, 2013). Consistent with this view, the birth of twins usually requires significant family reorganization to deal with an overload of caregiving tasks (Beck, 2002) and difficulties in providing each child warmth and reciprocity (Feldman, Eidelman, & Rotenberg, 2004).

The developmental tasks associated with the transition to parenthood as proposed by Colman and Colman (1991) have been recently reviewed and expanded by Figueiredo and Lamela (2014). According to these authors, the transition to parenthood encompasses a series of developmental tasks, including 1) to accept the pregnancy, 2) to accept the reality of the fetus, 3) to initiate the relationship with the baby (emotional and representational), 4) to reassess the identity and to add a new identity as parent, 5) to reevaluate and establish a new relationship with the extended family, 6) to reevaluate and establish a new marital relationship, 7) to build the coparental system (to establish an emotional and behavior alliance with the other parent, to share experiences and decisions in accordance with child's best interest and to respond in an articulated way to the child's needs), 8) to reevaluate the relationship with the other children, 9) to accept the baby as a separate person and 10) to operationalize parenthood (provide the necessary care to infant physical and psychological development). These are argued to be sequential and mutually influencing tasks.

In our view, developmental psychopathology provides an adequate framework for this work given that it is a broad, integrative perspective of understanding individual development and functioning (Cicchetti, 2006). The defining feature of developmental psychopathology is the study of the continuities and discontinuities between normality and psychopathology. Therefore, it has a dual focus on normal and abnormal, adaptative and unadaptative, developmental processes (Cicchetti & Toth, 2009; Toth & Cicchetti, 2013). This perspective emphasizes that normative development provides a crucial and necessary comparison for determining whether behavioral and emotional outcomes are atypical or problematic (Drabick & Kendall, 2010). Likewise, researchers of fetal behavior have been able to detect variations in fetal central nervous system functioning at different stages of prenatal development due to previous knowledge on normal behavioral development (Mulder & Visser, 2016).

In addition, developmental psychopathology has been pointed out as especially suited for investigating developmental transitions (Rutter, 1990; Schulenberg et al., 2004). While adaptation and development occur throughout the life course, during transitions and turning points the individual developmental trajectory might change (Rutter, 1996). Thus, these transitions provide an opportunity for studying developmental continuities and discontinuities, as well as the diversity of trajectories. Research conducted within a developmental psychopathology framework seeks to understand causal processes operating and interacting at multiple levels (biological, psychological and social context) and appreciates the role of developmental mechanisms involved (Cicchetti & Toth, 1998; Rutter & Sroufe, 2000; Sameroff, 2000). Next, we present several developmental psychopathology conceptual issues and principles that we believe are particularly relevant to this study.

Risk and protective factors

Risk factors refer to "conditions or variables associated with a lower likelihood of positive outcomes and a higher likelihood of negative or socially undesirable outcomes in a variety of life areas" (Jessor, Turbin & Costa, 1998, p. 195). In contrast, protective factors increase the likelihood of positive outcomes and decrease the likelihood of negative consequences from exposure to risk (Jessor, Turbin & Costa, 1998).

Research has shown that multiple risk and protective factors (genetic, biochemical, physiological, cognitive, affective, experiential, intrafamilial, socioeconomic, social and cultural) are associated with maladaptation or psychopathological outcomes. For adults and, specifically for parents, lack of partner or of social support, unplanned or unwanted pregnancy, adverse events in life and high perceived stress, present/past pregnancy complications, traumatic (violence and sexual abuse in childhood), personal (substance use disorder, bipolar disorder and history of suicide attempt) and family history of mental disorder, as well as the birth of a very low birth weight infant have been identified as risk factors for mental disorders (e.g., Biaggi, Conroy, Pawlby, & Pariante, 2016; Helle et al., 2016; Tebeka, Le Strat, & Dubertret, 2016). For children, family risk factors include parental age (McGrath, Petersen, Agerbo, Mors, Mortensen, & Pedersen, 2014), low socioeconomic status (e.g., Sameroff, Seifer, Zax, & Barocas, 1987), parental mental illness (e.g., Reck, Nonnenmacher, & Zietlow, in press), child maltreatment (Norman, Byambaa, De, Butchart, Scott, & Vos, 2012), harsh parenting (e.g., Wiggins, Mitchell, Hyde, & Monk, 2015), parental conflict (Yap & Jorm, 2015), unsupportive family relations (Repetti, Taylor, & Seeman, 2002), and parental substance use (Hill, Tessner, & McDermott, 2011). Although, previously mentioned risk factors have an independent contribution for maladaptative or psychopathological outcomes, they often co-occur and interact (Rutter, 1990; Sameroff, Seifer, Barocas, Zax, & Greenspan, 1987). Risk factors may opperate additively (Sameroff et al., 1987) or synergistically, such that the co-occurrence of two or more factors generates a greater deleterious effect than the sum of the factors considered in isolation (Evans, Li, & Whipple, 2013; Rutter, Tizard, & Whitmore, 1970). It is essential to realize that risk factors for parental mental disorders, such as high stress and poor social support are also risk factors for poor child development, suggesting that the relation between parental mental disorders and impaired early child development is multilevel and cumulative (Walker et al., 2011).

As for protective factors, they may 1) promote development (e.g., early learning and caregiver–child interaction facilitate early development; Walker et al., 2011), 2) counterbalance the impact of risks factors (e.g., secure mother-infant attachment reduce the impact of paternal alcoholism on toddler behavior problems; Edwards, Eiden, & Leonard, 2006) or 3) interact with them, reducing the probability of maladaptative or psychopatological outcomes mainly for specific groups (e.g., marital satisfaction, parenting self-efficacy, community engagement, higher social support, and daily parent-child interaction reduced the risk of developmental delay for children in 'at-risk' environment[1]; McDonald, Kehler, Bayrampour, Fraser-Lee, & Tough, 2016).

According to an ecological-transactional development model, human functioning and development over time is determined by bi-directional, reciprocal patterns of effect among the multiple levels of the ecology (Cicchetti, 2006). Not only the quality of the mutual influence within the individual and between the individual and external factors shape the individual development and adaptation over time, but also the dynamic balance between risk and protective factors within and between levels of the ecology (Cicchetti, 2006). A negative or positive balance between risk and protective factors over time lead to opposite levels of competence in the individual that, in turn, may result in different levels of vulnerability to mental disorders (Cicchetti, 2006).

In line with this view, chronic and recurrent stresses heighten risks for maladaptation (Walsh, 2016). Cumulative stress derived from a pileup of multiple stressors (internal and external) can drain resources (Walsh, 2016) and overwhelm individuals and families at some point, heightening the risk for subsequent problems (Patterson, 2002; Sameroff, 2000). According to McCubbin and Patterson (1983) conceptualizion, five types of stressors and strains (i.e., demands) contribute to a pile-up in the family system "(a) the initial stressor and its hardships, (b) normative transitions, (c) prior strains, (d) the consequences of family efforts to cope; and (e) ambiguity, both

[1] "At risk environment" was defined as having having a mother with poor mental health (presence of at least two of the following: a history of abuse, a history of a mental health disorder, depression during pregnancy, or anxiety during pregnancy) or as having socio-demographic risk (at least of the following: mother <25 years of age at delivery, low education, low family income, single marital status, new Canadian/lived in Canada for <five years, or experienced food insecurity).

intra-family and social" (p. 11). For instance, a couple, with a history of infertility, expecting a child conceived with assisted reproductive techniques might experience at least three of the previosuly mentioned types of stressors given that difficulties in conceiving a child tend to be experienced as stressful (Cousineau & Domar, 2007). Other possible stressors might include a twin or higher-order multiple pregnancy instead of a singleton pregnancy, primiparity, etc. Mederer and Hill (1983) argued that a stress score might be constructed to reflect the pile-up of life events occuring over a limited period. Even though past experiences of adversity and coping can either generate negative expectations, they may also serve as models of resilience (Walsh, 2016). As Rutter (2012) pointed out, a negative experience may have either a sensitizing effect (increase vulnerability) or a strengthening effect (increased resistance) to later stress or adversity (**resilience**). Previously infertile couples who became parents after assisted conception may develop a higher resistence to the normative stress associated with the transition to parenthood, namely by a strengthened couple relationship (marital benefit). Research has shown that almost one-third of fertility patients report high marital benefit (Peterson, Pirritano, Block, & Schmidt, 2011). In addition, the positive or negative adaptation to the transition to parenthood may also depend on whether prior stressors (e.g., infertility, use of assisted reproduction techniques) were adequately mastered (Hill, 1973; cit Bodenmann, 1995), but also to the meanings attributed to experiences (Cicchetti & Rogosch, 1996). An important contribution of the developmental approach is on the recognition of the critical role of timing for the organization of behavior (Ciccetti & Toth, 2009). Challenging situations may have a different impact depending on individual and family life cycle passage. For example, expecting twins may have a different impact on first- or second-time parents.

In sum, a comprehensive understanding of risk and protective factors demands an integration of multiple levels and analysis of individual and contextual factors (Cicchetti & Dawson, 2002). Maladaptation and psychopathology are considered to be the result of the transaction between individual characteristics, adaptational history, and the present context (Boyce et al., 1998; Cicchetti, 2002).

Contextual Influences

For couples, a number of contextual factors influence fertility and the psychological well-being of parents, including parity, method of conception (spontaneous vs. assisted conception); single parenthood vs. two-parent family; singleton, twin or higher-order parenthood; couple relationship; marital instability; division of household labor; social support; (predictable) family income; access and duration of paid parental leave; child allowances; access to childcare services; employment status; flexible working hours; number of work hours and shifts; job insecurity; welfare regime (Balbo, Billari, & Mills, 2013; Barnet, Gareis, & Brennan, 2008; Figueiredo & Conde, 2011; Margolis & Myrskylä, 2011; Stavrova & Fetchenhauer, 2014; Strazdins et al., 2008). At the couple level, the psychological well-being of both members has been found to be related (Sels, Ceulemans, Bulteel, & Kuppens, 2016), namely during the transition to parenthood (Anding, Röhrle, Grieshop, Schücking, & Christiansen, 2016). In these close, intimate relationships partners have a mutual influence on each other's emotions, cognition and behavior over time (Agnew, Van Lange, Rusbult, & Langston, 1998; Anding et al., 2016; Sels et al., 2016; Zvara, Mills-Koonce, Heilbron, Clincy, & Cox, 2015). Similarity within couples may be primarily attributed to *assortative mating*, whereby people tend to select for partners individuals with similar characteristics (Kenny, 1996). According to Kenny (1996), interdependence within dyads (e.g., couples, twins) may also arise due to three different processes: partner effects, common fate and mutual influence. Partner effects refer to the influence of one person's characteristics or behavior on the other person. For instance, a recent study has shown that husband's depression has a negative influence on wife's relationship satisfaction (Li & Johnson, 2016). Mutual influence occurs when both partners' outcomes directly influence each other. Husband's and wife's marital satisfaction are an example of mutual influence. Common fate occurs when both partners are exposed to the same causal factor. The birth of a child and infertility are examples of these dyad-level stressors, because they affect both partners' lives.

For children, research has shown that external factors, such as maternal variables (e.g. age, diet, physical activity, stress), diseases, exposure to drugs and to environmental toxins, influence the course and character of their prenatal development (Bornstein, Arterberry, & Lamb, 2014; Doyle et al., 2015). Even though the placenta protects the fetus from many harmful factors, some cross the placental barrier and threat the integrity of the fetus (Rassmussen, 2012). While some of these effects are short-term, as demonstrated by research on the effects of prenatal maternal distress (including depression, anxiety and stress) on fetal behavior (DiPietro, Costigan, & Gurewitsch, 2003; Van den Bergh, Mulder, Visser, Poelmann-Weesjes, Bekedam, & Prechtl, 1989), others are long-term sleeper effects, as demonstrated by the link between prenatal maternal distress and offspring cognitive deficits and emotional problems in childhood and adolescence (Barker, Jaffee, Uher, & Maughan, 2011) and schizophrenia in adulthood (Khashan et al., 2008). Research from allied fields of developmental science have shown how early experience (e.g., prenatal maternal stress) influence developing biological systems, including stress neurobiology (e.g., Essex et al., 2011), imune system functioning (e.g. Miller, Chen, & Parker, 2011) and gene expression (e.g. Oberlander et al., 2008). The multilevel, long-term consequences of prenatal adversity to adult health and well-being (fetal programming) highlight the sensitivity of prenatal development to environmental exposures (Thompson, 2015).

According to the *predictive adaptative response model*, some of these changes can be viewed as an adaptation to an adverse environment. Even during pregnancy, the fetus is able to detect the characteristics of the environment and to adapt in a way that promotes survival (Gluckman & Hanson, 2004; Unternaehrer et al., 2016). According to this view, there is an increased risk for the emergence of biological or behavioral problems if the postnatal environmental conditions change. The results of Sandman, Davis and Glynn (2012) are consistent with this model as they have found that children exposed to maternal depression both pre- and postnatally showed similar developmental outcome as those exposed at neither time, and improved outcomes to those exposed at either time. This model contrasts with the more dominant deviance or deficit model of psychopathology (O'Connor, Monk, & Fitelson, 2014). However, the long-term consequences of this adaptation remain unknown. These findings are in line with the developmental psychopathology perspective that recognizes that "adaptative and maladaptative may take on different definitions depending on whether one's time referent is immediate circumstances or long-term development" (Ciccetti, 2006, p. 3).

As previously shown, the link between prenatal maternal distress and child development is complex, depending on instrument, severity, duration, timing of exposure and infant gender (DiPietro, 2012). In fact, some studies even found that maternal distress is associated with positive outcomes (DiPietro et al., 2010; DiPietro, Novak, Costigan, Atella, & Reusing, 2006). Maternal pregnancy-specific stress was associated with higher neurologic maturation in neonates (DiPietro et al., 2010). Moderate maternal distress at mid-pregnancy (DiPietro et al., 2006) was also positively associated with cognitive development at 24 months. Research on this topic has been pointed out as a good example of translational research that advocates a multidisciplinary clinical research and integration (O'Connor et al., 2014).

Even though some of these results rise concerns about the intergenerational transmission of risk, two important notes should be made. Although children of the most prenatally anxious and depressed women are at a double risk (12%) of probable mental disorder at age 13 years, most children are not affected (Glover, 2015). In addition, the effects of early adversity on later mental health and psychopathology are likely mediated and sometimes reversed by later experiences (Schulenberg et al., 2004; Thompson, 2015)

Developmental pathways

Longitudinal studies from childhooh to adult life (see Rutter, 1989 for a review), with different at risk populations for developing a mental disorder (e.g., offspring of mothers with a schizophrenic disorder) and normative groups have shown a significant heterogeneity in origins, processes and outcomes of developmental pathways (Cicchetti, 2006). These findings are illustrative of the principles of equifinality and multifinality in the developmental course. The equifinality principle posits that "the same end state may be reached from a variety of different initial conditions and through different processes" (Cicchetti, 2006, p. 13). Children who develop a specific disorder may have had quite different contributing factors as demonstrated in the studies of Rutter (1989) and Rutter and Quinton (1984). The principle of multifinality (Wilden, 1980) "states that the effect on functioning of any component's value may vary in different systems. Actual effects will depend on the conditions set by the values of additional componentes with which it is structurally linked." (Cicchetti, 1990, pp. 18-19). As illustrated in the study of Quinton and Rutter (1988), some risk pathways of institution-reared children changed into more adaptative routes after specific events, such changing school and having more school positive experiences.

PSYCHOLOGICAL ADJUSTMENT TO TWIN PARENTHOOD

Twin birth rates have increased worldwide in the last decades, largely due to the increased use of assisted reproductive technology (ART), fertility enhancing treatments (e.g., ovulation induction agents) and to the increase in maternal age (Abramowicz, 2016; Pinborg, 2005). Recent statistics indicate that twins represent 20% of deliveries resulting from ART in Europe, namely in Portugal (Dyer et al., 2016). Based on a diverse range of studies, ART contributes between 2% and 24% to twin pregnancies (Blondel & Kaminski, 2002; Dickey, 2007).

Compared with the well-known maternal and child health risks associated with twin pregnancy, relatively little is known about the psychological adjustment of couples during the transition to twin parenthood. Parents of twins have to deal repeatedly with several stressful situations. Compared with singletons, twins have a 3-7 times higher rate of perinatal mortality and morbidity due to a higher incidence of antepartum complications, prematurity and low birth weight (Blondel et al., 2002). As a result, twins also show poorer neurodevelopmental outcomes than singletons (Lorenz, 2012).

To meet the needs of two or more children, parents may experience financial, childcare, physical and psychological issues (Chang, 1990; Choi, Bishai, & Minkovitz, 2009; Ellison & Hall, 2003; Klock, 2004; Robin, Josse, & Tourrette, 1991; Ross, McQueen, Vigod, & Dennis, 2011; Vilska & Unkila-Kallio, 2010). Inadequate sleep and fatigue are one of the major problems experienced by mothers of twins with a prevalence ranging from 49% (Chang, 1990) to 64% (Neifert & Thorpe, 1990). Other major difficulties include accomplishing daily household tasks (Neifert & Thorpe, 1990), lack of time to care of other children, disturbance in the marital relationship, financial strain and inadequate social support from relatives (Chang, 1990). However, most of the difficulties experienced in the first months appear to decrease over time (Neifert & Thorpe, 1990; Chang, 1990). For instance, mothers reporting inadequate sleep decreased from 66% at 3 months to 18% at 30 months (Chang, 1990). Similarly, mothers reporting lack of time to care of other children declined from 39% to 25% from 3 to 30 months postpartum (Chang, 1990). In a large study comprising 375 mothers of twins, 43% reported felling anxious and 30% depressed in the first 3 months postpartum (Hay & O'Brien, 1984). Negative maternal mood has found to be related with low parenting satisfaction, while positive mood was associated with low parenting distress and high parenting self-efficacy in a sample of 162 mothers of twins aged 2 to 23 months (Damato, Anthony, & Maloni, 2009). Social support appears to be associated with maternal stress (Baor & Soskolne, 2010, 2012; Lutz et al., 2012), but not with maternal mood (Damato et al., 2009). Overall, there is some evidence that the first 3 months after delivery seem to be a particularly vulnerable period for mothers due to the overload of caregiving tasks and different sleeping and feeding patterns of the twins (Beck, 2002).

When compared to mothers of singletons, mothers of twins display similar (Vilska *et al.*, 2009) or higher levels of anxiety (Jahangiri *et al.*, 2011) and similar levels of depression during pregnancy (Vilska *et al.*, 2009). As for fathers, similar levels of anxiety and depression between those expecting singletons and twins have been noted (Vilska *et al.*, 2009). After birth, evidence from studies with large samples suggests that mothers of young twins (≤ 5 years old) show increased levels of anxiety (Vilska *et al.*, 2009) and depression (Ellison *et al.*, 2005; Olivennes *et al.*, 2005; Thorpe *et al.*, 1991; Vilska *et al.*, 2009) than mothers of singletons. One of these studies showed that mothers of twins were significantly more likely to have clinically significant depression symptoms than mothers of singletons 5 years after childbirth (34.4% and 23.9%, respectively; Thorpe et al., 1991). During the postpartum period, fathers of twins showed similar levels of anxiety and higher levels of depression than parents of singletons (Vilska *et al.*, 2009).

Given the association between twin pregnancy and IT and the observation that infertility-related distress may persist after successful IT (Hjelmstedt, Widström, Wramsby, & Collins, 2004), some concerns about the potential cumulative negative effect of twin parenthood after IT have been raised (Baor, Bar-David, & Blickstein, 2004; Klock, 2004). In addition, IT parents of twins may have to deal with more stressful situations than SC parents of twins, especially in the first years of life. A recent meta-analysis has shown that IT pregnancies have a significantly higher risk of preterm and very preterm birth, low birth weight, and congenital malformations (Qin, Wang, Sheng, Xie, & Gao, 2016). Furthermore, IT twins are more likely to be admitted to a neonatal intensive care unit (NICU) and to be hospitalised in their first three years of life than spontaneously conceived (SC) twins suggesting that the former may have a frailer health status (Hansen et al., 2009). The existing evidence is, however, mixed. Some studies suggest that IT parents of twins have similar or even fewer psychopathological symptoms than SC parents of twins (Munro, Ironside, & Smith, 1990; Vilska et al., 2009). One of the few longitudinal studies that examined the impact of mode of conception on the psychological adjustment to twin parenthood found that IT mothers exhibited fewer depression symptoms at mid-pregnancy and similar levels of depression and anxiety symptoms to SC mothers at 3 months and 1 year postpartum; however, no differences were found between IT and SC fathers of twins at pregnancy or during the postpartum period (Vilska et al., 2009). However, other studies revealed that mothers of IT twins reported more psychopathological symptoms and poorer coping resources than mothers of SC twins (Baor et al., 2004; Baor & Soskolne, 2010; Colpin, Munter, Nys, & Vandemeulebroecke, 1999). This contrasting pattern of results might be explained, at least in part, by parity. While for most parents of twins no negative impact of IT on the psychological well-being was found (Munro et al., 1990; Vilska et al., 2009), more negative outcomes have been noted among the subgroup of first-time mothers (Baor et al., 2004; Baor & Soskolne, 2010; Colpin et al., 1999).

With regard to the marital relationship, the few studies that have compared IT and SC parents of twins have found lower self-reported marital quality in IT mothers at late pregnancy compared with mothers of SC twins (Baor & Soskolne, 2010), but similar marital relationship quality 9 months (Baor et al., 2004) and 1 year postpartum (Colpin et al., 1999).

Differences in pregnancy- and child-related attitudes between IT and SC parents of twins are largely unknown. Research with singletons suggests that IT mothers seem to have more idealized attitudes to pregnancy than SC mothers (McMahon, Tennant, Ungerer, & Saunders, 1999). It is unclear whether the prenatal maternal expectations of IT mothers of twins are unrealistic as well (Baor & Soskolne, 2010).

There is also a very limited body of research on gender differences in psychological adjustment to twin parenthood among IT and SC parents. Munro et al. (1990) found no gender differences in psychiatric morbidity in a sample of parents of twins conceived spontaneously, by hormonal treatment, or IVF. In contrast, mothers of twins showed lower psychological well-being than fathers, regardless of mode of conception (Baor et al., 2004).

So far, most of the available evidence on the psychological adjustment to twin parenthood comes from cross-sectional studies, carried out during the postpartum period, with mothers only. The only study that overcome most of the methodological limitations of previous studies (longitudinal design, carried out during pregnancy and the postpartum period, included both mothers and fathers, studied other variables than psychopathological symptoms), no over time analyses were described and data analysis was made separately for mothers and fathers. In the present study, all these factors were taken into account. Specifically, we tested the independent and combined effect of mode of conception and parent gender on the psychological adjustment (psychopathological symptoms, adjustment and attitudes) of both parents over pregnancy (13, 21 and 30 weeks) and the postpartum period (1, 4 and 8 weeks postpartum) using longitudinal dyadic analysis.

PAPER I

FETAL BEHAVIOR AND HEART RATE IN TWIN PREGNANCY

Tendais, I., Visser, G. H., Figueiredo, B., Montenegro, N., & Mulder, E. J. (2013). Fetal behavior and heart rate in twin pregnancy: A review. *Twin Research and Human Genetics, 16*, 619–628. doi:10.1017/thg.2012.149

Twin Research and Human Genetics
Volume 16 ▮ Number 2 ▮ pp. 619–628 ▮ © The Authors 2013 ▮ doi:10.1017/thg.2012.149

Fetal Behavior and Heart Rate in Twin Pregnancy: A Review

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Fetal movements and fetal heart rate (FHR) are well-established markers of fetal well-being and maturation of the fetal central nervous system. The purpose of this paper is to review and discuss the available knowledge on fetal movements and heart rate patterns in twin pregnancies. There is some evidence for an association or similarity in fetal movement incidences or FHR patterns between both members of twin pairs. However, the temporal occurrence of these patterns seems to be for the most part asynchronous, especially when stricter criteria are used to define synchrony. The available data suggest that fetal behavior is largely independent of sex combination, fetal position, and presentation. Conversely, chorionicity appears to have some influence on fetal behavior, mainly before 30 weeks of gestation. There is preliminary evidence for the continuity of inter-individual differences in fetal activity and FHR patterns over pregnancy. Comparisons between studies are limited by large methodological differences and absence of uniform concepts and definitions. Future studies with high methodological quality are needed to provide a more comprehensive knowledge of normal fetal behavior in twin pregnancy.

■ **Keywords:** twin pregnancy, fetal movement, fetal heart rate, fetal behavior

Twins have been the subject of great interest since ancient times. However, it was not until the second half of the 19th century that twins ceased to be regarded as an interesting phenomenon (natural wonder) and an obstetrical challenge. This attitude toward twins changed after the appearance in of Galton's study in 1876: 'The history of twins as a criterion of the relative powers of nature and nurture'. Galton was not only the first to postulate the existence of two types of twins (zygosity) but also laid the foundation of the (behavioral) genetic research carried out until now.

Because monozygotic twins are genetically identical, every difference between them is believed to be due to the environment (nurture). On the other hand, dizygotic twins share a similar genetic background as brothers and sisters, but like monozygotic twins they are of the same age, share the same uterus at the same time, and to a certain extent develop under similar circumstances. By quantifying similarities and differences in various outcomes between monozygotic and dizygotic twins, one may start to determine the relative contributions of heredity (nature) and environmental factors (nurture) during human development (Plomin & Asbury, 2005). Thus, prenatal observation of fetal movements simultaneously performed in both halves of monozygotic or dizygotic twins could be of great importance for behavioral genetics research, developmental neurobiology, and for the evaluation of the condition of fetuses, especially with respect to interfetal growth discordances and in cases of twin–twin transfusion syndrome (TTTS).

Besides genetic and environmental main effects, several forms of gene–environment interplay have been identified. Overall, they indicate that the influence of genes and environments on human development is not completely independent. The findings on epigenetic effects, for example, demonstrate that gene expression is influenced by environmental factors. Environmental risk exposure has also been found to be influenced by genetic factors (gene–environment correlations; Rutter et al., 2006). During ontogeny, there is a continuous interaction between genetic and environmental factors, which basically starts at

RECEIVED 22 March 2012; ACCEPTED 31 August 2012. First published online 14 January 2013.

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the zygotic stage. The study of prenatal behavior in twin pregnancies starting in early pregnancy may give insight into the causation of fetal behavior and factors that possibly play a role, such as maternal factors (hormones, heart rate and bowel sounds, stress), blood flow to the uterus, fetal position, and inter-twin contacts (physical or humoral, e.g., via a third circulation).

Today, the scientific study of twins represents a preferred method in behavioral genetics, developmental studies, matched-pair experimental designs and epidemiology. At the same time, important applications are found in an increasing number of areas, particularly in psychology and psychiatry, medical genetics, reproductive biology and obstetrics, and neonatology. For instance, advances in ultrasound monitoring have facilitated a shift of developmental psychology frontiers from the neonatal to fetal period. Knowledge about the origins and development of individual differences, as well as the factors that may influence this process, can now be expanded with the study of behavior throughout pregnancy.

Fetal movements and fetal heart rate (FHR) have been extensively analyzed to describe normal development over the course of pregnancy, especially in singleton pregnancies. These variables are considered indirect measures of the fetal central nervous system (CNS) functioning (de Vries & Fong, 2006). Fetal movements follow a developmental course over pregnancy (de Vries et al., 1988; Roodenburg et al., 1991; ten Hof et al., 2002) and abnormal fetal movements have been observed in fetuses with brain dysfunction (de Vries & Fong, 2007). Similarly, abnormal heart rate patterns are suggestive of fetal distress. The association of fetal movements with heart rate patterns into specific combinations characterizes fetal behavioral (sleep–wake) states that resemble those described in the neonate. Disturbed development of fetal sleep organization has, for instance, been found in fetuses of women with type-1 diabetes (Mulder et al., 1987) and in fetuses exposed to antidepressants (Mulder et al., 2011).

Fetal Movements and Fetal Heart Rate Research

General Considerations and Definitions

Throughout this review, the original terminology used by various researchers, such as fetal 'behavior', 'movements', 'motility', 'motor behavior' or 'motor activity', 'reactive', or 'evoked' movements, is maintained. These terms are often employed interchangeably.

Fetal behavior has been defined as 'any observable action or reaction (to an external stimulus)' (Hepper, 1996, p. 145). 'Fetal behavior' and 'fetal movements' are employed in some studies as synonyms (de Vries et al., 1982, 1985; Piontelli et al., 1999). In the literature, the assessment of fetal behavior has also been referred to as the simultaneous assessment of fetal activity and FHR patterns (Nijhuis, 2003). However, none of the reviewed studies adopted this last definition.

Spontaneous fetal movements have been described as non-reflexive, autonomous movements originating in the fetus itself whereas the evoked fetal movements were thought to be caused by a variety of stimuli (in the fetal environment), being reflex in origin (Goodlin & Lowe, 1974; Graves, 1980; Preyer, 1882/1888).

In the next section, we provide a brief overview of what is known about normal fetal functional development in singletons, followed by a review of studies that have focused on fetal movements and heart rate in twin pregnancy.

Fetal Movements

Research on singletons has shown that normal fetal movement is characterized by wide interfetal variations in incidence of fetal movement patterns (sideways bending, general movement, startle, hiccup, breathing movement, stretch, yawning, isolated arm or leg movements, hand–face contact, jaw opening, sucking and swallowing, and head retroflexion, rotation, and anteflexion) from its early appearance to the end of gestation. It is striking that all fetal movement patterns have their onset before 14 weeks of gestation, although their incidences change over pregnancy (de Vries et al., 1982; Roodenburg et al., 1991).

In twins, the onset and incidence of specific movement patterns has been less investigated than in singletons. In contrast, researchers were more interested in the overall activity level of twins as compared with singletons in the synchrony of fetal movements and in the rate of spontaneous and evoked fetal movements. Few studies have examined specific variables of twin pregnancy that may have an association with fetal movements, such as zygosity, chorionicity, sex combination (male–male, male–female, female–female), and other variables such as fetal position (longitudinal or transverse lie) or presentation (cephalic, breech, or transverse).

Ultrasound observations of fetal movements provide the most accurate means to analyze fetal activity, as an experienced observer is able to differentiate between spontaneous, evoked, and passive movements of twins. Hence, we will provide an overview of studies that employed this technique to study fetal movements in twins.

Quantitative analysis of fetal movements of twins was performed in eight studies using two-dimensional (2D) ultrasound (Arabin et al., 1996; Mulder et al., 2004, 2012; Piontelli et al., 1997, 1999; Sadovsky et al., 1987; Samueloff et al., 1991; Zimmer et al., 1988) and more recently with 4D ultrasound (Degani et al., 2009; Hata et al., 2011).

The incidence of spontaneous movement patterns studied in the first half of twin pregnancies has been found to be similar to that in singletons (Piontelli et al., 1997). However, others recently found that twins have a lower incidence of general movements than singletons over pregnancy, but

TABLE 1
Definitions of Synchrony/Simultaneity in Fetal Movements (FM) and Fetal Heart Rate (FHR) Adopted in Twin Studies

Definitions of synchrony/simultaneity	Studies
A movement/FHR acceleration of one twin is followed by a movement/FHR acceleration of the co-twin within 15 seconds.	Gallagher et al. (1992) (FHR, FM) Sherer et al. (1990, 1991, 1992) (FHR)
Movements/FHR accelerations of both twins start exactly at the same time.	Mulder et al. (2012) (FM) Sadovsky et al. (1987) (FM) Zimmer et al. (1988) (FM)

more breathing movements in the last trimester (Mulder et al., 2012).

The incidence of several fetal movement patterns seems to be fairly correlated within twin pairs over pregnancy, ranging from 0.30 for general movements to 0.72 for breathing movements (Mulder et al., 2012). Stronger associations were found for all studied fetal movement patterns before 30 weeks of gestation.

The temporal coincidence of fetal movements in both twins has been investigated in several studies. However, the adopted definition of synchrony/simultaneity varied between studies (see Table 1). Fetal movements or FHR accelerations were defined as synchronous/simultaneous when a fetal movement or an FHR acceleration of one twin was seen at the same time as in the co-twin up to 15 seconds apart. In this review, we will focus only on the results of studies in which synchrony was defined as the immediate start of fetal movements, heart rate patterns, or the association between both.

With 2D ultrasound, the reported rate of simultaneous movements in both twins studied in the first half of pregnancy was 5% (Samueloff et al., 1991), 24–26% in the last trimester of pregnancy (Sadovsky et al., 1987; Zimmer et al., 1988), and 10–80% throughout pregnancy, using different window lengths to define simultaneity (Mulder et al., 2012). Analysis of fetal movements revealed significant increase in breathing movement synchrony over pregnancy within twin pairs, while no gestational trend was found for general movements' synchrony (Mulder et al., 2012).

Spontaneous movements seem to be more frequent than evoked movements throughout twin gestation. As stated previously, spontaneous movements were defined as those originating in the fetus itself, while evoked movements were believed to be caused by a variety of stimuli. In the first half of pregnancy, spontaneous movements represent 88% of overall activity in twin pregnancies at 13 weeks and 71% at 20–22 weeks (Piontelli et al., 1997). A higher mean rate of spontaneous fetal movements (95%) was reported in a cross-sectional study that included twin pregnancies at 10 to 22 weeks (Samueloff et al., 1991). The same research team reported a mean proportion of 76% of spontaneous fetal movements in the second half of pregnancy (Sadovsky et al., 1987).

Based on a limited number of studies, the occurrence of fetal movement patterns seems largely independent of variables such as zygosity, chorionicity, sex combination, position, and presentation. No difference in fetal movements' synchrony or incidence of most fetal movement patterns has been found according to zygosity and chorionicity (Arabin et al., 1995; Hata et al., 2011; Mulder et al., 2012; Piontelli et al., 1997, 1999; Zimmer et al., 1988). Similar levels of spontaneous (Piontelli et al., 1999) and evoked fetal movements were found in monochorionic (MC) and dichorionic (DC) twins from 12 to 22 weeks of pregnancy (Hata et al., 2011; Piontelli et al., 1997, 1999). However, in one study MC twins showed a more substantial decline in spontaneous activity than opposite-sex DC twin pairs after 15 weeks (Piontelli et al., 1999). Earlier findings that pointed to an association between fetal position and breathing movement incidence in late pregnancy (Zimmer et al., 1988) were not confirmed by a recent longitudinal study (Mulder et al., 2012). No difference in total fetal activity (amount of time the fetus breathed and moved) has been found to be associated with fetal presentation (Zimmer et al., 1988) or position (Mulder et al., 2012).

Inter-Twin Contacts

The existence of contacts between both twins during pregnancy has been somewhat controversial in the late 1980s and early 1990s. Sadovsky et al. (1987) and Samueloff et al. (1991) on the basis of their ultrasound observations in each trimester of pregnancy rejected the possibility that movements of one twin stimulated the co-twin. However, Sherer et al. (1990, 1992) suggested that 'tactile communication' between twin fetuses could explain coinciding fetal heart accelerations during non-stress testing.

These contacts have been described in a small number of ultrasound studies carried out from the mid-1990s to the present time either by using conventional 2D (Arabin et al., 1996; Piontelli et al., 1997) or 4D ultrasound (Hata et al., 2011; Sasaki et al, 2010). A consistent finding across these studies is that inter-twin contacts seem to occur earlier in MC than in DC twins.

Arabin et al. (1996) investigated the onset and development of inter-twin contacts until 16 weeks of pregnancy and classified them according to their duration, reaction of the co-twin (present or absent), velocity, and body parts involved. The first contacts with a reaction of the co-twin were first observed in MC twins around 8 weeks (Arabin et al., 1996; Piontelli et al., 1997). Inter-twin contacts were found more frequently in MC than DC twins until 16 weeks of pregnancy. The onset of complex contacts (lasting >5 seconds) was observed earlier in the same-sex female twins, while an increased frequency of these contacts was found in the same-sex male pairs at 8–16 weeks (Arabin et al., 1996). Piontelli et al. (1997) observed steady increase in time spent in evoked movements from 11–13 weeks to 18–19 weeks followed by a plateau at 20–22 weeks. While no specific developmental trend is evoked, movements were identified by these authors, some movement patterns were observed more frequently at particular gestational periods. A lack of response to an unequivocal contact was noted at all studied ages from 10–22 weeks. Using 4D ultrasound, Hata et al. (2011) found that at 12–13 weeks most inter-twin contacts do not elicit a reaction by the co-twin, even though a wide variation in reaction rates was reported. In addition, no significant difference in the rate of reaction was found according to chorionicity. Consistent with the results obtained in previous studies using 2D ultrasound, MC twins showed significantly more contacts than DC twins at the end of the first trimester of pregnancy (10–11 weeks of gestation) studied by 4D ultrasound (Sasaki et al., 2010). However, no differences were found between both groups at 12–13 weeks, as these contacts increased significantly in DC twins whereas contacts in MC twins remained stable.

Inter-twin contacts assessed by ultrasound in the last trimester of pregnancy have not been described yet, probably due to limitations on simultaneous monitoring of the entire body of both twins.

Three studies investigated the existence of a 'dominant twin' in a twin pair during pregnancy (Piontelli et al., 1997, 1999; Sherer et al., 1992), although the definitions of 'dominance' were different. Sherer et al. (1992) analyzed repeated non-stress FHR tracings of twin pairs of at least 30 weeks' gestation to investigate whether inter-twin contacts were consistently initiated by the same twin ('dominant twin'). It was assumed that the presence of FHR acceleration in both twins occurring within a 15-second period was caused by a previous inter-twin contact. The results showed that the initiation of inter-twin contacts was not systematically associated to one of the twins. Similarly, Mulder et al. (2012) found no evidence of a 'dominant twin' as demonstrated by higher fetal activity than the co-twin throughout pregnancy. In contrast, in another study using 2D ultrasound, dominance by one of the twins was found to be consistent from 10 to 22 weeks of gestation, as indicated by higher overall spontaneous activity level (Piontelli et al., 1999). In addition, it was noted that most of the 'dominant twins' in each pair had significantly lower evoked movements than their co-twins. Although the authors interpret this finding as the possible result of lower fetal stimuli generated by the less active twin, the number of contacts initiated by each twin was not reported (Piontelli et al., 1999). The contradictory results of these studies are probably explained by the adoption of different definitions of 'dominance', and diverse methodologies (ultrasound vs. electronic fetal monitoring) and study periods during pregnancy.

Fetal Heart Rate

Studies on normal, low-risk singletons have revealed developmental trends in FHR and its variation: With advancing maturation there is gradual decline in resting FHR and increase in FHR variability and FHR accelerations (Nijhuis et al., 1998; Ohel et al., 1985; Serra et al., 2009; ten Hof et al., 1999). These characteristics are recognizable early in pregnancy and follow a continuous gestational trend (Serra et al., 2009). In addition, there is an evidence of diurnal variation in mean FHR and its variability from mid-gestation onwards (Visser et al., 1982; Vries et al., 1987).

Twins and singletons display a similar frequency of heart rate accelerations from 28 weeks onwards (Ohel et al., 1985).

Most of the research on FHR in twins has focused on the intra-pair coincidence of heart rate patterns and accelerations as a potential marker of zygosity type or evoked fetal movements resulting from inter-twin stimulation.

There is some evidence for the association between chorionicity and synchrony of accelerations. Twins with synchronous accelerations were more likely to be MC or have fused DC placentas and smaller intra-pair birth weight differences than asynchronous twin pairs (Devoe, 1985; Devoe & Azor, 1981). Overall, 58% of FHR patterns were classified as synchronous, although no specific definition of synchrony was provided (Devoe & Azor, 1981). In another study, Sherer et al. (1992) found that less than 10% of all accelerations were synchronous. Synchronous heart rate accelerations have also been noted immediately after vibroacoustic stimulation (Sherer et al., 1991).

Similar patterns in diurnal variation in FHR of twins have been consistently found regardless of chorionicity (Lunshof et al., 1997; Maeda et al., 2006; Muro et al., 2001). A significant intra-pair correlation in diurnal rhythms of FHR baseline was found (Maeda et al., 2006; Muro et al., 2001). In contrast, no intra-pair coincidence of sustained fetal tachycardia was noted (Maeda et al., 2006; Muro et al., 2001). Sustained fetal tachycardia is a characteristic of FHR pattern D, the specific heart rate pattern of the fetal behavioral state 4F described by Nijhuis et al. (1982) that corresponds to active awake state. These findings were drawn from the studies that included uncomplicated (Maeda et al., 2006; Muro et al., 2001) and mildly complicated twin pregnancies (mild maternal hypertension, discordant fetal growth; Lunshof et al., 1997).

Nonlinear analysis of heart rate variability revealed smaller intra-pair differences in nonlinear properties of FHR in MC than in DC twins before 30 weeks (Shiba et al., 2008). However, from 30 to 36 weeks, MC twins seem to become increasingly different in FHR-regulating mechanisms. It has been suggested that this could be explained by an increasing influence of maternal and environmental factors over pregnancy. Nevertheless, the results also indicate that both MC and DC twin fetuses followed a similar trend in the heart rate dynamics, which is progressively more chaotic with increasing gestational age.

Association Between FHR and Fetal Movements

With advancing gestation, the fetal quiescence and activity periods are progressively extended and some linkage between heart rate, body movement, and eye movements occurs between 25 and 32 weeks in singleton pregnancies (Drogtrop et al., 1990; Visser et al., 1987). The combination of the three state variables into the well-defined behavioral states that resemble those in the neonate can be recognized at 36–38 weeks of gestation (Nijhuis et al., 1982).

None of the few twin studies that examined association between FHR patterns and fetal movements have applied the strict criteria proposed by Nijhuis et al. (1982) to define behavioral states, which would require the use of two FHR monitors and four ultrasound transducers for body and eye movements. The simultaneous observation of FHR and fetal movements has mostly been studied in twins by means of a fetal actocardiograph. To assess the effects of maternal betamethasone administration in twin pregnancy, FHR and fetal movements have been collected simultaneously with the use of four ultrasound devices (Mulder et al., 2004). However, these variables were described separately and their association has not yet been reported.

Gallagher et al. (1992) examined fetal behavior patterns of twins, a modification of fetal behavioral states that relies on the FHR and fetal movement monitoring provided by actocardiograph without the use of permanent ultrasonography. Ninety-five percent of the fetal behavior patterns (sleep or awake states) were synchronous. Interestingly, the same-sex twins were found to have greater synchrony in fetal behavior patterns than opposite-sex twins, whereas no significant differences were found between MC and DC twins (100% and 92% of the time respectively).

Arabin et al. (1993) classified FHR and fetal movement patterns occurring at the same time in twin pairs as 'concordant' or 'discordant', using a 3-minute window. Fetal behavioral patterns were categorized as 'occasionally' or 'continuously' 'concordant' or 'discordant spontaneous behavior'. No systematic changes were identified in the distribution of all patterns from 26 to 36 weeks (Arabin & van Eyck, 2006). According to the authors, twins exhibit a higher percentage of active periods (mean 80%) than singletons, probably caused by inter-twin contacts.

Stability of Individual Differences

Intra-fetal consistency in fetal movements in singletons has been shown to be weak during pregnancy (de Vries et al., 1988; ten Hof et al., 2002), whereas less intra-fetal variation has been found for FHR parameters (Visser et al., 1982) and fetal behavioral states (Groome et al., 1995). A number of studies on singletons have documented a continuity of inter-individual differences in FHR and movement patterns observed in the second half of gestation and infant heart rate or motor activity (Almli et al., 2001; DiPietro et al., 2000; Groome et al., 1999), right handedness (Hepper et al., 2005), infant and child development (DiPietro et al., 2002, 2007), and infant temperament (DiPietro et al., 1996; Werner et al., 2007). In developmental psychology research, there is no widely accepted theoretical definition of temperament. Nevertheless, most authors agree that temperament is a set of genetically influenced traits thought to underpin behavioral individual differences seen after birth (e.g., irritability, emotionality, activity level, or fearfulness) that show some stability over time. According to the existing theories, these predispositions can be seen as more or less influenced by developmental and environmental conditions. Prenatal factors such as maternal stress have been associated with infant temperament (Buitelaar et al., 2003).

In fetal twin studies, there is also some evidence for the emergence of relatively stable inter-individual differences between twins in the first trimester (Degani et al., 2009), the first and second trimester (Piontelli et al., 1999), and in the third trimester of pregnancy (Sherer et al., 1990).

In a prospective study, Sherer et al. (1990) found that the number of FHR accelerations of each twin fetus remained constant throughout the third trimester, suggesting the existence of stable individual differences in FHR by the end of gestation. Degani et al. (2009) have reported an association between the intra-pair difference in total fetal activity observed at 11–14 weeks of pregnancy and the intra-pair difference in infant temperament at 3–6 months of age. According to the authors, maternal reports on infant temperament and the more active twin after birth were correlated with prenatal inter-twin differences in fetal activity.

Inter-pair differences in spontaneous and evoked fetal movements were maintained from 10–11 weeks to 21–22 weeks (Piontelli et al., 1999). In addition, MC twins were found to be more similar than opposite-sex DC twins in both types of fetal movements. Interestingly, this similarity was found to decrease with advancing gestation, suggesting that maternal supply guaranteed by the umbilical cord is not identical for both twins even in normally evolving MC pregnancies. Nevertheless, a sex effect may have accentuated the observed differences in fetal activity levels.

So far, no longitudinal studies have analyzed individual differences in fetal movements throughout gestation. These studies could provide fundamental knowledge about the onset and origins of individual behavioral differences.

Discussion

Thirty years after the publication of the first twin study on fetal behavior reviewed, no definite conclusions can be made about normal fetal movements and heart rate in twin pregnancy. Ultrasound studies comparing fetal activity in twins and singletons are scarce and the results are mixed. Overall, some association or similarity in fetal movement incidences or FHR patterns between both members of twin pairs can be detected. Nevertheless, the temporal occurrence of these patterns is for the most part asynchronous when shorter time intervals were selected. In two of the reviewed studies, it is unclear which criteria were used when the FHR accelerations were called synchronous. A similar problem arises with the determination of synchronous fetal movements. However, a wide range of estimates is found for simultaneous movement incidence during pregnancy even when a strict definition of synchrony is used (start at the same time). Asynchronous occurrence of rest–activity cycles and different observation periods can partly account for these differences.

From the available research, chorionicity is the only variable that appears to have some influence on fetal behavior. It shows an association with the onset and frequency of inter-twin contacts in early pregnancy, intra-pair differences in fetal movements and FHR during pregnancy. MC twins initiate inter-twin contacts earlier in pregnancy, have more contacts until 13–16 weeks, and demonstrate more similarities than DC twins in fetal movement and FHR patterns. However, differences between MC and DC twin pairs seem to decrease over pregnancy as MC twins become less similar, especially after 30 weeks. In line with these findings, research on the organization of sleep and awake cycles in the most extreme form of monozygosity — the Siamese twin — revealed that a particular behavioral state in one twin member (REM sleep, non-REM sleep, active awake, quiet awake) rarely occurs simultaneously in the co-twin after birth until five months of age (Lenard & Schulte, 1972; Sackett & Korner, 1993). The identical genotype, the shared environment, and the mutually experienced (tactile) stimulation through motor activity do not seem to be able to synchronize the temporal organization of the sleep and awake cycles. Even in the craniopagus, with a common cerebral blood circulation, the different sleep and awake periods occurred almost independent of each other. The existence of a humoral, sleep-inducing factor, which has been suggested based on experiments in animals with coupled circulations, should therefore be doubted (Lenard & Schulte, 1972). Altogether, these findings could indicate that intra-pair similarities in fetal activity expressed in fetal movement incidence and FHR patterns can reflect maturation as previously suggested for fetal behavioral states in the neonatal period (Sackett & Korner, 1993). On the other hand, the intra-pair differences demonstrated on the temporal occurrence of fetal movements and heart rate patterns could be an expression of each twin's individuality.

There is some evidence that the incidence of evoked movements is lower than that of spontaneous movements. This finding corroborates a previous conclusion that fetal movements are, for the most part, independent. It also suggests that not every inter-twin contact provoked by one of the twins is followed by a reaction from the co-twin.

Considering the small number of the reviewed studies that addressed the continuity of individual differences, we can conclude that there is only preliminary evidence for the emergence of relatively stable individual differences in activity and heart rate patterns during pregnancy.

This review highlights the limited number and the high heterogeneity of studies on fetal movements and heart rate patterns in twin pregnancy. The study of fetal behavior in twin and higher order pregnancies is even more time-consuming than in singletons, and also has additional methodological limitations that relate to the monitoring of twins simultaneously, the identification of each twin throughout pregnancy (can be problematic in studies beginning in early pregnancy before sex determination in monoamniotic twins and same-sex twins), the definition of synchrony, conceptual views on twins, and the interpretation of the data. In general, the available research evidence is based on small, selected samples (uncomplicated twin pregnancies), the recording time is (far too) short, and studies were carried out either in the first or the second half of pregnancy with a lack of longitudinal studies. Often, there is no information about the type of placentation or zygosity.

The definitions and procedures used have varied considerably from study to study. Comparison between most of the ultrasound studies describing evoked fetal movements and synchronous/simultaneous fetal movements is hampered by the various concepts and definitions used. Evoked fetal movements include those movements that occur within 1 second of a touch (Arabin et al., 1996) or 4 seconds following an unambiguous stimulus/response pattern in an otherwise immobile fetus (Piontelli et al., 1997). Synchronous/simultaneous fetal movements are thought to be caused either by inter-twin contacts or environmental stimuli. However, the conclusions of some studies of the same group (Sadovsky et al., 1986, 1987; Samueloff et al., 1991) and their a priori assumptions about spontaneous movements and movements induced by maternal and other environmental stimuli have been partly refuted or rendered out of date (Arabin et al., 1995; Visser et al., 1992).

In our opinion, the definitions of the two types of activities — synchronous and evoked fetal movements — and also the different time intervals between the stimulation and the reaction are probably reflecting different aspects of fetal movement patterns of twins. Synchronous activity does not require by definition previous intra-pair stimulation, while the evoked fetal movement include only the activity performed after a stimulus. Some additional problems can also be found when analyzing these definitions. It is not clear if a passive movement following a touch of the co-twin is included in the synchronous and evoked categories. In the ultrasound studies, the qualitative judgment of a body movement can give a definitive answer to the active or passive character of that movement, except probably in the third trimester and under pathological circumstances (large interfetal growth discordancy, whether or not accompanied by oligo- or polyhydramnion). Conversely, with the use of actograph it is not possible to differentiate between active and passive movements because passive movements are simply recorded as fetal activity. The same holds for maternal perception of each twin's fetal movements. Therefore, actograph and maternal perception of fetal movements cannot reliably be used for fetal movement observations in twin studies.

Arabin and van Eyck (2006) have recognized that some reactions could be merely passive movements, non-reactions to the contact of the co-twin that cause displacement of the total body or some body parts due to the intensity of contact. Still it is unclear whether these were included in the 'first reach and touch' pattern proposed by these authors as this movement pattern has been described to disappear at 13 weeks of gestation (Arabin et al., 1996). The absence of a reaction after stimulation, resulting in a passive movement or not, may have a different meaning according to the observed frequency and the stage of pregnancy and thus may be of potential clinical value. There is some evidence that the lack of response of one twin to a contact from the co-twin can be noted from 10–13 weeks onwards (Hata et al., 2011; Piontelli et al., 1997). The underlying causes of this phenomenon are unknown. The absence of response to a contact in late pregnancy in twin pregnancies might be a result of maturation, habituation to repeated contacts, lack of intrauterine space, or a non-responsive sleep state in the co-twin. Decrease in incidence and number of fetal movements has been found in the last trimester of pregnancy both in singletons (ten Hof et al., 2002) and twins (Mulder et al., 2012) and has been interpreted as a developmental phenomenon that could not be simply explained by the appearance of longer episodes of fetal quiescence until term (ten Hof et al., 2002). The use of 4D ultrasound in fetal behavioral studies is another technique that has also several important limitations, especially in the first trimester of pregnancy. Even with the latest 4D ultrasound machines with high frame rates, four movement patterns easily recognized with 2D ultrasound are not detected or documented with 4D ultrasound, namely sideways bending, breathing, hiccups, and facial movements (Kurjak et al., 2006).

Future twin studies could help clarify contradictory results from research with singletons and enhance our knowledge on a number of research topics. In our opinion, these pregnancies are a natural and valuable model to study the potentially adverse maternal psychological (anxiety, stress, depression) and physiological (cortisol) effects on fetal, neonatal, infant, and child behavior and development. Although a direct link has been established between maternal anxiety/stress and fetal behavior in the last trimester of pregnancy in singletons (van den Bergh et al., 2005), there is no such data available from twin pregnancies.

The 'fetal origins hypothesis' of the long-lasting effects of prenatal factors, namely maternal psychological state, on child development has been studied extensively in singletons (Gutteling et al., 2005; Huizink et al., 2004), but not in twins.

There is growing evidence from animal and human studies for a sex-dependent fetal programming by maternal stress (Brunton & Russell, 2010; van den Bergh et al., 2006). Investigations comparing the same-sex with opposite-sex twin pairs could give additional insight into this subject. In addition, twin studies could provide information regarding the prenatal predictors of maturation and temperament, especially when one of the twins has a recognized malformation or is growth-restricted.

Twin studies on the continuity of movement patterns and heart rate from the prenatal to the postnatal period are also lacking. In singletons, the entire repertoire of fetal movement patterns observed at the third trimester of pregnancy can also be seen after birth (Prechtl, 1984). There is also some evidence for the continuity of heart rate and its variability from prenatal life to postnatal lifespan of 1 year (Almli et al., 2001; Lewis et al., 1970) and 10 years (Thomas et al., 1989).

In addition, appropriately designed twin studies are needed to examine differences in FHR and behavior according to gender as the evidence from singletons is mixed. Although some authors observed gender-specific FHR (Bernardes et al., 2008; Buss et al., 2009) and fetal movements (Almli et al., 2001; Hepper et al., 1997), others have found no gender differences in these prenatal variables (de Vries et al., 1987; DiPietro et al., 2000; Robles de Medina et al., 2003). The same-sex twins were found to have more synchrony in behavior patterns than opposite-sex twins (Gallagher et al., 1992). However, the authors recognize that sex combination could be a confounding variable and chorionicity is probably the explaining cause for this finding. On the other hand, no difference in spontaneous (Arabin et al., 1995) or simultaneous fetal movement (Zimmer et al., 1988) has been found relative to sex combination.

Nevertheless, the considerable interfetal variability of behavior of both singletons and twins has made the identification of abnormal fetus still difficult. These individual differences can be a fundamental topic of research, especially as these have been noticed from early pregnancy onwards and are overall unstable over pregnancy. Methodological aspects, including disregard of the fetal rest–activity and sleep–wake cycles can, at least in part, explain the low intra-individual stability of fetal behavior. Behavioral state-dependent changes in the human fetus have been well documented in the last trimester of pregnancy (Pillai & James, 1990; Visser et al., 1992).

Acknowledgment

This research was funded by FCT doctoral grant (SFRH/BD/40146/2007) to the first author.

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OBJECTIVES

This thesis aims to contribute to existing knowledge on the psychological adjustment to twin parenthood and on twins' fetal development, by the accomplishment of the following objectives:

1) To describe the trajectories of parents' psychological adjustment (psychological distress, marital relationship, attitudes to pregnancy and the baby and attitudes to sex) during the transition to twin parenthood (Paper II).
2) To determine whether parents of twins and parents of singletons differ on psychological adjustment trajectories during the transition to parenthood (Paper III).

   To accomplish this goal, a control group of parents of singletons was derived from a previous study conducted by our research team.
3) To investigate whether psychological adjustment trajectories vary as a function of mode of conception (spontaneous vs. after infertility treatment) and parent gender during the transition to twin parenthood (Papers II and III).
4) To examine twins' fetal development (fetal movements and heart rate) and to investigate factors associated with it (Papers IV and V).

The main characteristics of the four empirical studies included in this thesis are presented in Table 1.

Table 1 *Main characteristics of the empirical studies included in this thesis*

Paper	Aims	Participants	Assessment times	Measures
Paper II	To examine whether mode of conception and gender are associated with parents' psychological adjustment across the transition to twin parenthood	41 couples expecting twins - 25 SC - 16 IT	Pregnancy - 8-15 weeks - 20-24 weeks - 28-34 weeks Postpartum - 1 week - 4 weeks - 8 weeks	Edinburgh Postnatal Depression Scale State-Trait Anxiety Inventory Maternal Adjustment and Maternal Attitudes Questionnaire Paternal Adjustment and Paternal Attitudes Questionnaire
Paper III	To examine the effects of mode of conception, type of pregnancy and parent gender - on anxiety and depression at each time point - on anxiety and depression trajectories	267 couples - 203 with SC singletons - 28 with SC twins - 17 with IT singletons - 19 with IT twins	Pregnancy - 8-15 weeks - 20-24 weeks - 28-34 weeks Postpartum - after childbirth - 3 months	Edinburgh Postnatal Depression Scale State-Trait Anxiety Inventory
Paper IV	To examine mean levels and developmental trajectories of fetal movements (general and breathing movements) in twins and associated factors	41 twin pairs	Pregnancy 12-15 weeks 20-23 weeks 28-32 week	Fetal data were analyzed from ultrasound recordings
Paper V	To evaluate the differences in linear and complex heart rate dynamics in twin pairs according to fetal sex combination (male-female, male-male, and female-female)	14 twin pairs - 6 male-female - 3 male-male - 5 female-female	31-36 weeks of gestation	Fetal data were collected using Philips Medical 50A fetal monitor connected by cable to the Omniview-SisPorto®3.5 system

METHOD

Participants

Forty-five couples with twins attending routine antenatal care appointments in three public hospitals in northern Portugal (Hospital São Sebastião, Hospital São João and Hospital Pedro Hispano) were enrolled in this longitudinal study at 12-15 weeks of pregnancy. The duration of pregnancy was calculated from the first day of the last menstrual cycle and confirmed by early ultrasound. Four parent-twin pairs were subsequently excluded due to death of one (1 *in utero* and 1 after birth) or both twins (2 twin pairs *in utero*). Thus, the final sample comprised 41 parent-twin pairs conceived spontaneously (SC; n = 25 couples) or after infertility treatment (IT; n = 16 couples).

In the IT group, 8 couples had conceived by intracytoplasmic sperm injection (ICSI), 7 by *in vitro* fertilization (IVF), and one after ovulation induction (OI). In this group, couples had been infertile for 3.8 years (SD = 2.2) and received an average of 1.8 (SD = 0.9) fertility treatments.

On average, parents were 30.7 years old (SD = 4.7, range 20-43 years), had been in the relationship for 8.3 years (SD = 4.7, range 1-21 years), and the majority were Caucasian (97.6%), primiparous (78%), married (75.6%), employed (84.5%), did not complete high school or equivalent (51.2%), and had medium and medium-low socioeconomic level (29.3% and 41.5%, respectively). More than half of the women had complications during pregnancy (53.7%) and delivered by cesarean section (63.4%). Most pregnancies were bichorionic biamniotic (93 %). The mean gestational age was 36.1 weeks (SD = 2.7, range 27.7-38.7).

Regarding twins, there were 17 female-female, 12 male-male and 12 male-female pairs, resulting in a total of 46 girls and 36 boys. Most of the twins were born full-term (51.2%), had low and very low birth weight (50% and 11%, respectively) and 1-min and 5-min Apgar scores ≥ 7 (79.3% and 94.3%, respectively). In more than a third of cases, at least one of the twins was admitted to a neonatal intensive care unit (NICU) (34.1%) with an average stay of 21.3 days (*SD* = 24.9).

Maternal and child differences were found between groups. All women in the IT group were primiparous, whereas in the SC group more than a third was multiparous (36.0%). IT twins had a significantly longer stay at NICU than SC twins (*U* = 102.0, *P* = .02). No statistically significant differences were found for parents' age, education, socioeconomic level, relationship length, pregnancy complications, delivery mode, gestational age or NICU admittance between SC and IT groups (32% vs. 37.5%, respectively).

Procedure

After approval from the Ethics Committee of each participating hospital, consecutive sampling of couples with less than 15-week twin pregnancies was carried out from June 2008 to August 2010. Potentially eligible couples were referred by their physicians. Inclusion criteria included carrying a twin pregnancy, having less than 15 weeks of gestational age, and knowing how to read and write in Portuguese. Of the 50 eligible couples approached, 45 agreed to participate in the study (80%). Written informed consent was obtained from all participating parents.

As depicted in Table 2, parents' psychological adjustment and twins' development was assessed three times before (T1-T3) and after (T5-T7) childbirth, on average, at 13, 21 and 30 weeks of pregnancy and 1, 4 and 8 weeks postpartum. Socio-demographic and clinical information of mothers and fathers was collected at T1 and T5. Fetal heart rate (FHR) was assessed at T3 and T4. Twins' temperament was assessed at 3 months corrected age (T8).

Table 2

Study design

	Pregnancy				Postpartum			
	T1 13 wks	T2 21 wks	T3 30 wks	T4 36 wks	T5 1 wk postpartum	T6 4 wks postpartum	T7 8 wks postpartum	T8 3 months corrected age
Twins	Fetal behavior	Fetal behavior	FHR Fetal behavior	FHR	Postnatal behavior and development	Postnatal behavior and development	Postnatal behavior and development	Temperament
Parents	SD Anxiety Depression Adjustment and attitudes	Anxiety Depression Adjustment and attitudes	Anxiety Depression Adjustment and attitudes		SD Anxiety Depression Adjustment and attitudes	Anxiety Depression Adjustment and attitudes	Anxiety Depression Adjustment and attitudes	

Observations of fetal behavior were carried out after routine ultrasound assessments. These observations were conducted for 20 min using real-time ultrasound (GE Voluson E8, GE Medical Systems, Zipf, Austria) with a multifrequency transabdominal probe and recorded on DVD for latter fetal movement analysis. A longitudinal view of the fetuses was preferred with the head, trunk and upper limbs visible, as well as the potential contact area of the twins. Whenever possible, both fetuses were monitored simultaneously (n = 82 recordings) to distinguish between active (spontaneous and evoked) and passive fetal movements. However, when an adequate view of both twins was not attained they were monitored in two consecutive periods (n = 80 recordings). Except for those who delivered before the last prenatal assessment (n = 3), the other participants completed all prenatal assessments (95%). Nevertheless, two recordings were not available for analysis due to technical problems.

Recordings of simultaneously monitored twins were scored by two researchers (I. T. and E. J. M), each scoring the fetal movements of one fetus. The scoring of the fetus positioned on the left or right side of the screen monitor was randomized at the first recording and was maintained at subsequent recordings whenever the identification of each twin was possible (e.g., gender difference). Recordings of twins separately monitored were all scored by one researcher (I.T.). Hand-held pushbuttons were used to score two fetal movement patterns – general and breathing movements - and the data was fed into a computer. General movements (spontaneous or evoked) were scored by pressing one button as long as the movement was performed (Mulder et al., 2012). Passive movements due to co-twin movement were overlooked. As fetal breathing movements last less than 1s, they were marked as discrete events.

Smoothing procedures previously applied to data from singletons (Mulder et al., 2004; ten Hof et al, 1999) and twins (Mulder et al., 2012) were performed with a software package (Poly 5, Inspector research Systems, Amsterdam, The Netherlands). Accordingly, a single burst was considered when consecutive GM occurred within 1 s of each other. Breathing movements occurring within 6 s apart were regarded as bouts of continuous breathing activity. The rate of each pattern was calculated for each fetus and fetal assessment. Fetal movement analyses of the 82 fetuses were based on 162 recordings with a total time of 4835.13 min. The median duration of each observation was 20 min (range 13-27 min).

Postnatal behavioral development was assessed 2-3 times at monthly intervals depending on the length of pregnancy and on the neonatal clinical status of each twin. The first assessment took place 48 hours after birth or when both twins were medically stable. While the first postnatal assessment was carried out at the hospital, the following were conducted during home visits. As recommended (Brazelton & Nugent, 1995), this assessement was conducted midway between feedings in a quiet and semi-darkened room by certified examiners,

Measures

Socio-demographic, clinical and perinatal data

Information on parents' age, ethnicity, parity, occupational and marital status, education level, history of infertility, mode of conception, pregnancy type, duration of pregnancy, medical complications, type of childbirth and twins' birth weight was collected by self-administered questionnaires.

Anxiety symptoms

The 20-item state subscale of the State-Trait Anxiety Inventory Form Y (STAI, Spielberger *et al.*, 1983) is a screening instrument used to measure current anxiety symptoms. Items are scored on a four-point Likert scale (1-4). The scores range from 20 to 80, with higher scores indicating higher anxiety. STAI Portuguese version has shown excellent internal consistency and high criterion validity (Tendais *et al.*, 2014). In this study, Cronbach's alphas ranged from .92 and .95 for women and .91 and .95 for men. A score of 40 or higher was considered clinically significant.

Depression symptoms

The Edinburgh Postnatal Depression Scale (EPDS, Cox *et al.*, 1987) is a self-report questionnaire composed of 10 items addressing depression symptoms within the previous seven days. The scores range from 0 to 30, with higher scores indicating more severe depression symptoms. The Portuguese version of this screening tool has shown good internal consistency and reasonable criterion validity (Tendais *et al.*, 2014). In the present study, Cronbach's alphas ranged from .77 and .85 for women and .72 and .79 for men. A score of 12 or higher was considered clinically significant.

Marital relationship, Attitudes to pregnancy and the baby and Attitudes to sex

Three subscales of the Maternal and the Paternal Attitudes and Adjustment questionnaire (MAMA, PAPA; Kumar, Robson, & Smith, 1984) were used. The marital relationship and the attitudes to pregnancy and the baby subscales comprise 11 items each (range 11 to 44). The attitudes to sex subscale comprises 12 items (range 12 to 48). Sample questions include "Has there been tension between you and your partner?" (marital relationship), "Have you found your partner sexually desirable?" (attitudes to sex), "Have you been worrying that you might not be a good mother/ father?" (attitudes to pregnancy and the baby). Each item is scored on a four-point Likert scale (1 = *never* to 4 = *very often*). MAMA Portuguese version has shown good internal consistency (α = .85, Figueiredo, Mendonça, & Sousa, 2004), as well as PAPA (α = .90 - .91, Pinto, Figueiredo, Samorinha, Tendais, & Nunes-Costa, 2015). In this study, Cronbach's alphas ranged from .79 to .82 for marital relationship, .51 to .54 for attitudes to pregnancy and the baby and .77 to .83 for attitudes to sex for women. For men, Cronbach's alphas ranged from .63 to .77 for marital relationship, .51 to .72 for attitudes to pregnancy and the baby and.72 to .74 for attitudes to sex. For the total score, Cronbach's alphas ranged from .74 to .84 for women and from .76 to .84 for men.

Fetal behavior

Fetal behavior was assessed through the observation of real-time ultrasound recordings. General movements (GM) involve the whole body (head, limbs, and trunk) and have variable amplitude, speed and patterning of body parts (de Vries et al., 1982). Breathing movements are characterized by a simultaneous inward movement of the thorax and an outward movement of the abdomen (de Vries et al., 1982). After training, interrater reliability on the identification of these fetal movement patterns was calculated using 6h recordings of fetal data. There was moderate to high agreement between the two raters (I.T. and E.J.M) for general movements and breathing movements (intraclass correlation coefficients: .65 and .94, respectively).

Fetal heart rate

FHR monitoring of both twins was performed using the Philips Medical 50A fetal monitor (Philips Medical, Eindhoven, The Netherlands) connected by cable to the Omniview-SisPorto®3.5 system (Speculum, Lisbon, Portugal) for computer analysis of cardiotocograms (Ayres-Campos, Sousa, Costa, & Bernardes, 2008). The computerized analysis of cardiotocographs provide a reproducible and objective interpretation of FHR tracings, calculating parameters that are difficult to assess by visual inspection (Ayres-de-Campos, Costa-Santos, & Bernardes, 2005). An additional advantage is the possibility of incorporating in these systems the latest clinical guidelines on fetal monitoring. In this study, several parameters of FHR variability were calculated, specifically mean and standard deviation of FHR, long-term irregularity, Delta FHR, short-term variation, interval index, low and very low frequency, movement frequency, high frequency, short- and long-term variability. A preprocessing algorithm was applied to reduce noise and artifacts as described elsewhere (Gonçalves, Rocha, Ayres-Campos, & Bernardes, 2008). Additionally, persistent segments of signal loss in the initial period of the tracings were visually identified and excluded.

Postnatal behavior

Postnatal behavior was assessed with the Neonatal Behavioral Assessment Scale (NBAS, Brazelton & Nugent, 1995). It includes 14 reflex items scored on a 4 point-scale, 28 behavioral items and 7 supplementary items scored on a 9 point-scale. Items are organized into 6 subscales, including Habituation (ability to respond to and inhibit discrete stimuli while asleep), Orientation (quality of alertness and ability to pay attention to visual and auditory stimuli), Motor (motor development and quality of movements and tone), Range of State (infant arousal and lability of states), Regulation of State (regulatory state control while facing high stimulation) and Autonomic Stability (signs of stress associated with homeostatic adjustments of the CNS) (Brazelton & Nugent, 1995). The NBAS has been shown to have adequate reliability and validity (Costa et al., 2010).

Temperament was assessed with the Infant Behavior Questionnaire - Revised (IBQ-R, Gartstein & Rothbart, 2003), a parent-report questionnaire developed to measure temperament of 3- to 12-month-old infants. In this 191-item questionnaire, the relative frequency of specific infant behavior in several situations during the previous week is assessed using a 7-point scale (1 - never to 7 - always or does not apply if the event did not occur). IBQ-R provides three broad dimensions of temperament that range from 1 to 7: (1) Surgency/Extraversion – an item-weighted sum of approach, vocal reactivity, high intensity pleasure, smiling and laughter, activity level and perceptual sensitivity subscales; (2) Negative Affectivity – an item-weighted sum of distress to limitation, fear and falling reactivity subscales; (3) Orienting/Regulation – an item-weighted sum of low intensity pleasure, cuddliness, duration of orienting and soothability subscales (Gartstein, & Rothbart, 2003). The IBQ-R shows good reliability with Cronbach's alpha of the broad dimensions ranging from .91 to .92 (Gartstein & Rothbart, 2003). The Portuguese version of IBQ-R demonstrated somewhat lower scores with Cronbach's alpha ranging from .70 to .93 (Costa & Figueiredo, submitted).

PAPER II

COUPLES' PSYCHOLOGICAL ADJUSTMENT TO TWIN PARENTHOOD: MODE OF CONCEPTION AND GENDER DIFFERENCES

Tendais, I., Figueiredo, B., Canário, C., Kenny, D. A. (2016). *Couples' psychological adjustment to twin parenthood: Mode of conception and gender differences*. Manuscript under review

Abstract

Introduction: To examine whether mode of conception and gender are associated with parents' psychological adjustment across the transition to twin parenthood.

Methods: Forty-one couples expecting twins conceived spontaneously (SC; n = 25) and by assisted reproduction techniques (ART; n = 16). Depressive and anxiety symptoms, marital relationship, attitudes to sex, and attitudes to pregnancy and the baby.

Results: ART parents showed a decline in marital relationship quality, no changes in attitudes to pregnancy and the baby and no changes in attitudes to sex over the postpartum. In contrast, SC parents did not change their perception of the marital relationship, reported more positive attitudes to pregnancy and the baby and more positive attitudes to sex over the postpartum. Compared to the other groups, ART women exhibited a higher increase in depressive and anxiety symptoms from pregnancy to postpartum and only anxiety symptoms exhibited a decline trend over the postpartum.

Conclusion: These findings suggest that ART parents may experience more psychological difficulties during the transition to twin parenthood than SC parents. ART mothers, in particular, appear to be more at risk of high levels of postpartum depressive symptoms.

Introduction

Twin birth rates have increased worldwide in the last decades, largely due to the increased use of assisted reproduction technology (ART). Parents of twins have to deal repeatedly with several stressful situations. Twins have higher risk of prematurity, low birth weight and perinatal mortality compared with singletons [1]. Twins also show poorer neurodevelopmental outcomes than singletons [2]. To meet the needs of two or more children, parents may experience financial, childcare, and physical and psychological issues [3, 4]. The first 3 months after delivery have been pointed out as a particularly vulnerable period for mothers due to the overload of caregiving tasks and different sleeping and feeding patterns of the twins [5].

Given that infertility-related distress has been observed during pregnancy and postpartum among parents of ART singletons [6], it would be expected that twin parenthood after ART may have a cumulative negative effect on parents' psychological adjustment. Contrary to expectations, some studies suggest that ART parents of twins have similar or even fewer psychological symptoms than parents of spontaneously conceived (SC) twins [7, 8]. One of the few longitudinal studies that examined the impact of mode of conception on the psychological adjustment of twins' parents found that ART mothers exhibited fewer depressive symptoms at mid-pregnancy and similar levels of depressive and anxiety symptoms to SC mothers at 3 months and 1 year postpartum; however, no differences were found between ART and SC fathers of twins at pregnancy or postpartum [8]. Other studies revealed that first-time mothers of ART twins reported more psychological symptoms and poorer coping resources than first-time mothers of SC twins [9-11]. Although some studies found that primiparous ART women had more psychological symptoms and lower psychosocial well-being than multiparous ART mothers [9-11], others found no evidence for negative effects of parity on parental mental health [8].

With regard to the marital relationship, the few studies that have compared ART and SC parents of twins have found lower self-reported marital quality in ART mothers at late pregnancy compared with mothers of SC twins [10], but similar marital relationship quality 9 months [9] and 1 year postpartum [11].

Differences in pregnancy- and child-related attitudes between parents of ART and SC twins are largely unknown. Research with singletons suggests that ART mothers seem to have more idealized attitudes to pregnancy than SC mothers [12]. It is unclear whether the prenatal maternal expectations of ART mothers of twins are unrealistic as well [10].

There is also a very limited body of research on gender differences in psychological adjustment to twin parenthood among ART and SC parents. Munro et al. [7] found no gender differences in psychiatric morbidity in a sample of parents of twins conceived spontaneously, by hormonal treatment, or IVF. In contrast, mothers of twins showed lower psychological well-being than fathers, regardless of mode of conception [9].

The inconclusive evidence regarding parents' psychological adjustment to twin parenthood after ART may be at least in part explained by differences in studied samples. Some studies have included only primiparous women [10, 12], whereas in others multiparous women were also included [7-9, 11]. ART couples appear to be a heterogeneous group in terms of their socio-demographic characteristics and fertility difficulties [13].

The purpose of this study was to examine whether mode of conception and gender are associated with parents' psychological adjustment (anxiety and depressive symptoms, marital relationship quality, attitudes to pregnancy and the baby and attitudes to sex) across the transition to twin parenthood, taking into account the interdependence of data within couples. Based on previous research, we hypothesized that ART parents (and ART mothers in particular) would have poorer psychological adjustment than SC parents during the transition to twin parenthood.

Method

Procedures and participants

After receiving ethical approval from the Ethics Committee, consecutive couples expecting twins were recruited at the antenatal Obstetrical Units of three public hospitals in Northern Portugal. Inclusion criteria included twin pregnancy, less than 15 weeks gestational age, and knowing how to read and write in Portuguese. Information on couples' psychological adjustment was collected three times during pregnancy (T1-T3) and three times after childbirth (T4-T6). The measures were completed separately by women and men, on average, at 13, 21 and 30 weeks of pregnancy and 1, 4 and 8 weeks after birth.

The sample included 41 couples with twins conceived spontaneously (SC; *n* = 25 couples) or by assisted reproduction techniques (ART; *n* = 16 couples). Of the 50 eligible couples approached, 45 agreed to participate in the study (80%). Four couples were excluded due to death of one or both twins. Two couples dropped out the study after childbirth for personal reasons. Within the ART group, 7 couples conceived through in vitro fertilization, 8 by intracytoplasmic sperm injection, and one by ovarian stimulation medication.

Measures

Couples' information was collected using a socio-demographic questionnaire. The state subscale of the State-Trait Anxiety Inventory Form Y (STAI) [14] was used to measure anxiety symptoms. It comprises 20 items that are scored on a four-point Likert scale. The scores range from 20 to 80, with higher scores indicating higher anxiety. The Portuguese version of the STAI has been validated for use during pregnancy and the postpartum period [16].

The Edinburgh Postnatal Depression Scale (EPDS) [15] was used to measure depressive symptoms. The EPDS is a self-report questionnaire composed of 10 items scored on a four-point Likert scale addressing depressive symptoms within the previous seven days. The scores range from 0 to 30, with higher scores indicating more severe depressive symptoms. The Portuguese version of the EPDS has been validated both for pregnancy and the postpartum period [16].

Marital relationship, attitudes to sex and to pregnancy and the baby were assessed with the subscales of the Maternal and the Paternal Attitudes and Adjustment scale (MAMA, PAPA) [17]. The attitudes to sex subscale comprises 12 items (range 12 to 48). The marital relationship and the attitudes to pregnancy and the baby subscales comprise 11 items each (range 11 to 44). Sample questions include "Has there been tension between you and your partner?" (marital relationship), "Have you found your partner sexually desirable?" (attitudes to sex), "Have you been worrying that you might not be a good mother/ father?" (attitudes to pregnancy and the baby). Each item is scored on a four-point Likert scale (1 = never to 4 = very often). MAMA Portuguese version has shown good internal consistency (α = .85) [18], as well as PAPA (α = .90 - .91) [19].

Data analysis strategy

Pearson correlations were used to assess couples' interdependence at baseline. In addition, unconditional multilevel models with gender and time as fixed effects were run for all outcome measures to estimate couples' interdependence over time.

Dyadic linear growth curve models were estimated using multilevel modeling [20], a method for studying hierarchically nested data structure such as repeated measures from dyads. Growth curve models were used to evaluate the degree of change in an outcome variable using time as the predictor variable and the influence of other variables in the moderation of change over time [21]. The data consist of 492 potential observations: 41 couples by 6 time points by 2 people (husband and wife). Two-level models were estimated for each psychological adjustment variable (dependent variables) in which observation is level 1 and couple is level 2.

Based on the examination of mean scores over time for men and women who conceived spontaneously and by ART, the following growth curve models were developed for depression, anxiety and attitudes to sex, and we found that change over time was different before and after the first assessment postpartum. Thus, for these variables, we used piecewise models with two different slopes: the first, from 3 (T1) to 9 months (T4) and the second, from 9 months to the last assessment (T6). In addition, the model contains two intercepts. The first called "intercept" represented where a person started at T1, and a second intercept named "pre-postnatal transition" was added to reflect change from pregnancy to the postpartum period. For the remaining outcome variables (marital relationship and attitudes to pregnancy and the baby), linear time changes were found from the first assessment during pregnancy to the last assessment postpartum. Thus, the postpartum intercept was set to zero and the two slopes were set equal. The models included main effects for time, mode of conception and gender. All interactions were examined, resulting in a possible three-way interaction between time, mode of conception and gender. Three multivariate outliers for anxiety and two for depression were detected and excluded from the analysis.

The time variable was defined in months and Time 0 was the beginning of the study (T1, $M = 3.05$, $SD = 0.17$) and so the intercept or baseline score represents the estimated mean level of each outcome variable at 3 months of pregnancy and the slope corresponds to the average linear change for each 1 month.

Results

In the ART group, couples had been infertile for 3.8 years ($SD = 2.2$) and received an average of 1.8 ($SD = 0.9$) fertility treatments. On average, participants were 30.7 years old ($SD = 4.7$, range 20-43 years), had been in the relationship for 8.3 years ($SD = 4.7$, range 1-21 years), and the majority were Caucasian (97.6%), primiparous (78%), married (75.6%), employed (84.5%), did not complete high school or equivalent (51.2%), and had medium and medium-low socioeconomic level (29.3% and 41.5%, respectively). More than half of the women had complications during pregnancy (53.7%) and delivered by cesarean section (63.4%). The mean gestational age was 36.1 weeks ($SD = 2.7$, range 27.7-38.7).

Most of the twins were born full-term (51.2%) and had low and very low birth weight (50% and 11%, respectively). In more than a third of cases, at least one of the twins was admitted to a neonatal intensive care unit (NICU) (34.1%) with an average stay of 21.3 days ($SD = 24.9$).

ART twins had a significantly longer stay at NICU than SC twins, $U = 102.0$, $p = 0.02$. A significant association was found between mode of conception and parity, $\chi^2(1) = -7.38$, $p < 0.01$. All women in the ART group were primiparous, whereas in the SC group more than a third was multiparous (36.0%). No statistically significant differences were found for age, education, socioeconomic level, relationship length, pregnancy complications, delivery mode, gestational age or NICU admittance between SC and ART groups (32% vs. 37.5%, respectively).

Means and standard deviations for the outcome variables are presented separately for women and men and for spontaneous and ART groups in Table 1.

Table 1. Mean and Standard Deviation of Outcome Variables for Women and Men and for Spontaneous and ART Groups at all Time Points.

	Women (*n* = 41) *M* (*SD*)	Men (*n* = 41) *M* (*SD*)	Spontaneous (*n* = 25) *M* (*SD*)	ART (*n* = 16) *M* (*SD*)
STAI-T	37.2 (7.2)	32.4 (6.6)	34.5 (6.6)	35.3 (8.3)
STAI-S				
T1	37.4 (10.3)	34.6 (8.3)	35.6 (10.2)	36.7 (8.3)
T2	34.0 (8.0)	32.0 (9.1)	32.4 (8.9)	34.1 (8.0)
T3	35.9 (9.4)	33.6 (9.4)	34.0 (9.0)	36.1 (10.1)
T4	40.6 (12.7)	35.5 (12.4)	35.1 (12.1)	42.9 (12.4)
T5	36.4 (11.4)	34.0 (10.7)	33.0 (8.5)	40.4 (14.1)
T6	37.8 (13.5)	32.1 (8.4)	33.8 (10.9)	36.9 (12.6)
EPDS				
T1	6.3 (3.7)	4.6 (2.9)	5.8 (3.4)	5.0 (3.4)
T2	5.1 (3.0)	4.1 (3.1)	4.2 (3.0)	4.8 (2.9)
T3	5.8 (3.2)	4.6 (3.5)	4.8 (3.2)	5.3 (3.4)
T4	6.7 (5.2)	4.6 (3.5)	4.3 (3.6)	7.9 (5.0)
T5	6.1 (4.7)	4.0 (4.2)	4.3 (3.7)	7.1 (5.5)
T6	6.2 (4.5)	3.6 (3.2)	4.0 (3.5)	6.5 (4.6)
Attitudes to sex				
T1	36.6 (4.3)	39.0 (3.8)	37.9 (3.6)	37.7 (5.1)
T2	36.2 (4.2)	37.7 (4.3)	37.0 (3.9)	36.9 (4.8)
T3	35.7 (4.2)	36.9 (4.6)	36.9 (4.0)	35.4 (5.1)
T4	36.8 (4.9)	37.4 (4.6)	36.7 (5.1)	37.8 (4.0)
T5	36.8 (4.6)	40.5 (3.1)	39.5 (4.0)	36.3 (4.6)
T6	37.8 (4.9)	39.6 (3.5)	38.7 (4.7)	38.7 (4.0)
Attitudes to pregnancy and the baby				
T1	32.4 (2.8)	33.7 (3.9)	33.1 (3.6)	32.8 (3.2)
T2	33.9 (3.4)	34.1 (4.0)	34.2 (3.8)	33.7 (3.7)
T3	33.3 (2.9)	34.3 (3.5)	34.0 (2.8)	33.5 (3.8)
T4	34.3 (2.5)	34.5 (3.0)	34.6 (2.5)	34.1 (3.2)
T5	34.3 (3.5)	35.0 (3.3)	34.9 (2.4)	33.7 (5.1)
T6	34.6 (3.2)	35.2 (3.0)	35.2 (2.7)	34.3 (3.6)
Marital relationship				
T1	38.8 (4.4)	37.4 (3.5)	37.8 (3.9)	38.4 (4.1)
T2	38.4 (4.2)	37.1 (3.6)	37.6 (3.6)	37.9 (4.4)
T3	38.5 (4.2)	36.4 (3.6)	37.8 (3.9)	37.0 (4.3)
T4	38.3 (4.2)	37.2 (4.5)	38.1 (3.8)	37.1 (5.3)
T5	36.4 (5.9)	37.8 (4.5)	37.6 (4.9)	36.0 (6.3)
T6	37.0 (4.6)	37.0 (4.0)	36.9 (4.5)	37.2 (4.2)

Note. STAI-T, State-Trait Anxiety Inventory – Trait; STAI-S, State-Trait Anxiety Inventory – State; EPDS, Edinburgh Postnatal Depression Scale. M, mean; SD, standard deviation; ART, assisted reproduction techniques. Data was collected at 13 (T1), 21 (T2) and 30 (T3) weeks of pregnancy and 1 (T4), 4 (T5) and 8 (T6) weeks after birth.

Positive and significant correlations within couples were found at baseline and over time indicating within-couple similarity in psychological adaptation, i.e., interdependence. At baseline, Pearson correlation between couples' scores were significant for anxiety, $r = 0.29$, $p = 0.01$, depression, $r = 0.46$, $p < 0.001$, marital relationship, $r = 0.26$, $p = 0.03$, attitudes to sex, $r = 0.31$, $p = 0.01$, and marginally significant for attitudes to pregnancy and the baby, $r = 0.23$, $p = 0.05$. Time-specific correlations of the residuals indicated that couples' anxiety, $r = 0.28$, $p < 0.001$, depression, $r = 0.23$, $p < 0.01$, marital relationship, $r = 0.18$, $p = 0.02$, attitudes to sex, $r = 0.28$, $p < 0.001$, and attitudes to pregnancy and the baby, $r = 0.23$, $p = 0.05$, scores were significantly correlated over time.

Tables 2 and 3 include detailed information on estimates, standard errors and effect sizes of variables for the piecewise growth curve models adjusted for twins' length of stay at NICU and mothers' parity. The statistical information of additional analyses and random effects is presented in the text.

Table 2. Piecewise Growth Models for Depressive and Anxiety Symptoms, Attitudes to Pregnancy and the Baby and Attitudes to Sex from Pregnancy to Postpartum.

	Anxiety			Depression			Attitudes to sex		
Fixed effects	*b*	*SE*	Effect size *r*	*b*	*SE*	Effect size *r*	*b*	*SE*	Effect size *r*
Intercept	32.77***	1.38		4.89***	0.53		37.99***	0.73	
Time pregnancy	-0.09	0.23	0.03	-0.14†	0.08	0.16	-0.28*	0.11	0.22
Pre-postnatal transition	3.90*	1.72	0.24	1.41*	0.56	0.25	1.43*	0.66	0.19
Time postpartum	-1.41*	0.62	0.19	-0.24	0.22	0.10	0.81**	0.28	0.24
Gender	-1.25	0.82	0.17	-0.72**	0.27	0.30	1.18**	0.38	0.37
Conception Mode	0.97	1.18	0.11	-0.12	0.45	0.04	-0.06	0.61	0.01
Gender x Conception Mode	0.47	0.82	0.07	0.15	0.27	0.07	0.35	0.38	0.12
Time pregnancy x Gender	0.14	0.22	0.06	-0.04	0.07	0.05	-0.11	0.09	0.10
Pre-postnatal transition x Gender	-1.72	1.27	0.11	-0.36	0.48	0.08	0.39	0.53	0.06
Time postpartum x Gender	0.14	0.60	0.02	-0.26	0.19	0.12	0.35	0.24	0.13
Time pregnancy x Conception Mode	-0.09	0.23	0.03	0.14†	0.08	0.15	-0.03	0.11	0.03
Pre-postnatal transition x Conception Mode	1.89	1.72	0.12	0.55	0.56	0.10	0.43	0.66	0.06
Time postpartum x Conception Mode	-0.20	0.62	0.03	-0.07	0.22	0.03	-0.55†	0.28	0.17
Time pregnancy x Gender x Mode	0.24	0.22	0.10	0.01	0.07	0.02	0.003	0.09	0.00
Pre-postnatal transition x Gender x Mode	-3.06*	1.27	0.19	-1.04*	0.48	0.21	-0.03	0.53	0.00
Time postpartum x Gender x Mode	1.24*	0.60	0.18	0.20	0.19	0.10	0.26	0.24	0.10

Random effects	Anxiety		Depression		Attitudes to sex	
Variances	Women	Men	Women	Men	Women	Men
Intercept	41.77**	47.84***	7.47***	5.10***	11.55***	13.46***
Slope	71.45**	42.94**	12.14***	3.55**	7.07*	2.29
Residual	40.55***	24.38***	4.22***	2.84***	6.82***	5.72***

Notes. Gender was coded as men = 1, women = -1; Conception mode was coded as ART = 1, spontaneous = -1; Effect size $r = \sqrt{(t^2/[t^2 + df])}$. Models are adjusted for twins' length of stay at NICU and mothers' parity. † $p < 0.10$ * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Table 3. Growth Models for Attitudes to Pregnancy and the Baby and Marital Relationship from Pregnancy to Postpartum.

Fixed effects	Attitudes to pregnancy and the baby			Marital relationship		
	b	*SE*	Effect size *r*	*b*	*SE*	Effect size *r*
Intercept	33.14***	0.57		38.37***	0.67	
Time	0.17**	0.05	0.45	-0.19*	0.07	0.38
Gender	0.41	0.33	0.19	-0.88*	0.36	0.36
Conception Mode	0.14	0.50	0.04	0.09	0.56	0.03
Gender x Conception Mode	-0.04	0.33	0.02	0.02	0.36	0.01
Time x Gender	-0.03	0.04	0.11	0.11†	0.06	0.27
Time x Conception Mode	-0.10†	0.05	0.29	-0.15†	0.07	0.30
Time x Gender x Conception Mode	0.01	0.04	0.04	0.04	0.06	0.11
Random effects						
Variances	Women		Men	Women		Men
Intercept	6.62**		15.19***	15.89***		8.15**
Slope	0.08*		0.13**	0.28**		0.10†
Residual	3.59***		2.53***	4.90***		5.78***

Notes. Gender was coded as men = 1, women = -1; Conception mode was coded as ART = 1, spontaneous = -1. Effect size $r = \sqrt{t^2 / [t^2 + df]}$.
Models are adjusted for twins' length of stay at NICU and mothers' parity.
† $p < 0.10$ * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

A significant main effect of gender was found for depression, marital relationship and attitudes to sex. Women had higher depression scores, less positive attitudes to sex, and a more positive perception of their marital relationship than men at baseline.

Marginally significant two-way interactions were found between mode of conception and time for marital relationship, attitudes to pregnancy and the baby and attitudes to sex. Tests of simple slopes revealed that ART parents reported a decline in marital relationship over time, $b = -0.25$, $p = 0.02$, $r = 0.36$, no changes in attitudes to pregnancy and the baby over time, $p > 0.10$, and a slow improvement in attitudes to sex over postpartum, $b = 0.65$, $p = 0.09$, $r = 0.13$. In contrast, SC parents reported no changes in marital relationship over time, $p > 0.10$, more positive attitudes to pregnancy and the baby over time, $b = 0.27$, $p < 0.001$, $r = 0.58$, and more positive attitudes to sex over postpartum, $b = 1.40$, $p < 0.001$, $r = 0.35$. A marginally significant two-way interaction was also found between gender and time for marital relationship indicating that women reported a decline in marital relationship over time, $b = -0.24$, $p = 0.03$, $r = 0.34$, while men showed no changes in marital relationship over time, $p > .10$.

Significant three-way interactions were found between mode of conception, gender and time for anxiety (see Figure 1) and depression. Tests of simple slopes revealed that ART women showed a significant increase in both anxiety, $b = 10.57$, $p = 0.01$, $r = 0.16$, and depression, $b = 3.35$, $p = 0.02$, $r = 0.16$, scores from pregnancy to the postpartum period. Smaller but significant increases in anxiety scores, $b = 1.55$, $p = 0.04$, $r = 0.13$, were also noted for SC men from pregnancy to the postpartum period. During the postpartum period, ART women showed a decline trend in anxiety scores, $b = -2.99$, $p = 0.05$, $r = 0.12$, and no changes in depression scores, $p > 0.10$, whereas a significant decrease in anxiety, $b = -2.30$, $p = 0.02$, $r = 0.13$, and a decline trend in depression scores, $b = -0.63$, $p = 0.05$, $r = 0.12$, was observed among SC men. Anxiety or depression scores remained unchanged for ART men and SC women from pregnancy to the postpartum period and during the postpartum period, all p's > 0.10. Compared with the other groups, ART women had a higher increase in anxiety, $p = 0.03$, and depression scores, $p = 0.07$, from pregnancy to the postpartum period.

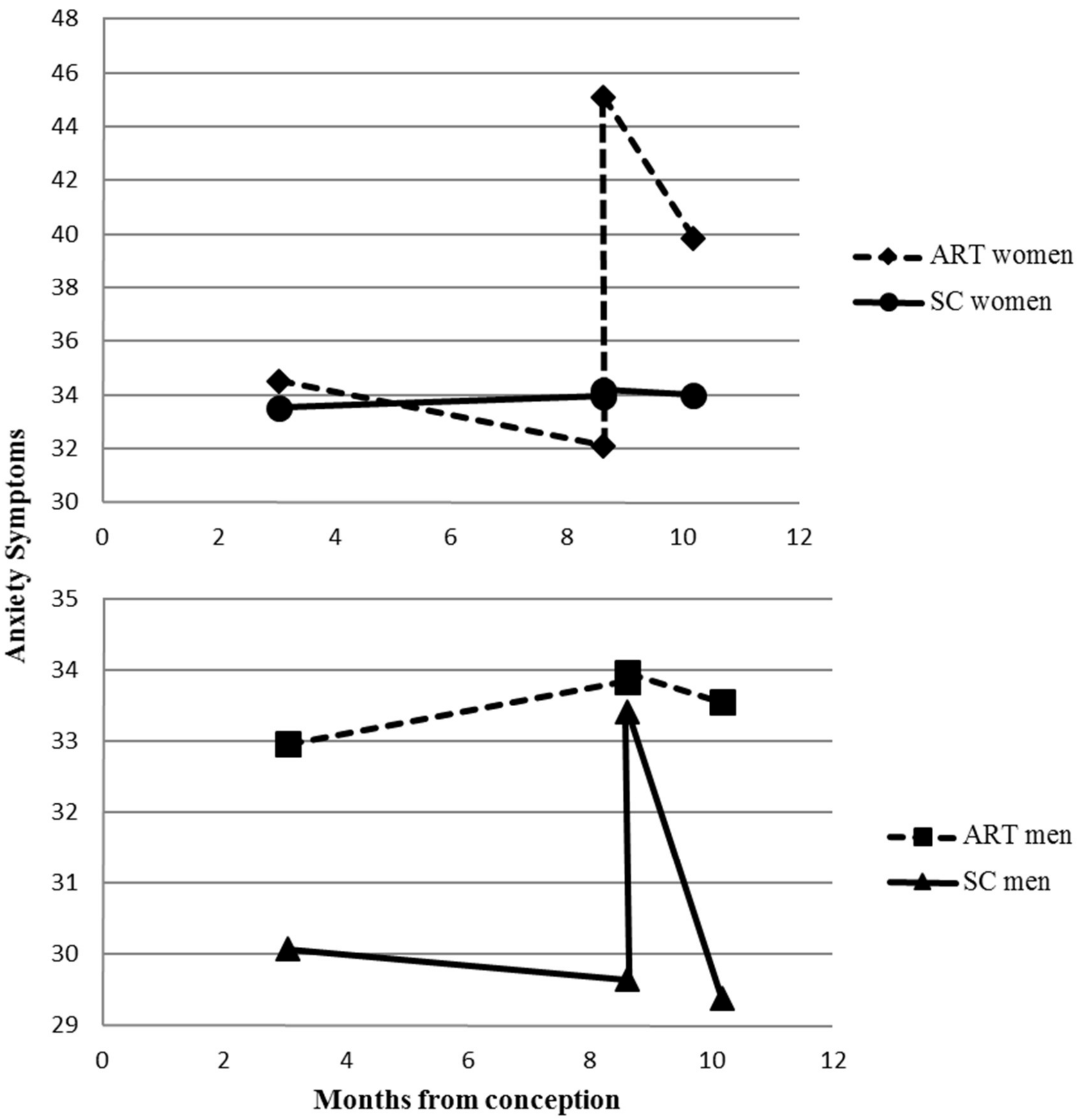

Figure 1. Mean predicted anxiety scores from pregnancy to the postpartum period for parents of twins by mode of conception and parents' gender. ART, assisted reproduction technology; SC, spontaneous conception.

Discussion

The results of this study show that both mode of conception and parents' gender are associated with parents' psychological adjustment across the transition to twin parenthood. Consistent with our hypothesis, ART parents in general and ART mothers in particular showed poor psychological adjustment over the transition to twin parenthood.

ART parents reported a decline in marital relationship quality, no changes in attitudes to pregnancy and the baby and no changes in attitudes to sex over the postpartum. In contrast, SC parents did not change their perception of the marital relationship, reported more positive attitudes to pregnancy and the baby and more positive attitudes to sex over the postpartum. These results suggest that ART couples experience interpersonal difficulties during the transition to twin parenthood that could result from several factors, including accumulating stress. Baor and Soskolne [10] also found that ART mothers of twins report lower marital quality, as well as lower self-efficacy and social support than mothers of SC twins at late pregnancy.

Given that a large proportion of couples undergoing fertility treatments desire having twins [22], it is surprising that ART parents' attitudes to pregnancy and the baby remained stable, whereas SC parents reported more positive attitudes to pregnancy and the baby over time. However, research with singletons showed that formerly infertile patients tend to experience increased anxiety about the security of the pregnancy and fetal survival [13]. We can speculate that these anxieties are higher in case of ART twin pregnancy which can ultimately influence the attitudes to pregnancy and the baby. Because the literature on the psychological adjustment in ART and non-ART parents of twins is scarce, the obtained results still need further exploration.

Compared with the other groups, ART mothers exhibited a significantly higher increase in anxiety and depressive symptoms from pregnancy to the postpartum period and only anxiety levels exhibited a decline trend over the postpartum. Similar results were obtained even when multiparous SC parents were excluded from the analyses. Our results are consistent with previous studies with mothers of twins showing that ART mothers have lower psychosocial well-being than SC mothers [9-11]. These adjustment difficulties may be due to a cumulative negative consequence of the prior experience of infertility and fertility treatment [13]. As previously noted, ART parents are a heterogeneous group regarding socio-demographic characteristics [13], namely because funding policies for fertility treatments vary significantly across countries. In Portugal, ART procedures provided in public clinics/hospitals are fully reimbursed, whereas medications are only partially reimbursed (40%). In the present study, most parents had medium socio-economic level and medium educational level and no differences have been found between ART and SC groups.

At the couple level, women and men showed similar results at each time period as demonstrated by the correlations at baseline and over time. These results show that couples' psychological adjustment tends to covary over the transition to twin parenthood. A previous study with singletons has shown that couples' psychological adjustment is significantly correlated across the transition to parenthood [23].

This study brings several clinical and developmental contributions. The longitudinal design starting at early pregnancy and comprising multiple time points covering pregnancy and the neonatal period provided detailed information on changes over time. This information can be helpful for tailoring interventions to the specific needs of parents during the transition to parenthood. In addition, a broad understanding of the psychological adjustment process was gained with the inclusion of measures (marital relationship and pregnancy and child-related attitudes) other than psychological symptoms. Our results also showed that women's and men's psychological adjustment is interdependent. Therefore, couple-based interventions may have better results for both women and men than those directed at the individual level. These interventions may be helpful for preventing and treating depression and anxiety, especially for ART mothers.

However, the current study has also limitations that need to be acknowledged. First, the small sample size, especially of the ART group, warrants cautious interpretation and generalization of the results. Nevertheless, effect sizes indicate that the identified differences are small to moderate. Second, the SC group included first- and second- time parents, whereas the ART group included only first-time parents and because of this estimates were adjusted for parity. Third, self-report measures may have increased the reporting bias.

Further research is needed to investigate whether the identified early postpartum adjustment difficulties are transient or persist over time. In addition, future studies should examine the contribution of declining marital relationship reported by ART couples to the increased anxiety and depressive symptoms during postpartum noted in ART women.

In conclusion, the findings of the current study suggest that ART couples may experience more adjustment difficulties during the transition to twin parenthood than SC couples, especially ART mothers. Although the sample size for the ART group was small, poorer psychological adjustment than the SC group was consistently observed.

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

PAPER III

PARENTS ANXIETY AND DEPRESSION SYMPTOMS AFTER SUCCESSFUL INFERTILITY TREATMENT AND SPONTANEOUS CONCEPTION: DOES SINGLETON/ TWIN PREGNANCY MATTER?

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The version of record Tendais, I., & Figueiredo, B. (2016). Parents anxiety and depression symptoms after successful infertility treatment and spontaneous conception: does singleton/ twin pregnancy matter? *Human Reproduction*, *31*(10), 2303-2312. doi: 10.1093/humrep/dew212 is available online at: http://humrep.oxfordjournals.org.sci-hub.cc/content/31/10/2303.full.pdf+html.

Title: Parents' anxiety and depression symptoms after successful infertility treatment and spontaneous conception: does singleton/ twin pregnancy matter?

Running title: Psychological adjustment to parenthood

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Running title: PSYCHOLOGICAL ADJUSTMENT TO PARENTHOOD

Abstract

Study question: Does mode of conception (spontaneous/ after infertility treatment), type of pregnancy (singleton/ twin) and parent gender have an effect on anxiety and depression levels and trajectories during pregnancy and the postpartum period?

Summary answer: Conception after infertility treatment (IT) was associated with a transitory increase in anxiety during the perinatal period for parents of singletons, while for IT parents of twins higher levels of psychopathological symptoms tended to persist during pregnancy and the postpartum period.

What is known already: Most previous studies have shown that successful IT is not associated with poor psychological well-being during pregnancy and the postpartum period, but there is also some evidence for heightened pregnancy-related anxiety, lower self-esteem and lower self-efficacy. Parents of twins, experience increased postnatal anxiety and depression.

Study design, size, duration: This prospective longitudinal study assessed 267 couples (N= 534) at each trimester of pregnancy, after childbirth and at 3 months postpartum.

Participants/materials, setting, methods: The sample comprised 36 couples who had conceived after IT (19 twin pairs and 17 singletons) and 231 couples who had conceived spontaneously (28 twin pairs and 203 singletons). Couples were recruited at four public hospitals in Portugal, and self-report measures of anxiety and depression symptoms were administered.

Main results and the role of chance: IT parents reported higher anxiety after childbirth than parents who conceived spontaneously (SC), regardless of pregnancy type. IT parents of twins showed higher anxiety at mid-pregnancy, as well as higher anxiety and depression at 3 months postpartum than IT parents of singletons. Among IT mothers, those who had twins exhibited higher depression after childbirth than those who had singletons. Differences according to mode of conception, pregnancy type and parents gender over time were also noted. During pregnancy, IT parents of twins showed no significant change in depression scores, while the other groups depression scores statistically significantly decreased over time. From pregnancy to the postpartum period, 1) IT parents showed an increase in anxiety scores, whereas SC parents exhibited no changes in anxiety scores; 2) IT women exhibited an increase in depression scores, while SC women depression scores decreased. During the postpartum period, IT and SC parents of twins showed no changes in anxiety scores, while IT and SC parents of singletons anxiety scores declined.

Limitations, reasons for caution: Due to the small number of IT couples, the interpretation and generalization of the results should be done with caution.

Wider implications of the findings: The adverse impact of IT on psychopathological symptoms depends mostly on time and type of pregnancy, and is greater for twin pregnancies. These findings are important for tailoring interventions that address parents' specific needs at different moments.

Study funding/competing interest(s): This work was supported by a grant (SFRH/BD/40146/2007) to the first author from the FCT, Foundation for Science and Technology (Portuguese Ministry of Education and Science), by the Operational Program Science and Innovation 2010 (POCI 2010) of the Community Support Board III, by FEDER Funds through the Operational Competitiveness Programme, COMPETE, and through national funding from the FCT under the projects: POCI/SAU-ESP/ 56397/2004 and PTDC/SAU/SAP/116738/2010. The authors have no conflicts of interest.

Key words: twins / depression / anxiety / infertility treatment / couples / longitudinal / dyadic growth models

Introduction

Evidence concerning parents' psychological well-being after successful infertility treatment (IT) is mixed (Hammarberg *et al.*, 2008). Most studies have shown that IT parents report similar or lower levels of anxiety and depression than controls who conceived spontaneously (SC) (Baor *et al.*, 2004; Cohen *et al.*, 2001; Colpin *et al.*, 1999; Gressier *et al.,* 2015; Jongbloed-Pereboom *et al.*, 2012; Klock and Greenfeld, 2000; Repokari *et al.*, 2005; Vilska *et al.*, 2009). Nevertheless, some studies have found that women who conceive after IT display higher levels of anxiety and depression during late pregnancy and after childbirth (McMahon *et al.*, 1997; Monti *et al.*, 2008, 2009), higher pregnancy-focused anxiety (Hjelmstedt *et al.*, 2003; McMahon *et al.*, 1997, 2011, 2013), lower self-efficacy and self-esteem (Gibson *et al.*, 2000) during the postpartum period than SC women. Previous findings have also indicated that IT parents are more likely to underreport negative affect (McMahon *et al.*, 2003) as they may feel less entitled to complain about the difficulties experienced (Hammarberg *et al.*, 2009).

Parents of twins display similar or higher levels of prenatal anxiety and depression (Jahangiri *et al.*, 2011; Vilska *et al.*, 2009) than parents of singletons. Evidence from studies with large samples suggests that parents of young twins (≤ 5 years old) show increased levels of anxiety and depression (Ellison *et al.*, 2005; Olivennes *et al.*, 2005; Thorpe *et al.*, 1991; Vilska *et al.*, 2009).

Most studies on multiple births do not control for IT and vice versa, and this is problematic because there is an association between the use of IT and multiple births (Ross *et al.*, 2011). It is important to examine the independent and the combined effects of IT and multiple birth on parents' psychological well-being. Results from a previous study suggest that twin parenthood, but not IT, is associated with poor psychological well-being during the postpartum period both for mothers and fathers (Vilska et al., 2009). Even though this study followed couples from mid-pregnancy to 1-year postpartum, no longitudinal data analysis was described.

The purpose of this study was to examine the effects of mode of conception, type of pregnancy and parent gender on anxiety and depression levels at each trimester of pregnancy, childbirth and 3 months postpartum. The effects of these factors on anxiety and depression symptoms trajectories over time were also examined.

Materials and methods

Participants

Consecutive sampling of couples with less than 15-week twin pregnancies was carried out. Couples were invited by the researchers to participate in a prospective longitudinal study at the antenatal Obstetrical Units of three public hospitals in Northern Portugal. Couples who did not speak Portuguese were excluded. Of the 55 couples contacted, 51 agreed to participate in the study (92.7%). Four of these couples were excluded due to death of one or both twins during the study period. Therefore, data from 47 couples was available for analysis.

Couples with singletons were derived from a larger prospective longitudinal study conducted in an Obstetrics Out-patients Unit in Oporto, Portugal. Mothers and fathers of at least 18 years of age who completed socio-demographic and clinical measures at baseline were selected. Detailed information on this study design is described elsewhere (Figueiredo and Conde, 2011).

Procedure

The Ethics Committee of the participating hospitals gave their approval for the study. The aims and procedures were explained to couples attending routine antenatal care appointments at 8-14 weeks of pregnancy and those who were willing to participate provided written informed consent. Information on parents' anxiety and depression symptoms was collected at each trimester of pregnancy (T1, 8-15 weeks; T2, 20-24 weeks;T3, 28-34 weeks) in the clinic, during hospital stay after childbirth (T4) and at 3 months postpartum (T5) during a home visit. Socio-demographic and clinical information of mothers and fathers was collected at T1 and T4. Mothers and fathers completed the paper-based measures separately.

Measures

Socio-demographic, clinical and perinatal data

Information on parents' age, ethnicity, parity, occupational and marital status, education level, history of infertility, mode of conception, pregnancy type, duration of pregnancy, medical complications, type of childbirth and singletons'/ twins' birth weight was collected by self-administered questionnaires.

Anxiety symptoms

The 20-item state subscale of the State-Trait Anxiety Inventory Form Y (STAI, Spielberger *et al.*, 1983) is a screening instrument used to measure current anxiety symptoms. Items are scored on a four-point Likert scale (1-4). The scores range from 20 to 80, with higher scores indicating higher anxiety. STAI Portuguese version has shown excellent internal consistency and high criterion validity (Tendais *et al.*, 2014). In this study, STAI was used at each time point and Cronbach's alphas ranged from .92 and .95 for women and .91 and .95 for men. A score of 40 or higher was considered clinically significant.

Depression symptoms

The Edinburgh Postnatal Depression Scale (EPDS, Cox *et al.*, 1987) is a self-report questionnaire composed of 10 items addressing depression symptoms within the previous seven days. The scores range from 0 to 30, with higher scores indicating more severe depression symptoms. The Portuguese version of this screening tool has shown good internal consistency and reasonable criterion validity (Tendais *et al.*, 2014). In the present study, EPDS was used at each time point and Cronbach's alphas ranged from .77 and .85 for women and .72 and .79 for men. A score of 12 or higher was considered clinically significant.

Data Analysis

Multilevel modeling was used to assess the effect of mode of conception, type of pregnancy and parent gender and their interactions on anxiety and depression symptoms at each time point while controlling for age, parity and couples' interdependence. Pairwise comparisons were performed when the interactions were significant. One-sided Fisher's Exact Test was also used to test the association between group membership and high anxiety (STAI $\geq$ 40) or depression symptoms (EPDS $\geq$ 12) when significant between-group mean differences were found.

Examination of mean scores over time revealed a linear change in anxiety and depression scores during pregnancy and the postpartum period. However, a significant increase was observed for some groups from T3 to T4. To capture this discontinuity, piecewise dyadic growth models were estimated using multilevel modeling to assess changes in anxiety and depression levels from the first trimester of pregnancy (T1) to the first assessment postpartum (T4), from pregnancy to postpartum (pre-postnatal transition) and from childbirth (T4) to 3 months postpartum (T5). In these models, observation is level 1 and couple is level 2. Time 0 was defined at T1 and time was scored in months. Model selection is described in Supplementary Methods.

Data was analyzed with IBM SPSS 23 Windows version (PASW Statistics for Windows, SPSS Inc, Chicago). *Post hoc* power calculations showed that the attained sample size permitted the detection of medium to large effects (effect size $f^2 = 0.18$) in full models with all predictors (power > 0.80, G*Power, Faul *et al.*, 2007).

Results

Participants' characteristics

The sample included 267 couples, 36 who had conceived after IT (19 with twins and 17 with singletons) and 231 who had SC (28 with twins and 203 with singletons) (Supplementary Fig. 1). In the IT group, 12 conceived by intracytoplasmic sperm injection (ICSI), 11 by *in vitro* fertilization (IVF), eight after ovulation induction (OI), four by artificial insemination (AI) and one after surgery. All couples in this group had previous infertility.

On average, participants were 30.0 years old (SD = 5.6, range 18-46 years), the majority were Caucasian (95.3%), married (62.8%), employed (86.1%), primiparous (56.4%), had < 12 years of education (60.5%), and had medium to medium-low socioeconomic level (53.5%). The mean gestational age was 38.4 weeks (SD = 2.1, range 27.7-42.0). IT couples had a mean duration of infertility of 3.6 years (SD = 2.7, range 0-10.0).

Socio-demographic and perinatal characteristics according to mode of conception and pregnancy type for mothers and fathers are presented in Table I. No differences were found for marital status, education level, occupational status and socio-economic level. No differences were found for infertility duration between those who delivered singletons and twins. However, there were significant differences in parity for both mothers and fathers between the 4 groups and in age for mothers whilst for fathers differences in age were close to statistical significance. Further analysis showed that IT mothers of twins were more likely to be older (≥ 30 years of age) than SC mothers of singletons or twins (both *P*'s = 0.01). IT mothers and fathers of twins were more likely to be primiparous than SC mothers and fathers of singletons (both *P*'s = 0.05).

One-hundred and eighty nine couples (70.8%) completed all questionnaires. No differences were found between IT and SC parents on full response rate (75.0% versus 70.1%, $P > 0.10$). Additional analysis showed that parents of twins had a lower response rate than parents of singletons [$\chi^2_{(1,267)} = 6.60$, $P < 0.001$].

Anxiety

a) at each pregnancy trimester, childbirth and 3 months postpartum

Mean anxiety scores at each time point for the different groups are displayed in Table II. The prevalence of anxiety is depicted in Table III.

During pregnancy and the postpartum period, significant mode of conception x pregnancy type interactions emerged. At T2 and T5, IT parents of twins showed higher anxiety scores than IT parents of singletons and were also more likely to score above the cut-off (all *P's* < 0.05). Among SC parents, no differences were found between parents of singletons and twins (both *P's* > 0.05). No significant differences were found between IT and SC parents of twins (both *P's* > 0.05). At T3, no significant difference was found between IT parents of twins and singletons ($P > 0.05$).

At T4, mode of conception and parent gender were significantly related with anxiety. IT parents reported higher scores than SC parents and were also more likely to score above the cut-off ($P < 0.001$). Women had higher scores than men, but were not more likely to score above the cut-off than men ($P > 0.05$).

b) trajectories over time

As shown in Table IV, a significant mode of conception x pregnancy type interaction predicted change in anxiety from pregnancy to the postpartum period and over the postpartum period. IT parents of singletons had a higher increase in anxiety from pregnancy to the postpartum period than IT parents of twins (both P's < 0.001), while SC parents of singletons and twins showed a non-significant increase (both P's > 0.05; Fig. 1). Over the postpartum period, IT parents of singletons showed a steeper decrease in anxiety than SC parents of singletons (both P's < 0.001), whereas no significant changes were found for IT and SC parents of twins (both P's > 0.05; Fig. 1). Gender also predicted change in depression during the postpartum period, with women reporting greater decreases than men.

After accounting for the predictors in the final model, couples' residuals were positively correlated ($r = 0.14$, $Z = 3.13$, $P < 0.001$) suggesting that if one member of the couple was more anxious at a particular time point, the other tended to be more anxious at that time. The final model explained 63.3% and 53.1% of the variance of change for mothers and fathers, respectively.

Depression

a) at each pregnancy trimester, childbirth and 3 months postpartum

Mean depression scores at each time point for the different groups are displayed in Table II. The prevalence of clinically significant depression is depicted in Table III.

Although women presented higher depression scores than men at each pregnancy trimester, there was a similar proportion of women and men scoring above the cut-off (all P's > 0.05).

After childbirth (T4), a significant interaction effect was found between conception mode, type of pregnancy and parent gender. IT mothers of twins showed higher depression scores than IT mothers of singletons and were also more likely to score above the cut-off (both *P's* = 0.01). No differences were found between mothers of SC singletons and twins ($P > 0.05$) or between IT and SC fathers of singletons and twins (both *P's* > 0.05). IT mothers of twins exhibited also higher depression scores than SC parents of twins ($P = 0.001$), but the proportion scoring above the cut-off in both groups was similar ($P > 0.05$).

At T5, a significant mode of conception x pregnancy type interaction emerged. IT parents of twins showed higher depression scores than IT parents of singletons and SC parents of twins and were also more likely to score above the cut-off (all *P's* < 0.05). In contrast, no difference was found between SC parents of singletons and twins ($P > 0.05$). A significant pregnancy type x parent gender interaction was also found. Mothers of twins exhibited higher depression scores than mothers of singletons ($P = 0.01$), even though the proportion scoring above the cut-off in both groups was similar ($P > 0.05$). As for fathers, no difference was found between those who had singletons and twins ($P > 0.05$).

b) trajectories over time

As shown in Table IV, the predicted depression score decreased during pregnancy. This trend was statistically significant ($P < 0.05$) in all groups except the IT parents of twins (Fig. 2).

A significant mode of conception x parent gender interaction predicted change in depression from pregnancy to the postpartum period, such that IT mothers showed a statistically significant increase in depression from pregnancy to the postpartum period (*P*'s < 0.001), while the other groups showed a non-significant increase (all *P*'s > 0.05) (Fig. 3).

Although all groups showed a decrease in depression scores during the postpartum period, IT parents decreased more quickly than SC parents. A significant pregnancy type x parent gender interaction also predicted change in depression over the postpartum, such that mothers of singletons decreased ($P < 0.001$), whereas no significant changes were found for the other groups (both P's > 0.05).

After accounting for the predictors in the final model, couples' residuals were positively correlated ($r = 0.16$, $Z = 3.70$, $P < 0.001$) indicating that if one member of the couple was more depressed at a particular time point, the other tended to be more depressed at that time. The variance of change of depression explained by the final model was 64.1% for mothers and 60.9% for fathers.

Discussion

This prospective longitudinal study examined the effect of mode of conception, pregnancy type and parent gender on anxiety and depression levels and trajectories during pregnancy and the postpartum period. We observed that the effect of IT on parents' psychopathological symptoms depends mostly on time and type of pregnancy. IT parents of singletons exhibited no increased risk of clinically significant anxiety and depression during pregnancy and the postpartum period when compared to spontaneous controls (data not shown), except after childbirth. In contrast, IT parents of twins appear to be at higher risk for clinically significant psychopathological symptoms during pregnancy and the postpartum period than IT parents of singletons, as well as at higher risk for postnatal depression than SC parents of twins.

Our findings suggest that IT parents, regardless of pregnancy type, may experience more adjustment difficulties to the perinatal period than SC parents as they experience an increase in anxiety from pregnancy to the postpartum and higher anxiety than SC parents after childbirth. Monti *et al.* (2008) also found a significant increase in manifest anxiety among IT mothers both during the third trimester of pregnancy and 1 week after childbirth. Previous research has shown that IT mothers present more child-related worries than SC mothers (McMahon *et al.*, 2011), namely, about the survival, normality and well-being of the baby, and separation from the child after birth (McMahon *et al.*, 1997). IT parents are likely to be confronted with most of their fears after childbirth and these feelings may increase their anxiety. An increase in levels of depression from pregnancy to the postpartum period was observed among IT mothers, whereas SC mothers, IT and SC fathers showed smaller, statistically non-significant changes. Our findings confirm that changes in depression levels from pregnancy to postpartum are moderated by mode of conception (Hammarberg *et al.*, 2008). and gender (Repokari *et al.* 2005). Previous studies have shown that IT mothers report less confidence in parental competence than SC mothers (Gibson et al., 2000), and this may have a negative effect on mood.

Our longitudinal study provides evidence for the combined effects of IT and twin pregnancy on anxiety and depression levels and trajectories over time. IT parents of twins experienced higher anxiety than IT parents of singletons during pregnancy and the postpartum period. In addition, IT parents of twins exhibited higher depression than IT parents of singletons and SC parents of twins during the postpartum period. Our results are consistent with previous research showing that primiparous IT mothers of 9-12-month-old twins report lower psychological well-being than primiparous mothers of SC twins (Baor *et al.*, 2004; Colpin *et al.*, 1999). In contrast, Vilska *et al.* (2009) found no interaction effects between IT and type of pregnancy on mothers' and fathers' well-being during pregnancy and the postpartum period. Ross *et al.*'s (2011) acknowledged that IT multiple birth is associated with increased risk for postpartum maternal depression. With regard to change over time, even though IT parents of singletons experienced a higher increase in anxiety than IT parents of twins from pregnancy to the postpartum period, anxiety levels of IT parents of singletons decreased over the postpartum period, while those reported by IT parents of twins remained stable. In contrast to the results of Jahangiri *et al.* (2011), we found that depression levels of parents declined significantly during pregnancy except for IT parents of twins, for whom the apparent trend was less steep and failed to achieve statistical significance. While expectations of parents of twins are focused on two infants, those of parents of singletons are concentrated on one child only. If the most feared thoughts related to childbirth injury and the well-being of the child would have been confirmed, the investment would have been lost. For most parents, these worries were not confirmed, and their anxiety level decreased sharply. However, for IT parents of twins, these worries are likely to have been followed by concerns regarding caring for two new-born infants (Jahangiri *et al.*, 2011), a decreased ability to provide each child with warmth and reciprocity (Feldman *et al.*, 2004), feelings of low self-efficacy and self-esteem (Gibson *et al.*, 2000).

This study has several limitations that need to be considered. Due to the small number of couples in the IT group, the interpretation and generalization of the results should be done with caution. This number was insufficient to compare the psychological well-being between those who conceived with IT (IVF, ICSI) and those who conceived with other methods (OI, AI). Because IT parents tend to underreport negative affect (McMahon *et al.*, 2003), the use of self-reported measures may have increased the reporting bias in the IT group. In addition, we were unable to distinguish the effects of previous infertility and use of IT on parents' well-being. Finally, in this study the extension of the trajectory during pregnancy to childbirth included in the model described in Table IV is an extrapolation and not a measurement. This extrapolation ignores the possibility that anxiety / depression may increase in the last weeks of pregnancy as birth becomes imminent.

Despite these limitations, the present study also has several important strengths. The inclusion of four different groups (parents of singletons and twins conceived spontaneously and after IT), into a prospective longitudinal design allowed us to study between-person differences in within-person change. The study yielded important and novel information about the effect of mode of conception on psychopathological symptoms over time according to the type of pregnancy. To date, little was known about the changes in psychological well-being experienced by these groups during the transition to parenthood. It also notes the specific time periods in which parents experience more psychopathological symptoms and extends previous research by assessing their clinical significance. In addition, by including both mothers and fathers, we were able to confirm that couples' psychological well-being is interdependent (Galhardo *et al.,* 2016).

Our findings underscore the need to provide special support to IT parents of twins to ensure the early detection and provision of psychosocial or specialized mental health care services to those experiencing poor psychological well-being. It should be noted that parents' depression and anxiety symptoms have been consistently associated with adverse outcomes on the fetus, neonate and child, including early emotional regulation and social problems and impairments in child cognitive development (Ross *et al.*, 2011; Stein *et al.*, 2014).

In conclusion, our results suggest that for those who conceived after IT, a twin pregnancy appears to be associated with more psychopathological symptoms over time. In contrast, the negative effects of IT on parents of singletons appear to be limited to increased anxiety during the perinatal period. These findings are important to the debate on the number of embryos for transfer.

Authors' roles

I.T. and B.F. designed the study. I.T. collected the data, undertook the statistical analysis interpreted the results and wrote the first draft of the manuscript. Both authors contributed to and have approved the final manuscript.

Acknowledgements

We thank Dr[a] Dulcina Ramada and Prof. Nuno Montenegro for their institutional support for this study. We also thank the families who collaborated in this study.

Funding statement:

This work was supported by a grant (SFRH/BD/40146/2007) to the first author from the FCT, Foundation for Science and Technology (Portuguese Ministry of Education and Science), by the Operational Program Science and Innovation 2010 (POCI 2010) of the Community Support Board III, by FEDER Funds through the Operational Competitiveness Programme, COMPETE, and through national funding from the FCT under the projects: POCI/SAU-ESP/ 56397/2004 and PTDC/SAU/SAP/116738/2010.

Conflict of interest

None to declare.

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Figure legends

Figure 1: Predicted anxiety (State-Trait Anxiety Inventory Form Y; STAI) scores in each trimester of pregnancy (T1-T3), the day of delivery (T4) and 3 months later (T5) as a function of mode of conception (infertility treatment [IT] versus spontaneous conception [SC]) and pregnancy type.

IT parents of singletons and IT parents of twins showed a significant increase in anxiety at T4 (both P's < 0.001), while the other groups showed a non-significant increase (P's > 0.05). Over the postpartum period, IT and SC parents of singletons showed a steep decrease in anxiety (both P's < 0.001), whereas the other groups showed a non-significant change (P's > 0.05).

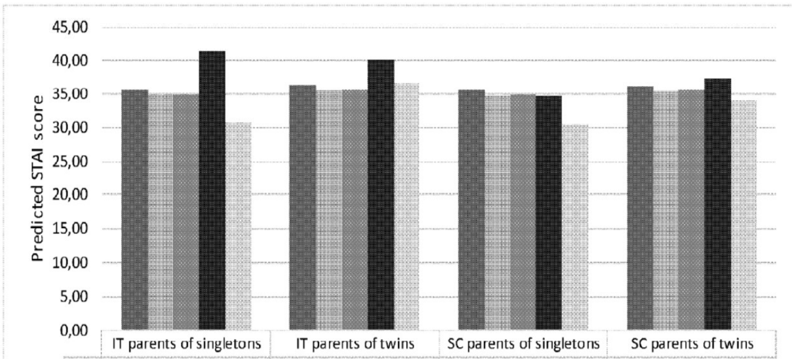
Figure 2: Predicted depression (Edinburgh Postnatal Depression Scale; EPDS) scores in each trimester of pregnancy (T1-T3), the day of delivery (T4) and 3 months later (T5) as a function of mode of conception (infertility treatment [IT] versus spontaneous conception [SC]) and pregnancy type.

All groups showed a significant decrease in depression scores during pregnancy (all P's < 0.05) except the parents of twins conceived after infertility treatment (IT parents of twins).

Figure 3: Predicted depression (Edinburgh Postnatal Depression Scale; EPDS) scores in each trimester of pregnancy (T1-T3), the day of delivery (T4) and 3 months later (T5) according to conception mode (infertility treatment [IT] versus spontaneous conception [SC]) and parent gender.

Mothers who conceived after infertility treatment (IT mothers) showed a significant increase in depression at T4 (*P*'s < 0.001), while the other groups showed a non-significant increase (*P*'s > 0.05).

Supplementary Figure 1: Flowchart with numbers and percentages of participants at each trimester of pregnancy (T1-T3), after childbirth (T4) and at 3 months postpartum (T5) according to mode of conception (spontaneous [SC] versus after infertility treatment [IT]) and type of pregnancy (singleton/ twin).



	IT parents of singletons	IT parents of twins	SC parents of singletons	SC parents of twins
T1	35,68	36,37	35,52	36,20
T2	34,91	35,60	34,75	35,44
T3	34,99	35,68	34,83	35,51
T4	41,48	40,12	34,62	37,36
T5	30,69	36,68	30,62	34,02

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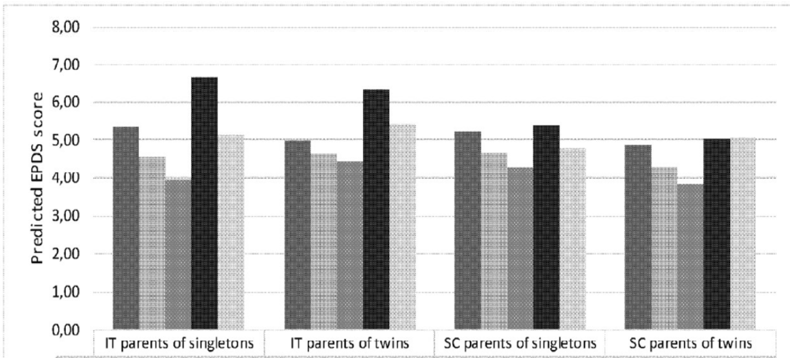

	IT parents of singletons	IT parents of twins	SC parents of singletons	SC parents of twins
T1	5,34	5,00	5,22	4,89
T2	4,57	4,64	4,68	4,29
T3	3,92	4,44	4,29	3,84
T4	6,67	6,34	5,37	5,03
T5	5,17	5,42	4,81	5,06

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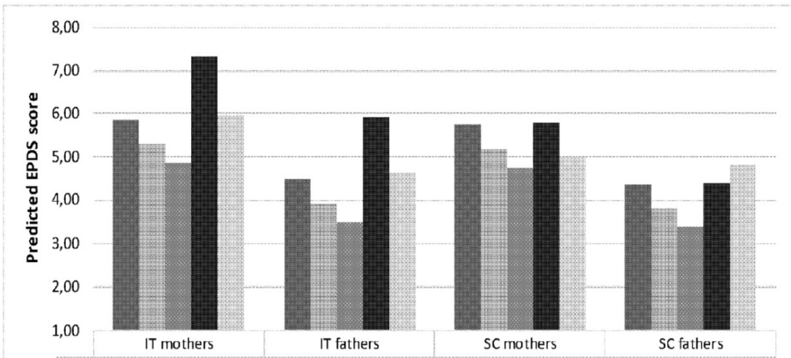


	IT mothers	IT fathers	SC mothers	SC fathers
T1	5,86	4,48	5,74	4,36
T2	5,30	3,91	5,18	3,80
T3	4,87	3,49	4,76	3,37
T4	7,31	5,93	5,78	4,39
T5	5,97	4,62	5,03	4,84

160x90mm (300 x 300 DPI)

Table I Comparison of socio-demographic and perinatal characteristics according to mode of conception (Spontaneous [SC] n=231 or Infertility treatment [IT] n=36 couples) and pregnancy type for mothers and fathers.

	Mothers				χ^2	Fathers				χ^2
	SC		IT			SC		IT		
	Singletons *n* (%)	Twins *n* (%)	Singletons *n* (%)	Twins *n* (%)		Singletons *n* (%)	Twins *n* (%)	Singletons *n* (%)	Twins *n* (%)	
Age (years)					7.93 *					7.72 †
< 30	115 (56.7)	18 (64.3)	8 (47)	5 (26)		83 (40.9)	11 (39.3)	3 (18)	3 (16)	
≥ 30	88 (43.3)	10 (35.7)	9 (53)	14 (74)		120 (59.1)	17 (60.7)	14 (82)	16 (84)	
Education (years)					1.56					2.67
< 12	113 (55.7)	13 (46.4)	11 (65)	10 (53)		138 (68.0)	16 (57.1)	9 (53)	13 (68)	
≥ 12	90 (44.3)	15 (53.6)	6 (35.3)	9 (47.4)		65 (32.0)	12 (42.9)	8 (47)	6 (32)	
Parity					9.30 *					11.08 *
Primiparous	104 (51.2)	18 (64.3)	11 (65)	16 (84)		106 (52.2)	18 (64.3)	11 (65)	17 (90)	
Multiparous	99 (48.8)	10 (35.7)	6 (35)	3 (16)		97 (47.8)	10 (35.7)	6 (35)	2 (11)	
Employed					0.15					0.26
No	158 (77.8)	21 (75.0)	13 (77)	15 (79)		193 (95.1)	26 (92.9)	16 (94)	18 (95)	
Yes	45 (22.2)	7 (25.0)	4 (24)	4 (21)		10 (4.9)	2 (7.1)	1 (6)	1 (5)	
Cohabiting					3.48					
No	18 (8.9)	2 (7.1)	0 (0)	0 (0)						
Yes	185 (91.1)	26 (92.9)	17 (100)	19 (100)						
Socio-economic level					3.78					
≤ Medium low	96 (47.3)	10 (35.7)	6 (35)	6 (32)						
≥ Medium	107 (52.7)	18 (64.3)	11 (65)	13 (68)						

$^{\dagger}P < 0.10$; $^{*}P < 0.05$. Chi square 3/267 degrees of freedom.

Table II Effects of mode of conception, pregnancy type and parents' gender on anxiety (State-Trait Anxiety Inventory Form Y; STAI) and depression (Edinburgh Postnatal Depression Scale; EPDS) scores at each time point controlling for age and parity.

	Adjusted scores (Mean ± SEM)				*t* values						
	Group				Main effects			Interaction effects			
	Spontaneous Conception (*n*=192-231)		Infertility Treatment (*n*=31-36)		Mode of conception (A)	Type of pregnancy (B)	Gender (C)	A*B	B*C	A*C	A*B*C
	Singletons	Twins	Singletons	Twins							
STAI											
T1	34.6 (0.47)	34.2 (1.28)	33.9 (1.59)	37.0 (1.52)	1.00	1.19	-1.53	1.37	-0.59	1.01	-1.65
T2	33.1 (0.49)	31.8 (1.27)	30.0 (1.69)	34.8 (1.58)	0.21	1.60	-1.93†	2.42*	-0.06	1.93†	0.35
T3	34.9 (0.53)	33.9 (1.43)	31.6 (1.87)	36.5 (1.72)	0.20	1.67†	-2.80**	2.09*	0.22	1.25	1.10
T4	34.1 (0.64)	36.1 (1.77)	41.8 (2.11)	39.8 (2.14)	3.10**	-0.08	-4.26***	-1.13	-0.92	-0.88	-0.30
T5	30.0 (0.52)	32.0 (1.37)	28.3 (1.65)	36.0 (1.71)	1.16	3.75***	-0.03	2.20*	-1.46	1.41	0.74
EPDS											
T1	5.5 (0.20)	5.5 (0.54)	5.6 (0.67)	5.2 (0.65)	0.42	0.01	-3.12**	-0.09	-0.64	0.14	-0.69
T2	5.1 (0.21)	3.8 (0.55)	4.8 (0.72)	5.1 (0.67)	1.13	-0.65	-2.73**	1.45	0.13	1.51	0.29
T3	4.8 (0.21)	4.5 (0.56)	3.7 (0.73)	5.5 (0.67)	0.33	1.69†	-3.20**	1.87†	-0.83	0.21	-0.09
T4	4.7 (0.24)	4.7 (0.67)	4.8 (0.82)	7.0 (0.81)	1.73†	1.67†	-5.47***	1.61	-1.34	-2.50*	-2.12*
T5	4.1 (0.24)	3.8 (0.62)	3.3 (0.77)	6.3 (0.79)	1.82†	2.24*	-0.76	2.79**	-2.78**	-0.42	0.08

$^{\dagger}P < 0.10$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table III Prevalence of high anxiety (State-Trait Anxiety Inventory Form Y; STAI ≥ 40) and high depression (Edinburgh Postnatal Depression Scale; EPDS ≥ 12) in spontaneous conception (SC) and infertility treatment (IT) mothers and fathers at all time points. Data are n(%).

	Mothers				Fathers			
	SC		IT		SC		IT	
	Singletons	Twins	Singletons	Twins	Singletons	Twins	Singletons	Twins
STAI ≥ 40								
T1	69 (34.0)	7 (25.0)	2 (12)	7 (37)	51 (25.1)	11 (39.3)	4 (24)	3 (16)
T2	62 (30.5)	4 (14.3)	2 (12)	6 (32)	58 (28.6)	3 (10.7)	2 (12)	5 (26)
T3	69 (34.0)	9 (32.1)	5 (29)	8 (42)	65 (32.0)	6 (21.4)	3 (18)	9 (47)
T4	78 (39.2)	15 (53.6)	9 (53)	13 (68)	60 (30.3)	10 (35.7)	9 (53)	11 (58)
T5	49 (25.7)	10 (35.7)	3 (18)	8 (42)	44 (23.0)	8 (28.6)	2 (12)	10 (53)
EPDS ≥ 12								
T1	22 (10.8)	4 (14.3)	1 (6)	4 (21)	23 (11.3)	4 (14.3)	1 (6)	0
T2	35 (17.2)	0	3 (18)	1 (5)	28 (13.8)	1 (3.6)	3 (18)	1 (5)
T3	25 (12.3)	2 (7.1)	3 (18)	4 (21)	31 (15.3)	2 (7.1)	2 (12)	2 (11)
T4	37 (18.6)	7 (25.0)	2 (12)	9 (47)	36 (18.2)	8 (28.6)	3 (18)	4 (21)
T5	37 (19.8)	4 (14.3)	2 (12)	7 (37)	37 (19.4)	6 (21.4)	1 (6)	6 (32)

Table IV Full and final trimmed piecewise growth curve models predicting anxiety and depression symptoms from pregnancy to the postpartum period as a function of mode of conception (spontaneous/ after infertility treatment), pregnancy type (singleton/ twin) and parent gender.

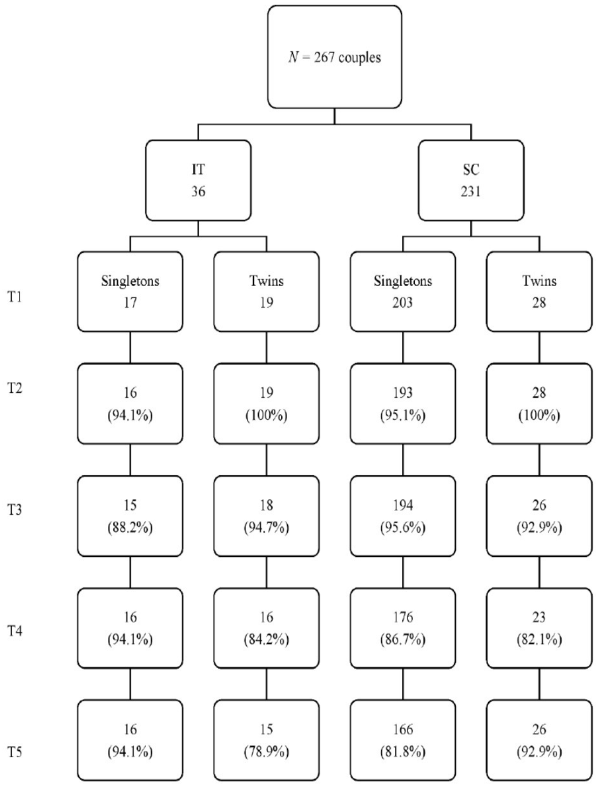
	Anxiety				Depression			
	Full model		Final		Full model		Final	
Fixed effects	*β*	*SE*	*β*	*SE*	*β*	*SE*	*β*	*SE*
Intercept	34.74***	1.81	35.09***	1.79	4.91***	0.76	4.92***	0.75
Time pregnancy	-0.11	0.12	0.04	0.07	-0.21***	0.05	-0.19***	0.03
Pre-postnatal transition	3.88***	0.65	3.25***	0.53	1.06***	0.24	0.93***	0.19
Time postpartum	-3.08***	0.35	-3.07***	0.35	-0.44***	0.12	-0.42***	0.12
Mode of conception	0.36	0.63	0.09	0.58	0.11	0.28	0.06	0.26
Pregnancy type	1.16	0.63	0.34	0.51	-0.06	0.28	-0.17	0.24
Gender	-1.04	0.41	-1.39	0.23	-0.74***	0.18	-0.69***	0.10
Mode of conception x Pregnancy type	1.17	0.63			0.18	0.28		
Mode of conception x Gender	0.56	0.40			0.01	0.17		
Pregnancy type x Gender	0.14	0.40			-0.14	0.17		
Mode of conception x Pregnancy type x Gender	-0.07	0.40			-0.09	0.17		
Time pregnancy x Mode of conception	-0.13	0.12			-0.03	0.05		
Pre-postnatal transition x Mode of conception	3.24***	0.65	2.30***	0.43	0.71**	0.24	0.59***	0.16
Time postpartum x Mode of conception	-1.10**	0.35	-0.97**	0.34	-0.28*	0.12	-0.27*	0.12
Time pregnancy x Pregnancy type	0.00	0.12			$0.08^{\dagger}$	0.05	0.04	0.03
Pre-postnatal transition x Pregnancy type	-0.86	0.65			-0.31	0.24		
Time postpartum x Pregnancy type	1.39***	0.35	1.14***	0.28	$0.24^{\dagger}$	0.12	$0.17^{\dagger}$	0.10
Time pregnancy x Gender	-0.01	0.10			0.01	0.04		
Pre-postnatal transition x Gender	-1.17*	0.57	-0.26	0.24	$-0.39^{\dagger}$	0.21	-0.40**	0.11
Time postpartum x Gender	1.24*	0.37	1.11*	0.23	0.43**	0.15	0.40***	0.11
Time pregnancy x Mode of conception x Pregnancy type	0.03	0.10			0.10*	0.05	0.06*	0.03
Pre-postnatal transition x Mode of conception x Pregnancy type	-1.71**	0.65	-1.02*	0.40	-0.36	0.24		
Time postpartum x Mode of conception x Pregnancy type	1.08**	0.35	0.96**	0.33	0.08	0.12		
Time pregnancy x Mode of conception x Gender	0.03	0.10			0.04	0.04		
Pre-postnatal transition x Mode of conception x Gender	-0.92	0.57			-0.73**	0.21	-0.29**	0.09
Time postpartum x Mode of conception x Gender	0.60	0.37			0.16	0.15		
Time pregnancy x Pregnancy type x Gender	-0.09	0.10			-0.02	0.04		
Pre-postnatal transition x Pregnancy type x Gender	-0.39	0.57			0.29	0.21		
Time postpartum x Pregnancy type x Gender	-0.09	0.37			-0.33*	0.15	-0.29***	0.07
Time pregnancy x Mode of conception x Pregnancy type x Gender	0.10	0.10			0.03	0.04		
Pre-postnatal transition x Mode of conception x Pregnancy type x Gender	-0.59	0.57			-0.28	0.21		

Time postpartum x Conception Mode x Pregnancy type x Gender	0.36	0.37		0.09	0.15

Mode of conception was coded as after infertility treatment = 1, spontaneous = -1. Pregnancy type was coded as twin = 1, singleton = -1. Gender was coded as men = 1, women = -1.

Models are adjusted for age and parity.

[†]*P*<0.10; [*]*P*<0.05; [**]*P*<0.01; [***]*P*<0.001.



Supplementary Figure SI Flowchart with numbers and percentages of participants at each trimester of pregnancy (T1-T3), after childbirth (T4) and at 3 months postpartum (T5) according to mode of conception (spontaneous/ after infertility treatment) and type of pregnancy (singleton/ twin).

SC, spontaneous conception; IT, infertility treatment.

209x297mm (300 x 300 DPI)

In a first step, unconditional models were estimated for each dependent variable to obtain intra-class correlations and to estimate individual and couple variance. In a second step, full models with all predictor variables and interactions were estimated. For final trimmed models, significant and marginally significant effects were retained, as well as main effects of predictors involved in significant and marginally significant interactions. Multivariate outliers were detected and excluded from full and final trimmed models.

Model significance and the explained variance of the predictors were assessed by comparing unconditional and final trimmed models estimated using the maximum likelihood method. Model significance was tested by χ^2 goodness of fit test.

PAPER IV

FETAL GENERAL MOVEMENTS AND BREATHING MOVEMENTS' DEVELOPMENTAL TRAJECTORIES IN TWINS AND ASSOCIATED FACTORS

Tendais, I., & Figueiredo, B., Mulder, E.J.H., Ramada, D., & Montenegro, N. (2016). *Fetal general movements and breathing movements' developmental trajectories in twins and associated factors*. Manuscript submitted for publication.

Abstract

This study examined the developmental trajectories of fetal general and breathing movements and associated factors in twins. Fetal movement patterns were assessed from real-time ultrasound recordings performed at 12-15, 20-23 and 28-32 weeks of gestation in 42 twin pairs. Both movement patterns followed a curvilinear, inverted U-shaped curve, increasing up to 18 and 26 weeks of pregnancy for general movements and breathing movements, respectively, and declining thereafter. Developmental trajectories were unrelated within-pairs of twins and were not associated with fetal sex, gestational age at delivery and birthweight. However, general movements incidence was associated with fetal sex at 21 weeks of pregnancy with male twins displaying less general movements than male twins. These findings support previous research showing that fetal movements reflect central nervous system maturation and suggest that twins' behavioral development is largely independent of co-twin development, fetal sex, gestational age at delivery and birthweight.

Keywords: fetal behavior; fetal movements; twins; general movements; breathing movements

Spontaneous fetal movements are a well-established marker of the maturation of the central nervous system (CNS) during pregnancy [1,2]. Fetal movements appear between 7.5 and 15 weeks of gestation, and most follow a developmental course [3,4]. For example, general movements (whole-body movements with variable amplitude, speed and patterning of body parts) incidence increases until the end of the first trimester and has a gradual decline during the second half of pregnancy [3-11]. This decreasing trend during the second half of pregnancy has been attributed to the development of inhibitory neural mechanisms and to the gradual development of different behavioral states [11,12]. Breathing movements are another example of a fetal movement pattern that appears to follow a clear developmental course during pregnancy. They are characterized by a simultaneous inward movement of the thorax and an outward movement of the abdomen [3]. Breathing movements gradually increase with advancing gestational age [6-8], and some studies report a decline near term [9,13,14]. However, there is wide variation in the percentage of time spent breathing in uncomplicated pregnancies (0.1 to 79.6% at 30-31 weeks of gestation and 0 to 86.2% at 38-39 weeks of gestation [15].

Spontaneous fetal movements are also a marker of the maturation of the CNS in twins [16]. However, very little is known about fetal movements in twins [17]. The developmental trend of twins' spontaneous activity from 10 to 22 weeks of gestation was described in a study [18]. Although the authors provided information on the spontaneous activity for each twin of the 15 pairs included in the sample, no overall developmental trend was reported for the 13 specific movement patterns studied. The developmental trends of general movements and breathing movements from 20 to 35 weeks of gestation, was analyzed in a sub-sample of 18 twin pairs showing that general movements decreased with advancing gestation, whereas breathing movements increased over time and then appeared to decline during the last weeks of gestation [19] as previously found in singletons. A significant intra-pair association of spontaneous fetal movements was found [19,20] suggesting that twins' fetal behavioral development is non-independent.

Fetal movements appear to be associated with a number of factors including fetal sex, length of gestation and birthweight. Regarding fetal sex, the results are inconsistent with some studies showing that male fetuses are more active than female fetuses [21-23], while most studies failed to find any sex differences in fetal movements incidence [19,24-27]. The absence of breathing movements has been identified as the best predictor of preterm birth [28]. Furthermore, it has been found that premature infants display less fetal body movements and an increase in fetal breathing movements that occurs earlier in gestation than full-term infants [8]. Similarly, reduced fetal movements at term have also been associated with the birth of small for gestational age (SGA) infants [29].

The present study extends prior research on studying twins' spontaneous fetal movements and their associated factors from ultrasound observations at each trimester of pregnancy by employing a prospective longitudinal design focused and the use of a dyadic statistical approach. Specifically, this study aimed to examine mean levels and developmental trajectories of fetal general movements and breathing movements in twins. and associated factors (fetal sex, interdependence, term vs. preterm birth and appropriate vs small for gestational age).

Methods

Participants

Forty-five twin pairs monitored in antenatal clinics of three public hospitals in northern Portugal (Hospital São Sebastião, Hospital São João and Hospital Pedro Hispano) were enrolled in this longitudinal study at 12-15 weeks of pregnancy. The duration of pregnancy was calculated from the first day of the last menstrual cycle and confirmed by early ultrasound. Three twin pairs were subsequently excluded due to *in utero* death of one or both twins. Thus, the final sample comprised 42 twin pairs (N=84).

The sample included 17 female-female, 13 male-male and 12 male-female twin pairs. Most pregnancies were bichorionic biamniotic (90.5%). More than half of the women had no complications during pregnancy (61.9%) and delivered by cesarean section (64.3%). The mean gestational age at delivery was 36.1 weeks (*SD* = 2.7, range = 27.7-38.7) and half were premature (< 37 weeks of gestation). More than a quarter (26.2%) was born small for gestational age (weight < 10th percentile). Most twins had normal 1-min and 5-min Apgar scores (89.3% and 98.8%, respectively). In more than a third of cases, at least one of the twins was admitted to an intensive care unit (31.0%).

Procedure

After approval from the Ethics Committee of each participating hospital, parents of twins were recruited from those referred by their physicians. Inclusion criteria included carrying a twin pregnancy, having less than 15 weeks of gestation, and knowing how to read and write in Portuguese. Written informed consent was obtained from both parents. Fetal movements were recorded after the routine ultrasound assessment at 12-15, 20-23 and 28-32 weeks of gestation for 20 min using real-time ultrasound (GE Voluson E8, GE Medical Systems, Zipf, Austria) with a multifrequency transabdominal probe. These observations were recorded on DVD for latter fetal movement analysis. A longitudinal view of the fetuses was preferred with the head, trunk and upper limbs visible, as well as the potential contact area of the twins. Whenever possible, both fetuses were monitored simultaneously (*n* = 81 recordings) to distinguish between active (spontaneous and evoked) and passive fetal movements. However, when an adequate view of both twins was not attained they were monitored in two consecutive periods (*n* = 40 recordings). Except for those who delivered before the last prenatal assessment (n = 3), the other participants completed all prenatal assessments. Nevertheless, two recordings were not available for analysis due to technical problems.

Recordings of simultaneously monitored twins were scored by two researchers (I.T. and E.J.M), each scoring the fetal movements of one fetus. The scoring of the fetus positioned on the left or right side of the screen monitor was randomized at the first recording and was maintained at subsequent recordings whenever the identification of each twin was possible (e.g., sex difference). Recordings of twins separately monitored were all scored by one researcher (I.T.). Hand-held pushbuttons were used to score two prenatal movement patterns – general and breathing movements - and the data was fed into a computer. General movements (spontaneous or evoked) were scored by pressing one button as long as the movement was performed [19]. Passive movements due to co-twin movement were overlooked. As fetal breathing movements last less than 1s, they were marked as discrete events.

Smoothing procedures previously applied to data from singletons [10,20] and twins [19] were performed with a software package (Poly 5, Inspector research Systems, Amsterdam, The Netherlands). Accordingly, a single burst was considered when consecutive general movements occurred within 1 s of each other. Breathing movements occurring within 6 s apart were regarded as bouts of continuous breathing activity. The rate of each pattern was calculated for each fetus and fetal assessment.

Measures

Twins' fetal behavior was assessed through the observation of real-time ultrasound recordings. General movements involve the whole body (head, limbs, and trunk) and have variable amplitude, speed and patterning of body parts [3]. Breathing movements are characterized by a simultaneous inward movement of the thorax and an outward movement of the abdomen [3]. After training, interrater reliability on the identification of these fetal movement patterns was calculated using 6h recordings of fetal data. There was moderate to high agreement between the two raters (I.T. and E.J.M) for general movements and breathing movements (intraclass correlation coefficients: .65 and .94, respectively).

Data Analysis

Dyadic growth curve models were estimated using multilevel modeling to examine the developmental trends of fetal movement patterns and to account for nonindependence of scores within twin pairs and across time. Twins were treated as indistinguishable dyads because no meaningful criterion could be used to systematically distinguish each twin member [30]. Accordingly, all parameters of the statistical models used were constrained to be the same across twins in the same pair [31]. Each twin was classified as Twin 1 or Twin 2, even though this classification was arbitrary [32]. Time was centered at 21 weeks of gestation so that the intercept represents the average incidence of fetal movements at mid-pregnancy. Slope coefficients represent the average change in fetal movements for each 1-week increase in time. Logarithmic transformation of fetal breathing movement variable was performed based on positively skewed distribution.

Model selection was based on a sequential process of comparing nested models using likelihood ratio tests [33]. Maximum likelihood estimation was used for significance assessment, whereas restricted maximum likelihood was used in reporting estimates of best fitting models. Fetal sex, gestational age at delivery (term vs. preterm) and birthweight (appropriate vs small for gestational age) were tested as predictors of mean levels and trajectories of fetal movements. Interdependence of fetal movements was assessed by time-specific correlation between the residuals at each time point with the significant predictors controlled in the model. No significant differences in inter-pair trajectories of change were identified on any of the outcome variables as tested by the inclusion of random slopes, suggesting a similar rate of change among twins. Thus, only intercepts were allowed to covary within and between twin dyads. These analyses were conducted with MLwiN 2.22 (Centre for Multilevel Modelling, University of Bristol).

Results

Descriptive analysis

Fetal movement analyses were based on 161 20-min (range 13-27 min) observation periods with a total time of 4069.92 min. A mean of 3 observations per twin pair was available for analysis (range 2-3).

General movements and breathing movements' developmental trajectories

The estimates of the best fitting dyadic growth curve models for each fetal movement are presented in Table 1. Figure 1 illustrates the developmental course of each fetal movement pattern.

Table 1

Dyadic Growth Models for Fetal Movements

	General movements		Ln Breathing movements	
Parameter	*b*	*SE*	*b*	*SE*
		Fixed effects		
Intercept	17.41***	0.79	2.98***	0.06
Fetal sex	-1.22**	0.47	-	-
Time	-0.32***	0.05	0.03***	0.01
Time^2	-0.06***	0.01	-0.003**	0.001
		Random effects		
Variances	Estimate	*SE*	Estimate	*SE*
Intercept	2.30	2.02	0.02	0.02
Residual	25.99***	2.92	0.22***	0.03
Correlations				
Intercept–intercept	.52		-.21	
Between residual	.01		.20	

** $p < .01$ *** $p < .001$

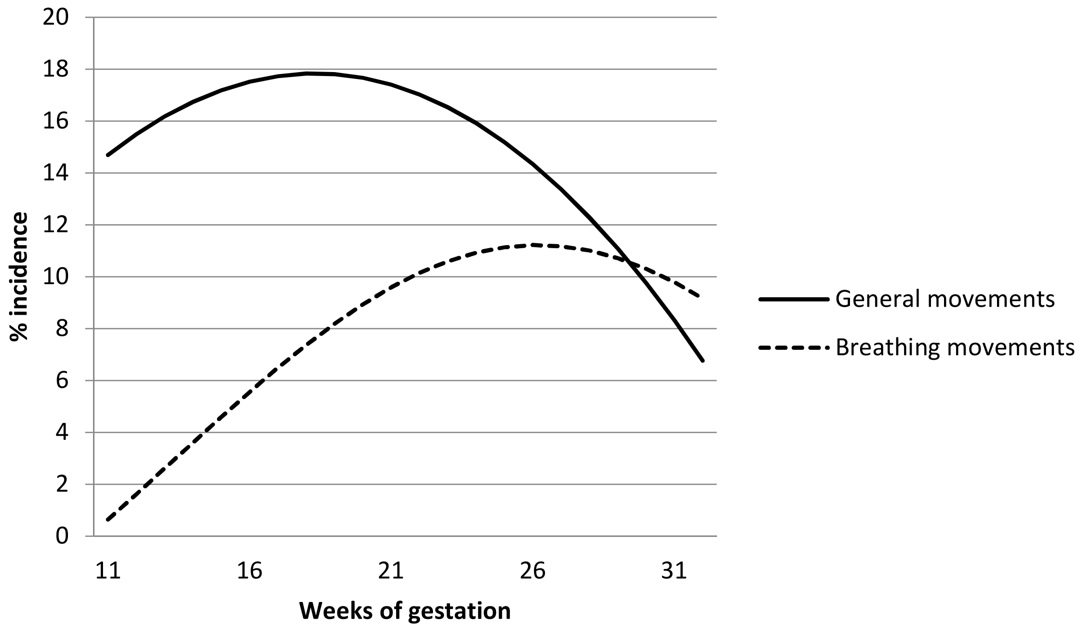


Figure 1. Developmental course of twins' fetal movement patterns

General movements At 21 weeks of pregnancy, the average incidence of general movements was 17.41. Significant linear and quadratic fixed effects were found for time. The incidence of general movements increased from 15.49% at 12 weeks to 17.83% at 18 weeks and, thereafter, decreased progressively reaching the lowest value (6.77%) at 32 weeks of pregnancy. No significant intercept variance was found at 21 weeks of pregnancy.

Breathing movements The average incidence of breathing movements was 9.59 (converted value of log transformed variable) at 21 weeks of pregnancy. Significant linear and quadratic fixed effects were found for time. The predicted incidence of breathing movements increased steeply from 1.62% at 12 weeks to 11.22% at 26 weeks of pregnancy and, thereafter, decreased progressively reaching 9.16% at 32 weeks of pregnancy. No significant intercept variance was found at 21 weeks of pregnancy.

Factors associated with general movements and breathing movements' developmental trajectories

Dyadic growth curve models showed no significant intra-pair correlation of the residuals for the overall incidence of general movements and breathing movements at the three time points of pregnancy (Table 1).

Fetal sex was a significant predictor of general movements at mid-pregnancy, such that male fetuses exhibited a lower incidence of general movements than female fetuses. However, female and male fetuses showed no differences in general movements' trajectory during pregnancy. No significant sex differences were found for breathing movements at mid-pregnancy or throughout the gestation.

There were no significant effects of prematurity and SGA on mean levels and developmental course of general movements and breathing movements.

Discussion

This study showed that general movements and breathing movements of twins followed a clear developmental trend providing further support to the notion that fetal movements reflect central nervous system maturation. Sex differences were found for mean levels of general movements at 21 weeks of pregnancy, but no significant differences were found in the developmental trajectories of general movements and breathing movements of male and female twins. General movements and breathing movements' developmental trajectories were independent of the co-twin, gestational age at delivery and birthweight.

Both fetal movement patterns followed a curvilinear developmental trend. General movements increased up 18 weeks of gestation and then showed a decline over the second half of gestation, whereas breathing movements increased up to 26 weeks of gestation and declined thereafter. These overall trends are generally in line with the developmental characteristics previously found in singletons [4-6,9-11,13,14,25]. However, some specific differences were noted. We found an earlier decrease in general movements and breathing movements incidence in twins than singletons as reported in the literature [11]. These results might be explained by accelerated maturation, lack of intrauterine space or anticipation of premature delivery [19]. It is also possible that the observed differences could be explained by the reduced observation period and the lack of control of prandial effects and circadian rhythms.

Our results are also partially consistent with those reported in a previous study on the spontaneous activity of twins during the second half of pregnancy [19]. These authors found a linear decrease of general movements and a linear increase of breathing movements from 20 to 35 weeks' gestation. We also found a linear decreasing trend for general movements from 18 weeks' gestation onwards. However, our results show that breathing movements follow a non-linear developmental trend. Several methodological differences may explain the different results, even though the same smoothing procedures have been used [10]. We were not able to control for prandial effects as Mulder et al. [19] and previous research indicates that breathing movements increase two to three hours after meals [15]. Second, Mulder et al. [19] monitored both twins simultaneously using two real-time ultrasound machines for 60 min, while in the current study the observation period was limited to 20 min and only one real-time ultrasound machine was used to monitor both twins simultaneously or one after the other when an adequate view of both fetuses was not accomplished. Given that breathing movements are less frequent than general movements and usually occur in bursts and not as single events [9], it is likely that different observation periods influence the obtained results. In addition, there is a wide variation on the percentage of time fetuses spend breathing movements [6,15]. Third, Mulder et al. [19]

study included only normal dichorionic twin pregnancies, whereas in the current study complicated twin pregnancies were also included.

In contrast with most previous research reporting no sex differences in fetal activity [19,24-27], we found that female fetuses were more active than male fetuses at 21 weeks of gestation. Sex differences in fetal activity have been found in small samples studies [21-23] and, therefore, it is also possible that they are due to Type I error [34]. However, other factors like differences in the rest-activity cycles that appear to emerge around 19-20 weeks [6,35] and in the amniotic volume could have also influenced the results reported here.

The lack of intra-pair association in fetal movement patterns during pregnancy was unexpected and contrasts with previous studies [19,20]. Methodological differences such as simultaneous vs. non-simultaneous observation periods of twins within pairs and short (20 min) vs. long (60 min) observation periods may explain differences in results between studies. Similarity in twins' fetal movements are probably noticeable with simultaneous monitoring and for extended periods of observation.

Fetal movements incidence and developmental trajectories were also unrelated with premature birth and SGA. While previous research has shown that the absence of breathing movements is a good short-term predictor of preterm labor [28] and reduced fetal movements after 36 weeks of gestation is associated with the birth of SGA infants [29], no significant developmental differences have been found among high- (threatening to deliver prematurely) and low-risk outcome groups in fetal body and breathing movements [8]. Altogether, these findings may suggest that fetal movements are proximal indicators of preterm labor or SGA infant given that altered fetal movements incidence appear to occur at late pregnancy.

The longitudinal assessment of fetal movements is a major strength of this study. However, several limitations need to be recognized, namely the small sample size, lack of simultaneous observation of both twins' fetal movements in a third of the recordings and the inability to control for possible confounders like diurnal rhythms and prandial effects.

This study provides new insights into age-dependent changes in twins' fetal general movements and breathing movements incidence using short ultrasound observations after routine ultrasound assessments. Future studies are needed to confirm the obtained results in normal pregnancies and those complicated by maternal and fetal conditions.

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*Acknowledgements***.** This study was supported by a doctoral grant (SFRH/BD/40146/2007) to the first author from Fundação para a Ciência e Tecnologia. We thank Prof. Carrapato, Dra Fátima Menezes and Profª Doutora Hercília Guimarães for their institutional support for this study. We thank Dr. Augusta Pinto, Profª Alexandra Matias, Dr. Eduarda Marques, Dr. Teresa Loureiro for performing several ultrasound recordings. We also thank Milton Severo for his contribution to statistical analysis. We especially thank the participating families for their time and willingness to share their experience with us.

PAPER V

SEX DIFFERENCES IN THE FETAL HEART RATE VARIABILITY INDICES OF TWINS

Tendais, I., Figueiredo, B., Gonçalves, H., Bernardes, J., Ayres-de-Campos, D., & Montenegro, N. (2015). Sex differences in the fetal heart rate variability indices of twins. *Journal of Perinatal Medicine*, *43*(2), 221-225. doi:10.1515/jpm-2014-0031

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Sex differences in the fetal heart rate variability indices of twins

Abstract

Aims: To evaluate the differences in linear and complex heart rate dynamics in twin pairs according to fetal sex combination [male-female (MF), male-male (MM), and female-female (FF)].

Methods: Fourteen twin pairs (6 MF, 3 MM, and 5 FF) were monitored between 31 and 36.4 weeks of gestation. Twenty-six fetal heart rate (FHR) recordings of both twins were simultaneously acquired and analyzed with a system for computerized analysis of cardiotocograms. Linear and nonlinear FHR indices were calculated.

Results: Overall, MM twins presented higher intrapair average in linear indices than the other pairs, whereas FF twins showed higher sympathetic-vagal balance. MF twins exhibited higher intrapair average in entropy indices and MM twins presented lower entropy values than FF twins considering the (automatically selected) threshold r_{Lu}. MM twin pairs showed higher intrapair differences in linear heart rate indices than MF and FF twins, whereas FF twins exhibited lower intrapair differences in entropy indices.

Conclusions: The results of this exploratory study suggest that twins have sex-specific differences in linear and nonlinear indices of FHR. MM twins expressed signs of a more active autonomic nervous system and MF twins showed the most active complexity control system. These results suggest that fetal sex combination should be taken into consideration when performing detailed evaluation of the FHR in twins.

Keywords: Cardiotocography; entropy; fetal monitoring; spectral analysis; twins.

DOI 10.1515/jpm-2014-0031

Received January 29, 2014. Accepted May 23, 2014. Previously published online June 19, 2014.

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Introduction

Consistent sex differences in health, behavior, and developmental outcomes after birth have been reported, but studies carried out during fetal life have yielded conflicting results. Previous studies on fetal heart rate (FHR) characteristics have reported sex-specific differences [2–4, 7, 9, 12], as well as no significant differences [5, 6, 8, 14, 17, 20], even when the same methodologies were used. In adults, women reveal a preponderance of vagal over sympathetic responsiveness in heart rate variability under basal conditions, especially before the age of 50 [13, 18, 22, 25]. One possible explanation for sex differences in the autonomic nervous system is the effect of sex-specific hormones [13].

Studies in twins provide a unique setting to examine sex-related differences in FHR patterns. A variation in the intrauterine hormonal environment has been consistently found in litter-bearing mammals according to the sex of the adjacent fetuses [23], and a small number of studies in human twins also support the existence of sex hormone transfer between fetuses [16, 24], even though hormonal effects appear to be less strong [23]. To date, few studies have evaluated the sex differences in FHR in human twins [26].

The aim of this study was to evaluate the differences in linear and complex heart rate dynamics in twin pairs according to fetal sex combination: male-male (MM), male-female (MF), and female-female (FF).

Methods

The study protocol was approved by the local Ethics Committee and written informed consent was obtained from pregnant women prior to study enrollment. The study sample comprised 14 twin pairs (6 MF, 3 MM, and

5 FF) from which 26 FHR recordings were simultaneously acquired in the antepartum period between 31 and 36.4 weeks of gestation. The average tracing length was 52.6 min (standard deviation, SD=45.2) and the average±SD signal loss was 11.6%±9.5%. Between one and three tracings of each twin pair were available for analysis such that, in total, 13 tracings were from MF, 5 from MM, and 8 from FF. Three sets of twins were monochorionic (MC) (one monoamiotic and two diamniotic) and eleven were dichorionic (DC). Chorionicity was determined during routine ultrasound scan at 11–13 weeks of gestation [19] and confirmed by placental histology after birth. Pregnant women had an average of 29.4 years (SD=4.1, range 24–39 years) and 42.9% had pregnancy complications such as preeclampsia, gestational diabetes, and premature rupture of membranes. Mean gestational age at delivery was 37.0 weeks (SD=1.7, range 32.1–38.7) and 43.1% delivered before 37 weeks of gestation. Mean newborn birth weight was 2478.8±442.7 g (range 1395–3290 g) and 42.0% had birth weight below 2500 g. All newborns had normal 5-min Apgar scores, and only two second twins had a 1-min Apgar score below 7. Admission to a neonatal intensive care unit occurred in 22%.

The FHR monitoring of both twins was performed using the Philips Medical 50A fetal monitor (Philips Medical, Eindhoven, The Netherlands) connected by cable to the Omniview-SisPorto®3.5 system (Speculum, Lisbon, Portugal) for computer analysis of cardiotocograms [1]. A preprocessing algorithm was applied to reduce noise and artifacts as described elsewhere [11]. Additionally, persistent segments of signal loss in the initial period of the tracings were visually identified and excluded.

Linear and nonlinear FHR analysis were performed in the initial four 5-min segments of each tracing, after the exclusion of initial periods with signal loss. Time-domain linear analysis included mean FHR (mFHR), standard deviation of FHR (sdFHR), long-term irregularity (LTI), Delta FHR (Δ), short-term variation (STV), and interval index (II). Frequency domain (spectral) linear analysis included very low frequency (VLF), low frequency (LF), movement frequency (MF), and high frequency (HF). LF and HF are mainly associated with the influence of the sympathetic and parasympathetic branches of the autonomic nervous system, respectively, on heart rate variability (HRV). MF reflects fetal movements, as well as maternal breathing. LF/HF and LF/(MF+HF) reflect sympathetic-vagal balance, and was also considered.

For nonlinear analysis, approximate entropy (ApEn) and sample entropy (SampEn) indices were computed, considering the embedding dimension (m) equal to 2 and the values 0.1 SD, 0.15 SD, 0.2 SD, and r_{Lu} [15] for the threshold parameter (r). These indices measure the complexity and irregularity of time series and have been linked to complex cortical nervous system activity [21]. Short- and long-term variation were also assessed by the SD1 and SD2 indices, respectively, extracted from the Poincaré plot analysis, as well as the SD1/SD2 ratio.

Linear and nonlinear indices were calculated as described elsewhere [10, 11]. Intrapair average [$(A+B)/2$] and absolute differences ($|A-B|$) for each calculated FHR index, between MF, MM and FF twins, were evaluated using 95% bootstrap (B=1000) percentile confidence intervals (CIs) (95% CI) for the median, and nonparametric Mann-Whitney and Kruskal-Wallis statistical tests, setting significance at $P<0.05$. Data were analyzed with IBM SPSS 19 Windows version (PASW Statistics for Windows, SPSS Inc., Chicago, IL, USA).

Results

Male-male twins presented a significantly higher intrapair average of time-domain linear FHR indices than the others, as well as for the spectral indices VLF, LF, MF, and HF. A significantly lower sympathetic-vagal balance of MM twins was also observed through LF/(MF+HF). The intrapair average of entropy indices with the threshold values 0.1, 0.15, and 0.2 was consistently found to be decreasing from MF, MM and FF twins, but significance was only found between MF and FF, whereas MMs presented significantly lower entropy values considering the threshold r_{Lu}. Short- and long-term variations as measured by indices SD1 and SD2, respectively, were significantly higher for MM twins, and the ratio SD1/SD2 decreased significantly with declining numbers of male fetuses.

Intrapair absolute differences of sdFHR, Δ, and STV indices were significantly higher between MM twins than between the others. Moreover, these differences were more pronounced as the number of male fetuses in the pair increased. Similar results were achieved with spectral indices VLF, LF, MF and HF, as well as for SD1 and SD2 variation indices of the Poincaré plot. The intrapair absolute differences of ApEn and SampEn were significantly lower between FF twins than between MF or MM twins. The intrapair average and absolute difference for each FHR index are presented in Table 1. The evolution of FHR indices STV, LF/(MF+HF), and SampEn(2, r_{Lu}), as a function of the gestational age, is shown in Figure 1.

The three MC FF pregnancies were compared with two DC FF twins. In general, both intrapair averages and absolute differences were similar, with the exception of a significantly (P=0.013) lower II average and lower (P=0.041) absolute difference of LF/HF in MC pregnancies.

Discussion

The FHR indices were significantly influenced by sex combination in twins, both in intrapair average and absolute differences. Previous research in singleton pregnancies already indicated that fetal sex significantly influences FHR indices [2, 3].

In general, MM twins presented higher linear indices than FF pairs, a finding in accordance with the previous results, and this may be related with a higher predominance of behavioral FHR pattern B (active sleep) in male fetuses [2]. The MM twins also presented higher short- and long-term variability than others. These findings are in line with the results of singleton studies showing a more active autonomous nervous system [2] and a higher FHR variability in male fetuses [7]. A lower sympathetic-vagal balance was observed in MM twins than in FF. Although MM twins consistently presented a higher intrapair

Table 1 Bootstrap percentile 95% CIs for the intrapair average [(*A*+*B*)/2] and intrapair absolute differences (|*A*–*B*|) of linear and nonlinear FHR indices for the MF, MM, and FF groups.

	(*A*+*B*)/2				|*A*–*B*|			
	MF	MM	FF	P-values	MF	MM	FF	P-values
mFHR	137.39–144.54	145.66–151.30	138.96–147.59	a,b,d	5.10–7.91	2.02–5.98	3.67–6.02	b
sdFHR	4.92–6.40	6.70–9.12	6.09–8.04	a,b,c	1.45–3.02	2.76–6.63	0.77–3.06	a,b,d
LTI	6.69–9.59	9.43–14.26	9.64–14.10	a,b,c	2.03–4.69	1.25–8.71	2.16–5.30	
Δ	17.56–21.38	24.66–30.89	18.05–25.75	a,b,d	3.98–7.85	8.38–24.30	2.40–9.20	a,b,d
STV	2.22–2.65	3.26–4.06	2.14–3.15	a,b,d	0.62–1.05	1.02–2.82	0.26–0.52	a,b,c,d
II	0.44–0.47	0.40–0.51	0.36–0.42	a,c,d	0.06–0.15	0.07–0.11	0.04–0.10	
TP	16.53–26.83	35.59–62.67	14.70–37.97	a,b,d	7.40–18.66	24.84–83.87	4.61–20.09	a,b,d
VLF	6.27–11.84	12.10–17.92	7.33–15.39	a,b	3.05–10.06	8.48–21.88	2.60–7.36	a,b,d
LF	6.90–12.30	18.69–42.4	7.41–18.64	a,b,d	3.38–8.54	12.06–64.43	2.56–5.28	a,b,d
MF	0.73–1.42	3.07–5.50	0.61–1.73	a,b,d	0.44–0.90	3.49–6.68	0.21–0.94	a,b,d
HF	0.21–0.35	0.67–1.16	0.17–0.31	a,b,d	0.10–0.20	0.54–1.40	0.07–0.13	a,b,d
LF/(MF+HF)	6.17–8.32	4.89–6.95	6.38–10.37	a,b,d	2.70–4.07	0.98–3.31	1.85–4.92	
LF/HF	32.82–50.43	23.02–44.62	35.15–61.88	d	13.53–36.13	10.17–25.47	16.02–45.30	
ApEn(2, 0.1)	0.74–0.82	0.61–0.83	0.67–0.74	c	0.13–0.26	0.12–0.24	0.09–0.21	
ApEn(2, 0.15)	0.60–0.67	0.47–0.70	0.49–0.55	a,c	0.13–0.25	0.13–0.26	0.08–0.18	
ApEn(2, 0.2)	0.44–0.53	0.39–0.56	0.36–0.42	a,c	0.14–0.19	0.11–0.21	0.05–0.14	a,c,d
ApEn(2, r_{Lu})	0.81–0.89	0.64–0.83	0.79–0.86	a,b,d	0.10–0.19	0.08–0.24	0.04–0.18	
SampEn(2, 0.1)	0.64–0.73	0.48–0.66	0.55–0.63	a,b	0.17–0.32	0.10–0.28	0.11–0.21	
SampEn(2, 0.15)	0.48–0.56	0.35–0.53	0.37–0.44	a,c	0.13–0.25	0.10–0.22	0.09–0.19	
SampEn(2, 0.2)	0.35–0.42	0.28–0.42	0.28–0.32	a,c	0.12–0.19	0.09–0.21	0.05–0.12	a,c,d
SampEn(2, r_{Lu})	0.71–0.82	0.48–0.66	0.66–0.82	a,b,d	0.15–0.28	0.10–0.30	0.09–0.31	
SD1	0.68–0.93	1.21–1.62	0.66–0.89	a,b,d	0.15–0.29	0.49–1.18	0.11–0.25	a,b,d
SD2	6.93–9.00	9.41–12.95	8.59–11.35	a,b,c	1.99–4.37	3.37–9.23	1.04–4.35	a,b,d
SD1/SD2	0.10–0.11	0.11–0.14	0.07–0.09	a,c,d	0.03–0.04	0.01–0.04	0.01–0.05	

[a]$P<0.05$ MF vs. MM vs. FF; [b]$P<0.05$ MF vs. MM; [c]$P<0.05$ MF vs. FF; [d]$P<0.05$ MM vs. FF.

average of spectral indices, FF twins showed a marked decrease in parasympathetic activity (measured by the HF component), thus leading to an increase in the sympathetic-vagal balance. The latter was accomplished by higher intrapair absolute differences in MM twins. Similar results were obtained for the indices total power and SD1/SD2. Although a similar trend was previously found in the singleton fetuses [2], these findings contrast with those reported in the majority of studies in adults [13, 18, 22].

Consistent with findings from singletons [2], FF pairs exhibited more complex FHR activity than MM twins as measured by $ApEn_{Lu}$ and $SampEn_{Lu}$ suggesting a more active complexity control system. However, no differences were found between FF and MM twin pairs in the other entropy indices. This inconsistency may indicate that signal complexity is not being captured when the recommended values for tolerance threshold (0.1–0.2 SD of the data) are applied. Nevertheless, MF pairs showed a consistently higher complexity of FHR data as measured by all entropy indices. The reasons for this result are not clear. Future studies comparing heart rate dynamics between males and females from same and opposite-sex twin pairs are, therefore, recommended.

The largest intrapair absolute differences were observed in MM twins for most linear indices, as well as in the short- (SD1) and long-term (SD2) variability measured by the Poincaré plot. This suggests a lower synchronization in the activity of MM twins. The mFHR index was an exception, where MF pairs presented the largest intrapair differences, probably reflecting a consistently higher mFHR in MMs and lower mFHR in FFs. MFs tended to be closer to FFs than to MMs in linear indices, while the opposite occurred for nonlinear indices. These findings are somewhat unexpected and suggest that factors other than sex may contribute to HRV. If fetal sex was to be a major factor to HRV, then MF would have the largest intrapair differences, and no difference would be found between the same-sex twin pairs. Several factors could explain the differential effect of sex combination on linear and nonlinear indices, such as fetal hormone transfer, fetal presentation, and intertwins contacts. We speculate that the largest intrapair differences in MM pairs may be due to higher male reactivity

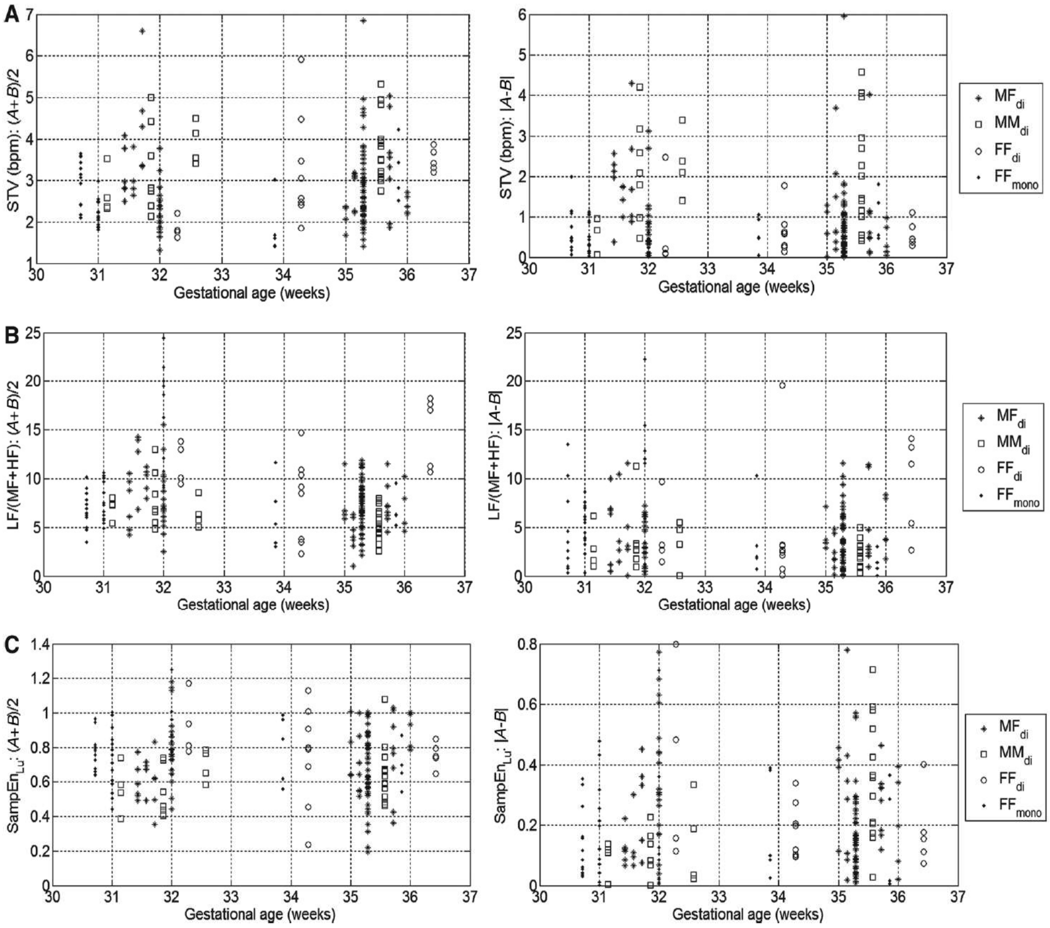

Figure 1 Evolution of FHR indices (A) STV, (B) LF/(MF+HF), and (C) SampEn(2, r_{Lu}) as a function of the gestational age, with respect to the twin pairs MF (DC), MM (DC), and FF (DC and MC). Left plots: intrapair average, $(A+B)/2$; right plots: absolute differences $|A-B|$.

associated with their increased autonomic activity, as well as to intrapair differences in presentation, as most MM had cephalic/breech presentation and breech presentations may had been more exposed to the noise produced by the maternal heart rate and gastric movements (Bernardes et al., unpublished observations). The smallest intrapair differences in FFs are probably related to the inclusion of more MC twins in this group. Chorionicity has been shown to influence FHR patterns with MC twins showing more similarities in FHR patterns than DC twins [26].

The automated analysis of cardiotocograms is a major strength of this study given its objectivity, reproducibility, and predictive validity. Conversely, the visual interpretation of the tracings has high inter- and intraobserver variability. Nevertheless, several limitations to this study need to be acknowledged, namely the small sample size and the inability to control for other possible confounders.

The results of this exploratory study suggest that fetal sex needs to be taken into consideration when performing a detailed evaluation of HRV in twins, as significant differences in heart rate dynamics may be found according to sex combination. Further research is needed to evaluate the influence of gestational age, type of presentation, and fetal behavior state on FHR indices, in line with the previous research in singletons. This may help to further clarify the complex relations that exist between twins and improve perinatal care in twin pregnancies.

Acknowledgments: This work was supported by a grant (SFRH/BD/40146/2007) to the first author from Fundação para a Ciência e Tecnologia. Hernâni Gonçalves is financed by a postdoctoral grant (SFRH/BPD/69671/2010) from the Fundação para a Ciência e a Tecnologia (FCT), Portugal.

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The authors stated that there are no conflicts of interest regarding the publication of this article.

DISCUSSION

This thesis aimed to contribute to existing knowledge on the psychological adjustment to twin parenthood and on twins' fetal development using a longitudinal design, collecting data from parents and twins at multiple time points and employing dyadic data analysis.

Summary of main findings

Our results suggest that the psychological adjustment to parenthood assessed using a broad range of measures (anxiety and depression symptoms, marital relationship, attitudes to pregnancy and the baby and attitudes to sex) is complex and depend of time, individual (gender) and dyad-level factors (mode of conception and type of pregnancy). On average, we found that parents of twins reported increasingly positive attitudes to pregnancy and the baby over time, a decrease in marital relationship and increasingly positive attitudes to sex from pregnancy to the postpartum period and over the postpartum period (Objective 1, paper I). No significant differences in anxiety and depression trajectories between parents of twins and parents of singletons were found, except for anxiety during the postpartum period, with parents of twins showing no significant decrease in anxiety levels, in contrast with the significant decrease noted among parents of singletons (Objective 2, paper II). While systematic differences in anxiety and depression trajectories were found between IT and SC parents of twins in two reports (papers II and III), significant gender differences in these trajectories emerged only in the smaller sample (paper II) (Objective 3). Accordingly, IT parents of twins showed no significant decrease in depression during pregnancy and a significant increase in anxiety from pregnancy to the postpartum period, whereas SC parents of twins showed a decline in depression during pregnancy and no significant increase in anxiety from pregnancy to the postpartum period (paper III). In addition, IT parents of twins showed a significantly higher risk for clinically significant depression symptoms than SC parents of twins and IT parents of singletons at 3 months postpartum (paper III). Using a smaller sample, we found that the adverse effect of IT on anxiety and depression trajectories during the transition to twin parenthood was limited to women and to the transition from pregnancy to the postpartum period (Objective 3, paper II). Regarding twins, age-dependent changes in studied fetal movement patterns were observed supporting the view that fetal movements reflect central nervous system maturation (Objective 4, paper IV). While the developmental course of fetal movements was found to be largely independent of a number of factors (co-twin, fetal sex, gestational age at delivery and birthweight), fetal sex differences were found in one fetal movement pattern at mid-pregnancy and heart rate parameters at late pregnancy (Objective 4, papers IV and V).

Consistent with the developmental psychopathology perspective, the results on parents' psychological adjustment to the transition to twin parenthood underscore the importance of studying the effect of multiple factors at multiple levels and their interactions to have a better understanding of adaptative and maladaptative patterns. A dynamic interplay between individual and dyad-level influences was seen over this developmental time. Some of these pathways revealed shades or degrees of psychopathology. For instance, the persistence of subthreshold-level anxiety among parents of twins over the postpartum period may indicate significant distress, but not necessarily a mental disorder. In a stressful environment characterized by constant demands from both twins, a vigilant behavior may be adaptative to immediate circumstances, but may render parents more vulnerable to higher levels of depression. The results of the first two reports also illustrate how different periods associated with specific developmental tasks may lead to increased difficulties. The transition from pregnancy to the postpartum period was associated with mean increases in anxiety and depression symptoms. During this period, parents begin to build the coparental system (to establish an emotional and behavior alliance with the other parent, to share experiences and decisions in accordance with child's best interest and to respond in an articulated way to the child's needs), have to accept the baby as a separate person and to operationalize parenthood. However, this transition appears to be particularly stressful for those who conceived after IT. This demonstrates how challenging situations may have a different impact depending on individual and family life cycle passage. However, a significant heterogeneity on levels and trajectories of anxiety and depression in women and men from pregnancy to the postpartum period was found demonstrating the diversity of patterns of individual development.

In the context of the developmental reorganization that occurs during the transition to parenthood, parents of twins and particularly those who conceived after IT, may experience several risk factors over a limited period of time. Twin pregnancy might be considered a nonnormative stressor (unexpected) and, therefore, may be more disruptive and stressful (Walsh & McGoldrick, 2013). In addition, a potential cumulative negative effect of twin parenthood after IT may arise. Previous (e.g., infertility, infertility treatment) and subsequent risk factors (e.g., pregnancy complications, prematurity, low birth weight, poor neonatal health status, long periods of birth admission and NICU admission) can drain resources (Walsh, 2016) and overwhelm parents, heightening the risk for subsequent problems (Patterson, 2002. Previous research has shown that IT mothers exhibit higher pregnancy-focused anxiety (Hjelmstedt *et al.*, 2003; McMahon *et al.*, 1997, 2011, 2013), lower self-efficacy and self-esteem (Gibson *et al.*, 2000) during the postpartum period than SC women. It is expected that these problems may be more problematic in the context of twin parenthood, especially during the first 3 months postpartum, due to the overload of caregiving tasks and different sleeping and feeding patterns of the twins (Beck, 2002). However, this pile-up of internal and external stressors may depend on whether prior stressors (e.g., infertility, infertility treatment) were adequately mastered (Hill, 1973; cit Bodenmann, 1995), but also to the meanings attributed to these previous experiences (Cicchetti & Rogosch, 1996). Other factors like availability and effectiveness of personal and social resources (instrumental and emotional support from family and friends), financial strain, job insecurity may add up or buffer the effects of having twins on parents' psychological adjustment during the transition to parenthood. These factors may partially explain the differences in findings between the current study and the longitudinal study carried out in Finland by Vilska et al. (2009). Some methodological differences between the two studies could also explain differences in findings, namely regarding sample characteristics (maternal age, parity, duration of infertility), time points and instruments to assess anxiety and depression symptoms. Cross-cultural differences regarding access and duration of paid parental leave, child allowances, access to childcare services and welfare regime might also explain the contrasting results.

Advances in ultrasound monitoring have facilitated a shift of developmental psychology frontiers from the neonatal to fetal period. Knowledge about the origins and development of individual differences, as well as the factors that may influence this process, can now be expanded with the study of behavior throughout pregnancy (paper I). Fetal studies have provided information on the emergence and maturation of sensory systems and fetal behavioral patterns. These studies have demonstrated that the behavioral repertoire of a full-term fetus was virtually the same as the newborn infant (DiPietro, Costigan, & Voegtline, 2015). By 15 weeks of pregnancy, the human fetus already shows a remarkable behavioral repertoire (de Vries, Visser, & Prechtl, 1985). Over the second half of gestation, fetal movements decrease likely due to inhibitory neural mechanisms (ten Hof et al., 2002) and get progressively clustered with other fetal variables (FHR patterns and eye movements) into fetal behavioral states at 36-38 weeks of gestation (Nijhuis, Martin, & Prechtl, 1984; Nijhuis, Prechtl, Martin, & Bots, 1982) that resemble neonatal behavioral states. Fetal studies have also found that fetal movements have important developmental functions (Adolph & Berger, 2005). Fetal body and breathing movements contribute to the normal musculoskeletal and lung growth and development, respectively (for an updated review on musculoskeletal development see Koos & Rajaee, 2014; Nowlan, 2015). In addition, fetal movements are essential for in utero survival (e.g., fetal swallowing to prevent an excessive accumulation of amniotic fluid) and for adaptation to extrauterine life (e.g., breathing movements, sucking) (Prechtl, 1984). The study of prenatal behavior in twins may give insight into the differential impact of maternal factors (hormones, depression, anxiety) according to fetal sex and other characteristics. However, studies that describe twins' normative behavioral development are needed to provide a necessary comparison for determining whether fetal movement patterns are atypical.

The results of our first report on twins' fetal development provide new insights into the developmental trends of fetal movements over the three trimestres of pregnancy supporting the view that fetal movements reflect central nervous system maturation. These trends had been described in detail for singletons under normal and abnormal conditions using several methods (ultrasound, actograph) at short intervals throughout pregnancy (weekly, biweekly) with extended periods observation (60-120 min) and rigorous control of potential confounders (diurnal and prandial effects). Similar studies in twins are scarce and highly heterogeneous (paper I).

Our results are consistent with the view that fetal movement patterns are not easily influenced by fetal and environmental factors, such as the co-twin, fetal sex, gestational age at delivery (term vs. preterm birth) and birthweight (appropriate vs small for gestational age). The obtained results also confirm that fetal sex is associated with both fetal movement incidence and FHR (papers IV and V).

The developmental trajectories found for twins are generally in line with the developmental characteristics previously found in singletons (e.g., D'Elia, Pighetti, Moccia, & Santangelo, 2001; de Vries et al., 1985; Lüchinger, Hadders-Algra, van Kan, & de Vries, 2008; Roodenburg, Wladimiroff, van Es, & Prechtl, 1991; ten Hof et al., 1999, 2002). However, some specific differences were noted. We found an earlier decrease in general movements and breathing movements incidence in twins than singletons as reported in the literature (ten Hof et al., 2002). These results might be explained by accelerated maturation, lack of intrauterine space or anticipation of premature delivery (Mulder, Derks, de Laat, & Visser, 2012). It is also possible that the observed differences could be explained by the reduced observation period and the lack of control of prandial effects and circadian rhythms.

Our results are also partially consistent with those reported in a previous study on the spontaneous activity of twins during the second half of pregnancy (Mulder et al., 2012). Our results are consistent regarding the developmental course of general movements, characterized by a linear decline from 18 weeks' gestation onwards. In contrast with Mulder et al. (2012), our results show that breathing movements follow a non-linear developmental trend. Several methodological differences may explain the different results, even though the same smoothing procedures have been used (ten Hof et al., 1999). While Mulder et al. (2012) included only normal dichorionic twin pregnancies, controlled for prandial effects and monitored both twins simultaneously using two real-time ultrasound machines for 60 min, in the present study normal and complicated twin pregnancies were included, we were not able to control for prandial effects and the observation period was limited to 20 min and only one real-time ultrasound machine was used to monitor both twins simultaneously or one after the other when an adequate view of both fetuses was not accomplished.

The introduction of fetal behavioral assessment into routine screening is limited by a number of factors, including the excessive time required to observe all fetal movement patterns and there is considerable inter-fetal variance which makes the identification of the compromised fetus very complex (Nijhuis, 2016). Nevertheless, it has been recommended that the introduction of fetal behavior assessment of the most frequently occuring fetal movement pattern into routine screening. Our results provide some support for this recommendation by showing that a brief assessment of fetal behavior can provide information on the developmental course of two clinically relevant fetal movement patterns.

One important note should be made regarding the analyses of twins' data (behavioral development and temperament) collected during the postpartum period and on the relationship between parents' and twins' data over time. The manuscripts with these analyses are still in preparation and, therefore, were not included in this thesis.

Limitations

Findings derived from this thesis have important limitations that need to be taken into account when interpreting results. First, the small sample size of parents of twins, especially of the IT group, reduce the statistical power and can result in a failure to achieve statistical significance. Second, the SC group included first- and second- time parents, whereas the IT group included only first-time parents. While a recent meta-analysis has shown that the role of parity in increasing the risk of antenatal depression and anxiety remains unclear (Biaggi et al., 2016), previous studies with IT mothers of twins have shown higher levels of psychopathological symptoms among primiparous than multiparous mothers (Colpin et al., 1999; Baor et al., 2004). Third, because IT parents tend to underreport negative affect (McMahon *et al.*, 2003), the use of self-reported measures may have increased the reporting bias, especially in the IT group. Fourth, in papers II and III, the extension of the trajectory during pregnancy to childbirth is an extrapolation and not a measurement. This extrapolation ignores the possibility that anxiety/ depression may increase in the last weeks of pregnancy as birth becomes imminent. Finally, we found that two subscales of MAMA/ PAPA (marital relationship and attitudes to pregnancy and the baby) had low internal consistency. Previous studies had shown the multidimensionality nature of these measures and good psychometric properties in samples of portuguese mothers and fathers (Pinto et al., 2015). Our results suggest that the total score of both instruments may assure acceptable to good reliability with parents of twins over the transition to parenthood.

The sample size was also a limitation of both studies with twins (paper IV and V, especially for the study of FHR (paper V). In addition, we were unable to monitor both twins simultaneously in a third of the recordings of fetal movements (paper IV). Moreover, we were also unable to study systematically the effect of maternal and fetal conditions on fetal behavioral development due to high heterogeneity of these complications. Lastly, we had no information on twins' zygosity to estimate the relative effects of genetic and environmental influences on child outcomes.

Practical implications

Our longitudinal design starting at early pregnancy provided detailed information on parents' psychological adjustment over time. It notes the specific time periods in which parents experience high distress and, therefore, a greater need for support. This information can be helpful for tailoring interventions to the specific needs of parents during the transition to twin parenthood.

Findings from this thesis have implications for couples undergoing infertility treatments, mental health practitioners working with them and policy makers. For couples, the results drawn from this study and others (e.g., Vilska et al., 2009) may help them make a more informed decision about the number of embryos to be transferred. Our findings underscore the need to provide special support to IT parents of twins during pregnancy and the postpartum period to ensure the early detection and provision of psychosocial or specialized mental health care services to those experiencing poor psychological well-being. It should be noted that parents' depression and anxiety symptoms have been consistently associated with adverse outcomes on the fetus, neonate and child, including early emotional regulation and social problems and impairments in child cognitive development (Ross *et al.*, 2011; Stein *et al.*, 2014). Therefore, preventive and supportive interventions directed to parents may have lasting effects on the offspring. Policy makers should be aware that in addition to the increased perinatal risks associated with an IT twin pregnancy, IT parents of twins may be at risk for postnatal depression. Changes in reimbursement policies like the one carried out in Belgium have resulted in a significant reduction of twin and higher order multiples (Ombelet, De Sutter, Van der Elst, & Martens, 2005).

Our results also showed that women's and men's psychological adjustment is interdependent. Therefore, couple-based interventions may yield better results for both women and men than those directed at the individual level.

Regarding twins' data, if future studies confirm our results in normal pregnancies and those complicated by maternal and fetal conditions, the introduction of a brief assessment of fetal behavior into routine screening at three time points during pregnancy can provide information on the developmental course of at least two clinically relevant fetal movement patterns.

Our results and those obtained with singletons pointed out to the need to take fetal sex into consideration when performing detailed evaluation of the FHR. Accordingly, a recent study has presented percentile curves for cardiotocographic parameters separately for male and female fetuses after finding consistent evidence for sex differences in FHR througout pregnancy in normal pregnancies (Amorim-Costa, Cruz, Ayres-de-Campos, & Bernardes, 2016).

Suggestions for future research

Further research is needed to investigate whether the identified early postpartum adjustment difficulties among parents of twins in general and IT parents of twins in particular are transient or persist over time. In addition, future studies should examine the contribution of declining marital relationship and depression trajectory during pregnancy to high depression symptoms during postpartum.

Considering that most couples who have been exposed to potential multiple stressors (e.g., IT parents of twins) did not score above the depression cut-off at 3 months postpartum, it would be important to identify the multiple trajectories during the transition to twin parenthood. This would enable the identification of at risk and resilient individuals, the identification of moments of discontinuity and the factors associated with adaptation and maladaptation. Correctly identifying individuals at risk for poor psychological adjustment would give the opportunity to target those who would benefit the most from preventive and supportive interventions. Recent studies have identified effective non-pharmacological interventions for depression and anxiety (Bowen, Baetz, Schwartz, Balbuena, & Muhajarine, 2014, Su et al., 2008; Thomas, Komiti, & Judd, 2014). On the other hand, the identification of factors associated with resilience could lead to the development of effective preventive-intervention strategies that promote the psychological well-being of parents during the transition to twin parenthood. Therefore, research focused on studying the underlying processes that lead to adaptation and maladaptation during the transition to twin parenthood is warranted.

Considering that our results contrast with those reported by Vilska et al. (2009) using a large sample of Finnish couples, cross-cultural studies could shed some light on the importance of factors at the macro- (e.g. the trend of childlessness, reimbursement policies of infertility treatments), exo- (e.g., parent's workplace policy regarding maternity/ paternity leave) and meso-systems (e.g., organizations that provide support to parents of twins, to parents who conceived after infertility treatment) on the psychological adjustment to twin parenthood achieved with or without infertility treatment. In addition, it would be important to study the dynamic interplay between these variables and those at the microsystem level.

Regarding twins' behavioral development, future studies in normal twin pregnancies and those complicated by maternal and fetal conditions are needed to confirm the obtained results. Research would benefit from investigating fetal behavioral development of more homogeneous groups (e.g., fetal growth restriction, fetuses of diabetic mothers) and pregnancies with twin discordant anomalies as it is expected that different conditions are associated with distinct behavioral changes.

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