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CONSECUENCIAS DE LA ACTIVACIÓN DEL EJE HIPOTALÁMICO-HIPOFISARIO-ADRENAL SOBRE LA SALUD MATERNO-FETAL DURANTE EL EMBARAZO, PARTO Y PUERPERIO

TESIS DOCTORAL INTERNACIONAL-INTERNATIONAL PhD THESIS

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En Granada, a 25 de febrero de 2018.

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RESUMEN

Esta tesis consta de doce capítulos estructurados como se muestra a continuación: a) introducción (capítulos I, II, III, IV); b) justificación y objetivos (capítulo V); c) estudio de revisión sistemática (capítulo VI); d) estudio de validación (capítulo VII); e) estudios empíricos (capítulos VIII, IX, X y XI), y f) discusión general, conclusiones y perspectivas futuras (capítulo XII). Estos capítulos se agrupan en dos partes, Parte teórica y Parte empírica.

El primer apartado se compone de la introducción teórica de esta tesis, dividida en cinco capítulos. En el capítulo I, se ofrece información sobre el proceso normal del embarazo, desarrollo embrionario y fetal, así como las adaptaciones físicas y psicológicas de la mujer durante el embarazo. En el capítulo II se define el estrés psicológico y su evaluación, estrés materno prenatal, activación del eje Hipotalámico-Hipofisario-Adrenal y cortisol como hormona de estrés y tipos de estrés materno prenatal. En el capítulo III, se muestran las consecuencias maternas del estrés durante el embarazo y en el capítulo IV se describen las consecuencias sobre el desarrollo fetal e infantil del estrés materno prenatal.

El segundo apartado se compone del capítulo V, que incluye la justificación, los objetivos tanto generales como específicos y las principales hipótesis de los estudios incluidos en esta Tesis Doctoral.

El tercer apartado lo forman seis capítulos (VI, VII, VIII, IX, X y XI) que incluyen cada uno de los estudios que forman esta tesis. El capítulo VI es un estudio de revisión sistemática que intenta dar luz sobre cuál de los métodos para evaluar estrés durante el embarazo (medidas psicológicas o cortisol) es un mejor predictor de consecuencias negativas en la embarazada o en la descendencia. Los resultados mostraron que los niveles de cortisol eran el mejor predictor de estas consecuencias

negativas. El capítulo VII incluye el estudio de adaptación y validación de la medida psicológica de estrés específico del embarazo *Prenatal Distress Questionnaire* (PDQ) en una población de embarazadas españolas. En este estudio se encontró que el PDQ tiene buenas propiedades psicométricas y una estructura factorial de tres factores, concordando con la versión original. El capítulo VIII está formado por el protocolo del estudio *GESTASTRESS* sobre los efectos del estrés durante el embarazo en la salud de la mujer y del recién nacido. *GESTASTRESS* es el acrónimo del estudio al que se le concedió un proyecto I+D en 2015 y con una duración de 3 años. En el protocolo se describen el diseño del estudio que se ha realizado con el objetivo de evaluar el estrés durante el embarazo, así como la evaluación infantil de cortisol y neurodesarrollo que se están aplicando a los hijos/as de esas embarazadas. El capítulo IX lo forma un estudio que tuvo como objetivo comprobar que variables psicológicas, hormonales, obstétricas y sociodemográficas a lo largo del embarazo predicen la depresión postparto. Los resultaron mostraron que aquellas embarazadas que después desarrollan depresión posparto tienen previamente mayores niveles de cortisol en pelo durante todo el embarazo y mayores niveles de estrés psicológico y síntomas psicopatológicos en el primer y segundo trimestre, en comparación con aquellas embarazadas que no desarrollan depresión posparto. En el capítulo X aparece un estudio que tuvo como objetivo comprobar si las embarazadas mediante técnicas de reproducción asistida (TRA) (inseminación artificial y fecundación *in vitro*) tenían mayores niveles de estrés que las embarazadas de manera espontánea. Los hallazgos revelaron mayores niveles de cortisol en pelo en el primer trimestre de embarazo en el grupo de embarazadas mediante TRA con respecto a embarazo espontaneo. No se encontraron diferencias en el nivel de cortisol en el segundo y tercer trimestre. Además, las embarazadas por TRA tenían mayores niveles de estrés psicológico en el tercer trimestre, que el grupo de

embarazadas de forma natural. El capítulo XI está formado por un estudio cuyo objetivo fue evaluar si los niveles de estrés materno durante el embarazo y los niveles de estrés neonatal predicen el neurodesarrollo infantil. Los resultados mostraron que altos niveles de cortisol materno en el primer trimestre predecían el elevado neurodesarrollo cognitivo infantil a los 6 meses de edad; altos niveles de cortisol materno en el primer, segundo y tercer trimestre predecían un bajo neurodesarrollo motor infantil a los 6 meses de edad. Además, altos niveles de cortisol materno en el posparto predecían un mejor neurodesarrollo cognitivo infantil a los 6 meses de edad.

El apartado final lo forma el capítulo XII que incluye la Discusión General, Conclusiones y Perspectivas Futuras con los hallazgos principales que se han encontrado en los seis estudios de esta Tesis Doctoral e implicaciones clínicas.

CAPÍTULO 1: EMBARAZO

1.1 Introducción

Cada año se producen 211 millones de embarazos y 134 millones de nacimientos, de los cuales 123 millones de bebés nacen vivos, según el más reciente informe sobre salud materna e infantil de la Organización Mundial de la Salud (OMS, 2005). El modelo de cuidado centrado en la mujer y el *screening* clínico de anomalías fetales tienen como objetivo minimizar los riesgos obstétricos y perinatales en la díada materno-infantil (NICE, 2017). Aunque el embarazo es un proceso normal, se producen multitud de modificaciones físicas y psicológicas que tratan de aumentar las probabilidades de supervivencia tanto de la madre como de su embrión, feto y bebé en desarrollo, tratando igualmente de favorecer el nacimiento de un bebé sano, una lactancia materna exitosa, y la capacitación materna para el cuidado del recién nacido (Fabre-Gonzalez, 2001; SEGO, 2010). Estas modificaciones pueden verse alteradas por diferentes agentes que afectan el curso del embarazo y el desarrollo infantil (Gary Cunningham, 2015), siendo el estrés psicológico uno de los mayores agentes tóxicos relacionado con la enfermedad y asociado con consecuencias negativas en la embarazada y en la descendencia (Babenko, Kovalchuk y Metz, 2015; Cohen, Janicki-Deverts y Miller, 2007).

Durante milenios, altos niveles de estrés nos han salvado la vida como especie. Sin embargo, en la actualidad, el estrés constituye uno de los mayores problemas de salud (Robles Ortega y Peralta-Ramírez, 2010). Niveles elevados de estrés materno prenatal se relaciona con efectos a largo plazo en la descendencia como esquizofrenia, asma y un menor neurodesarrollo infantil; efectos a medio plazo en los recién nacidos como nacimiento prematuro y bajo peso al nacer; efectos maternos como depresión posparto, diabetes gestacional y pre-eclampsia (Class y cols., 2014; Lobel y Dunkel Schetter, 2016). Sin embargo, altos niveles de estrés en determinados períodos del

desarrollo fetal podrían tener consecuencias favorables, promoviendo un neurodesarrollo más acelerado en la descendencia (Davis y Sandman, 2010). Se desconoce cuál es el período crítico más sensible a la influencia del estrés en el embarazo (Medsker, Forno, Simhan y Celedón, 2015; Zijlmans, Riksen-Walraven y Weerth, 2015). Además, no existe consenso en cuanto a qué proporción de estrés prenatal es responsable de la existencia de resultados negativos en la madre y en la descendencia (Glover, 2014), ya que los hallazgos en torno a las consecuencias específicas del estrés prenatal sobre la salud infantil son inconsistentes (Schetter y Tanner, 2012).

1.2 El embarazo como proceso normal

Un embarazo normal tiene una duración de 280 días aproximadamente, siendo a término cuando se produce entre la semana 37 a la 42 de gestación (Fabre-Gonzalez, 2001). Cuando el embarazo tiene una duración menor a las 37 semanas, tiene como resultado el nacimiento de un recién nacido prematuro, que es la mayor causa de mortalidad neonatal e infantil (Delnord, Blondel y Zeitlin, 2015). El peso normal para un recién nacido es entre 2500-4000 g. Recién nacidos con un peso inferior a 2500 g se asocian a déficits del neurodesarrollo y elevada mortalidad infantil (Howe, Sheu, Hsu, Wang y Wang, 2016) .

Desde el momento de la fecundación comienza en la mujer una serie de fenómenos coordinados que se suceden de forma armoniosa (Bajo-Arenas, 2007). Esta sucesión de eventos prenatales comienza a determinar las características del futuro recién nacido desde antes de nacer (ver Figura 1).

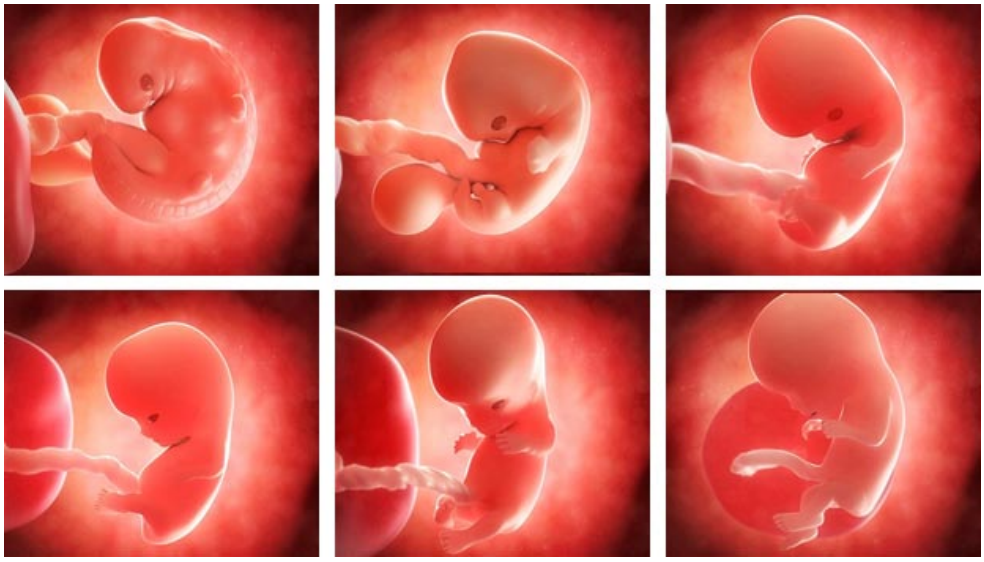


Figura 1. Desarrollo embrionario y fetal. Imagen tomada de https://www.menudoembarazo.es/images/etapas-embarazo/embarazo_primer_trimestre.jpg

Estos fenómenos son:

a) *Recorrido del zigoto por las trompas*: El zigoto o huevo fecundado entre el espermatozoide y el óvulo, viaja desde las trompas de Falopio hasta el útero, donde crecerá y se desarrollará.

b) *Implantación*: EL zigoto llega a la pared del útero donde anida y se implanta. Es el momento en que los vasos sanguíneos de la madre comienzan a alimentar al zigoto para que éste pueda desarrollarse con normalidad.

c) *Desarrollo embrionario*: Desde la segunda semana de embarazo y hasta la 9ª, lo que en apariencia era sólo un cúmulo de células, da como resultado a un embrión en el que comienzan a formarse y desarrollarse sus órganos, incluido el corazón y el sistema nervioso.

d) *Desarrollo placentario*: La placenta es el órgano encargado de proveer alimento y protección al bebé antes de nacer. Su desarrollo comienza en la 2ª semana de embarazo y va paralelo a las demandas del embrión y feto.

e) *Desarrollo fetal*: A partir de la 9ª semana de embarazo (segundo mes) el feto se encuentra unido a su madre a través del cordón umbilical por la placenta y está rodeado por el líquido amniótico. Es característico en esta etapa la maduración de los tejidos y un rápido crecimiento.

1.3 Desarrollo embrionario y fetal

Las 40 semanas de embarazo se dividen en 3 trimestres durante los cuales el embrión y posterior feto se va formando y desarrollando, para dar a luz un bebé sano y con altas probabilidades de supervivencia extrauterina (Sadler, 2016):

a. Primer trimestre de embarazo: Abarca desde la fecundación hasta la semana 12 de embarazo. En esta etapa se produce el desarrollo del embrión u organogénesis, es decir, la formación de todos los órganos. Una vez el zigoto se ha implantado en la pared uterina, y a partir de la semana 3, aparecen una serie de capas germinativas (endodermo, mesodermo y ectodermo) cada una de las cuales dará paso a los distintos órganos.
   - La capa interna, el endodermo, dará lugar al aparato respiratorio, glándula tiroides, hígado, vejiga y sentido de la audición.
   - La capa intermedia, el mesodermo, formará a los músculos, cartílagos y huesos.
   - La capa externa, el ectodermo, dará lugar a la formación de aquellas estructuras que favorecen el contacto con el mundo exterior, como son la piel, sentidos, sistema nervioso central y

sistema nervioso periférico, a través de un complejo proceso denominado neurulación.

Este primer trimestre es un período decisivo para el normal desarrollo. Dado que es cuando se están formando los rudimentos de los órganos, existe en esta etapa una elevada susceptibilidad a los eventos ambientales, incluido el estrés (Glover, 2015).

b. Segundo trimestre de embarazo: Abarca desde la semana 12 a la 28, y se caracteriza por la primera fase del período fetal, con la maduración de los órganos que se formaron en el primer trimestre. En el segundo trimestre, aunque el feto aumenta considerablemente de tamaño, al final del quinto mes su peso apenas llega a los 500 g. Dada su complejidad, el sistema nervioso, junto con el aparato respiratorio, son los últimos en madurar. Por consiguiente, un bebé que nace durante el segundo trimestre de embarazo tiene muy limitadas probabilidades de vida extrauterina, al carecer de pulmones maduros y de un sistema nervioso poco desarrollado, que harían imposible funciones básicas como la respiración y actividades motoras como la succión y deglución de alimentos.
c. Tercer trimestre de embarazo: Desde la semana 28 al nacimiento, se produce en el feto un gran aumento de peso, desde 500 g a 3500 g aproximadamente. Aunque la mielinización nerviosa comienza a partir del tercer trimestre, la mayor parte de este proceso se produce tras el nacimiento. Sin embargo, el desarrollo del sistema nervioso es suficiente para permitir la vida extrauterina normal a partir de la semana 37 de embarazo, período en los que los pulmones también están preparados

para respirar fuera del útero (Cunningham, 2015). Al final del tercer trimestre los niveles cortisol deben estar elevados respecto a los trimestres anteriores, lo que favorecerá un adecuado desarrollo fetal. Es tal la importancia de este aspecto, que aquellas embarazadas que van a tener un parto prematuro, se les administra de manera exógena corticoides, para asegurar la correcta maduración pulmonar y la posibilidad del bebé de realizar una correcta respiración tras el nacimiento (Fabre-Gonzalez, 2001).

1.4 Adaptaciones psicológicas durante el embarazo

Muchas embarazadas tienen problemas de atención, memoria y sueño durante el embarazo y tras el parto. Aunque el embarazo produce en muchas ocasiones emociones positivas, los elevados niveles de estrés pueden favorecer la aparición de trastornos psicológicos durante el embarazo y el posparto (NICE, 2014).

Durante esta etapa, las causas, el curso, pronóstico y riesgo de recaídas relacionadas con enfermedad psicológica son las mismas que en cualquier otra etapa de la vida, siendo los más comunes los trastornos del estado de ánimo y lo trastornos de ansiedad, que afectan al 12% de embarazadas (*American Psychological Association*, APA, 2013). La depresión posparto, que en algunos casos se asocia con suicidio y maltrato infantil, alcanza al 15-20% de mujeres durante el primer año tras el nacimiento del bebé. Es tal la importancia, que entre 2006 y 2008 hubo 1,27 muertes maternas por cada 100.000 nacimientos, como consecuencia de trastornos psicológicos en este período (NICE, 2014).

Según Dunkel Schetter (2010) para una mejor comprensión de los procesos psicológicos que ocurren durante el embarazo y posparto, es importante tener una perspectiva multinivel, en la que se tengan en cuenta el aspecto individual, de relaciones sociales y de nivel comunitario:

- Nivel individual: Se refiere a factores médicos (p.e. diabetes gestacional, pre-eclampsia, abortos previos, multiparidad), genéticos (p.e. cromosomopatías, enfermedades hereditarias), neuroendocrinos (p.e. tumor de hipófisis, hipertiroidismo) psicológicos (p.e. depresión, ansiedad) y de estrés (p.e. estrés psicológico, niveles elevados de cortisol).
- Nivel social: Se refiere a la exposición de la embarazada a estresores en la pareja (violencia de género), en el trabajo o en el vecindario. Un meta-análisis informó que aquellas embarazadas expuestas a violencia de género tenían 1,5 veces más posibilidades de tener recién nacidos prematuros y más incidencia de pre-eclampsia, diabetes gestacional, y placenta previa, que las que no estaban expuestas a violencia (Shah y Shah, 2010).
- Nivel comunitario: Raza/etnia, aculturación (recepción y adaptación a una cultura nueva, generalmente como consecuencia de un proceso migratorio) y nivel socio-económico.

Aunque históricamente, el foco de atención en investigación en torno a los procesos psicológicos en el embarazo y posparto ha estado en los factores médicos, en las últimas décadas la investigación ha tomado en consideración los factores culturales y sociales. Más recientemente, el estrés psicológico ha tomado importancia y es actualmente una de las causas de trastornos psicológicos durante el embarazo y posparto

con más peso (Dunkel Schetter, 2010). Niveles elevados de estrés materno se han relacionado con diabetes gestacional, trastornos hipertensivos, crecimiento intrauterino retardado y parto prematuro. De este modo existe un gran consenso en que aquellas embarazadas con mayores niveles de estrés presentan más riesgos durante el embarazo (Roy-Matton, Moutquin, Brown, Carrier y Bell, 2011).

1.5 Adaptaciones físicas durante el embarazo

Las adaptaciones en la mujer durante el embarazo son diversas. La mayoría de estas adaptaciones comienzan tras la fecundación y continúan durante todo el embarazo debido al estímulo fetal. Resulta imprescindible para los profesionales que se dedican al cuidado de las embarazadas, conocer los cambios normales del embarazo, con el fin de no catalogar como patológico lo que en realidad es una adaptación normal al proceso. Estas adaptaciones vuelven a su estado basal casi completamente, una vez finaliza el embarazo y la lactancia. Así los cambios principales según Cunningham (2015) serían los siguientes:

a) *Sistema cardiovascular*: Se produce un aumento del volumen sanguíneo y de la frecuencia cardiaca. A pesar del aumento progresivo de los niveles de la hormona del estrés, cortisol, durante el embarazo, se produce una disminución de la tensión arterial, para favorecer el paso de nutrientes a través de la placenta hasta el bebé en desarrollo. Estos cambios hacen que la postura de la embarazada influya en la tensión arterial. El fenómeno de la hipotensión en decúbito dorsal está fuertemente asociado con la posición de la embarazada. Así, el útero grávido comprime firmemente los grandes vasos venosos y arteriales posteriores, reduciendo el llenado cardíaco y el gasto cardíaco, provocando una marcada disminución de la tensión arterial, lo que normalmente se denomina síndrome de hipotensión supina.

b) *Aparato respiratorio*: El crecimiento paulatino del útero eleva al diafragma y los pulmones unos 4 cm y ensancha la circunferencia torácica, lo que aumenta la cantidad de oxígeno que llega a los pulmones. Además, aumenta la capacidad de transporte de oxígeno y así la cantidad del mismo que llega al feto.

c) *Cambios metabólicos*: Se produce un incremento de la retención hídrica, aumento de la formación proteica de estructuras (placenta, feto, útero, sangre materna) y un aumento del depósito de grasas (para proteger al feto y a la madre de potenciales períodos de inanición y favorecer la producción de leche materna). Estos cambios tienen como resultado el aumento de peso materno a lo largo del embarazo, que se muestran en la Tabla 1.

d) *Función inmunitaria:* Con el objetivo de albergar al feto, diversas funciones inmunitarias se encuentran deprimidas. De lo contrario, el sistema inmunológico materno haría imposible el desarrollo y progresión de los tejidos placentarios, embrionarios y fetales, al considerarlos extraños y distintos al materno. Este hecho es la base de la mejoría experimentada por las mujeres embarazadas que padecen alguna enfermedad autoinmune, y la cierta susceptibilidad a sufrir diversas infecciones.

e) *Sistema endocrino*: El progresivo desarrollo de la glándula tiroidea fetal, que es autónoma de la función tiroidea materna desde la semana 10 de embarazo, es vital para el normal neurodesarrollo fetal. De este modo, en los primeros días de nacimiento, la detección de metabolopatías mediante la prueba del talón en recién nacidos, como el hipotiroidismo, favorecen el diagnóstico y tratamiento precoz de las mismas, para promover un normal desarrollo infantil. Como se detalla en el capítulo siguiente de esta tesis, el eje Hipotalámico-Hipofisario-

Adrenal aumenta la producción de cortisol a lo largo del embarazo, favorecido por la estimulación placentaria.

Tabla 1. Aumento de peso durante el embarazo por trimestres

	AUMENTO DE PESO (gramos) DURANTE EL EMBARAZO EN CADA TRIMESTRE:		
Tejidos y fluidos	**1er trimestre**	**2º trimestre**	**3er trimestre**
Feto	*5*	*1500*	*3500*
Placenta y líquido amniótico	*50*	*1200*	*1800*
Útero y mamas	*200*	*900*	*1500*
Sangre	*100*	*1300*	*1500*
Depósitos grasa materna	*300*	*3400*	*3700*
Total	*500*	*5600*	*12000*

1.6 Embarazo mediante técnicas de reproducción asistida

Según la Organización Mundial de la Salud (OMS), en la actualidad existen casi 50 millones de parejas infértiles (Mascarenhas, Flaxman, Boerma, Valderpoel y Stevens, 2012), lo que ha hecho que el uso de las técnicas de reproducción asistida (TRA) haya aumentado recientemente (Kupka y cols., 2016).

Las TRA comprenden un conjunto de técnicas que permiten que muchas mujeres puedan ser madres. Entre estas técnicas se encuentran la inseminación artificial y la fecundación *in vitro* (FIV) (Villasante, Duque y García-Velasco, 2005).

Mediante la inseminación artificial, se produce un depósito de espermatozoides en el tracto reproductor de la mujer, de manera no natural. En aquellos casos en los que el origen del semen es la propia pareja, denominamos al procedimiento inseminación artificial conyugal u homóloga. En los casos en los que el semen es de un donante, la denominación es inseminación artificial de donante. El método más recomendado y habitual en la actualidad para llevar a cabo la inseminación artificial es a través del depósito intrauterino con cánulas transcervicales. Son menos recomendada la inseminación intraperitoneal DIPI (*direct intraperineal insemination*), intratubárica ó intracervical. El uso previo de técnicas de capacitación espermática mediante los gradientes de *Percoll* o *Swim-up*, permite seleccionar los espermatozoides con una mayor movilidad, lo que aumenta las probabilidades de embarazo.

En aquellas situaciones en las que existe obstrucción tubárica, y por lo tanto los espermatozoides no pueden pasar a través de las trompas de Falopio, existe un recuento de espermatozoides bajo, abortos de repetición, parejas portadoras de cromosomopatía o alguna enfermedad genética, se indica la realización de FIV. Para la fecundación de los ovocitos, que son las células precursoras de los óvulos, se utiliza la técnica FIV convencional o la inyección intracitoplasmática de espermatozoides (ICSI), lo que facilita la fecundación del óvulo directamente por los espermatozoides seleccionados.

Aunque las TRA facilitan que muchas mujeres queden embarazadas, según el Instituto Valenciano de Infertilidad-Madrid (IVI), la tasa de gestación por paciente se

sitúa en torno al 50%, la tasa de abortos es del 20% y la tasa de embarazo múltiple del 17% (IVI, 2003).

Existe actualmente cierta controversia en cuanto a la seguridad de las TRA. En este sentido, se han descrito una mayor incidencia de pre-eclampsia en aquellas mujeres embarazadas mediante inseminación artificial de donante (Gonzalez-Comadran y cols, 2014) , así como un aumento de prematuridad y bajo peso al nacer (Adams y cols., 2017). Además se ha informado de la relación entre embarazos por TRA y déficits en el neurodesarrollo infantil, un mayor riesgo de autismo, parálisis cerebral, déficits intelectuales y alteraciones de la visión y audición (Hediger, Bell, Druschel y Louis, 2012).

CAPÍTULO II: ESTRÉS MATERNO PRENATAL

2.1. Introducción

En los últimos años se ha descubierto que elevados niveles de estrés psicológico durante el embarazo tiene un fuerte impacto negativo a corto y largo plazo sobre la salud materna e infantil. Sin embargo, el embarazo en sí mismo es un período que genera estrés, debido a las múltiples demandas, cambios en los roles familiares y las modificaciones físicas y psicológicas que ocurren en este período. De este modo, de forma habitual, las embarazadas se preocupan por la salud de su bebé, por el parto y nacimiento, por el embarazo y por los riesgos médicos.

Además del estrés específico del embarazo que padecen las mujeres, las embarazadas están expuestas como cualquier persona a estresores en su vida diaria, en el hogar, en el trabajo e incluso a grandes eventos estresantes como una guerra, atentados terroristas o la muerte de un ser querido (Lobel y Dunkel Schetter, 2016), pero con mayores niveles de vulnerabilidad. Por ello, se necesita seguir profundizando mediante la investigación en los efectos del estrés materno prenatal sobre la salud materna e infantil (Glover, 2014).

2.2. Modelos de estrés

El *modelo de estrés basado en el proceso de interacción estímulo-respuesta* es el más completo al tener en cuenta las variables biológicas y psicológicas (cognitivas) que intervienen en el proceso de estrés (Lazarus y Folkman, 1984). Otros modelos sólo tienen en cuenta variables biológicas (Selye, 1964) ó variables ambientales (Dohrenwend y Dohrenwend, 1981) de manera independiente. En concordancia con la propuesta de Lazarus y Folkman (1984), la evaluación cognitiva del individuo sobre la situación de estrés media entre el estímulo y la respuesta, dividiéndose en tres tiempos:

- Evaluación primaria: Esta primera evaluación que el individuo realiza de la situación de estrés puede ser irrelevante, benigna o estresante, en cuyo caso existe riesgo de amenaza y pérdida.
- Evaluación secundaria: Aquí el individuo determina si los recursos personales de los que dispone son suficientes para enfrentarse a la situación de estrés.
- Reevaluación: Tras realizar la evaluación primaria y valorar los recursos de los que dispone, se produce un cambio encaminado a hacer frente al estrés.

Sin embargo, el modelo de Lazarus y Folkman (1984) no está libre de críticas, y se ha hecho hincapié en la falta de especificidad en cuanto a los mecanismos por los que la respuesta que el individuo da al estrés puede afectar la salud del mismo (Robles-Ortega y Peralta-Ramírez, 2010).

2.3 Evaluación del estrés durante el embarazo

2.3.1 Medidas psicológicas

La evaluación psicológica de los niveles de estrés durante el embarazo se encuentra con dos importantes retos (Nast, Bolten, Meinlschmidt y Hellhammer, 2013):

- El *concepto de estrés,* como hemos comentado en el apartado anterior, resulta de una desregulación entre las demandas del ambiente y los recursos personales para hacer frente a esas demandas (Lazarus y Folkman, 1984). En este sentido, la evaluación psicológica de estrés necesita tener en cuenta la percepción individual de estrés, las demandas, recursos y respuestas de estrés.
- Por otro lado, no todos los *instrumentos psicológicos* son totalmente apropiados para evaluar durante el embarazo, período en el cual se producen ciertos cambios, como en los hábitos de sueño, alimentación, etc., que están presentes también en otros trastornos psicopatológicos como por ejemplo la depresión. Por

este motivo, el uso de cuestionarios psicológicos que generalmente se aplican a la población general pueden no ser los más adecuados al subestimar los niveles de estrés durante el embarazo.

Teniendo en cuenta estas consideraciones, se conocen un total de 43 cuestionarios psicológicos que se han usado para la evaluación psicológica de los niveles de estrés y otras psicopatologías asociadas a éste (Nast y cols., 2013). Entre todos ellos, a continuación, se describen los más ampliamente utilizados por sus excelentes propiedades psicométricas y por su capacidad de predecir resultados negativos maternos y en la descendencia:

- *Inventario de Ansiedad Estado-Rasgo (STAI)* (Guillén-Riquelme y Buela-Casal, 2011; Spielberger, 1983) para evaluar la tendencia a dar una respuesta ansiógena, de manera habitual (rasgo) o en un momento puntual (estado). Consta de dos escalas de 20 ítems cada una con un formato de respuesta tipo *Likert*. Cada escala presenta una estructura formada por dos factores y uno de segundo orden. El coeficiente de fiabilidad alfa de *Cronbach* de la versión española oscila entre $0,87 < \alpha < 0,93$. A pesar de tratarse de un instrumento desarrollado hace más de 30 años para medir ansiedad en población general y no estrés durante el embarazo, su elevada asociación con resultados negativos hacen de él un cuestionario con elevada validez para evaluar la ansiedad en mujeres embarazadas (Nast y cols., 2013).
- *Inventario de Estrés Percibido (PSS)* (Cohen, Kamarck y Mermelstein, 1983; Remor, 2006) se usa para la evaluación general de estrés percibido durante los últimos 30 días de las participantes a través de 14 ítems de respuesta tipo *Likert* de 0 (nunca) a 4 (muy a menudo). El coeficiente de

fiabilidad alfa de *Cronbach* de la versión española es de α = ,81. Esta escala es la mejor medida de autoinforme para medir estrés percibido durante el embarazo (Nast y cols., 2013).

- *Cuestionario de 90 Síntomas Revisado* (Caparrós-Caparrós, Villar-Hoz, Juan-Ferrer y Viñas-Poch, 2007; Derogatis, 1994): Está formado por 90 ítems de respuesta tipo *Likert* de 0 (nunca) a 4 (extremadamente) para evaluar nueve dimensiones psicopatológicas: somatizaciones, obsesión-compulsión, sensibilidad interpersonal, depresión, ansiedad, hostilidad, ansiedad fóbica, ideación paranoide y psicoticismo. Además, la escala ofrece tres índices globales de malestar psicológico: índice global de severidad (GSI), índice positivo de malestar (PSDI) y total de síntomas positivos (PST). El coeficiente de fiabilidad alfa de *Cronbach* de la versión española de las sub-escalas oscila entre ,67 < α < ,94. Es un instrumento adecuado para evaluar aspectos psicopatológicos de los que carece otras medidas diseñadas específicamente para el embarazo, como la *Abreviated Scale for the Assessment of Psychosocial Status in Pregnancy* (Goldenberg y cols., 1997).

En relación a medidas de *estrés específico del embarazo* los más usados y con mayores propiedades psicométricas son:

- *Cuestionario de Preocupaciones Prenatales* (PDQ) (Caparros-Gonzalez y cols., 2015; Yali y Lobel, 1999). Este instrumento de 12 ítems fue inicialmente diseñado para embarazadas de habla inglesa en los EEUU (Yali y Lobel, 1999). Se centra en la evaluación de las preocupaciones e inquietudes específicas del embarazo en relación a los síntomas físicos, relaciones interpersonales, crianza de hijos, problemas médicos, parto y

nacimiento y la salud del bebé (Yali y Lobel, 1999). Sus excelentes características psicométricas se han informado en varios estudios (Alderdice y cols., 2013; Gennaro, Shults y Garry, 2008; Pluess, Bolten, Pirke y Hellhammer, 2010) y se ha usado en los EEUU, Irlanda, Reino Unido y Alemania (Alderdice y cols., 2013; Koletzko, La Marca-Ghaemmaghami y Brandstätter, 2015; Lynn, Alderdice, Crealey y McElnay, 2011). Además, su estructura factorial se ha evaluado satisfactoriamente en embarazadas de bajo y alto riego (Alderdice y Lynn, 2011; Alderdice et al., 2013). Así se encontraron tres factores: "Preocupaciones sobre el Nacimiento/Bebé", "Preocupaciones sobre el Peso corporal/Imagen Corporal" y "Preocupaciones sobre las Emociones/Relaciones". Este cuestionario ha sido descrito como el mejor instrumento psicológico para evaluar los niveles de estrés durante el embarazo (Nast y cols., 2013). Ha sido descrito como un buen predictor de resultados negativos del embarazo, como ansiedad, bajo peso al nacer, recién nacidos prematuros y un bajo neurodesarrollo infantil (Duthie y Reynolds, 2013; Lobel y Dunkel Schetter, 2016). De hecho, aparece como un mejor predictor de resultados negativos del embarazo que el estrés general (Lobel y Dunkel Schetter, 2016). Sin embargo, este instrumento psicológico aún no se ha adaptado para su uso con embarazadas en España. Por ello uno de los objetivos de esta tesis pretende validar y adaptar dicho instrumento para la población española.

- *Pregnancy Related Anxieties Questionnaire* (PRAQ) (Van den Bergh, 1990): Este cuestionario, aunque diseñado para medir miedos y preocupaciones propias del embarazo, es más extenso que el PDQ,

teniendo 34 ítems. Además, carece de capacidad predictiva de neurodesarrollo infantil. La versión original de este instrumento, ha sido traducido a Holandés (Huizink, Mulder, Robles de Medina, Visser y Buitelaar, 2004). Está compuesto por tres factores y el coeficiente de fiabilidad alfa de *Cronbach* de los factores oscilan entre 0,79-0,83. Actualmente no contamos con una versión adaptada al castellano.

2.3.2 Medidas biológicas del estrés: Cortisol

Cuando una persona experimenta una situación de estrés se produce un incremento en sus niveles de cortisol. El cortisol es un glucocorticoide que se sintetiza en las glándulas suprarrenales como consecuencia de la activación del eje Hipotalámico-Hipofisiario-Adrenal. Una vez el cortisol alcanza el torrente sanguíneo, su naturaleza altamente lipofílica favorece su paso a través de la placenta para alcanzar al feto (Rakers, Rupprecht, Dreiling, Bergmeier, Witte y Schwab, 2017). Sin embargo, la existencia de la enzima placentaria 11beta hidroxysteroidea dehidrogenasa tipo 2 (11-HSD-2) es capaz de inactivar hasta el 90% del cortisol materno. Además, teniendo en cuenta que el feto no es capaz de producir cortisol por sí mismo hasta el final del embarazo, las concentraciones plasmáticas fetales de cortisol en condiciones basales son hasta 10 veces inferiores a las concentraciones plasmáticas maternas de cortisol (Chapman, Holmes y Seckl, 2013). Aunque este mecanismo protege al feto de los elevados niveles de cortisol materno, la barrera placentaria se altera ante la presencia de estrés psicológico, con lo que el feto estaría expuesto a niveles más altos de cortisol. Esta alteración de la barrera placentaria se traduce en una reducción de la actividad y expresión de la enzima 11-HSD-2 tanto en modelos animales como en humanos (Chapman, Holmes y Seckl, 2013; Monk, Feng, Lee, Krupska, Champagne y Tycko, 2016). Además, la actividad de 11-HSD-2 se reduce a partir del tercer trimestre de

embarazo, lo que aumenta la vulnerabilidad del feto ante los niveles elevados de cortisol materno (Murphy y Clifton, 2003). En este entorno, elevados niveles de cortisol materno se han relacionado con consecuencias negativas maternas, fetales e infantiles (Glover, 2015). En concreto, se relaciona con parto prematuro, bajo peso al nacer, afectación del neurodesarrollo y depresión posparto (Lobel y Dunkel Schetter, 2016). Esta situación ha promovido la evaluación de los niveles de cortisol desde diferentes muestras biológicas (Wosu, Valdimarsdóttir, Shields, Williams y Williams, 2013):

- *Pelo*: Los niveles *de cortisol en pelo* en embarazadas ofrecen una medida fiable y retrospectiva de los niveles de estrés crónico (D'Anna-Hernandez, Ross, Natvig y Laudenslager, 2011). Se trata de una medida no invasiva, segura, libre de influencias por variables situacionales, factores individuales o ritmos circadianos, y que puede almacenarse a temperatura ambiente (Wosu y cols., 2013). La tasa de crecimiento del pelo es de aproximadamente 1 cm al mes. Al tomar una muestra de pelo de 1 cm de longitud de la zona más cercana del cuero cabelludo, podremos conocer los niveles de cortisol durante el mes previo. Esta regla puede aplicarse para diferentes longitudes de pelo siendo la más utilizada la medida de los 3 cm que representan los tres últimos meses (Wennig, 2000). A través de los vasos sanguíneos, glándulas sebáceas y sudoríparas que drenan en los folículos pilosos, el cortisol se va depositando a lo largo del tiempo en las fibras de pelo, tal como se muestra en el modelo propuesto por Russel y cols. (Russell, Koren, Rieder y Van Uum, 2012) de la Figura 2.

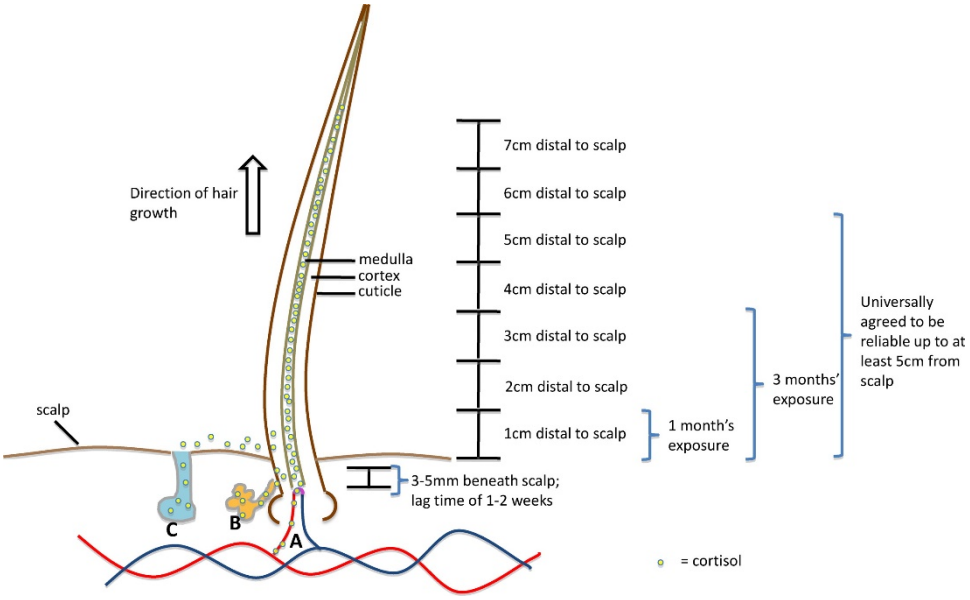


Figura 2. Modelo de depósito de niveles de cortisol en pelo desde vasos sanguíneos y glándulas adyacentes. Imagen tomada de Russell y cols. (2012).

El cortisol en pelo está siendo ampliamente evaluado en el ámbito del embarazo, encontrando que niveles elevados de cortisol en pelo durante la gestación se han asociado a nacimiento prematuro (Hoffman, Mazzoni, Wagner, Laudenslager y Ross, 2016; Kramer y cols., 2009), y aculturación en inmigrantes (D´Anna-Hernandez, Hoffman, Zerbe, Coussons, Ross y Laudenslager, 2012). Además se ha usado con éxito como biomarcador de estrés restrospectivo en embarazadas diagnosticadas de asma (Smy y cols., 2016).

- *Sangre*: El uso de sangre para la medición de niveles de cortisol en embarazadas ofrece información de estrés puntual y por lo tanto está influenciado por ritmos circadianos, precisa de un profesional sanitario para la toma de la muestra y requiere de una técnica relativamente dolorosa (Goedhart, Vrijkotte, Roseboom, van der Wal, Cuijpers y

Bonsel, 2010). Además, es necesario la toma de varias muestras a lo largo del día y en distintos momentos temporales para poder hacer una aproximación de los niveles de cortisol a lo largo del tiempo, lo que dificulta aún más su recolección (Bergman, Sarkar, Glover y O'Connor, 2010). Niveles elevados de cortisol en sangre se han asociado a un menor peso al nacimiento y un mayor riesgo de ser Pequeño para la Edad Gestacional (PGA), parámetros relacionados a un menor desarrollo infantil (Goedhart y cols., 2010).

- *Saliva*: Los niveles de cortisol de saliva durante el embarazo, aunque no precisa de una técnica invasiva ni dolorosa, ofrecen información de estrés agudo y está influenciado por ritmos circadianos. Así, con el objetivo de obtener información de la activación crónica del eje Hipotalámico-Hipofisiario-Adrenal, son necesarias la toma de varias muestras diarias durante un período de tiempo (Bolten, Wurmser, Buske-Kirschbaum, Papoušek, Pirke y Hellhammer, 2010). Niveles elevados de cortisol en saliva durante el embarazo aparecen como predictor del peso al nacimiento y del índice de masa corporal de los recién nacidos (Hompes y cols., 2012). Para medir el estrés en el embarazo mediante el cortisol en saliva se utiliza el Cortisol Awakening Response (CAR). Está técnica consiste en la recogida de cortisol de los primeros 30-45 minutos tras despertarse mediante un *Salivette® Cortisol (Sarstedt, Numbrecht, Alemania, Ref.51.1534)*. El *Salivette®* es un tubo dentro de otro de mayor tamaño con un algodón higiénico que permite la recogida de la muestra de saliva sin la asistencia de ningún profesional (D´Anna-Hernandez, Ross y Natvig, 2011).

- *Orina*: Los niveles de cortisol obtenidos de muestras de orina poseen los mismos inconvenientes que las muestras de sangre y saliva, al estar sus niveles determinados por variables situacionales e informar de estrés agudo. Mediante el uso de información de ultrasonidos, altos niveles de cortisol en orina se han relacionado con mayor actividad fetal, con fetos con una circunferencia cefálica y abdominal menor (Field, Hernandez-Reif, Diego, Figueiredo, Schanberg y Kuhn., 2006) y un menor diámetro biparietal (Diego y cols., 2006).
- *Líquido amniótico*: Los niveles de cortisol en líquido amniótico que rodea al feto en desarrollo se han asociado con los niveles de cortisol maternos (Glover, 2015). La extracción de líquido amniótico para la obtención de niveles de cortisol precisa de una técnica invasiva por parte de un médico obstetra, que punciona el abdomen hasta llegar a la cavidad amniótica mediante control ecográfico y con especial precaución de no dañar ninguna de las estructural fetales (ver Figura 3). Además, la técnica en sí conlleva un mayor riesgo de aborto y pérdida fetal (Bergman y cols., 2010). A pesar de sus inconvenientes, esta técnica se ha usado previamente aprovechando el momento de la amniocentesis para la valoración del riesgo de desarrollo de anomalías genéticas en el feto, como trisomía 21 (Síndrome de Down) entre otras (Bajo-Arenas, 2007). Elevados niveles de cortisol en líquido amniótico se han asociado a un menor peso al nacimiento y mayores niveles de estrés infantil (Baibazarova y cols., 2013).

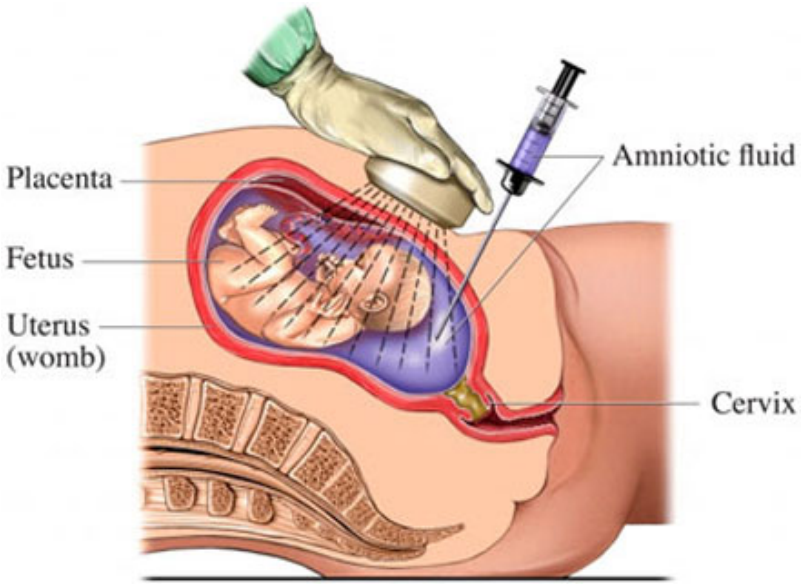
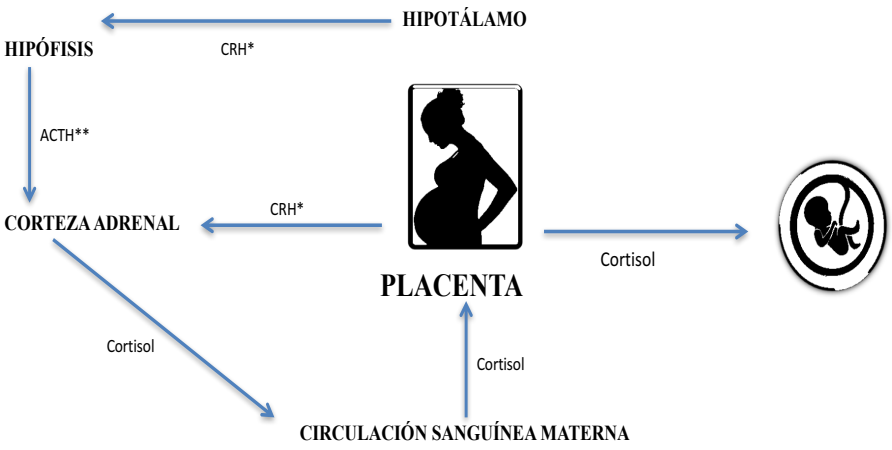


Figura 3. Técnica de toma de muestras de líquido amniótico con guía ecográgica. Tomado de http://www.fragmenthealth.com/reproductive-system/amniocentesis-procedure-test-risks.html.

2.4 La hormona del estrés en el contexto materno prenatal: El cortisol

Los pioneros trabajos del epidemiólogo David Barker sobre los Orígenes Fetales de la Enfermedad Adulta, pusieron las bases para el estudio de la relación entre los eventos ocurridos durante el embarazo, como el estrés, y su relación con riesgos posteriores en la descendencia (Barker, 1995). Altos niveles de estrés durante el embarazo, como los producidos por la hambruna en invierno ocurrida en Holanda al final de la Segunda Guerra Mundial, tienen efectos devastadores transgeneracionales a largo plazo (Sapolsky, 2004).

El estrés prenatal implica una desregulación del eje Hipotalámico-Hipofisiario-Adrenal que ocurre durante el embarazo como consecuencia de la exposición materna a cortisol (Lobel y Dunkel Schetter, 2016) (ver Figura 2).



Nota: *CRH: Hormona Liberadora de Corticotropina.
** ACTH: Hormona Adenocorticotropa.

Figura 2. Eje Hipotalámico-Hipofisiario-Adrenal durante el embarazo

Las preocupaciones específicas del embarazo en torno al proceso de gestación (estrés específico del embarazo) y la exposición de la embarazada a eventos estresantes (hogar, trabajo, guerras, muerte de un familiar), hacen que el hipotálamo sintetice y secrete la hormona liberadora de corticotropina (CRH) como consecuencia de la respuesta biológica al estrés. De este modo, el CRH estimula la liberación de cortisol por parte de las glándulas suprarrenales, lo que prepara al organismo para hacer frente al estímulo estresante (Sandman, Glynn y Davis, 2016). En parte debido a la presencia de la placenta, el eje Hipotalámico-Hipofisiario-Adrenal está profundamente alterado durante el embarazo. La placenta, que es un órgano endocrino de origen fetal, favorece el aumento de la liberación de cortisol por las glándulas suprarrenales debido al

incremento del CRH de origen placentario (Glynn, Davis y Sandman, 2013). Aunque existe evidencia de que los niveles de cortisol regulan de forma negativa la liberación de CRH desde el hipotálamo, el cortisol favorece la secreción de CRH placentario durante el embarazo. Además, el hecho de que la hipófisis duplica su tamaño durante el embarazo, favorece que aumenten aún más los niveles de cortisol a lo largo de la gestación y posparto (Glynn et al., 2013) (ver Figura 3).

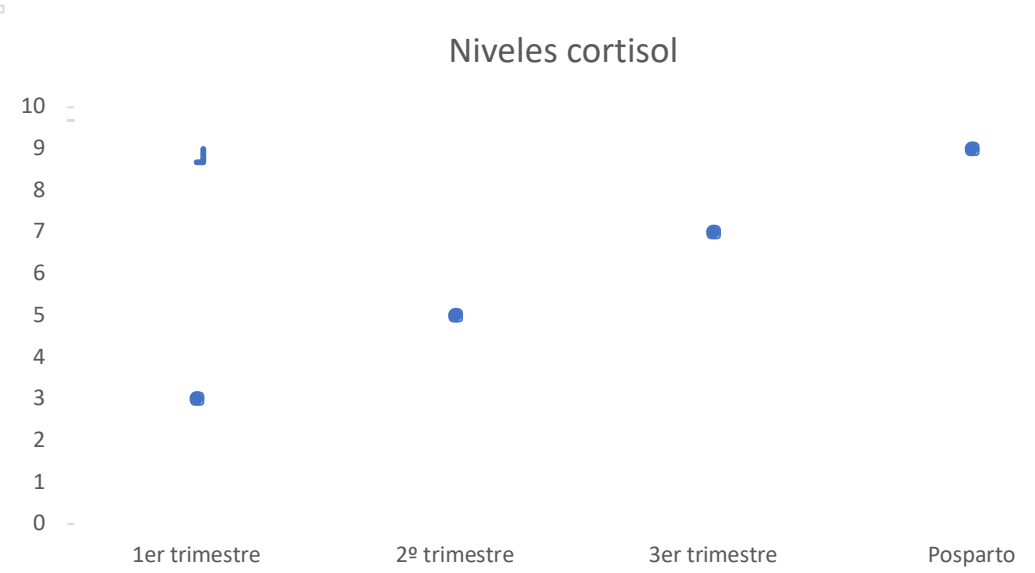


Figura 3. Evolución de la secreción de cortisol materno durante los tres trimestres de embarazo y posparto, adaptado de Glynn et al, (2013)

2.5 Tipos de estrés materno prenatal y su relación con las variables de embarazo y del neonato

Como describen Lobel y Dunkel- Shetter (2016) la investigación en torno al estrés materno prenatal se ha centrado principalmente en:

- *Eventos de vida mayores*: Se refiere a la exposición individual de la embarazada a pérdida de empleo, o exposición de la comunidad entera a desastres naturales, guerras o atentados terroristas. También a eventos de

vida mayores individuales, como el fallecimiento de un familiar durante el embarazo que tienen importantes consecuencias. En concreto, en un gran estudio de cohortes basado en 1,35 millones de nacimientos llevado a cabo en Dinamarca, la pérdida de un familiar querido durante el embarazo se asoció a bajo peso al nacer (Khashan y cols., 2008). Es interesante señalar que estudios como este sólo se pueden realizar en países nórdicos con una gran tradición de registros epidemiológicos (Sapolsky, 2004). Respecto a eventos estresantes que afecten a una comunidad entera de individuos, destacar el de la cohorte *Project Ice-Storm,* que aún hoy en día continúan estudiando los efectos maternos y en la descendencia de una gran tormenta de hielo ocurrida en Canadá en 1998. Entre los hallazgos informados por este estudio, las embarazadas que informaron de mayores niveles de estrés percibido en relación a esta gran tormenta de hielo tuvieron descendencia con un peor neurodesarrollo motor a los 5 años, especialmente enlentecido en las niñas (Cao, Laplante, Brunet, Ciampi y King, 2014). Estos hallazgos van en consonancia con los estudios que muestran cómo las adversidades que ocurren durante la vida intrauterina tienen consecuencias a largo plazo (Meinlschmidt & Tegethoff, 2015). Una revisión sistemática encontró que el estrés materno prenatal como consecuencia de eventos de vida mayores se asociaba a ansiedad, depresión antenatal y postnatal y mayores niveles de cortisol maternos, así como con malformaciones congénitas embrionarias, síntomas de hiperactividad, resistencia a la insulina y patologías cutáneas, musculo-esqueléticas y genito-urinarias en la descendencia (La Marca-Ghaemmaghami y Ehlert, 2015). Otra de las fuentes de estrés materno

prenatal es la percepción de riesgo durante el embarazo. Contrariamente a las expectativas generadas por el aumento de las tecnologías, especialmente en torno al cuidado prenatal del embarazo, el progreso en los avances tecnológicos ha ido paralelo a un aumento de la percepción de riesgo de las embarazadas, que ha elevado los niveles de estrés y sus importantes repercusiones clínicas importantes (Robinson y cols., 2015).

- *Emociones y percepciones de estrés relacionadas con el embarazo*: Niveles elevados de ansiedad y depresión se han relacionado con resultados negativos maternos y en el recién nacido. Sin embargo, los niveles de estrés específico del embarazo aparecen como un mejor predictor de resultados negativos (Alderdice, Lynn y Lobel, 2012; Lobel y Dunkel Schetter, 2016). El estrés específico del embarazo se refiere a las preocupaciones que tienen las embarazadas como consecuencia de la nueva situación de embarazo y el próximo nacimiento del bebé. Así, estas preocupaciones generadoras de estrés están en relación a síntomas físicos del embarazo, cambios en las relaciones interpersonales, cuidar del futuro bebé, problemas médicos, parto y nacimiento y la salud del bebé (Yali y Lobel, 1999). Estudios previos han evidenciado que el estrés específico del embarazo es un fuerte predictor de la actividad fetal, afectación del desarrollo y prematuridad (Lynn, Alderdice, Crealey y McElnay, 2011; Nast y cols., 2013).

2.6 Estrés en los tratamientos de reproducción asistida

Las dificultades de fertilidad generan en la mujer y en la propia pareja grandes dosis de estrés, que se extiende a todos los ámbitos de vida, transformando el placer del sexo en un procedimiento médico sin éxito (Sapolsky, 2004). En este contexto, las

técnicas de reproducción asistida (TRA) han supuesto un gran avance que posibilita ser madre y ser padre con la ayuda de esta formidable tecnología. Sin embargo, estos tratamientos resultan económicamente caros, requieren la administración de dolorosas inyecciones, administración de distintas hormonas, extracciones de sangre, ecografías y largas sesiones de espera que posiblemente informen sobre el fracaso del proceso, y consiguientemente haya que comenzar el ciclo de nuevo. Por este motivo, y acompañado por las elevadas tasas de abortos que muchas veces acompañan a las TRA, hacen que este proceso genere grandes dosis de estrés (Bailey, Ellis-Caird y Croft, 2017; Patel, Sharma, Narayan, Binu, Dinesh y Pai, 2016).

En relación al estrés que acompaña a las TRA, una cuestión a tener en cuenta es si los altos niveles de estrés aumentan o disminuyen las tasas de éxito de las TRA. La mayoría de los estudios apoyan que altos niveles de estrés psicológico se asocian con menores tasas de éxito de estas técnicas, como así han concluido varios meta-análisis (Matthiesen, Frederiksen, Ingerslev y Zachariae, 2011; Frederiksen, Farver-Vestergaard, Skovgard, Ingerslev y Zachariae, 2014).

Además del estrés generado por las TRA, la infertilidad y TRA se han asociado con efectos negativos durante el embarazo y posparto (Hammarberg, Fisher y Wynter, 2008). Al comparar embarazadas mediante TRA con embarazadas de forma natural algunos estudios han concluido que aquellas embarazadas mediante TRA tienen mayores niveles de estrés que las embarazadas de forma natural (Gourounti y cols., 2013; Mcmahon y cols., 2013). Sin embargo, otros estudios informaron de niveles de estrés equivalentes en mujeres que concibieron por TRA o de forma espontánea (Darwiche y cols., 2014; Shin y cols., 2015; Stevenson, Troter, Bergh y Sloane, 2016). Es tal la falta de consenso en torno al tema del estrés relacionado con TRA, que una reciente revisión sistemática concluyó que existen inconsistencias en cuanto a los

niveles de estrés durante el embarazo después de TRA (Gourounti, 2016), lo que implica la necesidad de seguir profundizando en este tema.

CAPÍTULO III: ESTRÉS MATERNO PRENATAL: CONSECUENCIAS MATERNAS

Introducción

Los altos niveles de estrés experimentados por la mujer durante el embarazo tienen importantes repercusiones en su salud física y psicológica, que van a determinar el desarrollo de un embarazo exitoso ó el fracaso del mismo. Entre las situaciones mórbidas relacionadas con la presencia de elevados niveles de estrés materno durante la gestación destacan los trastornos hipertensivos, prematuridad y depresión posparto.

3.1 Consecuencias del estrés materno prenatal sobre la salud física materna

Altos niveles de estrés psicológico durante el embarazo se han asociado a varios aspectos relacionados con la salud materna, el curso del embarazo, del parto y nacimiento y el puerperio. Incluso antes del embarazo, el estrés puede influir en el éxito de la concepción. En esta línea, Massey y cols (2016) encontraron asociación entre unos niveles elevados de cortisol en pelo con una menor tasa de embarazos tras un tratamiento de reproducción asistida.

3.1.1 Estrés durante el embarazo, pre-eclampsia y diabetes gestacional

Durante el embarazo, niveles elevados de estrés se han relacionado con mayor probabilidad de pre-eclampsia y diabetes gestacional (Shaw y cols., 2017; Valsamakis y cols., 2017). La pre-eclampsia es una patología propia del embarazo cuyos síntomas principales son hipertensión y proteinuria. Implica afectación de los riñones, retina, hígado e incluso el cerebro. La mujer puede desarrollar convulsiones, en cuyo caso pasaría a denominarse eclampsia, e incluso fallecer. Por otro lado, la diabetes gestacional es un trastorno que puede inducirse por el embarazo. Se detectan altos niveles de glucosa en la sangre materna durante el embarazo, existiendo un mayor riesgo de diabetes materna después del embarazo, hipertensión, macrosomía fetal,

mayor número de partos instrumentados y cesáreas, e incluso un mayor riesgo de muerte fetal (Gary Cunningham, 2015).

Una reciente revisión sistemática que evalúo a 2532 estudios, ha relacionado la existencia de altos niveles de estrés materno prenatal con un menor flujo sanguíneo utero-placentario (Levine, Alderdice, Grunau y McAuliffe, 2016). Así, se encontró relación entre elevados niveles de estrés y menores índices de resistencia y pulsatilidad de la arteria uterina, índice de resistencia y pulsatilidad de la arteria umbilical, índice de pulsatilidad de la arteria cerebral media y el flujo de la vena umbilical. Estos hallazgos muestran evidencia de la relación entre estrés psicológico y los mecanismos biológicos mediante los cuales se afecta a la salud materna y fetal.

Como se explicó en el anterior capítulo, la enzima placentaria 11beta hidroxysteroidea dehidrogenasa tipo 2 (11-HSD-2), es la responsable de metabolizar el paso de cortisol materno al feto. Esta hormona protege al feto de los excesos de cortisol materno, siempre que su actividad se mantenga intacta y los niveles de cortisol materno no superen su capacidad metabólica. Se ha comprobado que niveles elevados de estrés materno durante el embarazo se asocia con una menor expresión de genes placentarios relacionados con la (11-HSD-2), responsable del paso de cortisol materno al feto (Capron, Ramchandani y Glover, 2018). En este sentido, altos niveles de estrés materno reducirán la capacidad de "filtro" de la placenta y el feto estará expuesto a mayores concentraciones de cortisol.

3.1.2 Estrés en el embarazo y variables del parto y nacimiento

En lo que respecta al parto y nacimiento, altos niveles de estrés durante el embarazo se han asociado a nacimiento prematuro y un mayor miedo al parto (Alderdice y cols., 2012; Lynny cols., 2011). A pesar de los beneficios que supone un

parto vaginal en contraposición al nacimiento por cesárea (Fabre-Gonzalez, 2001), aquellas embarazadas a las que se les práctica cesárea, tienen menores niveles de cortisol en sangre de cordón umbilical y sus bebés presentan menores niveles de cortisol en saliva (Sapolsky, 2004). Estos hallazgos deben interpretarse con precaución y alejarse de la idea de que el nacimiento por cesárea reduce los niveles de estrés infantiles. Contrariamente, unos óptimos niveles de cortisol en el recién nacido son imprescindibles para el desarrollo pulmonar que le permitirán un adecuado ajuste a la vida extrauterina (Roberts, Brown, Medley y Dalziel, 2017).

3.2 Consecuencias del estrés materno prenatal sobre la salud psicológica en el puerperio

El puerperio, o posparto, comprende el período de tiempo tras el nacimiento del bebé, durante el cual remiten casi totalmente las adaptaciones maternas al embarazo (Gary Cunningham, 2015; Fabre-Gonzalez, 2001). Las molestias físicas tras el parto o cesárea, la falta de sueño y las grandes demandas del bebé, favorecen la aparición de depresión transitoria o *blues* posparto en aproximadamente el 50% de las mujeres. Se trata de un problema autolimitado que en la mayoría de los casos cede en 2-3 días de forma espontánea. Cuando los síntomas continúan, puede agravarse en un cuadro más grave como la depresión posparto.

- *Depresión posparto*: Afecta al 15% de las mujeres tras el nacimiento de sus bebés y se caracteriza por alteración del estado de ánimo que puede llegar a suicidio (Yim, Tanner Stapleton, Guardino, Hahn-Holbrook y Dunkel Schetter, 2015). Aunque pasa usualmente inadvertida y no recibe tratamiento, la *National Institute for Health and Care Excellence* (NICE, 2016) requiere la implantación de medidas urgentes que minimicen sus efectos negativos en la madre y su descendencia. Un número creciente de

estudios la asocian con un menor apego, un menor neurodesarrollo infantil (O'Hara y McCabe, 2013) y un menor desarrollo de la capacidad de imitación infantil (Perra, Phillips, Fyfield, Waters y Hay, 2015). La detección temprana de aquellos factores relacionados con la depresión posparto pueden prevenir su desarrollo y sus consecuencias negativas, como la psicosis puerperal (Sockol, Epperson y Barber, 2013). Por ello, es imprescindible realizar una completa evaluación psicológica durante el posparto (Pérez Ramírez, García-García y Peralta-Ramírez, 2013; Perez-Ramirez, Garcia-Garcia, Caparros-Gonzalez y Peralta-Ramirez, 2017), para poder así identificar las variables que mejor predicen la posterior aparición de sintomatología de depresión posparto (Leigh y Milgrom, 2008; Robertson, Grace, Wallington y Stewart, 2004). Diversos factores de riesgo se han relacionado con la aparición de depresión posparto (ver Tabla 2) (Caparros-Gonzalez y cols., 2017). Sin embargo, aún no existe consenso en cuanto a la relación entre la activación del eje HHA y la aparición de depresión posparto (Yim, Stapleton, Guardino, Hahn-Holbrook y Schetter, 2015).

- *Síndrome de Estrés Postraumático tras el parto y nacimiento*: Aproximadamente 4,3 millones de mujeres en todo el mundo padecen esta entidad tras el parto (Ayers, McKenzie-McHarg y Slade, 2015). Se caracteriza por una re-experimentación repetida del parto que se acompaña de una elevada activación fisiológica y de alto nivel de estrés psicológico; estos síntomas pueden durar incluso varios años en ausencia de tratamiento y a veces co-existe con depresión posparto (Ayers, Bond, Bertullies y Wijma, 2016; Ayers, Harris, Sawyer, Parfitt y Ford, 2009).

Existen diversos factores de vulnerabilidad que durante el embarazo favorecen la aparición del Síndrome de Estrés Postraumático (PTSD) tras el parto y nacimiento, como la existencia de problemas psicológicos, exposición a altos niveles de estrés por abusos sexuales y violencia de género, ansiedad y estar embarazada por primera vez (Ayers, 2004). Los efectos negativos del PTSD maternos pueden tener consecuencias transgeneracionales en la descendencia relacionadas con una menor activación del eje HHA (Yehuda y cols., 2005).

	SOCIODEMOGRÁFICOS	**OBSTÉTRICOS**	**PSICOLÓGICOS**	**BIOLÓGICOS**
Factores de riesgo	*-Edad < 25 años* *-Sueldo bajo* *-Baja educación* *-Violencia física*	*-Abortos previos* *-Muerte fetal anteparto* *-Ausencia analgesia parto* *-Embarazo no deseado*	*-Depresión antenatal* *-Ansiedad antenatal* *-Bajo apoyo social*	*-Cortisol elevado*

Tabla 2. Factores de riesgo de depresión posparto.

CAPÍTULO IV: ESTRÉS MATERNO PRENATAL: CONSECUENCIAS SOBRE EL DESARROLLO FETAL E INFANTIL

Introducción

Además de las consecuencias negativas que el estrés tiene sobre la salud materna, el estrés materno prenatal provoca efectos perjudiciales sobre la descendencia. Durante el embarazo, los niveles de estrés psicológico materno afectan al desarrollo fetal (Glover, 2014). Como se explicó en el apartado 3.2 de esta tesis, la placenta ejerce un efecto protector a modo de filtro, que impide el paso de la madre al feto de ciertas cantidades de cortisol. Cuando esas cantidades sobrepasan el límite que la placenta tiene como filtro, el cortisol pasa al feto, iniciándose el proceso de Programación Fetal de la Salud y la Enfermedad (Glover, 2015), que se engloba en el concepto previamente señalado como Orígenes Fetales de la Enfermedad Adulta. De este modo aquellos niños y niñas que durante su etapa fetal estuvieron expuestos a elevados niveles de estrés (psicológico y cortisol) tienen una mayor probabilidad de que su neurodesarrollo y desarrollo físico se vea perjudicado. Igualmente, estos infantes tendrán una mayor probabilidad de desarrollar distintas patologías físicas y psicológicas a lo largo de su ciclo vital (La Marca-Ghaemmaghami y Ehlert, 2015).

Si una mujer está embarazada durante un período en el que en su país se está experimentando una hambruna (propia de guerras, refugiados, aislamientos estatales, etc), ese feto en desarrollo está programándose para cuando nazca, poder sobrevivir en un ambiente extrauterino con falta de alimento. El feto se desarrolla con una programación para ahorrar cada caloría que ingiera durante toda su vida, teniendo mayor probabilidad de desarrollar obesidad, hipertensión y diabetes en la edad adulta (Sapolsky, 2004). Estos hallazgos se comprobaron en los estudios, referidos anteriormente, derivados de la hambruna holandesa ocurrida al final de la Segunda Guerra Mundial. En estos estudios se hizo un seguimiento de los bebés nacidos en ese momento, entre el 01 de noviembre de 1943 y 28 febrero de 1948, durante 60 años,

encontrando un mayor riesgo de enfermedades cardiovasculares en la edad adulta de aquellos que estuvieron expuestos a la hambruna durante su etapa fetal (Roseboom, van der Meulen, Ravelli, Osmond, Barker y Bleker, 2001).

Los efectos fetales e infantiles de la exposición a estrés materno prenatal se muestran en los siguientes apartados:

4.1 Peso del bebé al nacer

El peso del recién nacido es un indicador de bienestar. Recién nacidos con un peso < 2500 g presentan problemas de salud y alta incidencia de mortalidad infantil (Gary Cunningham, 2015). La relación entre estrés materno y bajo peso al nacer se ha estudiado extensamente. Niveles elevados de cortisol y de estrés específico del embarazo (preocupaciones de las embarazadas en torno al proceso de embarazo, parto y cuidados del recién nacido) durante el embarazo se han asociado a un bajo peso al nacimiento (Alderdice y cols., 2012; Baibazarova y cols., 2013). Un reciente meta-análisis basado en casi 6 millones de recién nacidos encontró una relación significativa entre altos niveles de estrés materno prenatal, producidos por la exposición materna a estrés específico del embarazo, eventos de vida estresantes y exposición a desastres naturales, tanto en embarazadas de bajo riesgo como aquellas de alto riesgo, con el bajo peso al nacer (Bussieres, Tarabulsy, Pearson, Tessier, Forest y Giguere, 2015).

4.2 Edad gestacional al nacimiento

Aunque se han producido importantes avances terapéuticos relacionados con recién nacidos prematuros (< 37 semanas de gestación) en la última década, la prematuridad está asociada con graves problemas de salud (Caparros-Gonzalez, de la Torre-Luque, Diaz-Piedra, Vico y Buela-Casal, 2017). Aquellas embarazadas con altos niveles de estrés psicológico tienen una mayor probabilidad de tener bebés prematuros

(Sandman y cols., 2016). Además, la prematuridad lleva consigo un gran gasto económico. A falta de datos fiables en países europeos (EFCNI, 2010) , se estima que en EEUU el gasto por prematuridad asciende a más de 26 billones de dólares al año (Butler, 2007). Niveles elevados de estrés durante el embarazo se asocian a nacimiento prematuro y bajo peso al nacer (Baibazarova, Van De Beek, Cohen-Kettenis, Buitelaar, Shelton y Van Goozen, 2013). Los niveles de estrés del entorno intrauterino en el que se desarrolla el bebé influye en gran medida en su óptimo desarrollo a partir de las primeras fases de embarazo y tiene repercusión en la edad gestacional al nacimiento (Glover, 2015).

4.3 Crecimiento intrauterino fetal

La técnica de ultrasonido, originariamente utilizadas para el sonar de los submarinos, ha aportado importantes hallazgos a la investigación en salud materna e infantil. Mediante el uso de ultrasonidos, existe evidencia de la relación entre altos niveles de estrés y ansiedad y un menor crecimiento fetal; concretamente un menor diámetro biparietal de aquellos fetos que en el segundo trimestre de embarazo sus madres informaron mayores niveles de estrés (Diego y cols., 2006). Un menor flujo placentario parece que es la causa de este menor crecimiento intrauterino. Así, al llegar una menor cantidad de sangre y nutrientes al feto, éste presenta un crecimiento menor de lo que le correspondería para su edad gestacional (Levine y cols., 2016).

4.4 Neurodesarrollo infantil

Altos niveles de estrés psicológico y cortisol en el líquido amniótico que rodea al feto se ha asociado a un menor desarrollo cognitivo infantil (Glover, 2015). Paradójicamente, niveles de estrés entre leve a moderado, se han relacionado con un neurodesarrollo motor y cognitivo acelerado (O'Connor, Heron, Golding, Beveridge y

Glover, 2002). Teniendo en cuenta el momento en el que ocurren los eventos estresantes, aquellas embarazadas con niveles elevados de cortisol en el primer o tercer trimestre, tuvieron bebés con neurodesarrollo motor acelerado al ser evaluados a los 12 meses de edad (Davis y Sandman, 2010). Por otro lado, mayores niveles de cortisol en el tercer trimestre de embarazo se relacionó con un menor desarrollo motor a los 3 y 8 meses de edad (Buitelaar, Huizink, Mulder, de Medina y Visser, 2003). En este sentido, existe incongruencias en cuanto a los efectos del estrés prenatal sobre el neurodesarrollo infantil. Por otro lado, mayores niveles de estrés específico del embarazo se ha asociado con un menor neurodesarrollo motor a los 12 meses (Davis y Sandman, 2010), siendo el PDQ el instrumento psicológico para medir estrés específico del embarazo el mejor predictor de neurodesarrollo infantil (Alderdice y cols., 2012).

4.5 Enfermedades físicas y psicológicas en la descendencia

Los efectos a largo plazo de la exposición prenatal a estrés aún no están claros (Glover, 2015). Se ha evidenciado cómo altos niveles de cortisol durante el embarazo se asocian con alteraciones en el tamaño de la amígdala y con problemas emocionales en la descendencia a los 7 años de edad (Buss, Davis, Shahbaba, Pruessner, Head y Sandman, 2012). También cómo la pérdida de un familiar durante el embarazo se relaciona con la aparición de trastornos del espectro autista, déficit de atención con hiperactividad y aumento del riesgo de suicidio en mayores de 2 años (Class y cols., 2014). Sin embargo, un gran estudio epidemiológico basado en una cohorte de 54. 458 nacimientos en Dinamarca, no se encontró relación entre altos niveles de estrés durante el embarazo y enfermedades en la descendencia en los primeros 21 años de vida (Hyland, Shevlin, Elklit, Christoffersen y Murphy, 2016).

CAPÍTULO V: JUSTIFICACIÓN Y OBJETIVOS

5.1 Justificación

Existen numerosos estudios que evidencian los efectos negativos del estrés psicológico materno durante el embarazo sobre la salud materna e infantil. También se ha informado de los efectos negativos que tienen los niveles elevados de cortisol durante el embarazo, como respuesta a una activación del eje HHA, sobre la madre durante la gestación y sobre la su descendencia. Sin embargo, la mayoría de estos estudios son transversales, evalúan estrés psicológico con medidas no específicas del embarazo y miden cortisol de momentos puntuales en substratos biológicos invasivos e influenciables por ritmos circadianos.

De este modo, a pesar de que diferentes investigaciones han estudiado los efectos adversos del estrés durante el embarazo, no se encuentran muchos estudios que evalúen longitudinalmente a lo largo de todo el embarazo, los efectos del estrés psicológico general, del estrés específico del embarazo y niveles de cortisol en pelo, sobre diferentes variables de salud materna e infantil.

En este sentido, nuestra hipótesis inicial es que el mecanismo psiconeuroinmunológico que subyace al deterioro de la salud materna e infantil debido al estrés psicológico durante el embarazo está asociada a una excesiva activación del eje HHA.

Así, cuando una mujer embarazada está expuesta a elevados niveles de estrés psicológico, se produce una activación del eje HHA, lo que aumenta los niveles de cortisol a los que está expuesta la embarazada y el bebé. Este hecho se ve favorecido por la activación de la placenta que favorece una mayor producción de cortisol por parte del eje HHA materno. Elevados niveles de cortisol podrían tener importantes

repercusiones sobre la salud materna en distintos momentos del período de gestación y sobre el propio feto en desarrollo y recién nacido.

5.2 Objetivos e hipótesis

Objetivo general

Estudiar las repercusiones del estrés psicológico materno prenatal sobre la salud materna e infantil durante el proceso de embarazo, parto y puerperio, así como sus repercusiones en el neurodesarrollo del bebé.

Objetivos específicos

Con el fin de llevar a cabo el objetivo principal de esta tesis, se realizaron seis estudios que se engloban en tres bloques con los siguientes objetivos específicos:

BLOQUE I

Revisión sistemática

Este bloque lo forma el estudio 1, que se encuentra actualmente en proceos de revisión. Es un bloque necesario para determinar las mejores medidas de estrés que se usarán en el resto de estudios de esta tesis. El objetivo específico del estudio del presente bloque fue:

- **Objetivo específico 1**: Comprobar cuál de las medidas de estrés durante el embarazo (Psicológico vs. Cortisol), era un mejor predictor de resultados negativos maternos y en la descendencia.

La hipótesis planteada fue que las medidas de estrés psicológico eran mejores predictores de resultados negativos que las medidas cortisol.

Este estudio se encuentra en proceso de revisión en la revista *Psychoneuroendocrinology* (Caparros-Gonzalez, Lynn, Alderdice y Peralta-Ramírez, 2018).

BLOQUE II

Validación

El objetivo específico del estudio fue:

- **Objetivo específico 2**: Adaptar y validar en embarazadas españolas de habla castellano el cuestionario PDQ, para poder evaluar los niveles de estrés específico del embarazo en dicha población.

La hipótesis planteada fue que el PDQ presenta óptimas características psicométricas confirmando la estructura factorial del PDQ, presentada en estudios previos.

Este estudio está en proceso de revisión en la revista *Women & Health* (Caparros-Gonzalez, García-García, Perra, Alderdice, Lynn, Lobel y Peralta-Ramírez, 2018).

BLOQUE III

Evaluación psicológica y biológica de estrés

En este bloque se llevaron a cabo cuatro estudios (estudio 3, 4, 5 y 6) para investigar la relación psicológica y biológica (cortisol en pelo) del estrés durante el embarazo con diversas variables. Este bloque es necesario para conocer desde diferentes perspectivas (psicológica y biológica) los efectos del estrés durante el embarazo en la salud materna e infantil.

En el estudio 3 de esta tesis doctoral, se describe el *Protocolo* de evaluación del estrés a lo largo del embarazo y posparto, así como la evaluación del neurodesarrollo infantil. En el estudio 4 de esta tesis, se investigó la capacidad de diferentes medidas de estrés psicológico y cortisol en pelo, para predecir la ocurrencia de síntomas de depresión posparto. En el estudio 5 de esta tesis, se estudió mediante cuestionarios psicológicos y cortisol en pelo, los niveles de estrés de embarazadas mediante técnicas de reproducción asistida y de forma natural. En el estudio 6, se evaluó si los niveles de estrés materno y neonatales podían predecir el neurodesarrollo infantil a los 6 meses de edad. Los objetivos específicos que se persiguieron en estos estudios fueron:

- **Objetivo específico 3**: Diseño del protocolo del estudio, denominado *GESTASTRESS,* de evaluación de los niveles de estrés psicológico y cortisol en pelo en embarazadas y evaluación de los niveles de cortisol en pelo de los recién nacidos. El estudio 3 está actualmente aceptado y se encuentra *in press* en una revista *JCR*. Este estudio es necesario para conocer la metodología idónea de evaluación de los niveles de estrés en las embarazadas y sus recién nacidos. Nosotros hipotetizamos que este protocolo nos permitirá estudiar la relación entre el estrés psicológico y la medida de cortisol en pelo con diferentes variables de salud materna durante el embarazo, fetal e infantil.

Se realizó el diseño que describe el protocolo de evaluación de la activación del eje HHA durante el embarazo, mediante la aplicación simultánea de cuestionarios psicológicos y la medida de cortisol en pelo de las embarazadas y sus recién nacidos.

El artículo está aceptado y está *in press* en la revista *Revista Española de Salud Pública* (Caparros-Gonzalez, García-García, Mariñas-Lirola y Peralta-Ramírez, 2018).

- **Objetivo específico 4**: Analizar las variables sociodemográficas, obstétricas, psicológicas y niveles de cortisol en pelo, durante el primer, segundo y tercer trimestre de embarazo, que pudieran predecir síntomas de depresión posparto.

La hipótesis que se planteó fue que las variables psicológicas y los niveles de cortisol en pelo podían predecir los síntomas de depresión posparto.

Este estudio está publicado en la revista *Plos One* (Caparros-Gonzalez, Romero-Gonzalez, Strivens-Vilchez, Gonzalez-Perez, Martinez-Augustin y Peralta-Ramirez, 2017).

- **Objetivo específico 5**: Comparar los niveles de estrés psicológico y niveles de cortisol en pelo, en embarazadas mediante técnicas de reproducción asistida versus embarazadas de forma espontánea.

La hipótesis fue que las embarazadas por técnicas de reproducción asistida presentan mayores niveles de estrés psicológico y mayores niveles de cortisol en pelo, que las embarazadas de forma espontánea durante todo el embarazo.

Este estudio se encuentra en revisión en la revista *Journal of Reproductive and Infant Psychology* (Caparros-Gonzalez, Romero-Gonzalez, Quesada-Soto, Gonzalez-Perez, Mariñas-Lirola y Peralta-Ramírez, 2018).

- **Objetivo específico 6:** Comprobar si los niveles de cortisol en pelo materno durante el primer, segundo, tercer trimestre y posparto así como los niveles

de cortisol de los recién nacidos, podían predecir el neurodesarrollo infantil a los 6 meses de edad.

La hipótesis planteada fue que los niveles de cortisol en pelo materno y neonatal podían predecir el neurodesarrollo cognitivo y motor infantil.

Este estudio se encuentra en revisión en la revista *Journal of Developmental & Behavioral Pediatrics* (Caparros-Gonzalez, Romero-González, Gonzalez-Perez, Cruz-Quintana, y Peralta-Ramírez, 2018).

OBJETIVO GENERAL

Estudiar las repercusiones del estrés psicológico materno prenatal sobre la salud materna e infantil durante el embarazo, parto y puerperio

CAPÍTULO VI: "ESTUDIO 1"

Cortisol levels versus Self-Report Stress Measures as Predictors of Adverse Pregnancy Outcomes: A Systematic Review

Caparros-Gonzalez, Lynn, Alderdice y Peralta-Ramírez (Under review). Cortisol levels versus Self-Report Stress Measures as Predictors of Adverse Pregnancy Outcomes: A Systematic Review. *Psychoneuroendocrinology*

"Cortisol levels versus Self-Report Stress Measures as Predictors of Adverse Pregnancy Outcomes: A Systematic Review"

Introduction

Stress during pregnancy can have negative transgenerational effects in the offspring and has been related to prematurity and low-birth weight (Alderdice and Lynn, 2009; Cannella et al., 2013; Yehuda et al., 2005). Prenatal stress can be assessed using self-report measures or biological measures. Associations have been found between self-reported prenatal maternal stress and increases in adverse outcomes for the infant, including neurodevelopmental, behavioural and cognitive disorders (Glover, 2014; Lobel and Dunkel Schetter, 2016). Thus, the Prenatal Distress Questionnaire, a self-report measure of prenatal stress was found to be related to adverse pregnancy outcomes (Alderdice et al., 2012; Lynn et al., 2011). Biological measures, such as cortisol, have been associated with negative pregnancy consequences (D'Anna-Hernandez et al., 2012; Entringer et al., 2015). Cortisol can be used independently and alongside self-report measurement tools to assess stress during pregnancy (Harville et al., 2009; Obel et al., 2005).

Cortisol is a frequently used biomarker in psychoneuroendocrinology research to assess levels of stress. This glucocorticoid hormone is released through stimulation of the Hypothalamic–Pituitary–adrenal (HPA) axis in response to stressors. Cortisol is necessary for an adequate functioning of the organism and regulates a wide range of processes, such as immunity, inflammatory responses and metabolism. It is, therefore, involved in adjustment to environmental challenges (Marieb and Hoehn, 2007). Cortisol

helps coping with stress through the coordination of brain and body functions (Lazarus and Folkman, 1984). This process involves a temporary increase of cortisol secretion, which consequently boosts energy availability by incrementing muscle strength, memory, glucose and lipid metabolism, and pain threshold (Marieb and Hoehn, 2007). Additionally, it has been reported that cortisol levels increases over the course of pregnancy and, therefore, the function of the HPA is affected by pregnancy (Duthie and Reynolds, 2013; Obel et al., 2005).

Cortisol during pregnancy has been analysed from saliva, urine, blood serum, amniotic fluid and more recently from hair samples (Bergman et al., 2010b; D'Anna-Hernandez et al., 2011; Fan et al., 2016; Jung et al., 2011; Suglia et al., 2010). On one hand, assessing cortisol levels from serum, saliva, amniotic fluid or urine can have certain limitations: they only inform about short-term cortisol concentrations (24-36 hours), are influenced by personal or environmental features, may be invasive (mainly for blood serum and amniotic fluid), and repeated measurements are required over a certain period of time to obtain a mean chronic concentration of cortisol (Andersson et al., 2004; Gibson et al., 1999; Glover et al., 2009; Kidambi et al., 2007; Kobelt et al., 2003; Wolfram et al., 2013). On the other hand, hair cortisol levels are a non-invasive assessment method that offer information on chronic stress by revealing long-term cortisol concentrations (Wosu et al., 2013). It has been reported that hair cortisol levels are a retrospective biomarker of HPA axis activity by reflecting cortisol release over a three-month period (D'Anna-Hernandez et al., 2011; Kirschbaum et al., 2009), even in pregnant women with asthma (Smy et al., 2016). Overall, each method has its strengths and limitations, which can influence the measurement of cortisol levels and the results obtained. Seth and colleagues recommend that the choice of measurement have to be consistent with each study's objectives (Seth et al., 2016).

Higher cortisol levels during pregnancy have been related with low birthweight, prematurity, pregnancy loss (D'Anna-Hernandez et al., 2012; Goedhart et al., 2010; McCool et al., 1994; Nepomnaschy et al., 2006; Sandman et al., 2006; Stewart et al., 2015), reduced childhood intelligence (LeWinn et al., 2009) and a higher gain in body fat (Entringer et al., 2015; Hohwü et al., 2015). Nevertheless, no association was found between cortisol levels during pregnancy and new-born head circumference (Koubaa et al., 2015), infant fear reactivity (Bergman et al., 2010a) or child temperament (Bergman et al., 2010b).

Considerable evidence has accumulated on the application of self-report tools to measure psychological stress during pregnancy (Alderdice et al., 2012; Nast et al., 2013). It has been substantiated that psychological responses to the alternatively labelled pregnancy-specific distress, worry, anxiety, or stress, is a significant indicator of prenatal psychological stress (Alderdice et al., 2012; Brunton et al., 2015; Nast et al., 2013).

Several studies have reported the association between cortisol levels during pregnancy with self-report anxiety and stress measures (Evans et al., 2008; Kalra et al., 2007; Kane et al., 2014; LP Lovely, 2003; Obel et al., 2005; Pluess et al., 2010). In this regard, high cortisol levels appeared to be associated with perceived stress, life event stress (Kalra et al., 2007; Obel et al., 2005), anxiety (Kane et al., 2014; LP Lovely, 2003; Pluess et al., 2010), and depression and anxiety when comorbid (Evans et al., 2008). However, a number of studies have failed to find any significant associations between cortisol levels and self-report stress questionnaires during pregnancy (Braig et al., 2016; Doyle et al., 2015; Petraglia et al., 2001; Shea et al., 2007; Sikkema et al., 2001; Voegtline et al., 2013).

Self-reported pregnancy-specific stress appears as the more powerful predictor of negative pregnancy outcomes (DiPietro et al., 2004; DiPietro et al., 2002; Lobel et al., 2008; Mulder et al., 2002; Sandman et al., 2012; Wadhwa et al., 1993). Pregnancy-specific stress specifically relates to worries and concerns about the health of the fetus, relationships, diet, body weight, appearance, labour, and delivery (Alderdice and Lynn, 2011; Yali and Lobel, 1999). Pregnancy-specific stress measures are more sensitive than generic stress measures in predicting foetal behaviour, infant development and infant emotional regulation (DiPietro et al., 2002; DiPietro et al., 2006), and has been linked to preterm birth, shorter gestation, neurodevelopmental delay and disturbances in brain structures in childhood (Buss et al., 2011; Buss et al., 2010; Dole et al., 2003; Huizink et al., 2003; Kramer et al., 2009; Roesch et al., 2004).

Measuring psychological stress during pregnancy faces two major challenges: firstly, the fact that stress is a multidimensional concept and, secondly, that self-report generic questionnaires commonly used to assess stress might not be appropriate for use during pregnancy. Prenatal psychological stress, measured through self-report questionnaires, has been linked to stillbirth, preterm birth, low foetal weight, altered immune offspring response, foetal growth restriction and low birth weight (Coussons-Read et al., 2012; Ding et al., 2014; Lewis et al., 2016). However, it has been recently reported that some studies have found no relation between self-report measures during pregnancy on adverse outcomes such as gestational age at delivery, infant birth weight, or obstetric difficulties (for a review see Levine et al., 2016). A meta-analysis concluded there were no significant associations between self-reported anxiety measures during pregnancy and potential perinatal outcomes (Littleton et al., 2007). In this study it was reported that those studies using small samples and less-commonly used anxiety self-report measures were more likely to find a significant association.

Risk of bias was detected in this meta-analytic review. Only studies published in English were included and outcomes theoretically associated with anxiety during pregnancy were not included (small for gestational age infants and risk of developing preeclampsia).

Due to the lack of consensus in this area and the inconsistences between studies, this current review specifically focuses on the relationship between cortisol levels, self-report measurement tools for stress, and adverse pregnancy outcomes. The aim is to identify cortisol levels or self-report measurement tools for stress are better predictors of adverse pregnancy outcomes.

Method

A systematic review was conducted in accordance with PRISMA guidelines (Moher et al., 2009) in order to identify studies reporting relationships between cortisol levels during pregnancy, self-report stress measurement tools and adverse pregnancy outcomes.

Search strategy

A systematic search strategy was developed. Studies were eligible for inclusion in this review if they reported the use of both self-report stress measures and cortisol levels to assess stress levels during pregnancy, alongside an evaluation of the relationship with at least one adverse pregnancy outcome. We included primary research studies. Search terms focused on pregnancy, stress, cortisol and a range of adverse pregnancy, maternal or infant-related outcomes, for example, preterm birth, low birth weight, still birth and infant development. MeSH headings were used for search terms in addition to a number of free text terms, where needed. The electronic databases included in the search were MEDLINE, PsychInfo, CINAHL, EMBASE, Web of

Science, Scopus, LILACS, LatinIndex and Redalyc. Filters were put in place to limit the search to articles that were published in English or Spanish and contained human female participants. Date of publication was not considered as a restriction in the search. The initial search was carried out in March 2016 with an updated search conducted in January 2018.

The retrieved records were independently screened by two reviewers (R.C.G and F.L.) according to the following inclusion criteria: (1) studies reporting the use of self-report measurement tools of stress during pregnancy; (2) studies reporting biological measurements of cortisol during pregnancy; (3) studies reporting relevant pregnancy outcomes; (4) studies reporting relevant maternal or infant outcomes; (5) quantitative approach to the study design and empirical analysis. Each of these criterions had to be satisfied for inclusion in the review. Due to the fact that stress is often a poorly defined term in the literature, self-report measures of anxiety were deemed relevant for inclusion. Studies were excluded if they only reported either the associations between self-report measures and adverse outcomes, or cortisol levels and adverse outcomes. Record titles and abstracts were initially screened, followed by an assessment of the full text of papers that were deemed to be eligible for inclusion. Two reviewers (R.C.G. and F.L.) verified the eligibility of each record and extracted the pertinent information from the studies eligible for inclusion through the use of a data extraction form designed specifically for the purpose of this review. Discrepancies at this stage were adjudicated by the first and second author.

Data extraction of study characteristics and findings was performed prior to determining any patterns in the literature. Once all data was extracted, findings from each study were tabulated and described in terms of a narrative synthesis of evidence. To rate the quality of the studies included, the Quality Assessment Tool for

Observational Cohort and Cross-Sectional Studies (National Institute of Health, 2014) was used. This quality assessment tool provides carefully designed criteria to evaluate observational cohort and cross-sectional studies. The checklist consists of 14 items assessing the research question, sample size justification, independent and dependent variables' definitions, potential confounding factors and offers a guidance for classifying the quality of each study as good, fair or poor. The use of this checklist reduces threats to internal and external validity and assists reviewers and researchers in assessing the quality of the reported study (National Institute of Health, 2014).

Results

A total of 17 empirical research studies were identified as eligible for inclusion. Figure 1 presents the PRISMA flow diagram detailing the identification of records, screening and eligibility process for the search. The studies were published between 1997 to 2018 and were all conducted in developed countries, including the United States, Canada, United Kingdom, the Netherlands and Germany. Participants recruited for these studies were mainly low risk pregnant women with singleton pregnancies although vulnerable groups, such as pregnant women reporting depression or adolescent mothers, was reported by five of the included studies (Bolten et al., 2010; Doyle et al., 2015; Field et al., 2006; Pluess et al., 2010; Ponirakis et al., 1997).

Table 1 summarizes the characteristics of included studies, with details on the stress measures used (both self-report stress measures and cortisol levels), timing of assessment in pregnancy, maternal and infant outcomes assessed, main study results and a quality assessment of each study.

Table 1. Summary of Included Studies

Authors (year)	Study Sample	Stress Measures and Timing of Assessment		Pregnancy, Maternal and Infant Outcomes	Results	Quality of studies
		Self-report	Cortisol			
Observational longitudinal studies						
Baibazarova et al. (2013)	158 pregnant women and their infants (N= 316 dyads)	Perceived Stress Scale (PSS), Pregnancy related anxieties questionnaire-revised (PRAQ-R) and State-Trait Anxiety Inventory-State (STAI-S) at T_1 (18 weeks)	Amniotic fluid and blood serum at T_1	Gestational age at birth, infant birth weight, Infant Behavior Questionnaire-Revised (IBQ-R) at T_2 (3 months postpartum)	*Self-report/Cortisol* No significant association *Self-report/Outcomes* No significant association *Cortisol/Outcomes* High amniotic cortisol predicted a lower birth weight ($r=-0.25$; $p<0.01$) High amniotic cortisol related to high infant fear and distress ($t=1.78$, $p<0.05$; $t=1.94$, $p<0.10$) High prenatal cortisol related to low birth weight and high infant temperament at T_2 ($x^2=72$, $p=0.95$, RMSEA = 0.00, CFI = 1.00, GFI = 1.00, AGFI = 0.99)	Good quality
Bergman et al. (2010)	125 mothers and their 125 babies (N=dyads)	STAI and Stressful Life Events Questionnaire (SLEQ) at T_1 (18 weeks) and STAI at T_2	Maternal blood cortisol and amniotic fluid cortisol at T_1	BSID and Infant-mother attachment (Ainsworth's Strange Situation) at T_2	*Self-report/Cortisol* No significant associations *Self-report/Outcomes* Lower maternal postnatal STAI-S scores related to securely attached children ($F=4.20$, $p<0.05$) Postnatal high STAI-S scores significantly associated with low MDI scores ($r=-0.23$, $p<0.05$)	Good quality

		(17 months postpartum)			*Cortisol/Outcomes* Significant inverse association between amniotic fluid cortisol (ln transformed values) and BSID r=− 0.25, p<0.01) Prenatal amniotic cortisol strongly predicted MDI in children with an insecure attachment history (r=−0.47, p<0.001) Marginal association between amniotic fluid cortisol and MDI was more negative earlier in gestation (B=1.08, SE= 0.57, p=0.06)	
Bolten et al. (2010)	75 pregnant women and their new borns (N= 150 dyads)	PSS and Prenatal Distress Questionnaire (PDQ) at T_1 (18 weeks) and T_2 (37 weeks)	Salivary cortisol T_1 and T_2 (on awakening; 07.00 a.m; 30´-45´-60´)	Gestational age at birth, infant birth weight, APGAR score, new-born head circumference	*Self-report/Cortisol* No significant associations *Self-report/Outcomes* No significant associations *Cortisol/Outcomes* Higher maternal cortisol on awakening at T_1 predicted lower birth weight (β=-0.29, p<0.05) Higher maternal cortisol on awakening at T_3 predicted lower birth weight (β=-0.30,p<0.01) and body length (β=-0.28,p<0.05) Higher maternal cortisol on awakening (both at T_1 and T_3) predicted lower birth weight (F=6.91,p<0.001), smaller body length (F=3.29,p<0.01) and smaller head circumference (F=2.99,p<0.05)	Good quality
Buitelaar et al. (2003)	170 pregnant women and their infants (N= 340 pairs)	Everyday Problem List (everyday hassles), Pregnancy Related, PRAQ-R and	Salivary cortisol at T_1, T_2 and T_3, 7 samples every 2h between 8:00 a.m. and 8:00 p.m	Gestational age at birth, infant birth weight, Bayle Scale for Infant Development (BSID) (Mental Development Index-MDI,	*Self-report/Cortisol* No reported *Self-report/Outcomes* Higher fear of giving birth at T_2 related to lower 8-month scores of infant development (F=5.04, p<0.05) High amount of daily hassles at T_1 associated with lower MDI scores at 8 months (F=3.9,	Good quality

	PSS at T_1 (17 weeks), T_2 (28 weeks) and T_3 (38 weeks)	Psychomotor Development Index-PDI and Infant Behaviour Record-IBR) at 3 and 8 months, Infant Characteristics Questionnaire (ICQ) at 3 and 8 months	p<0.05) Strong fear of giving birth at T_2 associated with lower MDI and PDI scores at 8 months (F=5.58, p<0.05; F=7.67, p<0.01) Fear of giving birth at T_2 and negative correlation with PDI scores at 8 months (F=5.30, p<0.05) Daily hassles at T_1 an independent risk factor for low MDI scores in 8 months infants (SOR=1.1, 95% CI 1.02–1.18) High levels of fear of giving birth at T_2 increased the risk of having an infant with a low PDI score at 8 months (SOR=1.3, CI 1.12–1.56) Overall significant effect of fear of giving birth on low exploration, test-affectivity, and goal-directedness scores at 3 and 8 months (BSID sub-scales) (F=2.74, p<0.05) Overall significant effect of fear of bearing a handicapped child on low exploration, test-affectivity, and goal-directedness scores at 3 and 8 months (F=2.87, p<0.05) Overall significant effect of high PSS scores on low exploration, test-affectivity, and goal-directedness scores at 3 and 8 months (Bayley sub-scales) (F=2.45, p<0.05) A strong fear of giving birth at T_1 linked to lower test-affectivity at 8 months (F=1.38, p<0.001) Strong fear of bearing a handicapped child at T_1 associated with lower scores of test-affectivity at 3 months (F=5.93, p<0.05) High levels of fear of bearing a handicapped child at T_1 related to reduced goal-directedness of 8 months (F=6.27, p<0.05)

					PSS scores at T_1 the only significant predictor of mother-reported difficulty and inadaptability at 3 and 8 months ($F=4.44$, $p<0.05$) *Cortisol/Outcomes* High cortisol at T_3 related to low MDI and PDI scores at 3 and 8 months ($F=4.61$, $p<0.05$) High cortisol at T_3 related to lower MDI scores at 3 months ($F=6.38$, $p<0.05$) High cortisol at T_3 related to lower PDI scores at 3 and 8 months ($F=9.15$, $p<0.05$; $F=9.38$, $p<0.05$) A linear negative effect of cortisol at 8 a.m. at T_3 on the MDI scores at 3 months ($F=7.19$, $p<0.01$) A linear negative effect of cortisol at 8 a.m. at T_3 on the PDI scores both at 3 and 8 months of age ($F=5.16$, $p<0.01$; $F=7.08$, $p<0.01$)	
Davis et al. (2010)	125 pregnant women and their infants (N=250 dyads)	PSS, Center for STAI-S, Pregnancy Specific Anxiety (PSA), Parenting Stress Index (PSI) at T_1(15 weeks), T_2(20 weeks), T_3(26 weeks), T_4(32 weeks), T_5(	Salivary cortisol at T_1, T_2, T_3, T_4 and T_5	BSID at 3, 6 and 12 months of age.	*Self-report/Cortisol* No significant associations *Self-report/Outcomes* High PSA scores at T_1 and related to an overall change associated with low MDI ($t=2.4$, $p<0.05$) High PSA scores at T_1 related to low MDI scores (t's ranged from 2.1 to 2.9, p's < 0.05) Overall rate of change of PSA was the strongest predictor of MDI ($\Delta R^2=0.05$, $p<0.05$) PSA scores independently (from cortisol) predicted MDI ($\Delta R^2=0.05$, $p<0.05$) *Cortisol/Outcomes* Lower maternal cortisol at T_1 predicted accelerated MDI scores across the first postnatal year ($t=-2.5$, $p<0.01$) Higher maternal cortisol at T_5 predicted accelerated infant MDI scores over the first	Good quality

		37 weeks, T_6(3 months postpartum), T_7(6 months postpartum), T_8(12 months postpartum)			postnatal year (t=1.9, p<0.05) Lower maternal cortisol before T_2 related to infants scoring higher on the 12 month MDI (t's ranged from −2.1 to −2.7, p's < 0.05)	
Davis et al. (2012)	178 pregnant women and their infants (N=356 dyads)	PSS, STAI-S and PSA at T_1 (20 weeks), T_2 (26 weeks) and T_3 (32 weeks)	Salivary cortisol at T_1, T_2 and T_3	Gestational age at birth, Child anxiety at 6 to 9 years (Achenbach System of Empirically Based Assessment –ASEBA- and Child Behavior Checklist-CBCL)	*Self-report/Cortisol* No significant associations *Self-report/Outcomes* High average PSS and PSA scores associated with higher child anxiety at 6 to 9 years of age (F=7.1, p<0.05; F=7.3, p<0.05 respectively) PSS scores at T_1 the strongest predictor of child anxiety (F=5.8, p<0.05) PSA scores at T_2 the strongest predictor of child anxiety F=5.8, p<0.05) PSA scores associated with child anxiety (Odds ratio=1.1, 95% confidence interval=1.0 to 1.2, p=0.05) PSA independently predicted child anxiety (F=6.1, p<0.05) *Cortisol/Outcomes* Elevated average maternal cortisol associated with higher child anxiety at 6 to 9 years of age F=6.7, p<0.05) Higher maternal cortisol twice more likely related to children with anxiety ratings within the borderline/clinically significant range ratings (Odds ratio = 2.1, 95% confidence interval=1.1 to 3.9, p<0.05) Maternal cortisol independently predicted child	Good quality

					anxiety (F=6.1, p<0.05)	
De Weerth et al. (2013)	107 pregnant women and their infants (N=202 dyads)	STAI-S, Daily Hassles Questionnaire (APL) and PRAQ-R at T_2 (37 weeks)	Salivary cortisol at T_1 (33 weeks, 2 consecutive days)	Infant salivary cortisol level due to separation at T_3 (9 months of age). Prior to the stress separation paradigm at $T_{3.1}$ (baseline concentration), $T_{3.2}$ (35´ post-separation), $T_{3.3}$ (75´ post-separation) and at $T_{2.4}$ (90´ post-separation)	*Self-report/Cortisol* No significant associations, except for a marginally significant positive relation between the STAI-S and maternal cortisol at T_1 (r = 0.18, p<0.10) *Self-report/Outcomes* Signifcant positive correlation between STAI-S scores at T_2 and cry/fuss at $T_{3.1}$ (r=-0.21; p<0.05) *Cortisol/Outcomes* Higher maternal cortisol levels predicted higher infant cortisol responses over time (Estimate=10.03, SE=4.11, p<0.05). Higher maternal cortisol levels related to less crying and fussing in general as reaction to maternal separations (Estimate=-0.99, SE=0.44, p<0.05).	Good quality
Doyle et al. (2015)	125 pregnant adolescents	Ecological Momentary Assessment (EMA) and Rating of their current physical activity at T_1 (16 weeks), T_2 (27 weeks) and T_3 (37 weeks)	Salivary cortisol at T_2 and T_3	Maternal physical activity, maternal blood pressure, maternal heart rate at T_1, T_2 and T_3 Fetal heart rate (FHR) and fetal movements at T_2 and T_3 Gestational age, birthweight, infant´s sex and pregnancy complications at birth	*Self-report/Cortisol* No significant associations *Self-report/Outcomes* Negative mood at T_1 (F=4.16, p<0.05) and T_2 (F=6.77, p<0.05) significantly associated with lower overall FHR in males Negative mood at T_1, more significant in females (F=9.53, p<0.01) associated with higher levels of fetal coupling (correlation between FHR and movements) during gestation *Cortisol/Outcomes* Higher cortisol levels at T_2 related to higher FHR at T_2 (F=4.20, p<0.05) Higher cortisol levels at T_1 related to less of an increase in fetal coupling levels during gestation	Good quality

					(F=8.20, p<0.01), and found an interaction with sex, being more significant in males (F=5.14, p<0.05)	
Field et al. (2006)	300 pregnant women	Structured Clinical Interview on Diagnosis (SCID), STAI-T, Trait Anger Expression Inventory (STAXI), Behavioral Inhibition and Behavioral Approach System questionnaire (BIS/BAS), Obstetric Complications Scale (OCS) and Postnatal Complications Scale (PNS) at T_1 (20 weeks)	Urinary cortisol at T_1 (20 weeks)	Fetal activity, fetal growth measures (femur length, head circumference, abdominal circumference and biparietal diameter) at T_1 (20 weeks) Gestational age, infant birthweight, infant birth length, infant head circumference and Brazelton Neonatal Behavior Assesment Scale (BNBS) at T_2 (birth)	*Self-report/Cortisol* No significant associations *Self-report/Outcomes* No significant associations *Cortisol/Outocomes* High cortisol levels related with more active fetuses, (F=3.81; p<0.05) small fetus head circumference (F=11.66; p<0.05), small fetus abdominal circumference (F=11.43; p<0.05), small fetus biparietal diameter (F=9.27; p<0.05) and low fetal weight (F=5.36; p<0.05)	Good quality
Goedhart et al. (2010)	2810 pregnant	STAI-S, PRAQ-R	Blood cortisol at T_1	Gestational age at birth, Infant	*Self-report/Cortisol* No significant association	Good quality

	women and their infants (N=5620 dyads)	Parenting Daily Hassles (PDH) and Work Experience and Appreciation Questionnaire (VBBA) at T_1 (13 weeks)		birth weight and Fetal growth	*Self-report/Outcomes* No significant association *Cortisol/Outcomes* Maternal cortisol levels negatively related to infant birthweight, (B=0.35; p<0.001) Maternal cortisol levels positively related to Small for Gestational Age (SGA) (OR=1.00; p<0.05) Higher maternal cortisol levels independently related to lower birthweights (B=0.94; p<0.05) Higher maternal cortisol levels were independently related to a higher SGA risk (OR=1.01; p<0.05)	
Hoffman et al. (2016)	90 pregnant women	PSS and STAI-S at T_1 (16 weeks), T_2 (22 weeks), T_3 (28 weeks), T_4 (34 weeks) and T_5 (40 weeks)	Hair cortisol concentrations (HCC) at T_1, T_3 and T_5	Gestational age at birth	*Self-report/Cortisol* PSS scores at T_1 positively correlated with HCC at T_2 (r=0.28, p<0.05) *Self-report/Outcomes* PSS scores at T_1 positively correlated with earlier gestational age at delivery (r=0.30, p<0.05) *Cortisol/Outcomes* HCC at T_2 was negatively correlated with gestational age at birth (r=0.25, p<0.05)	Good quality
Hompes et al. (2012)	91 pregnant women	Hospital Anxiety Depression Scale (HADS) and PRAQ at T_1 (14 weeks), T_2 (27 weeks) and	Salivary cortisol (4 time points at each trimester; awakening, 30 min, 4 h, and 12 h) at T_1 (14 weeks), T_2 (27 weeks) and T_3 (37 weeks).	Intrauterine growth at 12, 20 and 30 weeks (through ultrasound) and infants´anthropometric measures at T_4 (birth): length, weight, Body Mass Index (BMI), Ponderal Index (PI)	*Self-report/Cortisol* No reported *Self-report/Outcomes* No significant associations *Cortisol/Outcomes* Basal cortisol levels at T_2 signficantly predicted the variance of weight (proportion of variance in growth variable explained (PVE) = 11.6%) and body mass index (BMI) at birth (PVE = 6.8%)	Good quality

		T_3 (37 weeks)		and Head Circunference (HC)		
Huizink et al. (2003)	170 mothers and their 170 newborns (N= 340 dyads)	Everyday Problem List (EPL), Pregnancy Related Anxieties Questionnaire-Revised (PRAQ-R), General Health Questionaire (GHQ) and PSS at T_1 (17 weeks), T_2 (28 weeks) and T_3 (38 weeks)	Salivary cortisol at T_1, T_2 and T_3	Gestational age at birth, Infant birth weight, BSID (MDI and PDI) at T_4 (3 months after birth) and T_5 (8 months after birth)	*Self-report/Cortisol* No reported *Self-report/Outcomes* Fear of giving birth at T_2 significantly associated with low scores of infant development at T_5, ($F=5.04$, $p<0.05$) High scores in EPL at T_1 significantly associated with lower MDI scores at T_5 ($F= 3.9$, $p<0.05$) Strong fear of giving birth (PRAQ-R) at T_2 significantly associated with lower MDI and PDI scores at T_5 ($F=5.58$, $p<0.05$; $F=7.67$, $p<0.01$) Strong fear of giving birth (PRAQ-R) at T_3 significantly associated with lower MDI scores at T_5 ($F=5.34$, $p<0.05$) Daily hassles at T_1 were an independent risk factor for low MDI scores of infants at T_5 (SOR=1.1, CI=1.02–1.189 Fear of giving birth (PRAQ-R) at T_2 increased the risk of having an infant with low PDI scores at T_5 (SOR= 1.3, CI=1.12–1.56) *Cortisol/Outcomes* High cortisol at T_3 related to the MDI and PDI scores at T_4 and T_5 ($F=4.61$, $p<0.05$) High cortisol at T_3 related to lower MDI scores at T_4 ($F =6.38$, $p<0.05$) High cortisol at T_3 related to lower PDI scores at T_4 and T_5 ($F=9.15$, $p < 0.05$; $F= 9.38$, $p<0.05$) Cortisol at T_1 or T_2 not related with infant development ($p>0.05$)	Good quality

Kivlighan et al. (2008)	98 pregnant women and their 95 newborns (190 dyads)	Pregnancy Experience Scale (PES), STAI and PSS at T_1 (36 weeks)	Salivary cortisol at T_1 (2 consecutive days): $T_{1.1}$ (08.00 am), $T_{1.2}$ (12.00 pm) and $T_{1.3}$ (16.00 pm)	Infant birth weight, gestational age at birth and Apgar scores at 1 and 5 minutes	*Self-report/Cortisol* Higher scores on STAI-T associated with flatter cortisol decline at $T_{1.3}$ (1.74% per +1 SD increase; 95% CI:0% to 3.3%) *Self-report/Outcomes* No significant associations *Cortisol/Outcomes* Low maternal cortisol at $T_{1.1}$ associated with higher infant birth weight in primiparous compare with multiparous (t=1.74, p<0.09)	Good quality
Kramer et al. (2009)	5092 pregnant women (Prospective study) 207 cases and 444 controls (case and control study)	Daily Hassles Scale (DHS), Perception of pregnancy risk (Single item from the scale of Taylor et al., 1992), Marital Strain Scale (MSS; chronic stress), Abuse Assessment Screen (AAS), Job-related Stress Scale	Hair cortisol (n=117 pregnant women; 31 cases and 86 controls) at T_2	Spontaneous preterm birth and placental histopatological features	*Self-report/Cortisol* No significant associations *Self-report/Outcomes* Only high PRAQ-R, perception of high pregnancy risk, was significantly associated with the risk of spontaneous preterm birth (OR=1.7, 95% CI=1.2-2.3) *Cortisol/Outcomes* Positive correlation between hair cortisol and gestational age (p<0.05)	Good quality

		(JSS), Prenatal Life Event Scale (PLES), PSS, PRAQ-R, Commitment to Pregnancy Scale (CPS) at T_1 (26 weeks) and T_2 (Pospartum, only the LES)				
Ponirakis et al. (1997)	27 adolescent pregnant women and their 27 babies (N=54 dyads).	NEO-AC Personality Inventory (, Anxiety sub-scale), STAI and Inventory of Socially Supportive Behaviors (ISSB) at T_1 (16 weeks), T_2 (34 weeks) and T_3 (4 weeks after delivery)	Salivary cortisol at T_1,T_2 and T_3	Infant birth weight, APGAR score at birth, abnormalities on newborn profile exam, resuscitation measures used at birth and infant electrocardiogram (EKG) at T_3.	Two composite factors (CF) reported: CF Trait variables (STAI-trait, STAS-trait, , NEO Anxiety) and CF State variables (STAI-state, STAS-state, and BDI) *Self-report/Cortisol* No reported *Self-report/Outcomes* Maternal trait composite score at T_1 positively correlated with infant Apgar at 5 min ($r=0.47$, $p<0.05$) Maternal state composite score at T_2 positively correlated with the number of abnormalities on the newborn profile exam ($r=0.59$, $p<0.001$) Maternal trait composite score at T_1 the only significant predictor of infant cardiac vagal tone ($r= 0.49$, $p<0.01$), accounting for 24% of the variance	Fair quality

					Cortisol/Outcomes Maternal mean cortisol at T_1 a significant predictor of infant Apgar at 1 min, 5 min and number of resuscitation measures on the infant (r=- 0.39, p<0.05; r=-0.45, p<0.05; r=0.45, p<0.05)	
Observational Cross-sectional study						
Diego et al. (2006)	41 pregnant women	Holingshead Four Factor Social Index (Socioeconomic status, SES), STAI-T and Daily Hasslels Scale (Field, unpublished scale) at T_1 (22 weeks)	Urinary cortisol at T_1	Fetal ultrasound biometry measures (femur length, head circumference, abdominal circumference, biparietal diameter and fetal weight estimation from fetal biometry) at T_1	*Self-report/Cortisol* Maternal STAI-T and hassles scores positively related to maternal cortisol (r=0.41, p<0.001; r=0.26, p<0.01; r=0.28, p<0.01) *Self-report/Outcomes* Maternal STAI-T scores negatively related to biparietal diameter, head circumference, and abdominal circumference (r=-0.28; p<0.001; r=-0.23; p<0.05; r=-0.21; p<0.05) Maternal SES and age not related to fetal biometry measurements (p>0.05) Maternal hassles scores negatively related to biparietal diameter (r=-0.29; p<0.01) *Cortisol/Outcomes* Maternal cortisol negatively related to biparietal diameter, head circumference, abdominal circumference, and fetal weight (r=-0.47; p<0.001; r =-0.43; p<0.001; r=-0.42; p<0.001;r=-0.33; p<0.01) Prenatal cortisol was the only significant predictor of fetal weight (X^2=42.46, p=0.28, CFI=0.99, RMSEA=0.03) Mothers with elevated maternal cortisol levels at risk for having below average estimated fetal weight foetuses (OR=12.81; CI=4.81– 34.09)	Fair quality

Associations between self-report stress measures and cortisol levels during pregnancy

A number of 12 studies reported significant associations between self-report stress measures and cortisol levels during pregnancy (Baibazarova, et al., 2013; Bergman et al., 2010; Bolten et al., 2010; Davis et al., 2010; Davis et al., 2012; De Weerth et al., 2013; Doylet et al., 2015; Goedhart et al., 2010; Hoffman et al., 2016; Kivlighan et al., 2008; Kramer et al., 2009; Diego et al., 2006). Three studies found a significant association between high maternal prenatal stress, assessed using the Perceived Stress Scale (PSS) (r=0.28), the State Trait Anxiety Inventory-Trait (STAI-T) (r=0.26-0.41) and the Daily Hassles Scale (r=0.28), with high cortisol levels, using hair, salivary and urinary cortisol respectively (Diego et al., 2006; Hoffman et al., 2016; Kivlighan et al., 2008). Considering these results are derived from studies using small samples, ranging from 41 to 98 pregnant women, and only one study rated as fair quality (Diego et al., 2006), makes these findings less valid. Nine studies, rated as good quality, found no significant associations between self-report stress measures and cortisol levels during pregnancy ($p>0.05$) (Baibazarova, et al., 2013; Bergman et al., 2010; Bolten et al., 2010; Davis et al., 2010; Davis et al., 2012; Doyle et al., 2015; Field et al., 2006; Goedhart et al., 2010; Kramer et al., 2009). These findings suggest that self-report measures of stress and the biomarker of stress, cortisol, reflect different underlying processes.

Infant birth weight

Eleven studies assessed associations between prenatal maternal stress, cortisol levels and infant birth weight. In relation to self-report stress measurement tools, the eleven studies found no significant associations with infant birth weight ($p>0.05$)

(Baibazarova et al., 2013; Bolten et al., 2010; Buitelaar et al., 2003; Doyle et al., 2015; Field et al., 2006; Goedhart et al., 2010; Hompes et al., 2012; Huizink et al., 2003; Kivlighan et al., 2008; Ponirakis et al., 1997; Diego et al., 2006). Nine of these 11 studies were rated as good quality (Baibazarova et al., 2013; Bolten et al., 2010; Buitelaar et al., 2003; Doyle et al., 2015; Field et al., 2006; Goedhart et al., 2010; Hompes et al., 2012; Huizink et al., 2003; Kivlighan et al., 2008).

These findings are in contrast to the use of cortisol levels to predict infant birth weight, where seven of these 11 studies reported significant associations between maternal cortisol levels during pregnancy and infant birth weight (Baibazarova et al., 2013; Bolten et al., 2010; Diego et al., 2006; Field et al., 2006; Goedhart et al., 2010; Hompes et al., 2012; Kivlighan et al., 2008). The remaining four studies found no association (Buitelaar et al., 2003; Doyle et al., 2015; Huizink et al., 2003; Ponirakis et al., 1997). The results suggest that observing higher maternal cortisol levels in the prenatal period is a risk factor for having an infant of lower than average weight at birth. The findings from the 11 studies indicate that maternal cortisol levels may be a more sensitive measure than self-report tools for identifying women at risk of having a low birth weight infant.

Gestational age at birth

Eleven studies assessed relations between prenatal maternal stress, cortisol levels and gestational age at birth (Baibazarova et al., 2013; Bolten et al., 2010; Buitelaar et al., 2003; Davis et al., 2012; Doyle et al., 2015; Field et al., 2006; Goedhart et al., 2010; Hoffman et al., 2016; Huizink et al., 2003; Kivlighan et al., 2008; Kramer et al., 2009). The totality of these 11 studies were rated as good quality. In respect to self-report stress measures, 9 studies found no significant association with gestational

age at birth ($p>0.05$) (Baibazarova et al., 2013; Bolten et al., 2003; Buitelaar et al., 2003; Davis et al., 2012; Doyle et al., 2015; Field et al., 2006; Goedhart et al., 2010; Huizink et al., 2003; Kivlighan et al., 2008). Only two studies reported a significant association between a self-report stress measures, assessed using the PSS and the PRAQ-R before 28 weeks of gestation, and gestational age at birth (Hoffman et al., 2016; Kramer et al., 2009). Thus, higher prenatal maternal stress was positively correlated with earlier gestational age at delivery ($r=20.30$, $p<0.05$) (Hoffman et al., 2016) and the risk of spontaneous preterm birth (OR=1.7, 95% CI=1.2-2.3).

The same two studies were also the only studies to report an association between cortisol levels during pregnancy and gestational age at birth, assessed through hair cortisol levels (Hoffman et al., 2016; Kramer et al., 2009). However, the direction of this association is unclear. Hoffman et al. (2016) reported higher hair cortisol levels at 22 weeks of gestation were related to lower gestational age at birth ($r=20.25$, $p<0.05$), while Kramer et al. (2009) identified higher hair cortisol levels at delivery, reflecting stress levels during the last trimester, to be associated with higher gestational age at birth ($p<0.05$). Although both studies (Hoffman et al., 2016; Kramer et al., 2009) were rated as good quality, the large sample assessed in Kramer et al.'s prospective study strongly support higher cortisol levels during pregnancy to be associated with a greater gestational age. The remaining nine studies, which report relatively smaller samples, found no significant association (Baibazarova et al., 2013; Bolten et al., 2010; Buitelaar et al., 2003; Davis et al., 2012; Doyle et al., 2015; Field et al., 2006; Goedhart et al., 2010; Huizink et al., 2003; Kivlighan et al., 2008). These results suggest the association between prenatal maternal stress, prenatal cortisol levels and gestational age remains unclear. The findings from the 11 studies indicate neither self-report stress nor cortisol levels are sensitive in identifying women at risk of preterm birth.

Intrauterine foetal growth

Four studies evaluated the correlation between self-report stress, cortisol levels and intrauterine foetal growth (Diego et al., 2006; Field et al., 2006; Goedhart et al., 2010; Hompes et al., 2012). Intrauterine foetal growth was measured by medical ultrasonography. Regarding self-report stress, three studies, rated as good quality, found no significant associations with intrauterine foetal growth (Field et al., 2006; Goedhart et al., 2010; Hompes et al., 2012). Goedhart and colleagues conducted a large good quality prospective study (Goedhart et al., 2010), which lends weight to the reliability of their findings. Only one study (Diego et al., 2006) found a significant association between self-report stress, assessed using the STAI-T and Daily Hassles Scale, and intrauterine foetal growth. In this study, high STAI-T scores were significantly related to low foetal biparietal diameter ($r=-0.21$; $p<0.001$), low foetal head circumference ($r=-0.23$; $p<0.05$) and low abdominal circumference ($r=-0.21$; $p<0.05$). High maternal daily hassles scores were associated with low foetal biparietal diameter ($r=-0.29$; $p<0.01$). However, this study (Diego et al., 2006) was rated as fair quality due to the cross-sectional design of the study, conducted with a small sample of pregnant women ($n=41$) and an absence of a sample size justification.

In respect to cortisol levels, two studies, rated as good quality, found no significant associations (Goedhart et al., 2010; Hompes et al., 2012); while two studies, one of which was rated as fair quality, reported a significant relation between high urinary cortisol levels with low intrauterine foetal growth (Field et al., 2006; Diego et al., 2006). According to these results, high cortisol levels (urinary) appeared to be a better predictor of low foetal growth than self-report measures however the evidence is very limited and should be interpreted with caution.

Infants´ neurodevelopment

Four studies in this review, rated as good quality, assessed self-report stress measures and cortisol levels with infant neurodevelopment at age 3, 6, 8, 12 and 17 months (Bergman et al., 2010; Buitelar et al., 2003; Davis et al., 2010; Huizink et al., 2003). Infants' neurodevelopment was assessed by using the Bayley Scales of Infant and Toddler Development (BSID) in all four studies. In relation to self-report stress measures the four studies reported significant associations (Bergman et al., 2010; Buitelar et al., 2003; Davis et al., 2010; Huizink et al., 2003). High maternal prenatal stress, assessed using the State Trait Anxiety Inventory-State (STAI-S), Pregnancy Anxiety Questionnaire-Revised (PRAQ-R), PSS and the Pregnancy-Specific Anxiety (PSA), was associated with low BSID scores ($p<0.05$). We planned to pool relevant data from studies to perform meta-regressions and assess the strength of associations between the stress measures and outcomes reported. However, following the process of data extraction, we observed that meta-regressions were not feasible as there were differences across the studies in the timing of assessments for the stress measures, timing of assessment for the outcomes measures and/or differences in the studies' samples.

In respect to cortisol levels to predict infants´ neurodevelopment, the same four studies (Bergman et al., 2010; Buitelar et al., 2003; Davis et al., 2010; Huizink et al., 2003) found significant associations. High amniotic and salivary cortisol levels before the third trimester were significantly related to low BSID scores in three studies (Bergman et al., 2010; Buitelaar et al., 2003; Huizink et al., 2003) and one study (Davis et al., 2010) reported low salivary cortisol levels at the third trimester of pregnancy predicted accelerated BSID scores (Mental Development Index) ($t=-2.5$, $p<0.01$). The findings for self-reported stress and cortisol are consistent across studies however the timing when cortisol is measured during pregnancy is a key factor that determines the

direction of the relation between cortisol levels and infant neurodevelopment (Glover, 2014; Glover et al., 2009; Hohwü et al., 2015).

Statistical analysis remained significant in all the studies after adjustments for confounders, such as maternal age, pre-gestational body mass index, level of education, marital status or infant gender.

Discussion

The aim of this review was to identify studies that assessed associations between psychological stress and biological stress with adverse outcomes as much research in the field uses either psychological or biological stress measures during pregnancy rather than both to assess associations with adverse outcomes.

Seventeen studies were included in this review, and only three studies reported a significant relation between high maternal self-report stress measures scores with high cortisol levels (Diego et al., 2006; Hoffman et al., 2016; Kivlighan et al., 2008). Three studies found a significant association between self-report stress measures and negative outcomes, including gestational age at birth (Hoffman et al., 2016; Kramer et al., 2009), and intrauterine foetal growth (Diego et al., 2006). Further four studies reported significant associations between self-report stress measures and infants' neurodevelopment (Bergman et al., 2010; Buitelar et al., 2003; Davis et al., 2010; Huizink et al., 2003).

These findings are in contrast to cortisol levels predicting negative outcomes in 12 studies, including infant´s birth weight (Baibazarova et al., 2013; Bolten et al., 2010; D'Anna et al., 2012; Diego et al., 2006; Field et al., 2006; Goedhart et al., 2010; Hompes et al., 2012; Kivlighan et al., 2008), gestational age at birth (Hoffman et al., 2016; Kramer et al., 2009), and intrauterine fetal growth (Field et al., 2006; Diego et al.,

2006). Cortisol levels were significantly associated with infant´s neurodevelopment in four studies (Bergman et al., 2010; Buitelar et al., 2003; Davis et al., 2010; Huizink et al., 2003).

This review found that the majority of studies failed to find an association between self-report stress measures and cortisol levels. There is minimal evidence to support the association between cortisol and self-report stress measures and that cortisol levels are a better predictor of negative outcomes than self-report stress measures. Nevertheless, they demonstrated similar results when predicting infants' neurodevelopment. Self-report stress questionnaires and cortisol levels may be measuring different constructs with self-report stress measures offer evidence of psychological distress according to subjective appraisals (Alderdice et al., 2012; Levine et al., 2016; Lobel and Dunkel Schetter, 2016; Lynn et al., 2011) while cortisol levels inform from the activation of the hypothalamic-pituitary-adrenal biological stress system (Glover, 2014; Sandman et al., 2016).

The studies included in this review were based predominantly on a unidimensional approach with general stress measured and aspects, such as the reliability and validity of the measurement tools should be taken into consideration when interpreting the findings. Future research would benefit by adding pregnancy-specific stress measures to their protocols so that higher quality evidence is available to make a judgement on measurement. Consideration of timing of assessment and method of measuring cortisol is essential when assessing stress levels during pregnancy (D'Anna-Hernandez et al., 2011; Sandman et al., 2016). The validity of hair cortisol levels as a biological measure of chronic stress has been successfully reported and offers beneficial aspects comparing to acute cortisol measures (Wosu et al., 2013). Regardless, only two studies used hair cortisol levels in its protocol (Hoffman et al.,

2016; Kramer et al., 2009). The fact that hair cortisol levels at the second trimester were associated with PSS scores at the first trimester (Hoffman et al., 2016), support the evidence existing on hair cortisol levels as a retrospective marker of chronic stress (D'Anna-Hernandez et al., 2011). Acute cortisol levels from blood, saliva, and urine limit the information obtained and are influenced by situational features (Wosu et al., 2013). Even when repeated acute cortisol levels are measured in pregnant women over a day, associations between self-report stress measures and cortisol levels remain insignificant (Entringer et al., 2011).

Regarding the association between cortisol levels with gestational age at birth, both studies finding significant associations used hair cortisol samples (Hoffman et al., 2016; Kramer et al., 2009), but reported contrary results. On one hand, Hoffman et al. (2016) found higher hair cortisol levels were related to lower gestational age at birth. These findings agree with previous studies reporting an association between high cortisol levels with preterm birth and short duration of gestation (Sandman et al., 2006; Stewart et al., 2015). On the other hand, Kramer et al. (2009) reported higher hair cortisol levels associated with higher gestational age at birth. Although the direction of the association between cortisol levels with gestational age at birth is still inconclusive, it appears that using hair cortisol levels (Wosu et al., 2013) can provide evidence of this relation. The large sample and prospective design used by Kramer et al. (2009) support the association between higher hair cortisol levels and higher gestational age.

Cortisol metabolism during pregnancy is beneficial to foetal maturation. Nevertheless, exposure to intense levels of cortisol can have detrimental maternal and infant effects (Hannerfors et al., 2015; Hellgren et al., 2016). A recent review reported significant associations between maternal stress and reduced foetal head growth, while other foetal growth measures showed inconclusive findings (Lewis et al., 2016). Even

though associations between self-report stress measures, cortisol levels and intrauterine foetal growth were studied in four studies in the present review, one study reported an association between self-report stress measures, assessed by the STAI-T and the Daily Hassles Scale and low foetal head growth (Diego et al., 2006). A more recent study reported an association between maternal stress and foetal growth restriction (Ding et al., 2014). Fetuses exposed to high levels of maternal stress while in the womb get lower volume blood flow (Levine et al., 2016), which may have detrimental consequences on foetuses growth. Other studies in this review including a large sample and assessing intrauterine foetal growth through ultrasounds at different time-points during pregnancy reported an absence of associations (Field et al., 2006; Goedhart et al., 2010; Hompes et al., 2012).

Far from the idea that high levels of stress can have adverse consequences, it has been suggested that certain levels of stress during pregnancy can accelerate motor and cognitive development (DiPietro et al., 2006). Thereby, high cortisol levels were associated with high MDI and PDI scores (Buitelaar et al., 2003; Huizink et al., 2003). In this respect, low levels of cortisol could predict an accelerated mental development (MDI) during the postpartum period and the first year (Bergman et al., 2010b; Bolten et al., 2010; Davis and Sandman, 2010). However, there is evidence that high levels of amniotic cortisol that surrounds the fetus while in the womb are related to low infant cognitive development (Glover, 2014). High levels of maternal stress were associated with low mental and psychomotor development (Buitelaar et al., 2003; Davis and Sandman, 2010; Huizink et al., 2003).

Associations between self-report stress measures, cortisol levels and pregnancy outcomes were different across and within studies. Adjustment for confounders was taken into consideration in all the studies. Besides, given statistical significance was

assumed at the level of $p < .05$, the alpha level was not corrected for comparisons. Thus, an optimal balance was established between the risks for type I and type II errors across all the studies in this review.

Further studies may benefit by applying longitudinal assessment of pregnant women from both a psychological and a physiological perspective. Pregnancy-specific stress measures would provide a more comprehensive and accurate approach of the associations with pregnancy outcomes (Alderdice et al., 2012; Lobel and Dunkel Schetter, 2016). Using chronic cortisol measures, as those provided through hair cortisol levels, would reduce the impact of invasive measurements and improve the timeframes assessed (Wosu et al., 2013).

Including psychological assessment and biological measures (i.e. cortisol) in clinical settings throughout pregnancy would reduce prenatal stress impact on the mother, the fetus and the infant, through applying optimal and adequate stress reduction therapy.

There was little association between self-report stress measures and cortisol levels in the studies reviewed, however many studies were low quality and the association between the two remain unclear. High self-report stress measures scores and high cortisol levels were associated with negative outcomes, this was most consistent for low infants´ neurodevelopment. However, certain levels of cortisol were associated with an accelerated mental and motor development. Cortisol levels appear to be a better predictor of negative outcomes than self-report stress measures.

CAPÍTULO VII: "ESTUDIO 2"

Psychometric validation of the Prenatal Distress Questionnaire in pregnant women in Spain

Caparros-Gonzalez, García-García, Perra, Alderdice, Lynn, Lobel y Peralta-Ramírez, M.I. (Under review). Psychometric validation of the Prenatal Distress Questionnaire in pregnant women in Spain. *Women & Health*

Introduction

Pregnancy is a time of adaptations, which can lead to considerable stress (Lynn et al., 2011; Gourounti et al., 2016) which in turn has been found to be related to adverse pregnancy outcomes such as anxiety, low birth weight, prematurity, and poor infant neurodevelopment (Duthie and Reynolds, 2013; Lobel and Dunkel-Schetter, 2016; Razurel et al., 2017).

As pregnancy is a major life event, it is thought that pregnancy-specific stress may be a better predictor of adverse outcomes than general stress (Alderdice and Lynn, 2009; Lobel et al., 2008; Lobel and Dunkel-Shetter, 2016). Pregnancy-specific stress refers to pregnant women's concerns about physical symptoms, health of the foetus, labour, relationships, and parenting (Alderdice, Lynn, and Lobel, 2012). Among 15 questionnaires identified to measure pregnancy-specific stress (Alderdice, Lynn, and Lobel 2012), the Prenatal Distress Questionnaire (PDQ) (Yali and Lobel, 1999) has been used in a wide range of studies and has been recommended to assess stress related to pregnancy (Nast et al. 2013). In addition to the original English version used in the U.S., Ireland and the United Kingdom (Alderdice et al., 2013; Gennaro, Shults, and Garry, 2008; Heberlein et al., 2016; Lobel et al., 2000; Lynn et al., 2011; Yali and Lobel, 1999), the PDQ has been translated in German samples (Bolten et al., 2011; Koletzko, La Marca-Ghaemmaghami, and Brandstätter, 2015; Pluess et al., 2010).

Two studies explored the factor structure of the PDQ in English-speaking samples, in low and high-risk pregnant women (Alderdice and Lynn, 2011; Alderdice et al., 2013), and it was found to be stable with three factors identified: "Concerns about Birth/Baby," "Concerns about Weight/Body Image," and "Concerns about Emotions/Relations". Convergent validity with the Perceived Stress Scale (PSS), the State-Trait Anxiety Inventory-State (STAI-S), and the Edinburgh Postnatal Depression

Scale (EPDS) has been demonstrated (Alderdice, Lynn, and Lobel 2012). The original PDQ (Yali and Lobel 1999) does not appear to have been administered to Spanish speaking samples.

The aim of this research was to translate the PDQ into Spanish, analyse its factor structure using Confirmatory Factor Analysis (CFA), and assess its convergent validity when applied to pregnant women in Spain. Since the PDQ dimensionality has been already explored across a number of studies (Alderdice and Lynn, 2011; Alderdice et al., 2013) and the three underlying dimensions identified by these studies are considered to display construct validity (Alderdice, Lynn, and Lobel 2012), we used Confirmatory Factor Analysis with the aim of examining how close the three-factor structure adequately represents the patterns of responses in our Spanish sample. In this way, our overarching aim was to investigate whether these factors embody significant facets of women's experience of pregnancy across a different cultural context.

Methods

Sample

This study is based on data from a longitudinal study of perinatal stress in Spain (GESTASTRESS). Eligible participants were low-risk pregnant women in the second trimester, proficient in the Spanish language, and over 18 years old.

Participants were identified and recruited by two community midwives (January 2015 – March 2016) while attending a prenatal appointment at two Health Centres in the south of Spain. The two midwives screened for eligibility potential participants according to medical data in the Pregnancy Health Document (Andalusian Ministry of Health, 2010), which is the official record of every pregnant woman in Spain. 305 pregnant women were approached to participate and 288 women consented to

participate. Subsequently, six were excluded (pregnancy ended in miscarriage), 37 declined to participate, after initially agreeing to collaborate, referring to lack of time, and 12 were excluded because they provided only sociodemographic information. This resulted in a final sample of 233 women, which was above the minimum sample size required to conduct a CFA: At least 10 participants per estimated item is the general consensus (Schreiber et al., 2006). All the n = 233 participants completed each of the PDQ items, therefore there were no missing data for which we had to account.

PDQ translation procedure

This process was performed in accordance with international guidelines for cross-cultural adaptations of questionnaires (Epstein, Santo, and Guillemin 2015; Martin and Savage-McGlynn, 2013).

An expert team in perinatal health and proficient in both languages translated the PDQ into Spanish separately (Version 1). Version 1 was translated back into English (Version 2) by a professional translator-editor. According to the expert team, Version 2 was grammatically and semantically equivalent to the original questionnaire. No variability was found among versions. A pilot sample of 40 participants completed the final translated version and found it easy to understand and adequately reflected their concerns. No further changes were made following piloting.

Study instruments

The Prenatal Distress Questionnaire (PDQ) is a 12-item scale to assess specific worries and concerns during pregnancy regarding physical symptoms, relationships, parenting, medical problems, labour and delivery, and the health of the baby (Yali and Lobel 1999). The psychometric properties reported for the PDQ substantiate its reliability and validity (Alderdice et al., 2013; Gennaro, Shults, and Garry, 2008; Lobel

et al., 2000; Lynn et al., 2011; Pluess et al., 2010; Yali and Lobel, 1999). Responses are on a 5-point Likert scale from 0 (Not at all) to 4 (Extremely). Responses to the 12-item PDQ are summed, providing a prenatal distress score ranging from 0 to 48.

The convergent validity of the PDQ was assessed through two psychosocial stress instruments:

The Perceived Stress Scale (PSS): The Spanish version of the 14-item Perceived Stress Scale (PSS) (Cohen, Kamarck, and Mermelstein, 1983; Remor, 2006) was utilised to assess perceptions of general stress during the last month. Responses are on a 5-point Likert scale from 0 (never) to 4 (very often). The Cronbach's alpha reliability coefficient of the Spanish version is $\alpha = 0.81$.

The Symptom Checklist-90-Revised (SCL-90-R) (Caparros-Caparros et al., 2007; Derogatis, 1994): This scale is used to assess psychopathological symptoms and has been translated into 26 languages. It consists of 90 items scored by a 5-point Likert scale from 0 (never) to 4 (extremely) and is used to assess nine dimensions: Somatization, Obsession Compulsion, Interpersonal Sensitivity, Depression, Anxiety, Hostility, Phobic Anxiety, Paranoid Ideation, and Psychoticism. The scale also has three global indexes of distress: GSI (overall psychological distress), PSDI (intensity of symptoms) and Positive Symptom Total (number of self-reported symptoms). The Cronbach's alpha reliability coefficients of the Spanish version range from 0.67 to 0.94.

Socio-demographic and obstetric data were collected from the Pregnancy Health Document (Andalusian Ministry of Health, 2010).

Data Analysis

Data were initially analysed using Stata 13 (StataCorp, 2013). Barlett's test of sphericity (χ^2 (1) = 541, $p < 0.001$) and the Kaiser-Meyer-Olkin ($KMO = 0.76$) to test the three-factor structure described previously (Alderdice and Lynn, 2011; Alderdice et al.. 2013) were satisfactory. Many items in the questionnaire displayed skewed and asymmetric distributions: to allow for this fact, we conducted Confirmatory Factor Analysis using a Weighted Least Squares Means and Variance adjusted (WLSMV) estimator: a WLSMV estimator is considered more adequate in analysing categorical data with non-normal distributions compared to Maximum-Likelihood (ML) estimators (Beauducel and Herzberg 2006; Rhemtulla, Brosseau-Liard, and Savalei, 2012). Analyses were run using Mplus 7.1 (Muthén and Muthén, 2013): the WLSMV estimator was invoked alongside specification of the items being categorical, and parametherization DELTA (Muthén and Muthén, 2013). Model fit was assessed by using the Likelihood ratio χ^2 of the model of interest compared against the saturated model: A non-significant result is indicative of good model fit with the data (Acock, 2013). We also used other goodness of fit indicators including the Comparative Fit Index (CFI), with values over 0.95 indicating good fit of the model; the Root Mean Squared Error of Approximation (RMSEA), with values less than 0.05 indicating a close fit; and The Weighted Root Mean Squared Residuals (WRMR), with values less than 1 indicating good fit (Hu and Bentler, 1999).

We estimated factor scores in the latent dimensions of the CFA model with the best fit. Relationships between these factor scores and other concurrent measures of anxiety and mental health variables were assessed using Pearson correlations to test convergent validity, which provides information about the extent to which two instruments measure a similar construct (Carlson and Herdman, 2012).

Ethics

The Human Ethics Research Committee of the University of Granada (reference 881), the Biomedical Ethics Research Committee and the Ethics Research Committee of the Health Centers, and the hospital where this study was implemented approved this study. This study was conducted according to the guidelines of the Helsinki Declaration (AMM, 2008) and the Good Clinical Practice Directive (Directive 2005/28/EC) of the European Union. Participation was voluntary and every participant read and signed an informed written consent document.

Results

Sample characteristics and descriptive statistics

Sociodemographic and health characteristics are displayed in Table 1. The PDQ mean item responses in our sample are presented in Figure 1, listed from the lowest to the most distressing.

Table 1. Sociodemographics and obstetric history (N = 233)

Variables	**Frequency (%)**
Sociodemographics	
Age (years)	
19-25	30(12.9)
26-35	155 (66.5)
36-42	48 (20.6)
Country of origin	

Spain	172 (73.8)
Latin America	46 (19.7)
Europe (not Spain)	12 (5.1)
Morroco	2 (0.9)
Russia	1 (0.4)
Marital status	
Married/Co-habiting	225 (96.6)
Single/Separated/Divorced	8 (3.4)
Employment situation	
Full-time job	36 (15.5)
Part-time job	119 (51.1)
Unemployed	62 (26.6)
Student	8 (3.4)
Student and employed	8 (3.4)
Level of education	
Primary School	15 (6.4)
Secondary School	71 (30.5)
University	147 (63.0)
Obstetric history	
Weeks of gestation	

7-15	16 (6.9)
16-30	206 (88.4)
31-37	11 (4.7)
Previous miscarriages	
None	169 (72.5)
1 miscarriage	49 (21.1)
2 miscarriages	11 (4.7)
3 or more miscarriages	4 (1.7)
Living children	
None	133 (57.1)
1 child	79 (33.9)
2 children	14 (6.0)
3 or more children	7 (3.0)
Wantedness	
I wanted to be pregnant	200 (85.8)
I did not want to be pregnant then or any time in the future	33 (14.2)
Pregnancy method	
Spontaneous	215 (92.3)
In vitro fertilisation	11 (4.7)

Artificial insemination	7 (3.0)

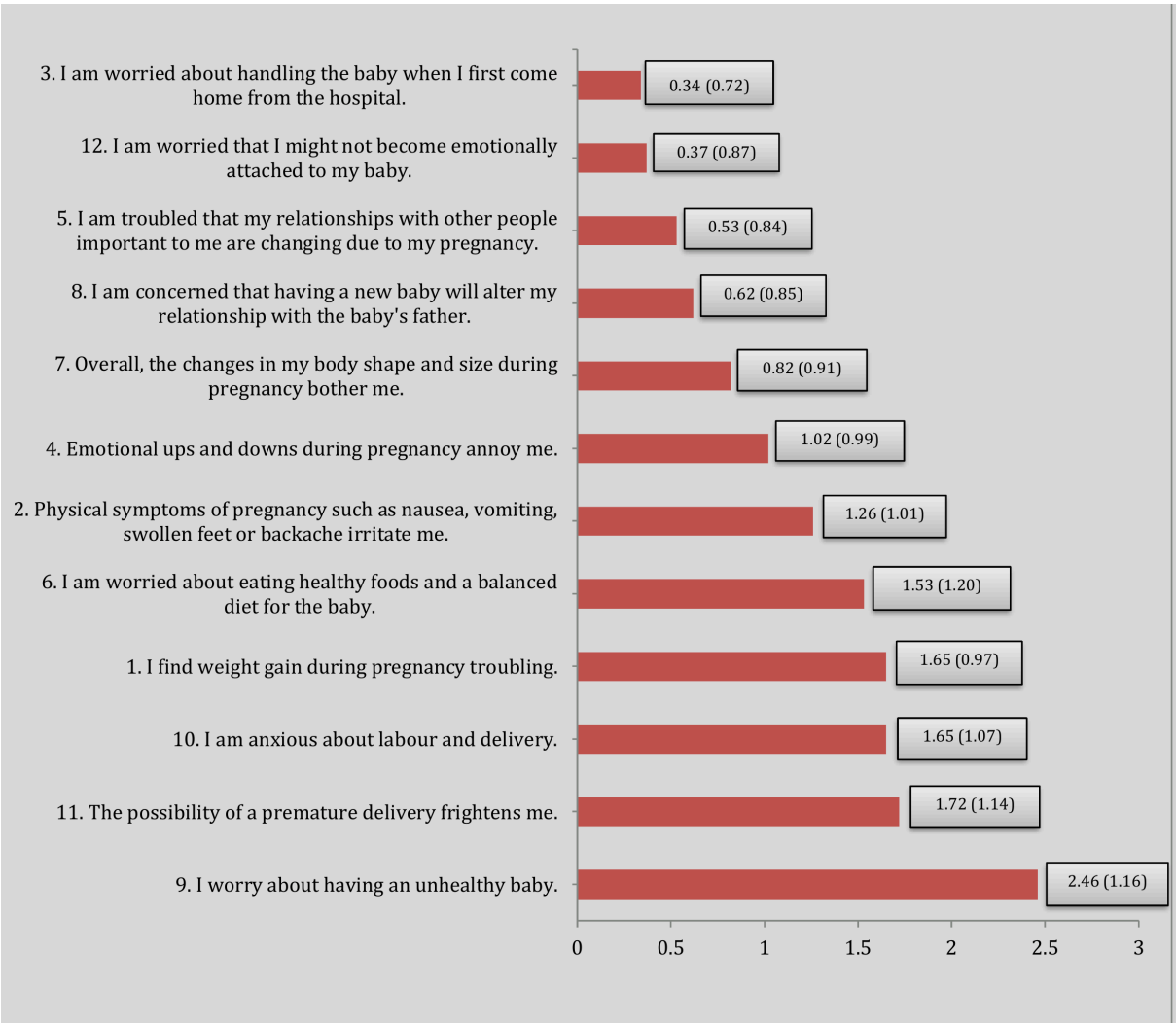


Figure 1. Spanish PDQ mean scores and standard deviations (in brackets) in the study sample for each item (N = 233)

Confirmatory Factor Analysis

We first tested the three-factor structure model reported by Alderdice and Lynn (2011). Factor 1 was made up of items reflecting concerns over the baby and giving birth (items 3, 6, 9, 10, 11 and 12); factor 2 was constituted from items reflecting worries about body and weight (items 1, 2, and 7); and factor 3, reflecting concerns

about relationships and emotions (items 4, 5 and 8). Our first run of the CFA (Model 1), shown in Table 2, suggested that item 2 (physical symptoms) was not related to any of the factors. This was similar to the analysis reported by Alderdice and Lynn (2011) so this item was excluded from further analyses. When we excluded item 2 the goodness of fit statistics did not yet indicate a good fit of this model. Successively, we considered item 6 (concerns about eating a healthy and balanced diet) may be a culturally-sensitive item: in countries with a traditional Mediterranean diet (such as Spain) that is traditionally low in saturated fat and whereby fresh fruit and vegetables are more easily available (Willett et al., 1995), there may be less concerns and emphasis on healthy eating during pregnancy compared to North-European countries, which traditionally adopted diets rich in saturated fat. Indeed, excluding item 6 from the scale resulted in an improvement of fit (Model 3 in Table 2), although the fit obtained was still not adequate. Data from a large cohort study on pregnant women confirmed the association between concerns about emotions and concerns about relationships (Rosand et al. 2011). For this reason, in Model 4 we tested the inclusion of a correlation between the error terms of item 4 (concerns about emotions) and item 5 (concerns about relationships). Due to reported associations between prematurity and the health of newborns (NICE, 2015), an association between the error terms of item 9 (concerns about health of newborn) and item 11 (concerns about birth before term) was assessed in Model 4. The results of this final model indicated a good fit of the model (see Model 4 in Table 2). All the associations between indicators and underlying factors were significant ($p < .001$). The standardized loadings are displayed in Figure 2. Bivariate correlations between the three factors were all significant ($p < .01$). Factor 1 (Birth Concerns) correlated with Factor 2 (Physical Concerns) ($r = .35$; $p < .001$). Factor 1 (Birth Concerns) correlated with Factor 3 (Relations Concerns) ($r = .71$; $p < .001$).

Factor 2 (Physical Concerns) correlated with Factor 3 (Relations Concerns) (r = .68; p < .001).

Table 2. Fit indices for the Spanish PDQ CFA for the 3-factor Models.

	Model 1	**Model 2**	**Model 3**	**Model 4**
	From Alderdice & Lynn (2011)	Excludes item 2	Item 2 and item 6 removed	Item 2 and item 6 removed; includes associations between items 4 and 5, as well as 9 and 11.
LR χ^2	$\chi^2(51)=164.8$, p< .001	$\chi^2(41)=101.1$, p< .001	$\chi^2(32)=65.1$, p< .001	$\chi^2(30)=47.1$, p< .001
CFI	0.86	0.90	0.94	0.97
RMSEA	0.098 (90%CI .081 to .115)	0.079 (90%CI .06 to .09)	0.067 (90%CI .04 to .09)	0.049 (90%CI .01 to .07)
WRMR	1.105	0.920	0.769	0.640

Although the CFA supported the presence of three underlying factors, the PDQ has been used as a uni-dimensional scale, assuming a single underlying factor. We also tested the fit of a model whereby all 10 items considered above were assumed as indicators of a single underlying factor. Once we modelled some additional associations between error terms (items 9 and 10; 9 and 11; 10 and 11; 1 and 7; 4 and 5), the fit indices overall indicated adequate model fit, χ^2 (30) = 55.7, p = .003; *CFI*= 0.95;

RMSEA = 0.061; *WRMR* = 0.710. In order to compare the three-factor model and the one-factor model, we ran these models again using an MLR estimator in Mplus 7.1 (Muthén and Muthén 2013), and thus obtain information criteria, while using a robust estimator to allow for asymmetric distributions of items. The information criteria of the uni-dimensional and tri-dimensional model were very similar (*BIC* = 6190.4 and *BIC* = 6190.3.8; *AIC* = 6069.6 and *AIC* = 6069.5 for the uni-dimensional and tri-dimensional models respectively), suggesting that overall the one-factor and three-factor models provided comparable fit to the data.

Considering the 12 items of the questionnaire, the Cronbach´s alpha was $\alpha = 0.734$.

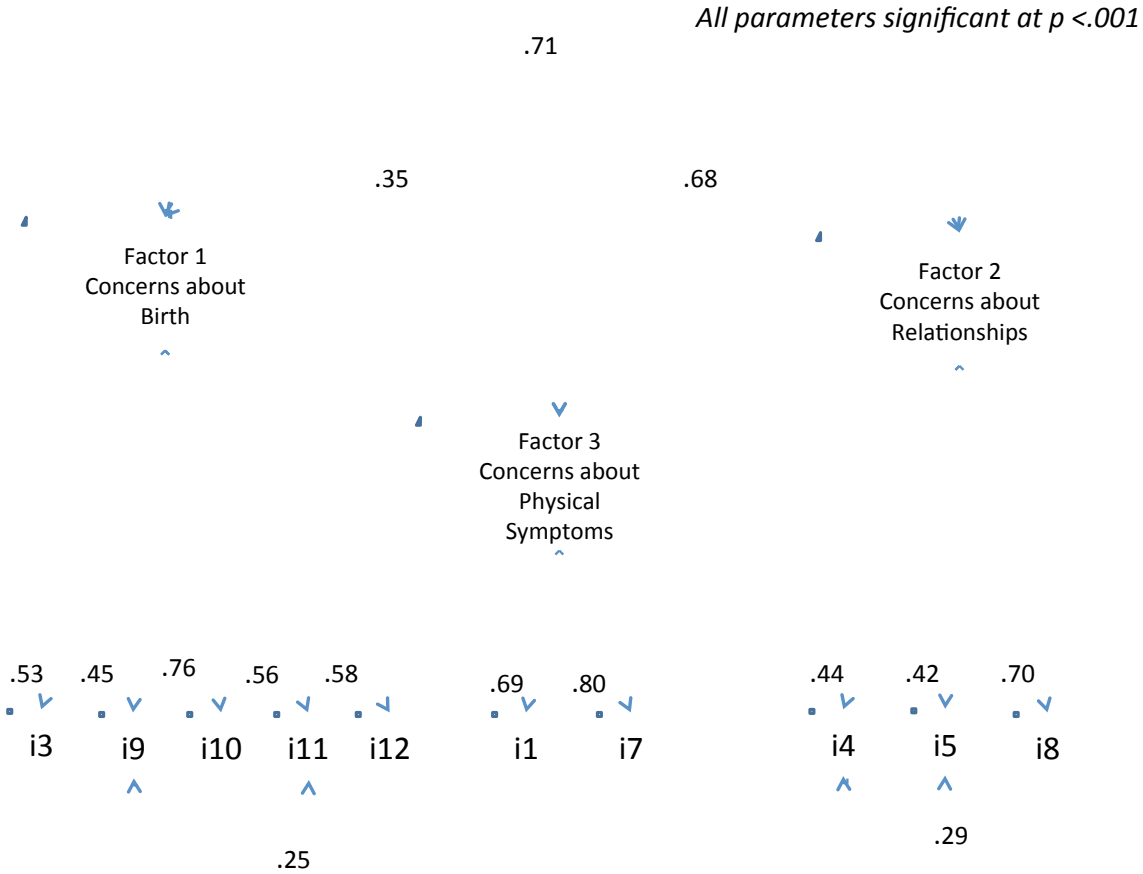


Figure 2. Standardized estimates of the final three factors model (Model 4)

Evidence of convergent validity

Convergent analysis indicates that the three PDQ factors and the one-factor solution were significantly (all *p* values < .01) positively correlated with the PSS and the SCL-90-R (Table 3).

		Three-factor Solution			**One-factor solution**
		Factor 1 (Birth Concerns)	**Factor 2 (Physical Concerns)**	**Factor 3 (Relations Concerns)**	
PSS		0.29**	0.18**	0.30**	.321**
SCL-90-R sub-scales					
	Somatization	.411**	.266**	.440**	.496**
	Obsession Compulsion	.267**	.269**	.337**	.352**
	Interpersonal Sensitivity	.296**	.271**	.338**	.344**
	Depression				

	.387**	.230**	.413**	.451**
Anxiety	.383**	.256**	.421**	.451**
Hostility	.356**	.149	.318**	.341**
Phobic Anxiety	.224**	.149	.241**	.266**
Paranoid Ideation	.246**	.195*	.285**	.282**
Psychoticism	.284**	.185*	.261**	.313**
GSI	.386**	.269**	.418**	.447**
PSDI	.333**	.138	.314**	.398**
Positive Symptom	.343**	.280**	.400**	.371**

Total				
Cronbach´s alpha	0.642	0.529	0.684	0.720

Note. p< 0.05*; p< 0.01**

Results did not indicate significant associations of factor scores in the three dimensions with age or marital status (all ANOVAs $p > 0.05$). Women pregnant for the first time reported higher Birth Concern (M = 7.56 compared to M = 5.72 for multiparous women), $t(150) = 3.63$, $p < .05$. Indeed, parity was a significant inverse predictor of Birth Concern factor scores (β = - 0.23, p = .007) even when we controlled for age and marital status, F (4, 146) = 2.85, p = .026, adjusted $R^2 = 0.05$. No significant differences were found between primiparous and multiparous women on the other factors.

Discussion

The PDQ is a valuable psychometric instrument used to assess stress related to pregnancy (Alderdice, Lynn, and Lobel, 2012; Kerry et al., 2015; Nast et al. 2013). Although it has not been adapted to Spanish speaking countries yet, the Revised Prenatal Distress Questionnaire (NuPDQ), a slightly expanded (17-item) version (Lobel et al., 2008), has already been translated and applied to Latin-American (Arroyo, 2013; Coussons-Read at al., 2012; Chaponniere, 2009) and Turkish (Yuksel, Akin, and Durna, 2014) women. Nevertheless, there are no publications reporting the use of the original PDQ in Spanish-speaking samples. The aim of this study was to confirm the three-

factor structure of the Spanish version of the original PDQ and report evidence of convergent validity among Spanish women.

Consistent with previous studies (Alderdice and Lynn, 2011; Alderdice et al., 2013), the CFA revealed a three-factor structure. Factor 1 (Birth Concerns) (items 3, 9, 10, 11 and 12); factor 2 (Relations Concerns) (items 4, 5 and 8); and factor 3 (Physical Concerns) (items 1 and 7). Covariances between errors in items 9 (unhealthy baby) and 11 (premature birth) and between items 4 (emotional concerns) and 5 (relationships) were included in our final model. Based on the content of these items, their overlap appears sensible and suggests that they share some common meaning beyond what they share with other items on the stress factor where they load.

We excluded item 2 (physical symptoms) since it did not load on its underlying factor (Physical Concerns). A similar result was reported by Alderdice and Lynn (2011) with low-risk pregnant women. A CFA conducted on high-risk pregnancies (Alderdice et al. 2013) included item 2 in the Physical Concerns factor, although exhibiting the lowest loading. These differences that correspond to sample characteristics suggest that worries about physical symptoms may not be related to other indicators of pregnancy-specific stress in low-risk pregnancies, particularly during the second trimester. Furthermore, in order to improve model fit in the present study, item 6 (eating healthy food) was not included, as it was not significantly associated with any of the 3 factors.

A uni-dimensional factor structure was found to have good fit, revealing this instrument can be used to evaluate a single underlying construct, namely pregnancy-specific stress. Studies have underscored the importance of assessing pregnancy-specific stress given its association with health-impairing behaviours during pregnancy and with adverse perinatal outcomes (Auerbach, Lobel, and Cannella, 2014; Coussons-

Read et al., 2012; Lobel et al., 2008). Incorporating brief and robust psychological questionnaires such as the PDQ into clinical care may improve the identification of women at risk of high levels of prenatal stress and promote healthier birth outcomes (Cannella, Auerbach, and Lobel, 2013).

The convergent validity between the Spanish version of the PDQ and other constructs appears to be good. All three factors were significantly correlated with the PSS. This finding corroborates previous research using the English version of the PDQ (Lobel et al., 2000; Yali and Lobel, 1999). Although previous studies have used the SCL-90-R on pregnant women (Peñacoba et al., 2017), the present study is the first to explore and find significant correlations of PDQ factors with the SCL-90-R. Even the strongest correlations are of moderate magnitude (approximately 0.4), suggesting that while pregnancy-specific stress is significantly related to general stress and to emotions such as anxiety and depression, this type of stress is still sufficiently independent to be defined as a unique construct.

Although the internal consistency of the three factors was below 0.7, the Cronbach's alpha is a measure that depends on the observed correlation of the items and the number of items, not taking into account error variance of the items. CFA allows isolating the variance items share with an underlying dimension from the error variance of the individual items, thus providing a reliable measure of the underlying dimension (Acock, 2013).

The Spanish version of the PDQ is a brief measure with good psychometric properties that provides information on the worries and concerns of pregnant women both in clinical and research settings. Although it was first developed to assess overall levels of pregnancy-related stress, the three-factor structure reported in this study gives

evidence of the additional detail that can be collected when using this measure. Although the results of this study suggest that 10 items were sufficient as indicators of the three underlying constructs, the two items excluded (physical symptoms and eating healthy foods) may be important independent contributors to prenatal stress and we would not recommend eliminating them from the instrument. The Revised PDQ, which has been successfully used in a variety of recent studies (Arroyo, 2013; Auerbach, Lobel, and Cannella 2014; Cannella, Auerbach, and Lobel, 2013; Coussons-Read et al. 2012; Lobel et al., 2008; Yuksel, Akin, and Durna, 2014) expands the instrument by the addition of several items that may slightly alter, and perhaps improve the factor structure observed for the original instrument.

Although the Spanish version of the PDQ was applied to a representative sample of low-risk pregnant women in the second trimester of pregnancy, this study had some limitations. Thus, Spanish was not the first language for a small portion of the women in this study (6.4%). Additionally, results only reflect measurement at one time point. Future studies should explore variations in responses and stability of the structure throughout pregnancy.

Evaluation of the psychometric properties of questionnaires is imperative. Although the development of psychological questionnaires is a long methological process, studies assessing reliability and validity of measures will improve the quality of psychological assessment and enable more appropriate and effective application to clinical settings (Gourounti et al., 2016).

Future research may investigate if the dimensions found in the present study are also adequate in representing the experience of women from other cultural backgrounds. Forthcoming studies need to look over gestation and assess potential capability of the

PDQ predicting adverse outcomes in a population based sample. Prospective studies could assess the psychometric properties of the PDQ on high-risk pregnant women (Alderdice et al., 2013). Good assessment is an important first step in both research and practice in developing effective care to support women experiencing stress in pregnancy (Gourounti et al., 2016). Health care providers can assess pregnancy-specific stress using the PDQ. The fact that the PDQ is a short pregnancy-specific stress measure may benefit clinical practice by starting conversations on specific aspects of pregnancy that women might want additional support.

The three-factor structure identified in a prior EFA was confirmed in this CFA using a sample of Spanish women with low-risk pregnancies. The results indicate that three factors explained the participants' pattern of responses to 10 questions. These factors represented concerns about birth and the baby, emotions and relationships, weight gain and bodily changes. These PDQ factors were significantly correlated with measures of general perceived stress and emotions, indicating good convergent validity. Results indicated that a one-factor structure also provides an adequate fit to the data; this factor represents an underlying pregnancy-specific stress construct. The Spanish version of the PDQ is a reliable and valid instrument to assess stress levels during pregnancy. The results of this study also provide evidence that the dimensions investigated by the PDQ represent significant dimensions of women's experience of distress during pregnancy across different western cultural contexts.

CAPÍTULO VIII: "ESTUDIO 3"

Protocolo del estudio de cohortes *GESTASTRESS* sobre los efectos del estrés durante el embarazo mediante la medida del cortisol en cabello de la mujer y del recién nacido

Caparros-Gonzalez, García-García, Mariñas-Lirola y Peralta-Ramírez (2018). Protocolo del estudio de cohortes *GESTASTRESS* sobre los efectos del estrés durante el embarazo mediante la medida del cortisol en cabello de la mujer y del recién nacido. *Revista Española de Salud Pública* (In press).

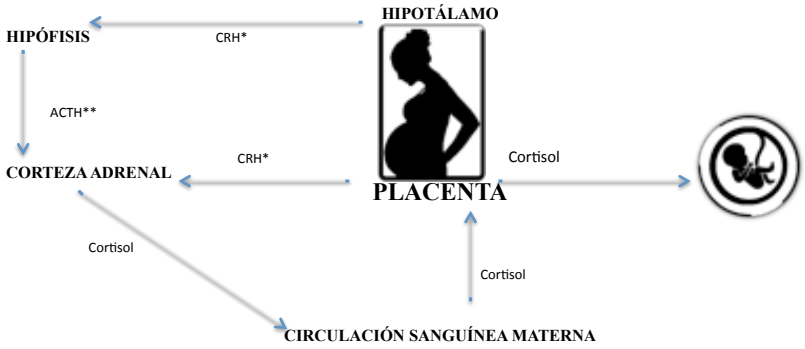
"Protocolo del estudio de cohortes *GESTASTRESS* sobre los efectos del estrés durante el embarazo mediante la medida del cortisol en cabello de la mujer y del recién nacido"

Introducción

El estrés psicológico, aunque nos ha salvado la vida como especie durante siglos, puede tener efectos devastadores en la salud (Sapolsky, 2008). Este, además de relacionarse con trastornos psicopatológicos es un buen predictor de enfermedades cardiovasculares, inmunes y endocrinas (Robles-Ortega y Peralta-Ramírez, 2010).

El estrés perinatal, aquel que ocurre en torno al embarazo, conlleva grandes modulaciones endocrinas e importantes repercusiones sobre la salud (Glover, 2015; Lynn, Alderdice y Crealey, 2011; Pérez-Ramírez, García-García, Caparrós-González y Peralta-Ramírez, 2017; Pérez-Ramírez, García-García y Peralta-Ramírez, 2013). Estudios longitudinales han evidenciado que los cambios ocurridos en el eje Hipotalámico-Pituitario-Adrenal (HPA) durante el embarazo producen alteraciones en la futura madre y en el feto que pueden perdurar hasta la edad adulta (Glover, 2014) (ver Figura 1). Los niveles de cortisol materno están mediados por la concentración de hormona liberadora de corticotropina (CRH) hipofisaria y placentaria, que elevan los niveles de cortisol a lo largo de la gestación, disminuyendo los mismos tras el parto (Sandman, Glynn y Davis, 2016). Distintos grados de activación del eje HPA se relacionan con diferentes estados de salud física y psicológica durante el embarazo, lo que afecta al normal curso del mismo, así como a diferentes resultados en el desarrollo del parto y puerperio (Alderdice, Ayers y Darwin, 2013).

Figura 1
Representación del eje HPA durante el embarazo



Nota: *CRH: Hormona Liberadora de Corticotropina.
** ACTH: Hormona Adenocorticotropa.

Figura 1. Representación del eje HPA durante el embarazo

Múltiples observatorios e instituciones relacionadas con la Salud Pública nacional e internacional incluyen en sus protocolos el control del estrés y ansiedad perinatal (Ministerio de Salud Pública de Ecuador, 2015; Ministerio de Sanidad España, 2014; NICE, 2016).

El objetivo de este artículo es presentar el protocolo del estudio longitudinal sobre el efecto del estrés perinatal desde la concepción hasta un año de vida en España. Este artículo sigue las recomendaciones de la declaración de la iniciativa STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) para la comunicación de estudios de cohortes en epidemiología observacional analítica (Von Elm, Altman, Egger, Pocock, Gotzsche y Vandenbroucke, 2008).

El estrés psicológico y la activación del eje hipotalámico hipofisario adrenal (HPA)

El estrés psicológico durante el embarazo se ha relacionado con consecuencias negativas para la madre y su descendencia (Alderdice, Lynn y Lobel, 2012). Como parte de la respuesta al estrés, el cortisol prepara al organismo para soportar y superar un estímulo estresante. En parte debido a la presencia de la placenta, el eje HPA está profundamente alterado durante la gestación. La placenta, órgano endocrino de origen fetal, favorece la secreción de cortisol (Glynn, Davis y Sandman, 2013). Además, durante el embarazo aumenta de tamaño la glándula pituitaria y aumenta la producción de cortisol desde la glándula adrenal (Sandman, Glynn y Davis, 2016).

Sin embargo, algunos estudios afirman que la percepción del estrés por la mujer embarazada no se correlaciona con la secreción de cortisol en sangre ni con prematuridad (Himes y Simhan, 2011), mientras otros sugieren que ciertos niveles de estrés durante el embarazo pueden incluso favorecer un más rápido desarrollo cognitivo y motor en la descendencia (DiPietro, Novak, Costigan, Atella y Reusing, 2005).

Estrés perinatal materno y sus consecuencias en el feto y el bebé

Modelos animales han encontrado relación entre estrés materno y la activación del eje HPA en su descendencia. Estos estudios mimetizan los efectos del estrés prenatal administrando a las hembras embarazadas corticoides y comprobando posteriormente sus efectos (Harris y Seckl, 2011;Weinstock, 2005). Así, se propone que altos niveles de estrés materno aumentan los niveles de cortisol en la madre, que al atravesar la placenta pueden afectar al desarrollo del cerebro fetal (Glover, 2015).

Niveles elevados de estrés materno durante el embarazo se relacionan con que sus bebés tendrán un mayor riesgo de alteraciones del neurodesarrollo, asma, bajo peso al nacer, prematuridad, autismo e incluso un mayor riesgo de esquizofrenia en la edad adulta (Glover, 2015; Sandman y cols., 2016; Pérez y cols., 2016). Por otro lado,

factores asociados con la duración de la lactancia materna pueden favorecer un mejor desarrollo cognitivo y motor de los bebés (Díaz-Gómez, Ruzafa-Martínez, Ares, Espiga y De Alba, 2016).

La evaluación de los niveles de cortisol materno se ha llevado a cabo en diferentes medios, como sangre, orina y líquido amniótico. Estas medidas informan de la cantidad de cortisol en un momento concreto mediante técnicas invasivas y están influenciadas por variables contextuales. Sin embargo, la medición de cortisol en pelo ofrece una serie de ventajas como informar de los niveles de estrés crónico de forma retrospectiva (Wosu y cols., 2015). Esta técnica innovadora, es altamente recomendada como método no invasivo y efectiva en la evaluación de la activación del eje HPA en la mujer embarazada al no estar influenciada por variables situacionales ni ritmos circadianos (D´Anna-Hernandez y cols., 2011; Stalder y Kirschbaum, 2012). Además por su carácter no invasivo y retrospectivo es también aplicable a recién nacidos, ya que puede informar de los niveles de estrés a los que han estado expuesto durante su vida intrauterina (Camille-Hoffman, D´Anna-Hernandez, Ross y Laudenslager, 2013).

Objetivos

1. Estudiar la relación entre la secreción de cortisol (eje HPA) en pelo materno y distintas variables de salud psicológica en la mujer embarazada a lo largo de los 3 trimestres de embarazo, parto y puerperio.
2. Describir si existe asociación entre los niveles de cortisol en pelo materno y la salud física (pre-eclampsia, diabetes gestacional, etcétera) en la mujer embarazada durante los 3 trimestres de embarazo, parto y puerperio.
3. Comprobar la relación existente entre el cortisol en pelo de la mujer a lo largo de todo el embarazo, lactogénesis y duración de lactancia materna.

4. Estudiar la relación entre la secreción de cortisol (eje HPA) en pelo materno a lo largo del embarazo con diferentes variables fetales: longitud del fémur, circunferencia abdominal, diámetro bi-parietal, peso estimado y riesgo de cromosomopatías.
5. Estudiar la asociación entre la secreción de cortisol (eje HPA) en pelo materno a lo largo del embarazo con diferentes variables de salud neonatal: semanas de gestación al nacimiento, peso al nacer, diámetro cefálico, diámetro torácico, talla y test de Apgar.
6. Comprobar la relación entre los niveles de cortisol en pelo materno durante todo el embarazo con los niveles de cortisol en el neonato en el puerperio.
7. Analizar si la secreción de cortisol de la madre durante todo el embarazo, así como su perfil psicopatológico, el cortisol del pelo del bebé al nacer y el cortisol de la madre en los meses posteriores son predictores del temperamento, estrés y neurodesarrollo del bebé a la edad de 6 y 12 meses.

Métodos

Diseño

Estudio prospectivo de cohortes, de base poblacional, en el que se evaluará a las mujeres embarazadas y a los fetos durante el primer, segundo, tercer trimestre de embarazo y en el puerperio. Además se realizará un seguimiento de los bebés a los 6 y 12 meses de edad.

El tamaño muestral se calculó con el programa G*Power 3.1.3 (Faul, Erdfelder, Lang y Buchner, 2007), para una potencia estadística del 80%, poder detectar diferencias estadísticamente significativas en el contraste de la hipótesis nula H_0:$\mu 1=\mu 2$

para 2 muestras independientes y asumiendo un nivel de significación del 5%. Con estos datos se estimó un tamaño muestral de 807 mujeres embarazadas.

Se captarán a las embarazadas en el primer trimestre de gestación en las consultas de control del embarazo de los centros de atención primaria. Posteriormente se les entregará el documento con la información del estudio y el consentimiento informado, que deberán leer, entender y firmar.

Los criterios de inclusión fueron mujeres embarazadas en el primer trimestre de gestación, mayores de 18 años y con un alto nivel del idioma castellano. Se excluirán aquellas embarazadas con alguna enfermedad previa al embarazo.

El estudio comenzó en Enero de 2016 y pretende tener una duración de 3 años. La selección de participantes se llevará a cabo durante 21 meses para asegurar la inclusión del total de la muestra.

Las instituciones colaboradoras de este estudio son la Universidad de Granada, el Centro de Investigación Mente, Cerebro y Comportamiento (CIMCYC), centro de salud Góngora (Granada), centro de salud Mirasierra (Granada), Hospital Clínico (Granada), Agencia Pública Empresarial Sanitaria Poniente de El Ejido (Almería), centro de salud Roquetas de Mar (Almería), Hospital Comarcal Antequera y Queen´s University Belfast.

Medidas

Para valorar la exposición al estrés se utilizarán las medidas de cortisol en pelo, que se tomarán y analizarán en concordancia con las guías recomendadas (D´Anna-Hernandez y cols., 2011; Stalder y Kirschbaum, 2012). Para la obtención de la muestra de pelo de donde se extraerá cortisol, se tomará un pequeño mechón de la parte

posterior de la cabeza, de un diámetro de medio centímetro, cortándose lo más cerca posible de la base del cráneo. La muestra se introducirá en un pliego de papel de aluminio y lo introduciremos en una bolsa de plástico con auto-cierre correctamente identificada con el código asignado a cada participante. Finalmente, se entregará en el laboratorio colaborador para su análisis mediante método ELISA. En el momento de nacimiento se tomará también una muestra de pelo del neonato siguiendo el mismo protocolo que el descrito. El procedimiento de toma de muestra de pelo es un método no invasivo, que no supone realizar ningún daño a los participantes y que está descrito en varios estudios (D´Anna-Hernandez, Ross y Natvig, 2011; Stalder y Kirschbaum, 2012).

Los datos sociodemográficos y obstétricos se obtendrán de la historia de salud de cada una de las mujeres seleccionadas La valoración de variables psicopatológicas y de estrés psicológico se basará en las escalas que se muestran en la Tabla 1.

Tabla 1. Descripción de las escalas psicológicas que se aplicarán a las mujeres embarazadas

Nombre de la escala	**Nombre abreviado**	**Descripción**	**Ítems**	**Opciones de respuesta**	**Fiabilidad (alpha Cronbach)**	**Cuándo se aplica**
Cuestionario de Preocupaciones Prenatales (Yali y Lobel, 1999)	PDQ	Diseñado para evaluar estrés específico del embarazo a través de la evaluación de síntomas físicos, relaciones, problemas médicos, parto,	12	5	0,71	1º, 2º y 3º trimestres de embarazo

		nacimiento y la salud del bebé				
Escala de Estrés Percibido (Cohen, Kamarck y Mermelstein, 1983)	EEP	Muy útil para evaluar el nivel de estrés percibido y el grado en que las personas encuentran su vida impredecible, incontrolable o sobrecargada. Formada por 14 ítems con cuatro alternativas de respuesta	14	4	0,81	1º, 2º y 3º trimestres de embarazo
Inventario de Síntomas (Derogatis, 1975)	SCL-90-R	Diseñada para medir trastornos psicopatológicos en base a nueve dimensiones primarias (somatizaciones, obsesiones y compulsiones, sensibilidad interpersonal, depresión, ansiedad, hostilidad, ansiedad fóbica, ideación paranoide y psicoticismo) y	90	5	0,73-0,88	1º, 2º y 3º trimestres de embarazo

		tres índices globales de angustia psicológica (índice de severidad global, síntomas positivos totales e índice de síntomas positivos de angustia)				
Escala de Resilencia (Connor y Davidson, 2003)	CD-RISC	Constituye una medida unidimensional de resiliencia que refleja la habilidad de tolerar experiencias de cambio, problemas personales, enfermedad, presión, fracaso y sensación de dolor	10	5	0,85	1º, 2º y 3º trimestres de embarazo
Escala de Depresión Posparto de Edimburgo (Cox, Holden y Sagovsky, 1987)	EPDS	De utilidad para evaluar riesgo de depresión posparto y suicidio	10	4	0,79	Posparto

Con el propósito de evaluar la variables fetales, se usarán las técnicas ecográficas y screening bioquímico de cromosomopatías que se indican en el Documento de Salud de la Embarazada (Junta de Andalucia, 2010). En lo referente a variables ecográficas fetales, se evaluarán los siguientes: biometría fetal en tres momentos diferentes; una por cada trimestre donde los principales parámetros que se calculan son: CRL (mm), longitud cráneo-caudal, TN (mm) translucencia nucal, DBP (mm) diámetro biparietal, LF (mm) longitud del fémur, peso (gramos), circunferencia abdominal, estática fetal (posición, presentación). De estas medidas se obtienen los siguientes índices: longitud, circunferencia cefálica, circunferencia abdominal y Peso estimado. En lo que respecta al screening bioquímico de cromosomopatías, se valorarán translucencia nucal (mm)**,** CRL (mm)**,** MoM Beta HCG-libre (Ul/L)**,** MoM PAPP-A (Ul/L)**,** MoM translucencia nucal y presencia o ausencia de hueso nasal. Estos parámetros generan tres índices de riesgo de cromosopatías: Índice Riesgo Síndrome de Down, Índice Riego Síndrome Edwards **,** Índice Riesgo Síndrome Patau. Esta prueba se realiza entre la semana 10 - 12 de embarazo.

En lo referente a datos del parto y nacimiento se tomarán de las historias de salud de las mujeres embarazadas.

Para la valoración de los bebés a los 6 y 12 meses se usarán las escalas de neurodesarrollo y conductuales que aparecen en la tabla 2.

Tabla 2. Descripción de las escalas de evaluación que se aplicarán a los bebés

Nombre de la escala	**Nombre abreviado**	**Descripción**	**Áreas que evalúa**	**Fiabilidad**	**Cuándo se aplica**
Escala Bayley	BSID	Diseñada para	-Desarrollo	0,88	A los 6 y

de Desarrollo Infantil (Bayley, 2006)		valorar el estado de desarrollo en niños con edades comprendidas entre un mes y tres años y medio	cognitivo (Percepción, Memoria, Aprendizaje, Vocalización) - Desarrollo Lenguaje (Expresivo y Receptivo) - Desarrollo Motor (Fino y Grueso) - Escala Socioemocional - Comportamiento Adaptativo		12 meses de edad
Cuestionario de Comportamiento Infantil (Rothbart, 1981)	IBQ	Diseñada para la evaluación del temperamento	- Nivel de actividad (frecuencia y amplitud del movimiento) - Ira/Malestar ante las limitaciones - Duración de la orientación sobre objetos y sucesos del ambiente - Miedo - Risa y sonrisa - Facilidad para tranquilizarse (componente autorregulador del temperamento)	0,70-0,79*	A los 6 y 12 meses de edad

Análisis estadístico

El análisis de los datos se llevará a cabo mediante Chi-cuadrado y t-student para evaluar si existen diferencias estadísticamente significativas en las variables descriptivas. Las variables longitudinales se analizarán mediante ANOVA de medidas repetidas, incluyendo como covariables aquellas variables estadísticamente significativas en el análisis descriptivo de los datos. Igualmente, el uso de regresión múltiple informará de aquellas variables de salud, psicológicas y de niveles de cortisol en pelo durante el embarazo que pueden predecir niveles de cortisol en pelo en recién nacidos y niveles de temperamento y neurodesarrollo en los bebés a los 6 y 12 meses de edad.

Cronograma

El cronograma del estudio desde su inicio en Enero de 2016 y con una duración de 3 años se muestra en la figura 2.

Figura 2
Cronograma del proyecto GESTASTRESS

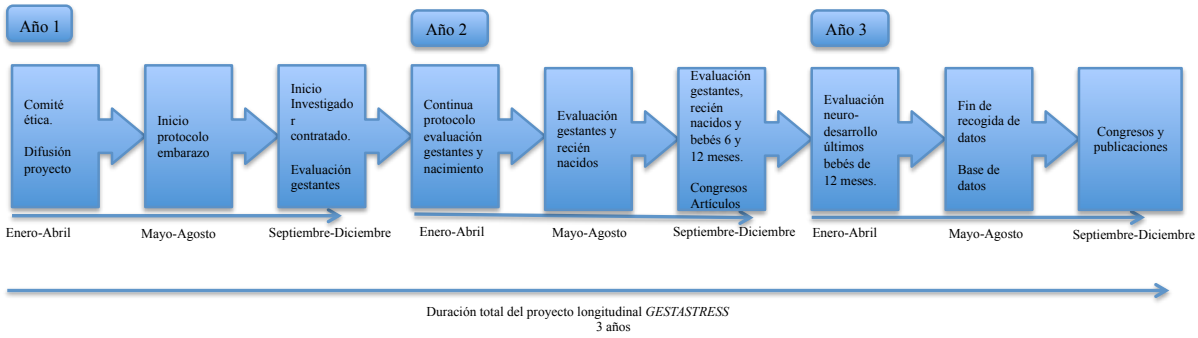


Figura 2. Cronograma de la cohorte *GESTASTRESS*

Consideraciones éticas

El protocolo de este estudio ha sido evaluado favorablemente por el Comité de Ética en Investigación Humana de la Universidad de Granada, el Comité de Ética de Centro de Granada y la conformidad de la dirección del Distrito Sanitario de Poniente de Almería.

Posibles impactos

La implantación de la técnica de cortisol en pelo, que actualmente solo se realiza en 5 centros de investigación en el mundo y no se ha realizado hasta ahora en España, supondrá un gran avance. Mediante esta técnica será posible medir el cortisol de las mujeres embarazadas a lo largo de los 3 trimestres. Además, es de gran innovación la posibilidad de valorar mediante la extracción de cortisol en pelo del bebé sus niveles de estrés intrauterino en el último trimestre y de forma inocua y no invasiva. Estudiaremos su relación con el estrés de la madre y con su posterior neurodesarrollo y temperamento.

Los resultados encontrados serán de gran importancia en el ámbito de la Salud Pública al permitir establecer puntuaciones promedio de cortisol en pelo de las mujeres embarazadas y sus bebés, para así hacer un screening de estrés en esta población y permitir su intervención. Estos factores tienen importantes implicaciones económicas en el sistema de salud a corto plazo y en la sociedad en general a largo plazo. Futuras investigaciones pueden incluir en sus protocolos la influencia del estrés fisiológico y psicológico durante el embarazo sobre determinantes tóxicos, nutricionales y genéticos en la salud maternal y en el desarrollo fetal e infantil (Ramón y cols., 2005).

CAPÍTULO IX: "ESTUDIO 4"

Hair cortisol levels, psychological stress and psychopathological symtoms as predictors of postpartum depression

Caparros-Gonzalez, Romero-Gonzalez, Strivens-Vilchez, Gonzalez-Perez, Martinez-Augustin y Peralta-Ramirez (2017). Hair cortisol levels, psychological stress and psychopathological symptoms as predictors of postpartum depression. *PLoS ONE, 12*(8): e0182817.

"Hair cortisol levels, psychological stress and psychopathological symptoms as predictors of postpartum depression"

Introduction

Although mental health problems during the postpartum period often go unrecognized and untreated, the National Institute for Health and Care Excellence (NICE, 2016) recommends an urgent intervention due to potential detrimental effects on the newborn and the mother's life. Postpartum depression affects from 10% to 15% of women after delivery and consists of emotional liability and sometimes suicidal ideation (Yim, Stapleton, Guardino, Hahn-Holbrook, & Schetter, 2015). An extensive number of studies have shown an association between postpartum depression and poor bonding between the mother and the newborn as well as lower infant neurodevelopment (Katon, Russso, & Gavin, 2014; O´Hara y McCabe, 2013). Early detection of factors associated with postpartum depression can prevent its appearance and negative outcomes (e.g. postpartum psychosis) (Sockol, Epperson, & Barber, 2013).

It has been previously reported that there is an association between sociodemographic risk factors during pregnancy (e.g. being younger than 25 years old) and postpartum depression symptoms (Leigh &Milgrom, 2008; Saligheh, Rooney, McNamara, & Kane, 2014). Furthermore, obstetric risk factors (e.g. previous miscarriages) have been found to be related to postpartum depression (Milgrom et al., 2008; Robertson, Grace, Wallington, & Stewart,, 2004). Past histories of psychopathological symptoms or suffering from depression, anxiety, or stress during pregnancy have been reported as psychological variables associated with postpartum

depression (Suhitharan et al., 2016; Turkcapar, Kadõoğlu, Aslan, Tunc, Zayõfoğlu, & Mollamahmutoğlu, 2016). Fig 1 shows a summary of risk factors associated with postpartum depression in previous studies.

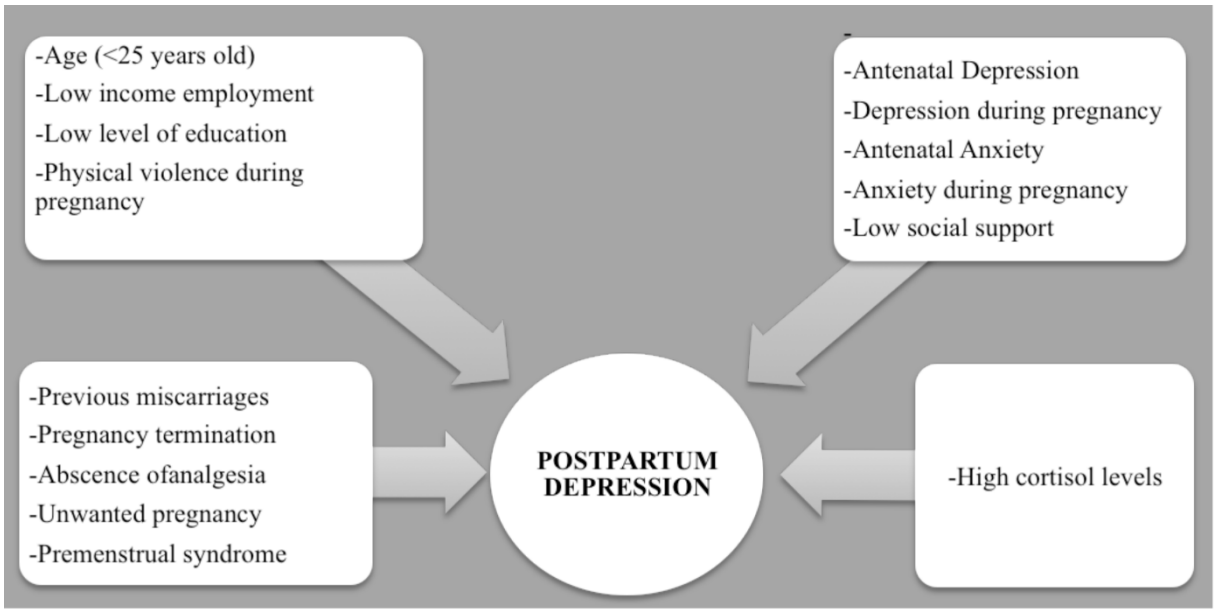


Figure 1. Summary of risk factors during pregnancy associated with postpartum depression (Glynn, Davis, & Sandman, 2013; Iliadis, Comasco, SylveÂn, Hellgren, SundstroÈm, & Skalkidou., 2015; Leigh & Milgrom, 2008; Milgrom et al., 2008; Robertson et al., 2004) Saligheh et al., 2014; Suhitharan et al., 2016; Turkcapar et al., 2015).

Another risk factor associated with postpartum depression is the dysregulation of the hypothalamic-pituitary-adrenal axis which results in an increased exposure of pregnant women to cortisol (Glyn et al., 2013; Illiadis et al., 2015). The hypothalamus synthesizes corticotrophin-releasing hormone (CRH) as part of the biological stress response. CRH stimulates the release of cortisol to prepare the organism to cope with stressful stimuli (Sandman, Glyn, & Davis, 2016; Wikenius et al., 2016). Due in part to

the presence of the placenta, the hypothalamic-pituitary-adrenal axis is deeply altered during pregnancy. The placenta, a fetal origin endocrine organ, promotes an increased release of cortisol from the adrenal gland through a dramatically increase of placental CRH over pregnancy (Glyn, Davis, & Sandman, 2013). Although cortisol negatively regulates the production of CRH from the hypothalamus, cortisol increases the release of placental CRH during pregnancy (Glyn, Davis, & Sandman, 2013). Other physical changes during pregnancy include how the pituitary gland doubles its size, and the level of production of cortisol from the adrenal gland increases (Sandman, Glynn, & Davis, 2016). Cortisol levels have been generally assessed from urine, saliva, blood, or amniotic fluid samples in pregnant women (Bergman, Sarkar, Glover, & O'Connor, 2010; De Rezende, 2016). Though each matrix offers information about the stress levels the women were experiencing at the time the sample was taken, these methods of assessment require an invasive technique and can be affected by situational variables or circadian rhythms (Sandman, Glynn, & Davis, 2016; Stalder & Kirschbaum, 2012).

Alternatively, testing via hair cortisol levels is an innovative technique that offers a retrospectively chronic stress measure of the preceding 3 months, is not invasive, is not affected by the time of the day, and is easy to transport and preserve (Mastorakos & Illias, 2003; Wikenius et al., 2016; Wosu, Valdimarsdotirr, Shields, Williams, & Williams, 2013).

The association between postpartum depression and the activation of the hypothalamic-pituitary-adrenal axis during pregnancy remains a challenge. On one hand, an association between postpartum depression and high hair cortisol levels during pregnancy has been reported (Braig et al., 2015). On the other hand, urine and blood cortisol levels were not associated with postpartum depression (Figueiredo & Costa; 2009; Yim et al., 2015). However, certain associations have been reported between low

blood cortisol levels and postpartum depression (Jolley, Elmore, Barnard, & Carr; 2007).

A recent review reported future research should improve the accuracy of cortisol measurements over time and use appropriate tools to assess depression (Seth, Lewis, & Galbany, 2016). More studies on risk factors associated with postpartum depression may reduce negative pregnancy outcomes (Milgrom, Schembri, Ericksen, Ross, & Gemmill, 2011; Sockol et al., 2013). Predicting those variables related to postpartum depression can improve pregnancy and infant health outcomes through tailored interventions during pregnancy (O'Hara & McCabe, 2013).

In this respect, the aim of this study was to analyze sociodemographic, obstetric, and psychological variables along with hair cortisol levels during the first, second, and third trimester of pregnancy that could predict postpartum depression symptoms.

Methods

Subjects

Participants were recruited at 3 health centers and a general hospital in the South of Spain, while attending a prenatal appointment. Fifty-seven pregnant women voluntarily agreed to participate in this study. Five women had a spontaneous miscarriage during the first trimester. Seven participants were unable to continue in the study during pregnancy due to lack of time. One participant moved to another city before giving birth. Finally, a total sample of 44 pregnant women was longitudinally assessed during the first trimester (M = 12.36 weeks of gestation; SD = 3.60), the second trimester (M = 25.32 weeks of gestation; SD = 3.24), and the third trimester (M = 34.94 weeks of gestation; SD = 3.34). Assessments took place while participants were attending a prenatal appointment with their midwives (health center) and obstetricians

(general hospital). Participants were followed up during a postpartum appointment with their respective health care practitioners (M = 15.79 days after birth; SD = 9.78) and divided into 2 groups: a group of women with postpartum depression symptoms (n = 16), scoring 10 or greater on the Edinburgh Postnatal Depression Scale, and a group of women with no postpartum depression symptoms (n = 28), scoring below 10 on the Edinburgh Postnatal Depression Scale. We used the cut off of 10, as it is the best cut-off score for European Spanish mothers. This cut off was indicative of highly likely to be suffering from postpartum depression (Garcia-Esteve, Ascaso, Ojuel, & Navarro, 2003).

Inclusion criteria was low-risk pregnant women above 18 years old with proficiency in the Spanish language. Participants were excluded if they presented any pathology before or during pregnancy. To minimize the confounding effect of risk variables, pregnancies with Cushing's disease, asthma, steroid medication, diabetes, and other conditions known to affect cortisol levels, were excluded.

This study was approved by the Human Ethics Research Committee of the University of Granada (reference 881), the Biomedical Ethics Research Committee and the Ethics Research Committee of the Health Centers, and the hospital where this study was implemented. Moreover, this study followed the guidelines of the Helsinki Declaration (AMM, 2008) and the Good Clinical Practice Directive (Directive 2005/28/EC) of the European Union. Participation was voluntary and an informed written consent document was read and signed by every participant.

Instruments

Sociodemographic and obstetrics data

Demographic information was collected my means of the Pregnancy Health Document (Andalusian Ministry of Health, 2010) since it is the official record of the health of every pregnant woman and her newborn.

Biological measures

For the purpose of assessing the activation of the hypothalamic-pituitary-adrenal axis, hair cortisol levels were measured through hair samples proximal to the scalp with a length no greater than 3 cm (assuming an average growth rate of 1 cm/month, a 3 cm segment contains cortisol that has been deposited over approximately the last 3 months). Samples consisting of approximately 150 strands of hair were collected from the posterior vertex of the head (Sauve, Koren, Walsh, Tokmakejian, & Van Uum, 2007). The hair samples were wrapped in a piece of aluminum foil to protect them from light and humidity and they were stored in an envelope at room temperature. Afterwards the hair samples were sent for analysis to the Faculty of Pharmacy at the University of Granada. The hair samples were weighed and ground to a fine powder to break up the hair's protein matrix and increase the surface area for extraction using a ball mill. Cortisol from the interior of the hair shaft was extracted into HPLC-grade methanol by incubation of the sample for 72 hours at room temperature in the dark with constant inversion using a rotator. After incubation, the supernatant was evaporated until completely dry using a vacuum evaporator and the extract was reconstituted in 150 ul of phosphate buffered saline at a pH of 8.0. The reconstituted sample was immediately frozen at -20ºC for later analysis (Chen et al., 2013; Meyer, Novak, Hamel, & Rosenberg, 2014; Russel et al., 2015).

The cortisol in the hair sample was measured using the a salivary ELISA cortisol kit with the reagent provided following the manufacturer's directions. Using a salivary

ELISA cortisol kit© is a validated method to assess hair cortisol levels and is highly positive correlated with liquid chromatograph–mass spectrometry (LC–MS/MS) (Russel et al., 2015). The sensitivity of the cortisol ELISA kit is 1.0 ng/ml as reported by the manufacturer and the cross reactivity is as follows: Prednisolone 13.6%, Corticosterone 7.6%, Deoxycosticosterone 7.2%, Progesterone 7.2%, Cortisone 6.2%, Deoxycortisol 5.6%, Pednisone 5.6% and Dexamethasone 1.6%. No cross-reaction was detected with DHEAS and Tetrahydrocortisone.

The intra- and inter-assay variations were analyzed on internal quality controls used for routine salivary cortisol measurement, measured in duplicate on eight consecutive assays. The intra-assay coefficients of variance (CV) were 2.7% at 10.7 ng/ml and 4.3% at 43.9 ng/ml. The inter-assay CVs were 4.4% and 6.3%, respectively.

Maternal perceived stress

Psychological stress was assessed by means of the 14-item Spanish version of the Perceived Stress Scale (PSS) (Cohen, Kamarck, & Mermelstein, 1983) to evaluate the perception of general stress during the preceding month. Each of the 14 items scores on a 5-point Likert scale (0 = never, 1 = almost never, 2 = once in a while, 3 = often, 4 = very often). The Cronbach's alpha reliability coefficient of the Spanish version is α = 0.81 (Remor, 2006).

Psychopathological symptoms

In this respect, the Spanish version of the SCL-90-R (Caparros-Caparros, Villar-Hoz, Juan-Ferrer, & Viñas-Poch, 2007; Derogatis, 1975) was used to assess psychopathological symptoms. This 90-item scale is scored using a 5-point Likert scale from 0 (never) to 4 (extremely). This instrument is used to assess 9 dimensions: Somatization, Obsession-compulsion, Interpersonal sensitivity, Depression, Anxiety,

Hostility, Phobic anxiety, Paranoid ideation, and Psychoticism. The scale also has 7 extra items distributed among 3 global indexes of distress: the GSI, which measures overall psychological distress; the PSDI, which is used to measure the intensity of symptoms; and Positive Symptom Total, used to measure the number of self-reported symptoms. The Cronbach's alpha reliability coefficients of the Spanish version range are between $.67 < \alpha < .94$ (Caparros-Caparros, Villar-Hoz, Juan-Ferrer, & Viñas-Poch, 2007).

Pregnancy-specific stress

For this purpose, the Spanish version of the Prenatal Distress Questionnaire (PDQ) (Caparros-Gonzalez et al., 2015; Yali & Lobel, 1999) was used to assess pregnancy-specific stress. It is a 12-item instrument scored on a 5-point Likert scale from 0 (none at all) to 4 (extremely) to assess specific worries and concerns pregnant women experience regarding medical problems, physical symptoms, body changes, labor, childbirth, relationships, and the baby's health. The Cronbach's alpha reliability coefficient of the Spanish version is $\alpha = .71$ (Caparros-Gonzalez et al., 2015).

Measurement of postpartum depression

The Spanish version of the Edinburgh Postnatal Depression Scale (Cox, Holden & Sagovsky, 1987; Maroto-Navarro, García-Calvente, & Fernandez-Parra, 2005) was used to assess the risk of postpartum depression. This 10-item instrument is scored on a 4-point Likert scale ranging from 0 (as always) to 3 (absolutely not). The best cut-off score for the Spanish version was 10/11 as highly likely to be suffering from postpartum depression (Garcia-Estevez, Ascaso, Ojuel, & Navarro, 2003). A cut off of 10 was also reported to be useful to screen for a posterior psyquiatric assessment in Spanish sample. Using a cut-off of 10 identified 100% of women with major depression, resulting in a

combined sensitivity of 79%, specificity of 95%. Although some authors recommend a 12/13 cut-off (Boyce, Stubbs, Todd, Boyce, Stubbs, & ToddCox, 1993; Harris, Huckle, Thomas, Johns, & Fung, 1989; Holden, & Sagovsky, 1987) even in Spanish Americans [42], the sensitivity for depression decreased to 62% and 14% of women with a major depression remained undiagnosed in European Spanish women during the postpartum period (Garcia-Estevez, Ascaso, Ojuel, & Navarro, 2003). A study lead by the Postpartum Depression: Action Towards Causes and Treatment (PACT) Consortium also used a cut-off of 10 to capture a wider range of severity of postpartum depression (minor to severe) (Reuland, Cherrington, Watkins, Bradford, Blanco, & Gaynes, 2009). The Cronbach's alpha reliability coefficient of the Spanish version is α =.79 (PACT, 2014).

Procedure

Pregnant women attending antenatal appointments at 3 public health centers in Granada and Roquetas de Mar, Spain, and a general hospital in El Ejido, Spain, (September 2015-July 2016) completed a battery of self-report questionnaires during their first, second, and third trimesters of pregnancy, and during the postpartum period. Participants received informative leaflets and stated their intention to participate at the next prenatal appointment. In our context, pregnant women attend an appointment with a General Practitioner (GP) before visiting a midwife. Clinical interviews performed by GPs reflected an absent of any decisional impairment that could affect their capacity to consent (IRB, 2017). Following the written consent, hair samples were obtained by a specifically trained midwife, according to suitable guidelines and participants completed all 3 previously mentioned questionnaires (PDQ, PSS, SCL-90-R) at home during each trimester, and returned the questionnaires at their next antenatal appointment. The Depression sub-scale of the SCL-90-R was used to assess antenatal

depression during pregnancy. Information regarding sociodemographic and obstetric data was obtained at the first antenatal appointment.

After delivery, participants attending a postnatal appointment (M = 15.79 days after birth; SD = 9.78) with a midwife at a public health center were assessed with the Edinburgh Postnatal Depression Scale.

Statistical analysis

Firstly, participants were divided into 2 groups: a group of women with postpartum depression symptoms (n = 16), scoring 10 or greater in Edinburgh Postnatal Depression Scale, and a group of women with no postpartum depression symptoms (n = 28), scoring below 10 in Edinburgh Postnatal Depression Scale. In order to verify both groups were equivalent in terms of the main sociodemographic, obstetric, and hair characteristics, 2-sample *t*-tests and a chi-square test were used to compare sociodemographic, obstetric, and hair characteristics between groups.

A mixed 2 × 3 analysis of ANOVA was conducted to check for statistically significant differences between both groups. The first factor includes two levels (women with postpartum depression symptoms and women with no postpartum depression symptoms) between the independent groups. The second was a repeated-measures within-subject factor during three trimesters: 1st trimester (hair cortisol, psychopathological symptoms and stress); 2nd trimester (hair cortisol, psychopathological symptoms and stress); 3rd trimester (hair cortisol, psychopathological symptoms and stress). The Greenhouse-Geisser correction was applied in the repeated-measures analyses. When a significant Group x Sampling Time interactions was found, *Bonferroni* analysis was conducted to determine the trimesters there were differences between. *Bonferroni* analysis is a conservative post hoc

procedure designed to compare different combinations while controlling the overall Type I error rate (α) (Field, 2009). A follow-up Student's *t*-test was conducted to determine whether there were differences in cortisol, psychopathological symptoms and stress levels between both groups Due to the fact that hair cortisol levels did not have a normal distribution, a natural log transformation (natural log; *LN* base e) was done.

Previous studies reported subjects with dyed hair presented lower hair cortisol levels (Abel et al., 2016; Manenschijn, Koper, Lamberts, & Van Rossum, 2011; Sauve, Koren, Walsh, Tokmakejian, & Van Uum, 2007). Participants with dyed hair were not excluded. Consequently, we controlled this factor on analysis. A previous study found that fetal sex could affect maternal cognition (Vanston & Watson, 2005). For this reason, a 2-sample *t*-test was conducted to test whether the sex of the fetus could influence cortisol levels.

Finally, with the purpose of testing which hair cortisol levels (first, second or third trimester) best explained the Edinburgh Postnatal Depression Scale scores, we carried out a multiple regression analyses using the introduction method. The independent variables were the hair cortisol levels on the first, second and third trimester. The dependent variables were the Edinburgh Postnatal Depression Scale scores.

Data analyses were performed using Statistical Package for Social Sciences 20.0 Mac version (SPSS, Armonk, NY). Differences were considered significant when $p < .05$.

Results

Descriptive sample characteristics

A total sample of 44 low-risk pregnant women between the ages of 24 and 39 years old (M = 32.38; SD = 3.96) participated in this study. As shown in Table 1, t-tests and a chi-square test indicate no differences between groups in respect to main sociodemographic data, obstetrics, and hair characteristics. Due to significant differences were found between groups on previous miscarriages ($X^2 = 4.71$, $p < .05$) and the sex of the fetus ($X^2 = 6.03$, $p < .05$), we included these variables as covariates on further analysis.

Table 1. Differences in sociodemographic, obstetrics variables and depression symptomatology between women with postpartum depression and without postpartum depression

		No depression X(SD)/%	**Depression X(SD)/%**	**Test a**	***p***
Socio-demographic variables					
Age		32.11(4.05)	32.94(3.62)	-.67	*.50*
Nationality	Spanish	24(85.70%)	4(25.0%)	7.86	.37
	Immigrant	4(14.3.60%)	12(75.0%)		
Marital status	Single/divorced/widow	10(35.7%)	8(50%)	.86	.35
	Married/cohabitant	18(64.3%)	8(50%)		
Employment situation	Working	23(82.1%)	11(68.8%)	1.04	.31
	Unemployed	5(17.9%)	5(31.2%)		
Occupation	Health	8(28.6.%)	5(31.2%)	-2.57	.79

	Education	7(25.0%)	2(12.5%)		
	Other	3(46.4%)	9(36.2%)		
Level of education	Secondary school	3(42.85%)	4(57.15%)	3.89	.14
	University	23(69.70%)	10(30.30%)		
Sport	Yes	19(67.9%)	7(43.8%)	2.45	.12
	No	9(32.1%)	9(56.2%)		
Pet	Yes	8(28.6%)	8(50%)	2.02	.15
	No	20(71.4%)	8(50%)		
Hair aspect	Nature	13(46.4%)	6(37.5%)	0.33	.56
	Dyed	15(53.6%)	10(62.5%)		
Obstetric information					
Primiparous	Yes	20(71.4%)	8(50%)	2.02	.15
	No	8(28.6%)	8(50%)		
Wanted pregnancy	Yes	24(85.7%)	13(81.2%)	1.52	.69
	No	4(14.3%)	3(18.8%)		
Pregnancy method	Spontaneous	22(78.6%)	13(81.2%)	0.45	.83
	Fertility treatment	6(21.4%)	3(18.8%)	4.71	.03*
Previous miscarriages	Yes	4(14.3%)	7(43.8%)	2.77	.78
Labor and delivery	No	24(85.7%)	9(56.2%)		
	Eutocic	20(74.1%)	9(56.2%)		

	Dystocic		4(14.8%)	2(15.4%)		
	C-section		3(11.1%)	1(7.7%)		
Pain relief in labor	None		2(8.0%)	3(23.1%)	4.11	.13
	Epidural		18(72.0%)	10(76.9%)		
	Warm bath		5(20.0%)	0(0%)		
Sex of the fetus	Female		7(25%)	10(62.5%)	6.03	.01*
	Male		21(75%)	6(37.5%)		
Depression						
Antenatal depression	Depression subscale clinical scores (> 70)	1st trimester	4(36,36%)	1 (10%)	0.65	.41
		2nd trimester	2(18,18%)	4(40%)	2.75	.09
		3rd trimester	5(45,45%)	5(50%)	1.04	.30
Postnatal depression	EPDS	< 10 scores	28(100%)	3(18.8%)	32.29	.001*
		10-12 scores	0(0%)	5(31.2%)		
		>12 scores	0(0%)	8(50%)		

Note. Significant at the *= $p \leq ,05$. [a] T-test of students used to quantitative variables and Chi-square test to categorical variables. Sport is presented to inform whether participants practiced or did not practice any regular physical activity during pregnancy.

Associations of maternal postpartum depression symptoms with indicators during pregnancy

We examined the associations of maternal postpartum depression symptoms with pregnancy-specific stress, perceived stress, and psychopathological symptoms during the first, second, and third trimester. The group with postpartum depression symptoms had higher scorers on the Edinburgh Postnatal Depression Scale (M = 13.50; range = 10-24) than the group with no postpartum depression symptoms (M = 4.75; range = 2-8), t = 9.92, p < .001. Five participants scored in the range of 10-12 on the Edinburgh Postnatal Depression Scale (see Table 1).

Regarding the between group analysis, an interaction effect was found between women with postpartum depression symptoms and women with no postpartum depression symptoms on pregnancy-specific stress throughout the three trimesters, $F(1, 41) = 4.08$, p = .05, which remained significant when including previous miscarriages, sex of the fetus and antenatal depression as covariates, $F(1, 37) = 5.67$, p < .05). *Bonferroni post hoc* analysis on the pregnancy-specific stress revealed no significant differences during the first, second or third trimester on the any of the two groups. Although pregnancy-specific stress levels were higher among participants with postpartum depression symptoms at the first, second, and third trimester of pregnancy, significant differences between both groups were found during the third trimester regarding pregnancy-specific stress (t = -2.67, p =.01) (see Table 2).

In respect to perceived stress, no significant interaction effect was found between both groups.

Table 2. Mean differences on stress and psychopathological symptoms with interaction effects between groups*trimesters

Trimester	**Questionnaires**	**Subscalesa**	**No depression**	**Depression**	***t***	***p***

			X(SD)	**X(SD)**		
Trimester 1	PDQ		13.96(6.44)	15.81(5.64)	*-1.79*	*.08*
	SCL-90-R	SOMS	57.14(26.29)	70.66(19.33)	*-2.70*	*.01**
		DEP	43.04(23.13)	44.28(18.79)	*-.89*	*.37*
		ANX	54.63(28.19)	60.01(17.95)	*-.74*	*.46*
		GSI	53.66(25.24)	57.59(19.83)	*-.53*	*.59*
Trimester 2	PDQ		12.57(5.54)	14.25(4.41)	*-1.03*	*.31*
	SCL-90-R	SOMS	41.90(17.78)	55.96(21.39)	*-2.34*	*.02**
		DEP	30.04(18.71)	47.33(23.60)	*-2.67*	*.01**
		ANX	40.41(21.06)	62.72(23.78)	*-3.22*	*.002**
		GSI	41.22(21.78)	58.22(25.16)	*-2.38*	*.02**
Trimester 3	PDQ		11.25(4.36)	14.75(3.78)	*-2.67*	*.01**
	SCL-90-R	SOMS	64.09(29.68)	74.42(27.29)	*-2.01*	*.051*
		DEP	44.24(27.15)	59.60(22.79)	*-1.90*	*.06*
		ANX	55.50(26.58)	66.43(25.89)	*-1.32*	*.19*
		GSI	55.63(29.83)	68.44(27.21)	*-1.41*	*.16*

Note.* Significant at the $p \leq .02$ level

[a]PDQ = Prenatal Distress Questionnaire; SCL-90-R = Symptom CheckList 90 Revised; SOMS = Somatisation; DEP = Depression; ANX = Anxiety; GSI = Global Severity Index.

Regarding psychopathological symptoms, the group with postpartum depression symptoms scored higher in every single SCL-90-R subscales during the first, second, and third trimester of pregnancy. This group had clinical scoring (score above 70) in the Somatization, Phobic anxiety, and Psychoticism subscales during the first trimester; Phobic anxiety sub-scale during the second and third trimester; Somatization, Obsessive-compulsive, Paranoid ideation and Psychoticism subscales at the third trimester (see Figure 2).

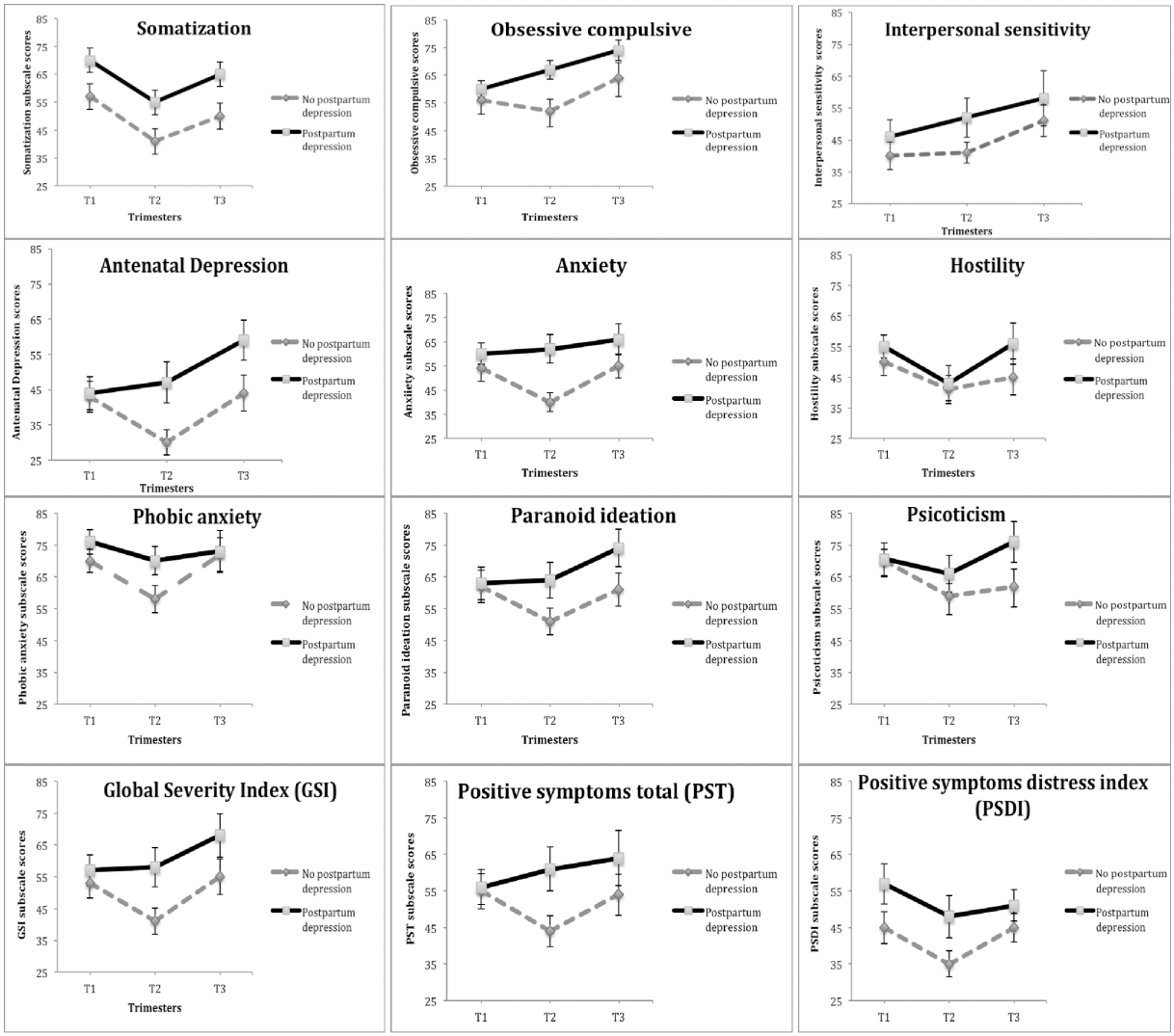


Figure 2. SCL-90-R scores throughout pregnancy in both groups.

Note. SOMS = Somatization; OBS = Obsessive-compulsive; SEN = Interpersonal sensitivity; DEP = Depression; ANX = Anxiety; HOS = Hostility; PHOB = Phobic anxiety; PAR = Paranoid ideation; PSI = Psychoticism; GSI = Global severity index; PST = Positive symptoms total; PSDI = Positive symptoms distress index; PPD = Postpartum depression; NO PPD = No postpartum depression.

The number of participants with clinical scores (above 70) on the Depression subscale of the SCL-90-R for both groups in the first, second and third trimester are displayed in Table 1.

Regarding the psychopathological symptoms subscales, an interaction effect between groups on the Somatization subscale, $F(1, 42) = 6.95$, $p < .05$, which remained significant when controlling for previous miscarriages, sex of the fetus and antenatal depression in analysis, $F(1, 37) = 8.54$, $p < .05$. Several repeated measures ANOVA revealed interaction of group*trimester on the Depression subscale, $F(1, 42) = 3.14$, $p < .05$, which remained partially significant when including previous miscarriages, sex of the fetus and antenatal depression as covariates, $F(1, 37) = 2.99$, $p = .059$; Anxiety subscale, $F(1, 42) = 4.21$, $p < .05$, even when controlling for previous miscarriages, sex of the fetus and antenatal depression in analysis, $F(1, 37) = 8.79$, $p < .05$, and the GSI subscale after including previous miscarriages, sex of the fetus and antenatal depression as covariates, $F(1, 37) = 5.10$, $p < .05$. Scales showing significant interaction effect group*trimester mean differences on psychopathological symptoms are displayed in Table 2. Regarding the postpartum depression symptoms group, the pairwise comparisons in for the main effect of trimesters using *Bonferroni* analysis showed significant differences on the Somatization subscale between trimester 1 and 2 ($p < .05$),

and trimester 2 and 3 (p <. 05); regarding the Depression subscale significant differences between trimester 2 and 3 (p <. 05); in respect to the GSI subscale significant differences between the trimester 2 and 3 (p <. 05). No significant differences were found in the no postpartum depression group throughout pregnancy.

Significant differences between both groups were found during the first trimester regarding the Somatization subscale (t = -2.70, p =.01); during the second trimester regarding the Somatization subscale (t = -2.34, p =.02), the Depression subscale (t = -2.67, p =.01), the Anxiety subscale (t = -3.22, p =.002) and the GSI global index (t = -2.38, p =.02). As shown in Table 2 psychopathological measures are higher within the group with postpartum depression symptoms. No significant differences were found between both groups during the third trimester.

Association between hair cortisol levels with postpartum depression symptoms

We examined the associations between hair cortisol levels during the first, second, and third trimester with postpartum depression symptoms. We found the group with postpartum depression symptoms obtained higher hair cortisol levels during the first, second, and third trimesters. A repeated measures ANOVA revealed a significant interaction between trimester*postpartum depression symptoms group on hair cortisol levels, $F(1, 41) = 7.96$; $p < .001$, which remained significant when including previous miscarriages, antenatal depression, sex of the fetus and dyed hair in the model, $F(1, 36) = 7.78$; $p < .001$.

In the group with postpartum depression symptoms, the pairwise comparisons for the main effect of trimesters using *Bonferroni* analysis showed a significant difference between trimesters 1 and 3 (p < .01), and a significant difference between

trimesters 2 and 3 ($p < .001$). No significant differences were found in this respect in the group with no postpartum depression symptoms.

A 2 sample *t*-test revealed significant differences between groups regarding hair cortisol levels in the first trimester ($t = -4.77$; $p < .001$) and the third trimester ($t = -2.06$; $p \leq .045$). No significant differences were found between groups in the second trimester (see Figure 3).

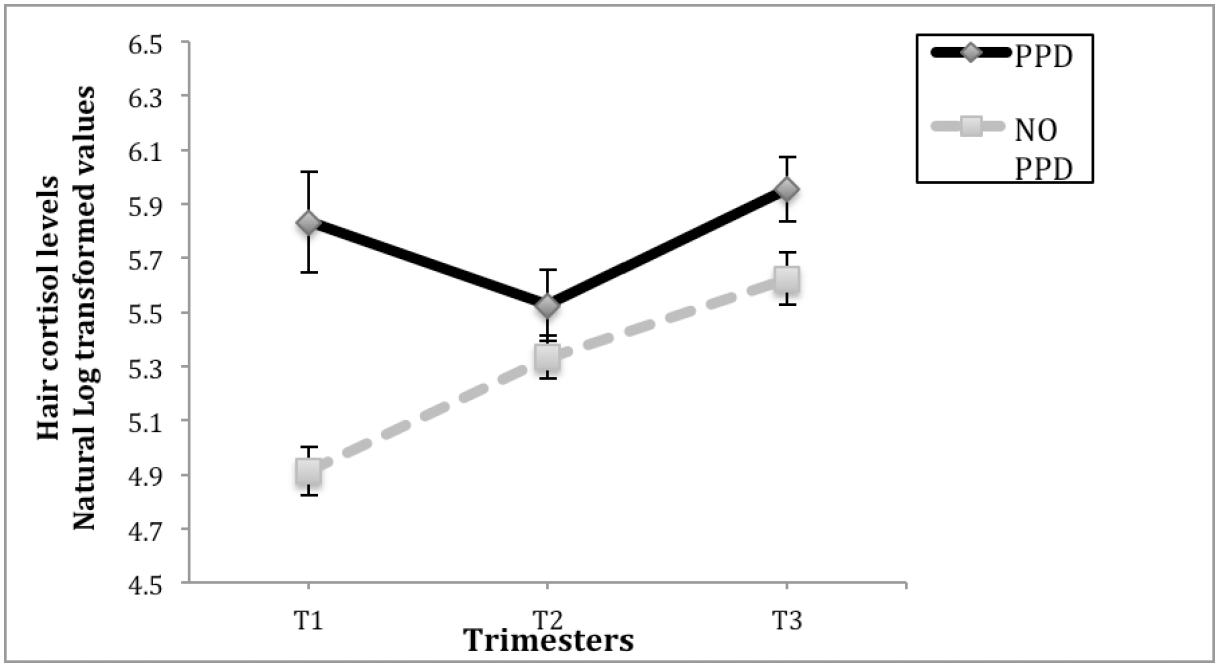


Figure 3. Hair cortisol levels differences (pg/mg) in each trimester between women with and without postpartum depression symptoms.

Note. *Significant at the $p \leq .05$ level.

A linear regression was carried out to test whether the mothers' hair cortisol levels could predict postpartum depression symptoms. Results of the regression revealed that hair cortisol levels could predict 21.7% of the variance of postpartum depression symptoms [$R^2 = .21$, ($F = 3.703$, $p < .05$)]. More precisely, hair cortisol at the

first trimester ($\beta = 0.32$, $p < .05$) and the third trimester ($\beta = 0.32$, $p < .05$) significantly predicted the Edinburgh Postpartum Depression Scale scores.

A 2-sample *t*-tests was used to assess whether the sex of the fetus could influence the release of cortisol during pregnancy. The independent variable was the sex of the fetus and the dependent variable were hair cortisol levels during the first, second and third trimester. No significant differences were found ($p > .05$) (see Table 3).

Table 3. Maternal hair cortisol levels and sex of the fetus.

Trimesters	**Female fetus *X*(*SD*)**	**Male fetus *X*(*SD*)**	***t***	***p***
1st trimester	5.49(.94)	5.08(.56)	-1.81	.07
2nd trimester	5.30(.55)	5.46(.40)	1.11	.27
3rd trimester	5.67(.54)	5.38(.52)	.69	.49

Note. Hair cortisol levels are log transformed values

As shown in Figure 3, hair cortisol levels increased from the first to the third trimester in the group with no postpartum depression symptoms, getting the higher hair cortisol levels at the third trimester. Nonetheless, in the group with postpartum depression symptoms, hair cortisol levels decreased from the first to the second trimester, and increased in the third trimester.

Discussion

The aim of this study was to study the sociodemographic, obstetric, psychological, and hormonal variables that may predict postpartum depression symptoms. For this purpose, we compared a group of pregnant women with postpartum

depression symptoms with a group of women with no postpartum depression. Sociodemographic variables have been informed to be relevant when assessing the psychological wellbeing of women in the postpartum period (Clout & Brown, 2015; Perez-Ramirez, Garcia-Garcia, Caparros-Gonzalez, & Peralta-Ramirez, 2016; Perez-Ramirez, Garcia-Garcia, 2013). Significant differences were found between groups in respect to previous miscarriages and the sex of the fetus. Therefore, these variables were included as covariates in further analysis. The group with postpartum depression symptoms had higher pregnancy specific stress, perceived stress, psychopathological symptoms and hair cortisol levels during the three trimesters of pregnancy.

However, significant differences were found in this study in respect to psychopathological symptoms during the first and second trimester, and in respect to pregnancy-specific stress during the third trimester between both groups. More precisely, during the first trimester, significant differences were found in respect to the Somatization SCL-90-R subscale. During the second trimester, significant differences were found in respect to Somatization, Obsessive-compulsive, Depression, Anxiety, IGS, and Positive Symptom Total subscales. Accordingly, it has been reported high correlations between the Anxiety and Somatization SCL-90-R subscales during the first and second trimester and the Edinburgh Postnatal Depression Scale (Bergink et al., 2011). In this respect, recent studies reported the presence of psychopathological symptoms through the SCL-90-R during pregnancy to be related with postpartum depression (Becker, Weinberger, Chandy, & Schmukle, 2016; Senturk, Yõldõz, Yõldõz, Yorguner, & Cakmak, 2016). More specifically, depression, anxiety, and stress during pregnancy have been related with negative pregnancy outcomes, including postpartum depression (Alderdice, Lynn, & Lobel, 2012; Peñacoba-Puente, Marín-Morales, Carmona-Monge, & Velasco-Furlong, 2016). Psychological stress during pregnancy has

been related to postpartum depression (Vliegen, Casalin, & Luyten, 2014). We could not find any significant differences between groups regarding perceived stress at any time point. These findings do not agree with those reported by Scheyer and Urizar (Scheyer & Urizar, 2016) who reported significant association between perceived stress during the 3 trimesters of gestation and postpartum depression. We hypothesize this could be due to the PSS being a general stress measure and that pregnancy-specific measures may likely improve pregnant women's psychological assessments. In this regard, pregnancy-specific stress was significantly different between groups during the second trimester. The fact that although both stress measures (PDQ and PSS) have been widely used when assessing stress levels during pregnancy, is noteworthy that the PDQ offers the opportunity to assess specific worries and concerns related to pregnancy and therefore is a more consistent pregnancy-specific stress measure and a better predictor of negative pregnancy outcomes (Alderdice, Lynn, & Lobel, 2012; Lobel & Dunkel-Schetter, 2016).

Biochemical measures as those provided through hair cortisol levels inform of chronic stress levels (Meyer et al., 2014). According to our results, hair cortisol levels in the group with postpartum depression symptoms descended from the first to the second trimester and ascended from the the second to the third trimester, resembling an U shape when plotted on a graph. This is the first study to report hair cortisol levels throughout pregancy in a group of women with postpartum depression sypmtoms. Although an upward course regarding cortisol levels throughout pregnancy has been previously reported (D'Anna-Hernandez, Ross, Natvig, & Laudenslager, 2011) these findings were reported in respect to pregnant women with no postpartum depression symptoms.

In the present study, hair cortisol levels were higher in the group with postpartum depression symptoms compared to the group with no postpartum depression

symptoms in the 3 trimesters and were significantly different in the first and the third trimester. A previous study reported that cortisol levels during the second trimester, but not in the third trimester, were higher and significantly different in those women with postpartum depression (Diego, Field, Hernandez-Reif, Cullen, Schanberg, & Kuhn, 2004). Our findings in this regard support the fact that high stress levels during pregnancy are related to postpartum depressive symptoms (Fan, Zou, Ma, Yue, Mao, & Ma, 2009; Nierop, Bratsikas, Zimmermann, & Ehlert, 2006). In fact, hair cortisol levels predicted postpartum depression symptoms in our study. More precisely, hair cortisol at the first trimester and the third trimester could predict postpartum depression symptoms. Our results do not support other studies reporting low cortisol levels during pregnancy with postpartum depression (Scheyer & Urizar, 2016; Seth et al., 2016)We hypothesize these differences may be due to the fact that previous studies have used acute stress biological measures (e.g. urine cortisol levels), and in our study hair cortisol samples were used which reflects levels of chronic stress within the last 3 months (Mastorakos & Illias, 2003; ; Wikenius et al., 2016; Wosu et al., 2013). Our study has several strenghts. First, the longitudinal design of our study offered an unique possibility of observing the range of psychological symptoms, including prenatal stress and cortisol levels throughout pregnancy in both groups. Second, we have used the PDQ, a pregnancy-specific stress measure which shows a consistent relation with negative pregnancy outcomes (Alderdice et al., 2012). More importanltly, the innovative assessment of stress through hair cortisol levels gives evidence of chronic stress through a single measurement (Mastorakos & Illias, 2003). Several studies have shown the benefits of knowing the risk factors involved in postpartum depression to improve maternal and infant outcomes (O'Hara & McCabe, 2013; Yim et al., 2015). Finnally, we considered the influence of a variety of sociodemographic and obstetric variables that

have been previously associated with pospartum depression (Leigh & Milgrom, 2008; Milgrom et al., 2008; Robertson et al., 2004; Saligheh et al., 2014). It is important to note that this study focused on assessing the association between sociodemographic and obstetric variables, psychological stress, psychopathological symptoms, and biological stress measured through hair cortisol levels, with postpartum depression symptoms. The percentage of women in the postpartum depression group (36%) in our study is quite high with respect to previous studies reporting percentages of 10-15% (Yim et al., 2015). In our context, it exits a lack of clinical screening and prevention related to postpartum depression, which might lead to greater numbers of women with postpartum depression symptoms. Nevertheless, the prevalence of postpartum depression can vary between studies, due to the different criteria used to define postpartum depression (Iliadis et al., 2015).

Although participants were longitudinally and prospectively assessed throughout pregnancy and the postpartum period, a limitation of the present study is the relatively small sample size, which should be considered for the interpretation of the data. A further potential limitation was that postpartum depression symptoms were only assessed at a single time point. A second follow-up after delivery would have offered the possibility to evaluate the participants' long-term psychological wellbeing and study possible associations with health variables during pregnancy.

In summary, according to our findings, high levels of maternal stress during pregnancy are associated with postpartum depression symptoms. Psychopathological symptoms in the first and second trimester, high pregnancy-specific stress in the second trimester, and high hair cortisol levels in the first and the third trimester were associated with postpartum depression symptoms. Since hair cortisol levels reflect stress levels during the previous 3 months preceding the time the sample was taken (Wosu et al., 2013), our

findings reflect that the preconception period, and the second trimester of pregnancy, are particularly sensitive periods related to postpartum depression symptoms. Our findings do not agree with a previous study reflecting changes in the HPA related with postpartum depression occur during the postpartum period (Duthie & Reynolds; 2013) instead, we found changes in the HPA may begin during the preconception period. In line with our findings, a recent study reported higher cortisol levels during pregnancy were related to postpartum depression symptoms (Illiadis et al., 2015). In this respect, prenatal effective stress screening interventions should be used widely during this time to reduce adverse outcomes (Schetter & Tanner, 2012). These findings are of clinical and research importance since they show the health variables that can predict postpartum depression symptoms among pregnant women. Assessing psychological health in the perinatal period can aid practitioners in making adequate decisions and provide valuable data on maternity care (Alderdice et al., 2013).

CAPÍTULO X: "ESTUDIO 5"

Hair cortisol in pregnant women who conceived using assisted reproductive technology affects their neonates´ development

Caparros-Gonzalez, Romero-Gonzalez, Mariñas-Lirola, Gonzalez-Perez y Peralta-Ramirez (Under review). Hair cortisol in pregnant women who conceived using assisted reproductive technology affects their neonates´ development. *Journal of Reproductive and Infant Psychology*.

"Hair cortisol in pregnant women who conceived using assisted reproductive technology affects their neonates´ development"

Introduction

Fertility problems affect 15% of women of reproductive age (Kupka et al., 2016). Consequently, recent years have witnessed an increase in the use of assisted reproductive technology (ART) (Cabrera-León, Lopez-Villaverde, Rueda, & Moya-Garrido, 2015; National Institute for Health & Care Excellence, NICE, 2014).

There is no consensus on the effect of emotional variables in women receiving ART. Some authors have reported that these women experience higher levels of psychological stress and anxiety (Bailey, Ellis-Caird, & Croft, 2017; Patel, Sharma, Narayan, Binu, Dinesh, & Pai, 2016), implying a lower probability of pregnancy (Matthiesen, Frederiksen, Ingerslev, & Zachariae, 2011). Other studies have found the same levels of psychological stress in women who conceived naturally or using ART (Shih et al., 2015; Stevenson, Trotter, Bergh, & Sloane, 2016). This is supported by a recent systematic review of stress levels following ART, which concluded that there are inconsistencies in the literature (Gourounti, 2015).

High levels of psychological stress during pregnancy have been associated with adverse outcomes such as low birth weight, pre-term birth and poor neonatal neurodevelopment (Lobel & Dunkel-Schetter, 2016). With respect to pregnancy-specific stress (worries and concerns of pregnant women regarding neonatal health, interpersonal relationships, physical health and childbirth) (Alderdice & Lynn, 2011; Alderdice, Lynn, & Lobel, 2012; Alderdice et al., 2013), some studies have reported that pregnant women who received ART presented higher stress levels in the first

(Hjelmstedt, Widström, Wramsby, & Collins, 2003), second (Gourounti, Anagnostopoulos, & Lykeridou, 2013) and third trimester of gestation (McMahon et al., 2013). However, other studies have found no such differences (Darwiche et al., 2014; Gameiro, Moura, Ramos, Canavarro, & Soares, 2010; Poikkeus et al., 2006).

Stress during pregnancy can be assessed from psychological questionnaires but also from cortisol levels, which yield biological data about stress levels (D'Anna-Hernandez, Ross, Natvig, & Laudenslager, 2011). Saliva, blood, urine and amniotic fluid are commonly used to measure cortisol levels during pregnancy (Massey, Campbell, Raine-Fenning, Aujla, & Vedhara, 2014). Such measurements provide information about cortisol at a specific time-point, are invasive and affected by situational variables or circadian rhythms (Wosu, Valdimarsdóttir, Shields, Williams, & Williams, 2013). In contrast, hair cortisol determination has been established as an innovative technique to measure activation of the hypothalamic-pituitary-adrenal (HPA) axis (Wosu, Valdimarsdóttir, Shields, & Williams, 2013). Furthermore, hair cortisol levels during pregnancy provide a retrospective measure of cumulative exposure to cortisol, can be obtained using a non-invasive procedure and are unaffected by fluctuations in circadian rhythms (Caparros-Gonzalez, et al., 2017a). Although several studies have assessed hair cortisol levels in pregnant women, only one has explored the relationship between hair cortisol and ART, finding that unlike cortisol levels in saliva, hair cortisol levels predict the success of pregnancy following ART (Massey et al., 2016).

In addition, neonatal hair cortisol levels reflect HPA axis activity during the third trimester of pregnancy (Hoffman, D'Anna-Hernandez Benitez, Ross, & Laudenslager, 2017). High cortisol levels during pregnancy have been associated with shorter gestation, lower birth weight (Baibazarova et al., 2013) and shorter neonatal

length (Bolten et al., 2011). Neonates conceived through ART are at higher risk of preterm birth and low birth weight (Henningsen et al., 2011; McDonald et al., 2009). No studies have analysed the relationship between maternal stress and hair cortisol levels during pregnancy on the one hand, and birth variables in women who received ART.

The main objective of this study was to compare stress levels throughout pregnancy in women who had conceived using ART and women who had conceived naturally, measuring stress by means of hair cortisol levels and psychological questionnaires.

We also studied the possible relationship between maternal and neonatal cortisol levels and somatometric values for newborn infants conceived naturally or through ART.

Methods

Participants

This study is part of a longitudinal study of perinatal stress in Spain named GESTASTRESS (Caparros-Gonzalez, Marinas-Lirola, Peralta-Ramirez, *in press*).

A total of 108 pregnant women from a health centre in the south of Spain showed interest in this study. Of these, 17 women decided not to participate because of lack of time and 18 were excluded from the analysis because they left the study before childbirth. It was not possible to take hair samples from 8 newborn infants because they did not have sufficient hair. The final sample consisted of 60 pregnant women and their 60 newborn infants. The mothers were assessed longitudinally (April 2016-May 2017) in the first (M = 10.97 weeks' gestation; SD = 3.57), second (M = 23.69 weeks'

gestation; *SD* = 3.37) and third trimester of pregnancy (*M* = 34.34 weeks' gestation; *SD* = 2.61); following delivery, we assessed mothers and their babies (*M* = 15.79 days after birth; *SD* = 9.78). Participants were divided into two groups: women who had conceived using ART (n = 13) and women who had conceived naturally (n = 47). The inclusion criteria were: pregnant women presenting a low risk pregnancy, being monitored in the public health system, over 18 years old and fluent in Spanish. We excluded pregnant women with a prior pathology. Impaired fertility was the only health condition present in the group of women who had conceived using ART. Of these, 10 women had conceived following in vitro fertilisation and 3 following artificial insemination.

All participants read and signed an informed consent form in accordance with the recommendations of the Helsinki Declaration (World Medical Association, 1964). The study was approved by the Ethics Committee for Human Research at the University of Granada (reference 881).

Hair cortisol

Biological analysis was performed using the enzyme-linked immunosorbent assay (Salivary ELISA Cortisol kit©, Alpco Diagnostics®, Windham, NH) to determine maternal and neonatal hair cortisol levels. The salivary ELISA cortisol kit is a validated method to assess hair cortisol levels and is highly positive correlated with liquid chromatograph-mass spectrometry (LC-MS/MS) (Russel et al., 2015). The sensitivity of the cortisol ELISA kit is 1.0 ng/ml as reported by the manufacturer and the cross reactivity is: Prednisolone 13.6%, Corticosterone 7.6%, Deoxycosticosterone 7.2%, Progesterone 7.2%, Cortisone 6.2%, Deoxycortisol 5.6%, Pednisone 5.6% and Dexamethasone 1.6%. No cross-reaction was detected with DHEAS and

Tetrahydrocortisone. The intra- and inter-assay variations were analyzed on internal quality control. The intra-assay coefficients of variance (CV) were 2.7% at 10.7 ng/ml and 4.3% at 43.9 ng/ml. The inter-assay CVs were 4.4% and 6.3%, respectively.

For each assessment, we took approximately 150 strands of hair and selected segments measuring three centimetres long, given that hair grows at the rate of one centimetre per month (Massey et al., 2016). The samples were taken from the crown of the head and cut close to the scalp in line with recommendations for hair sample collection (Sauvé, Koren, Walsh, Tokmakejian & Van Uum, 2007). These were then wrapped in aluminium foil and kept at room temperature until subsequent laboratory analysis. The samples were washed twice with isopropanol and left to dry for 48-72 hours. Next, they were weighed and ground in a ball mill (BulletBlender Storm, Swedesboro, NJ, USA) to break down the protein matrix. Cortisol was extracted by incubation in HPLC grade methanol for 72 hours at room temperature in darkness. The samples were centrifuged and the supernatant was evaporated using a vacuum evaporator (Centrivac, Heraeus, Hanau, Germany). The resulting extract was resuspended in 150 μL of phosphate buffered saline (PBS) at pH 8.0 and the reconstituted sample was kept at -20°C until subsequent ELISA analysis (Chen et al., 2013; Meyer, Novak, Hamel, & Rosenberg, 2014; Sauvé et al., 2007).

Instruments

We used the Prenatal Distress Questionnaire (PDQ; Yali & Lobel, 1999; Spanish version by Caparros-Gonzalez et al., 2015). It is a 12-item instrument scored on a 5-point Likert scale from 0 (none at all) to 4 (extremely) to evaluate specific worries pregnant women have regarding medical problems, physical symptoms, body

changes, labor, childbirth, interpersonal relationships, and the baby's health. The Cronbach's alpha reliability coefficient of the Spanish version is α = .71.

Besides, we used the Perceived Stress Scale (PSS; Cohen, Kamarck, & Mermelstein, 1983; Spanish version by Carrobles & Remor, 2001) to assess the general perception of stress during the last month. It consists of 14 self-report items scored on a five-point Likert scale from 0 (never) to 4 (very often). The Cronbach's alpha reliability coefficient of the Spanish version is 0.81.

Symptoms of psychopathology were assessed by means of the SCL-90-R (Derogatis, 1977; Spanish version by Caparros-Caparros, Villar-Hoz, Juan-Ferrer, & Viñas-Poch, 2007). It consists of 90 items rated on a five-point Likert scale of distress. The Cronbach's alpha reliability coefficient of the Spanish version is 0.80.

Sociodemographic, obstetric data, and somatometric values for newborn infants (birthweight, height and head circumference) were collected from the Pregnacy Health Document (Andalusian Ministry of Health, 2010), which is the official health document of pregnant women in Andalusia.

Procedure

Subjects were invited to participate in the study when attending their first antenatal appointment with a midwife, in the first trimester.

Data were collected on four occasions: during antenatal appointments in the first, second and third trimesters of pregnancy and during the first postnatal appointment with the midwife. During each antenatal appointment, the community midwife took a hair sample from participants and distributed the questionnaires (PDQ, PSS and SCL-90-R), which subjects completed at home and submitted at their next appointment.

During the postnatal appointment, the midwife took maternal and neonatal hair samples, and administered the PSS and SCL-90-R to mothers.

Data analysis

To determine whether the main sociodemographic, cortisol and obstetric variables were equally distributed in both groups, we conducted various Student's *t*-test and *chi*-squared test analyses.

To assess whether the two groups presented significant differences for psychological and cortisol variables, we performed various 2×4 mixed ANOVAs, where the first independent between-subjects factor had two levels (ART conception versus natural conception). The second within-subject repeated measures factor had four levels (first, second and third trimester and postpartum). The Greenhouse-Greisser correction was applied to the repeated measures factor. This analysis was repeated including as covariates the variables that were not equally distributed in the two groups. For variables showing a group*trimester interaction effect, we performed a difference in means test using the Student's *t*-test statistic.

Given that the scores for hair cortisol did not present a normal distribution, we conducted a natural log transformation (natural log; *LN* base e).

To determine the relationship between maternal hair cortisol levels in both groups and somatometric variables in newborn infants, we calculated Pearson's *r*. All statistical analyses were performed using the Statistical Package for Social Sciences version 20.0 for Mac (SPSS, Armonk, New York).

Results

Sample description

The sample consisted of 60 pregnant women aged between 23 and 44 years old (M = 32.62; SD = 4.49) who were divided into two groups, women who had conceived using ART (n = 13) and women who had conceived naturally (n = 47), and their 60 newborn infants (23 boys and 37 girls). Both groups presented similar values for the majority of sociodemographic variables, hair characteristics and obstetric history (see Table 1). However, significant differences were found between the two groups in maternal age, number of children, number of abortions and weight of newborn infants.

Table 1. Differences in sociodemographic and obstetric variables between pregnant women through assisted reproductive treatment and women with natural pregnancies.

		ART ***n* = 13** ***X*(*DT*)/%**	**Natural** ***n* = 47** ***X*(*DT*)/%**	**Contrast test***	***p*** **
Sociodemographic Variables					
Age		36 (4.12)	31.68 (4.16)	3.32	**0.02**
Nacionality	Spanish	12(92.30%)	39(83%)	0.69	0.40
	Inmigrant	1(7.70%)	8(17%)		
Marital status	Single/divorced/widow	0(0%)	0(0%)		
	Married/cohabitant	13(100%)	47(100%)		
Job situation	Working	11(84.60%)	40(85.10%)	0.002	0.96
	Unemployed	2(15.40%)	7(14.90%)		

Education level	Primary school	0(0%)	0(0%)	0.29	0.59
	High school	4(30.80%)	11(23.40%)		
	University	9(69.20%)	36(76.60%)		
	None	0(0%)	0(0%)		
Cigarette	Yes	1(7.70%)	3(6.40%)	0.03	0.87
	No	12(92.30%)	44(93.60%)		
Alcohol	Yes	0(0%)	1(2.10%)	0.28	0.60
	No	13(100%)	46(97.90%)		
Hair aspect	Nature	3(23.10%)	23(48.90%)	2.77	0.10
	Dyed	10(76.90%)	24(51.10%)		
Obstetric variables					
Primiparous	Yes	10(76.90%)	23(28.90%)	3.22	0.07
	No	3(23.10%)	24(51.10%)		
Living children	None	12(92.30%)	24(51.10%)	7.25	**0.03**
	One	1(7.70%)	20(42.60%)		
	Two or more	0(0%)	3(6.40%)		
Previous miscarriages	None	8(61.50%)	37(78.70%)	7.18	**0.03**
	One	2(15.40%)	9(19.10%)		
	Two or more	3(23.10%)	1(2.10%)		
Wanted pregnancy	Yes	13(100%)	46(97.90%)	.28	.6
	No	0(0%)	1(2.10%)		

Sex of the newborn	Male	8(61.50%)	15(31.9%)	3.96	0.13
	Female	5(38.50%)	32(68.1%)		
Height of the newborn (cm)		50.77 (1.29)	50.45 (1.62)	0.65	0.51
Weight of the newborn (g)		3508.05 (433.47)	3288.78 (300.67)	2.11	**0.04**
Head circumference of the newborn (cm)		36.21(1.97)	35.04(2.36)	0.89	0.37

*Contrast test were *Student´s t* (for quantitative variables) and *Chi-Square* χ^2 (for categorical variables).

**$p \leq ,05$ was considered significant (in bold).

Maternal and neonatal hair cortisol levels according to ART or natural conception

To determine differences in maternal cortisol levels throughout pregnancy according to ART or natural conception, we conducted a mixed ANOVA, which showed an interaction effect between group*trimester in hair cortisol levels $F(1.154) = 3.86$; $p < 0.05$. This interaction was maintained when age, previous abortions, previous children and weight of the newborn infant were included in the model as covariates $F(1.143) = 2.73$; $p < 0.05$ (see Figure 1).

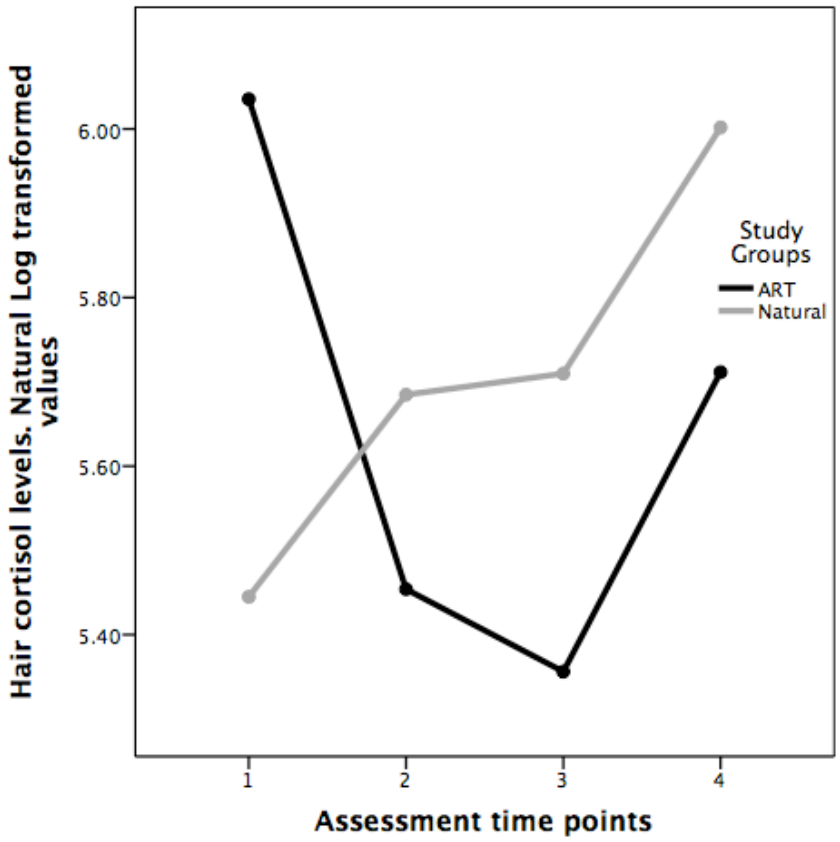


Figure 1. Hair cortisol levels during pregnancy and the postpartum in both groups. Note: 1 = 1st trimester; 2 = 2nd trimester; 3 = 3rd trimester; 4 = postpartum; ART = Assisted Reproductive Treatment group; Natural = Natural pregnancy group

A between-subjects analysis revealed a statistically significant difference between the two groups of women in the first trimester (t = -3.09; $p < 0.05$), whereby hair cortisol levels were higher in women who had used ART (M = 519.91 pg/mg) than in women who had conceived naturally (M = 281.52 pg/mg) (see Table 2). No differences were found between groups in the second and third trimesters.

With regard to the infants, we found a statistically significant difference in hair cortisol levels between the two groups (t = -2.03; $p < 0.05$) (see Table 2), whereby infants born to women who had used ART presented lower hair cortisol levels (M =

2071.95 pg/mg) than those born to women who had conceived naturally (M = 3421.12 pg/mg).

Table 2. Maternal and infant´s hair cortisol levels, pregnancy specific-stress and perceived stress depending on the method of pregnancy (ART or natural)

Pregnancy trimesters	Assessment	ART M(DT)	Natural M(DT)	*t*	*p*
1_{st} trimester	Maternal cortisol	519.91 (450.34)	281.52 (153.06)	-3.09	**0.01**
	PDQ	15.49 (3.11)	15.27 (4.69)	-0.15	0.87
	PSS	26,17 (0,98)	26,62 (1,16)	1.26	0.21
2_{nd} trimester	Maternal cortisol	276.31 (133.35)	381.52 (445.35)	0.83	0.41
	PDQ	13.03 (0.75)	12.97 (3.57)	-0.06	0.95
	PSS	27.02 (0.91)	26.55 (1.11)	-1.38	0.17
3_{rd} trimester	Maternal cortisol	297.31 (219.55)	372.11 (262.34)	0.94	0.35
	PDQ	12.90 (3.17)	12.87 (4.29)	-0.03	0.97
	PSS	27.87 (1.39)	26.69 (0.98)	-3.47	**0.01**
Pospartum	Maternal cortisol	385.18 (261.83)	850.01 (1634.89)	1.01	0.31

Newborn cortisol	2071.95 (1583.86)	3421.12 (3397.98)	2.03	**0.04**
PSS	27.78 (1.56)	26.49 (1.15)	-3.29	**0.01**

*p<.05 was considered significant (in bold).

Psychological stress and psychopathological symptoms in mothers according to ART or natural conception

A repeated measures ANOVA revealed an interaction effect between group*trimester in relation to perceived stress $F(1.155) = 6.29$; $p < 0.05$, which remained significant when age, previous abortions and previous children were included in the model as covariates $F(1.149) = 3.26$; $p < 0.05$ (see Figure 2). The results of a between-subjects analysis showed a statistically significant difference in the third trimester ($t = -3.47$; $p < 0.05$), whereby women who had used ART reported higher levels of perceived stress (M = 27.87; SD = 1.38) than those who had conceived naturally (M = 26.69; SD = 0.98). This difference was maintained in the postpartum period ($t = -3.29$; $p < 0.05$), whereby the women who had used ART again reported higher levels of perceived stress (M = 27.78; SD = 1.56) than those who had conceived naturally (M = 26.49; SD = 1.15), (see Table 2).

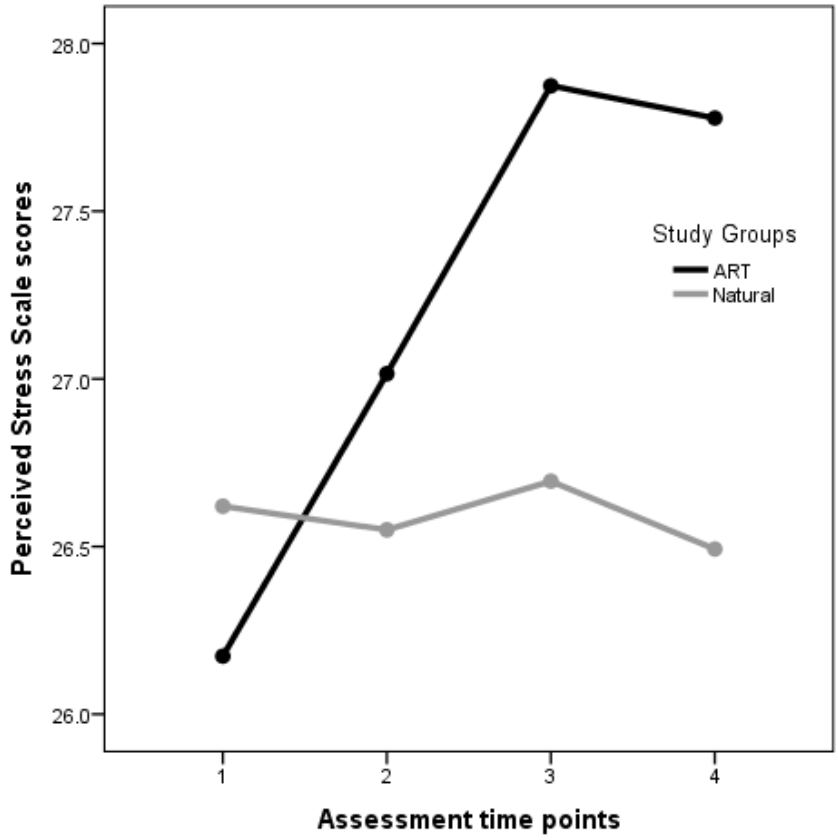


Figure 2. Perceived Stress Scale Scores during pregnancy and the postpartum in both groups. Note: 1 = 1st trimester; 2 = 2nd trimester; 3 = 3rd trimester; 4 = postpartum; ART = Assisted Reproductive Treatment group; Natural = Natural pregnancy group

No interaction was found between the two groups for the SCL-90-R psychopathological symptom subscales or the PDQ scale.

Maternal and neonatal cortisol levels and somatometric values for newborn infants

We performed various correlations to determine whether there was a relationship between maternal and neonatal cortisol levels and somatometric values for newborn infants, finding an inverse relationship between maternal cortisol levels in the second trimester and the length of the infant in the group of women who had conceived naturally ($r = -0.42$; $p < 0.05$). In addition, the results showed a significant inverse

correlation between neonatal hair cortisol levels and the length of infants born both to women who had used ART (r = -0.65, $p < 0.05$) and those who had conceived naturally (r = -0.42; $p < 0.05$) (see Tables 3 and 4). No significant correlations were found with the other somatometric variables.

Table 3. Pearson´s correlations between hair cortisol levels and anthropometric variables in the natural pregnancy group natural.

	1	2	3	4	5	6	7	8
1	1							
2	.21	1						
3	.23	**.75***	1					
4	-.01	-.01	.08	1				
5	-.15	-.07	-.09	-.03	1			
6	.09	-.23	-.06	-.14	-.02	1		
7	.13	**-.42***	-.26	-.08	-.01	**.46***	1	
8	.08	-.06	-.11	-.09	-.06	.28	.19	1

Note: 1 = Maternal hair cortisol levels at the 1_{st} trimester; 2 = Maternal hair cortisol levels at the 2_{nd} trimester; 3 = Maternal hair cortisol levels at the 3_{rd} trimester; 4 = Maternal hair cortisol levels at the postpartum; 5 = Newborn hair cortisol levels; 6 = Infant´s birthweight; 7 = Height of the newborn; 8 = Head circumference of the newborn.

*p<.05 was considered significant (in bold).

Table 4. Pearson´s correlations between hair cortisol levels and anthropometric variables in the assisted reproductive treatment group.

	1	2	3	4	5	6	7	8
1	1							
2	.15	1						
3	**.-56***	.09	1					
4	-.31	.15	**.69***	1				
5	-.23	-.12	-.39	-.08	1			
6	.03	.03	.03	-.01	-.43	1		
7	.19	.14	.17	-.17	**-.65***	**.92***	1	
8	.41	.02	.25	-.24	-.39	.22	.28	1

Note: 1 = Maternal hair cortisol levels at the 1_{st} trimester; 2 = Maternal hair cortisol levels at the 2_{nd} trimester; 3 = Maternal hair cortisol levels at the 3_{rd} trimester; 4 = Maternal hair cortisol levels at the postpartum; 5 = Newborn hair cortisol levels; 6 = Infant´s birthweight; 7 = Height of the newborn; 8 = Head circumference of the newborn.

*p<.05 was considered significant (in bold).

Discussion

The aim of this study was to determine whether one group of women who had conceived via ART and another which had conceived naturally presented differences in stress levels throughout pregnancy. Stress was assessed by means of psychological questionnaires and cortisol in hair samples. We also investigated the relationship between maternal and neonatal cortisol levels, and somatometric values for newborn infants in both groups.

This study revealed significant differences in cortisol levels between the two groups. In the first trimester, women who had used ART showed higher hair cortisol levels than those who had conceived naturally; however, this difference was not maintained in the second and third trimesters. In addition, we found that women who had used ART reported higher levels of perceived stress in the third trimester and postpartum. Our results also showed that hair cortisol levels in infants born to women who had used ART were lower than in those born to women who had conceived naturally.

The group of women who had used ART presented higher hair cortisol levels in the first trimester. Given that these cortisol levels also reflect stress levels prior to pregnancy, our results support previous studies reporting high levels of stress in women undergoing ART treatment (D'Anna Hernandez et al., 2011). One reason for these higher levels may be the high number of medical procedures to which these women are subjected, such as ultrasound, gynaecological check-ups and hormonal treatments (Patel et al., 2016). Related to this finding, we also observed that infants born to women who had used ART showed lower hair cortisol levels at birth. These results are consistent with studies on animal models such as rhesus monkeys, in which —as with our group of

women who had used ART— high maternal cortisol levels in the first trimester were associated with low hair cortisol levels in newborn infants (Kapoor et al., 2016). High levels of cortisol crossing the placenta in the early stages of foetal development may disrupt correct cortisol production in the foetus (Glover, Bergman, Sarkar, & O'Connor, 2009).

Although cross-sectional studies have reported higher levels of pregnancy-specific stress in women who have used ART (Gourounti et al., 2013; McMahon et al., 2013), our findings support the results obtained previously using a longitudinal design (Gameiro et al., 2010), namely that there are no differences in this respect between women who use ART and those who conceive naturally. One advantage of longitudinal over cross-sectional studies is that they provide the possibility of assessing changes in stress levels during pregnancy and optimise identification of stress as part of the transition to motherhood (Bassi et al., 2017).

In terms of perceived stress, women who had used ART reported higher general stress levels in the third trimester and postpartum than women who had conceived naturally. These results do not coincide with those reported in a recent systematic review, in which no differences in general stress levels were found between women who had used ART and those who had conceived naturally. However, it should be noted that this review was based on cross-sectional studies (Gourounti, 2015).

Besides women who have used ART, other groups of pregnant women (e.g. immigrants or those who develop postpartum depression) are exposed to high levels of stress (Lara-Cinisomo, Girdler, Grewen, & Meltzer-Brody, 2016; Perez-Ramirez, Garcia-Garcia, & Peralta-Ramirez, 2013; Perez-Ramirez, Garcia-Garcia, Caparros-Gonzalez & Peralta-Ramirez, 2017). In agreement with the study by D'Anna-

Hernandez, Ross, Natvig and Laudenslager (2011), hair cortisol levels in the group of women who had conceived naturally increased as their pregnancy progressed. In contrast, hair cortisol levels in the women who had used ART followed a similar pattern to that of pregnant immigrant women (Schreier et al., 2016) or to the U shape presented by women who subsequently develop symptoms of postpartum depression (Caparros-Gonzalez et al., 2017a).

In addition, hair cortisol levels were also associated with other variables. In particular, we found that maternal cortisol levels were related to neonatal length. Thus, in the group of women had conceived naturally, higher maternal cortisol levels in the second trimester were associated with a shorter neonatal length. In addition, we found that in the ART group, higher neonatal cortisol levels were associated with a shorter length at birth. Although it is difficult to establish the mechanisms involved in these results, our findings are consistent with an association between higher maternal stress levels during pregnancy and poorer neonatal development (Alderdice et al., 2013). Higher cortisol levels during pregnancy have also been associated with low birth weight, pre-term birth and smaller neonatal size (Glover, 2015; Lobel & Dunkel-Shetter, 2016).

Although the longitudinal design of the present study yielded valuable information throughout pregnancy and postpartum, the sample size was relatively small because the initially higher number of participants was subsequently reduced to mothers whose newborn infants had sufficient hair to analyse cortisol levels.

In addition, although questionnaires such as the PDQ were administered to measure pregnancy-specific stress (Alderdice et al., 2013; Yali & Lobel, 1999), our analysis would have been enhanced by the administration of postpartum-specific

questionnaires. Besides enabling long-term follow-up of the infants, these would have allowed us to verify the effects of stress during pregnancy on the subsequent neurodevelopment of infants (Glover, 2015; Sandman, Glynn & Davis, 2016).

This study has important implications for practice. Our results confirm the effects of stress in pregnant women, especially those who have received ART, and it is thus crucial to address this increase in stress and cortisol. An efficient screening system is necessary in order to detect women in need of psychological support and offer them the effective psychological treatment currently available to reduce stress levels during pregnancy (van Willenswaardy et al., 2015; van Willenswaardy et al., 2017) and prevent negative outcomes in neonates (Caparros-Gonzalez et al., 2017b). Having identified the type of pregnancy, midwives and psychology professionals should take steps to ensure that other health professionals and relatives promote the woman's health throughout pregnancy and early postpartum, in order to foster her mental health and the infant's development.

Women who had used ART presented higher hair cortisol levels in the first trimester and higher levels of perceived stress in the third trimester and postpartum than women who had conceived naturally. Infants born to women who had used ART presented lower hair cortisol levels than those in infants born to women who had conceived naturally. In addition, cortisol levels in ART infants were associated with length at birth.

Assessing stress levels during pregnancy and postpartum using psychological questionnaires combined with hair cortisol levels yielded more complete information, enabling us to gain a better understanding of the associated consequences than would

have been possible if only using only one or other of these measures (Tarullo-John & Meyer, 2017).

Women who have received ART treatment are vulnerable to stress, and it is necessary to be aware of this in order to provide psychological support throughout pregnancy and childbirth.

CAPÍTULO XI: "ESTUDIO 6"

Maternal and Neonates´ Hair Cortisol Levels Predict Human Infant Development at 6 Months of Age

Caparros-Gonzalez, R.A., Romero-Gonzalez, B., Gonzalez-Perez, R., Cruz-Quintana, F. y Peralta-Ramírez, M.I. (under review). Maternal and Neonates´ Hair Cortisol Levels Predict Human Infant Development at 6 Months of Age. *Journal of Developmental & Behavioral Pediatrics.*

"Maternal and Neonates´ Hair Cortisol Levels Predict Human Infant Development at 6 Months of Age"

Introduction

The impact of prenatal stress can have long-term adverse consequences for the unborn developing fetus (Herba, Glover, Ramchandani, & Rondon, 2016; Lobel & Dunkel Schetter, 2016). This concept has been named as `fetal programming´, and refers to the fact that exposure to antenatal stress increases the risk to develop behavioural, emotional and cognitive problems in later life (Rakers et al., 2017). Maternal stress during pregnancy affects fetal development during certain sensitive periods that can result in permanent changes and lifelong consequences for infants´ health (La Marca-Ghaemmaghami & Ehlert, 2015).

The underlying mechanism responsible for the transferation of maternal stress to the fetus involves maternal cortisol crossing the placenta to the fetal compartment (Glover, 2015). Stress during pregnancy promotes the hypothalamus synthesizes corticotrophin-releasing hormone (CRH), which increases the release of cortisol, so the organism can cope with the stressful situation (Caparros-Gonzalez et al., 2017). During pregnancy, the placenta, an endocrine organ of fetal origin, alters the hypothalamic-pituitary-adrenal axis. Through an intense increase of placental CRH during gestation, the placenta increases the release of cortisol from the adrenal gland (Duthie & Reynolds, 2013). Besides, the pituitary glands increasing its size and the positive effect cortisol have on increasing the placental CRH production, results on high cortisol levels circulating during pregnancy (Sandman, Glynn, & Davis, 2016).

Cortisol levels has been previously assessed from blood, urine, saliva, or amniotic fluid samples in pregnant women (Bergman, Sarkar, Glover, & O'Connor, 2010; de Weerth, Buitelaar, & Beijers, 2013; Jung et al., 2011). These matrixes inform about acute stress levels, require an invasive and painful technique and are influenced by circadian rhythms and environmental variables (Sandman et al., 2016; Stalder & Kirschbaum, 2012). As an alternative, testing cortisol levels from hair samples is an advanced technique that informs of chronic stress of the last three months, is not affected by situational variables, is not invasive, and is simple to transport and conserve (Mastorakos & Ilias, 2003; Wosu, Valdimarsdóttir, Shields, Williams, & Williams, 2013).

The development of the fetal brain can be altered as a consequence of exposure to high levels of cortisol (Glover, 2017). Elevated levels of amniotic cortisol surrounding the fetus in the womb were associated with low infant cognitive development (Glover, 2015). Maternal stress during pregnancy is a key determinant of delay in cognitive and motor development among infants (Huizink, Robles de Medina, Mulder, Visser, & Buitelaar, 2003). Nevertheless, not all studies have reported such relations (DiPietro, Novak, Costigan, Atella, & Reusing, 2006). In this respect, it has been reported an association between high cortisol levels during pregnancy with high motor and cognitive infants´ development (Buitelaar, Huizink, Mulder, de Medina, & Visser, 2003). Other studies have stated that low levels of cortisol may predict an accelerated cognitive development during the postpartum and the first 12 months of age (Bergman et al., 2010; Bolten et al., 2010; Davis & Sandman, 2010). The association between maternal cortisol levels during pregnancy and infants´ development is not clear (Davis & Sandman, 2010; La Marca-Ghaemmaghami & Ehlert, 2015). The exact time

when exposure to maternal stress impact on infants´development remains a challenge (Rakers et al., 2017).

In this regard, the aim of this study was to assess maternal hair cortisol levels during the first, second, and third trimester, along with neonate´s hair cortisol levels that could predict infants´ neurodevelopment.

Methods

Participants

An amount of 130 pregnant women were asked to participate (Figure 1). A sample of 117 women voluntarily consented to participate. After initially agreeing to participate, 12 women declined to collaborate referring to lack of time. Due to 4 pregnancies ended in miscarriage, we excluded those participants. Eleven were excluded because only sociodemographic data was collected. Eight pregnant women decided to leave the study either in the second or the third trimester. Immediately after delivery, seven newborns did not have enough hair to analyze cortisol and were excluded, which resulted in a final sample of 75 participants. Six months after delivery, thirty-four pregnant women did not attend the appointment with the psychologist referring to lack of time (consequently, the neurodevelopment of these 34 participants was not assessed). The final study sample was composed of 41 pregnant women with age ranging from 22 to 39 (M = 31.90 years; SD = 4.15) and their 41 infants (22 girls and 19 boys) who were full term at the time of delivery (M gestational age = 39.32 weeks, SD = 1.12 weeks; M weight = 3200.98 grams, SD = 377.06 grams). Infants in this study were stable at birth with 1-minute Apgar score ranging from 9 to 10 (M = 9.5, SD = 0.5).

Eligible participants were low-risk pregnant women in the first trimester, proficient in the Spanish language, over 18 years old and did not have steroid medication. Participants were recruited by two community midwives (March 2016 – May 2017) while attending a prenatal appointment at two Health Centres.

The present study was approved by the Biomedical Ethics Research Committee and the Ethics Research Committee of the Health Centers where this study was implemented. This study was approved by the Human Ethics Research Committee of the University of Granada (reference 881). This study followed the guidelines of the Helsinki Declaration (AMM, 2008) and the Good Clinical Practice Directive (Directive 2005/28/EC) of the European Union. All procedures were conducted in accordance with the American Psychological Association' s Ethical Principles of Psychologists and Code of Conduct (APA, 2002).

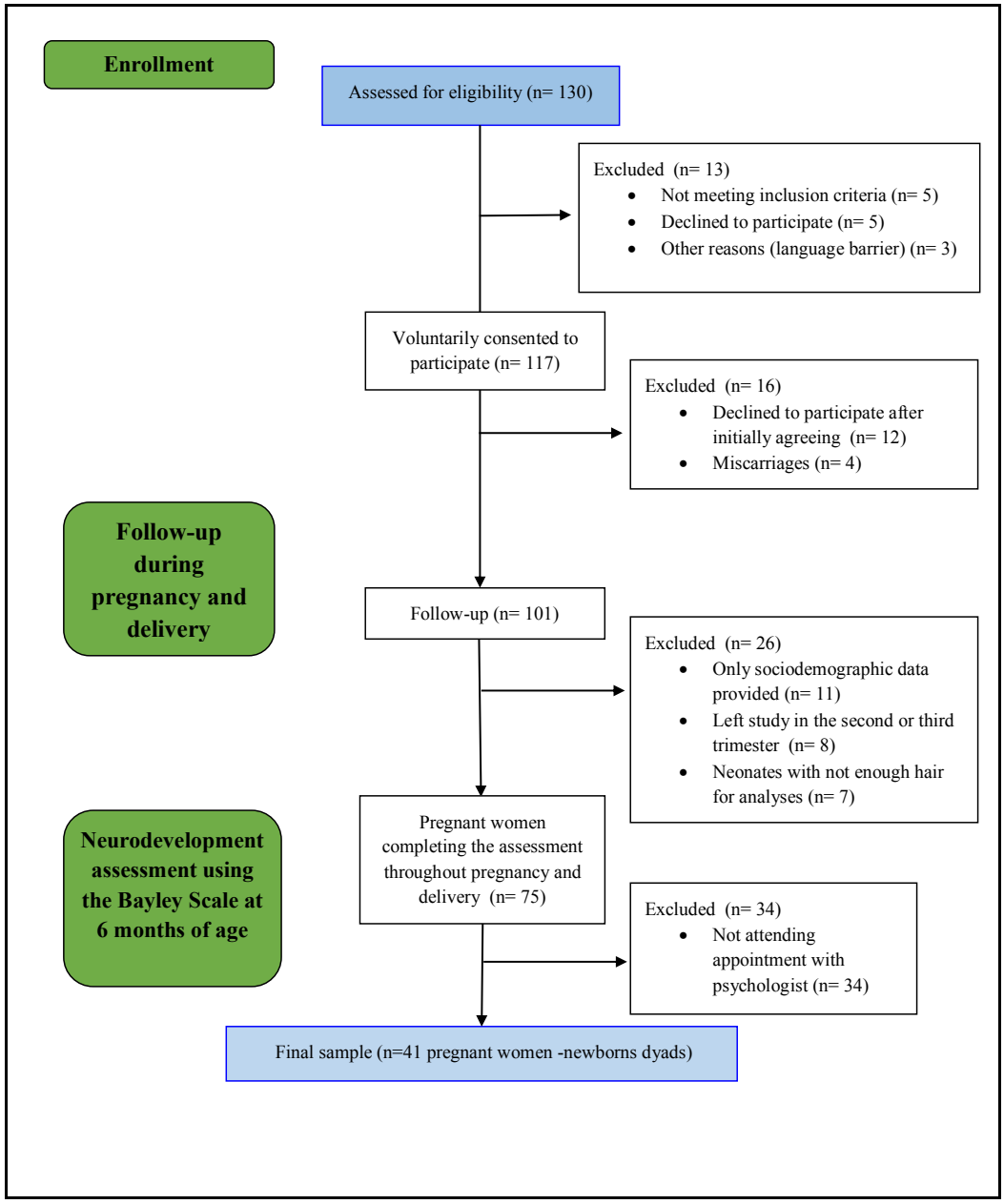


Figure 1. Participants´ follow up flow diagram.

Procedure

Maternal hair samples were evaluated at 3 time-points during pregnancy, coinciding with a prenatal appointment with a community midwife (Time 1: 12 weeks of gestation ± 3.31, Time 2: 24 weeks of gestation ± 3.09, Time 3: 34 weeks of gestation ± 2.69). After delivery, maternal hair samples, and newborn hair samples were assessed at 1 time-point, coinciding with a postnatal appointment with a community midwife (Time 4: 10 days after delivery ± 2.4). Six months after birth a trained psychologist assessed the infant´s neurodevelopment at our laboratory at the university (Time 5: 6.25 months ± 0.39). The assessment of the infant´s neurodevelopment took between 40-50 minutes. Participants´ received 10 ε and a report informing the level of neurodevelopment of their infants.

Additionally, medical, obstetric and sociodemographic information was obtained from the official pregnancy health document.

Predictor variables

Maternal, Newborns and Infants´ Hair Cortisol Assay

In order to assess the activation of the HPA axis, hair cortisol levels were obtained from hair samples proximal to the scalp with a maximum length of 3 cm (an average growth rate of 1 cm/month was assumed; a 3 cm segment contains cortisol deposited over the last 3 months). Each sample was collected from the posterior vertex of the head and consisted of approximately 150 strands of hair (Sauvé, Koren, Walsh, Tokmakejian, & Van Uum, 2007). Then, the hair samples were wrapped in a piece of aluminum foil, stored in an envelope at room temperature and sent for analysis to the

collaborative laboratory. After incubating the samples for 72 hours at room temperature in the dark with constant inversion using a rotator, cortisol from the hair samples was extracted into HPLC-grade methanol. At that time, the supernatant was dried using a vacuum evaporator (Centrivac, Heraeus, Hanau, Germany) and the resulting extract was reconstituted in 150 ul of phosphate buffered saline at a pH of 8.0. Afterwards, the reconstituted sample was frozen at -20ºC for later analysis (Chen et al., 2013; Meyer, Novak, Hamel, & Rosenberg, 2014; Sauvé et al., 2007).

Following the manufacturer's directions, the Salivary ELISA Cortisol kit© was used to measure hair cortisol levels (Alpco Diagnostics®, Windham, NH). The Salivary ELISA Cortisol kit© is a validated tool to measure hair cortisol levels and is highly positive correlated with liquid chromatograph–mass spectrometry (LC–MS/MS) (Russell et al., 2015). As reported by the manufacturer, the sensitivity of the Cortisol ELISA kit is 1.0 ng/ml and the cross reactivity is as follows: Prednisolone 13.6%, Corticosterone 7.6%, Deoxycosticosterone 7.2%, Progesterone 7.2%, Cortisone 6.2%, Deoxycortisol 5.6%, Pednisone 5.6% and Dexamethasone 1.6%. No cross-reaction with DHEAS and Tetrahydrocortisone was detected.

Eight consecutive assays measured in duplicate were applied on internal quality controls to check the intra- and inter-assay variations for routine salivary cortisol measurement. The intra-assay coefficients of variance (CV) were 2.7% at 10.7 ng/ml and 4.3% at 43.9 ng/ml. The inter-assay CVs were 4.4% and 6.3%, respectively.

Outcome variable

Infants´ Neurodevelopment

Levels of infants´ neurodevelopment were assessed using the Bayley Scales of Infant Development-Third Edition (BSID; Bayley, 2006; Spanish version by Bayley, 2015). The BSID is a standardized assessment tool to measure infants and toddlers´ ´neurodevelopment from 1 to 42 months. Measurements of neurodevelopment were obtained at 6 months using the Cognitive, Language, and Motor scales. All assessments were performed by the same psychologist at our research laboratory. Following the author´s instructions, the raw scores of the successfully completed items are converted into scaled scores which offers the percentile rank the infant is in regarding each of the domains assessed. For each sub-scale, scaled scores are derived from the total raw scores and range from 1-19 (M = 10; SD = 3). Percentile ranks indicate the standing of a certain child in respect to that of the children across the standardization sample and ranges from 1 to 99, being 50 the mean and median (Bayley, 2006).

Medical, Obstetric and Sociodemographic Information

Medical risk, obstetric history and sociodemographic information was obtained from the Official Health Pregnancy Document (Junta de Andalucia, 2003). It consists on an extensive health document on the health status of every pregnant woman regarding fetal ultrasound measures, prenatal diagnosis, genetic counseling and obstetric risk.

Statistical analyses

Due to the fact that hair cortisol levels did not present a normal distribution, a natural log transformation (natural log; LN base e) was performed.

In order to control for potential confounding variables that could influence infant neurodevelopment and may bias level of development acquired, we assessed level of maternal education, salary and infants´ birthweight. Maternal level of education and salary have been reported to be associated with infant´s neurodevelopment in previous studies (González-Alzaga et al., 2015; Rodríguez-Barranco et al., 2014). Infants´ birthweight is a major factor associated with infants´ long term development (Field et al., 2006; Glover, 2015).

For this reason, level of maternal education, salary and infants´ birthweight were included and controlled for in statistical analysis.

Aiming to compare whether there were any differences between those participants in the final sample (Group 1) and those pregnant women who left the study before completing all the assessment points (Group 2), descriptive analyses were done on maternal hair cortisol levels, infants´hair cortisol levels, infants´sex, maternal school level, family salary and infants´ birth weight, using a 2-sample t –test, between both groups.

Next, in order to examine the associations between predictors, predicted variables and covariates, Pearson´s partial correlations were performed. An absent of high correlations (> .80) between predictors and the variance inflation factor (VIF) < 3.08 indicated that multicollinearity was not present between predictors. Correlations < .80 and VIF ≤ 10 indicates lack of multicollinearity, which makes it easier to assess the importance of an individual predictor in a regression analysis (Field, 2009).

Finally, in order to assess the effect of maternal and infant hair cortisol levels on infants ´neurodevelopment, hierarchical linear regressions analyses were performed. The models for the regression analyses for each of the predictors were as follows: First,

the three covariates predictors (maternal school level, salary, infant birthweight) was entered into the model (step 1), followed by a single predictor (maternal or neonates´ hair cortisol levels at one time-point) (step 2). This procedure was followed to assure obtaining a reliable regression model and respecting the rule of having at least 10 participants per predictor (Field, 2009).

Analyses were conducted using SPSS v20 (IBM Corp) for Mac OSX version 10.12.6.

Results

Descriptive sample characteristics

Table 1 shows descriptive analyses of the main sociodemographic, obstetrics, and hair cortisol levels throught pregnancy. Descriptives analyses revealed no differences in sociodemographics, obstetrics and hair cortisol levels throughout pregnancy between the final sample (Group 1) and those participants that did not attend the neurodevelopment assessment appointment with the psychologist (Group 2) (Table 1).

Table 1. Differences in sociodemographic, obstetrics and hair cortisol levels between Group 1 (Final sample) and Group 2 (Participants not attending the neurodevelopment assessment appointment).

	Group 1*	**Group 2***	**Test****	***p***
	(n*=41*)	***(n*=34*)***		
	***X*(*SD*)/N(%)**	***X*(*SD*)/N(%)**		

Socio-demographic variables

Age (years)		32.90(4.15)	33.06(3.72)	-1.12	.21
Nationality	Spanish	35(85.40%)	29(85.30%)	0.01	.99
	Immigrant	6(14.60%)	5(14.7%)		
Marital status	Single/divorced/widow	4(9.80%)	6(17.60%)	1.01	.31
	Married/cohabitant	37(90.20%)	28(82.40%)		
Employment situation	Working	33(80.50%)	28(82.40%)	0.04	.83
	Unemployed	8(19.50%)	6(17.60%)		
Salary	< 1000 €	15(36.60%)	12(35.30%)	0.12	.93
	1000-2000 €	6(14.60%)	6(17.6%)		
	>2000 €	20(48.8%)	16(47.1%)		
School level (years)		13.90(2.09))	13.29(2.55)	1.13	.26
Sport	Yes	22(53.7%)	13(38.2%)	1.77	.18
	No	19(46.30%)	13(61.80%)		
Hair aspect	Nature	19(46.30%)	14(41.20%)	0.75	.38
	Dyed	22(53.70%)	20(58.80%)		

Obstetric information

Primiparous	Yes	19(46.3%)	16(47.1%)	0.01	.95
	No	22(53.70%)	18(52.90%)		
Wanted pregnancy	Yes	36(87.8%)	28(82.4%)	0.44	.51
	No	5(12.2%)	6(17.60%)	0.74	.86

Previous miscarriages	Yes	16(39.0%)	18(52.90%)	1.45	.23
	No	25(61.0%)	16(47.10%)		
Labor and delivery	Eutocic	34(82.90%)	30(88.20%)	1.72	.42
	Dystocic	5(12.20%)	4(11.8%)		
	C-section	2(4.90%)	0(0%)		
Pregnancy method	Spontaneous	36(87.80%)	31(91.20%)	0.22	.63
	Assisted Reproductive Technique	5(12.2%)	3(8.8%)		
Sex of the fetus	Female	22(53.70%)	19(55.90%)	0.04	.84
	Male	19(46.3%)	15(44.1%)		
Infant birth weight		3200(377.05)	3299(379.46)	-1.12	.26
Hair cortisol levels					
Maternal hair cortisol levels (pg/mg)	1st trimester	303.52 (392.35)	394.68 (497.01)	-0.88	0.38
	2nd trimester	422.64 (712.78)	373.88 (514.97)	0.33	0.74
	3rd trimester	386.46 (338.93)	375.38 (569.65)	0.11	0.92
	Postpartum (1 month)	919.27 (1536.71)	629.28 (1088.28)	0.92	0.36
Infant hair cortisol levels (pg/mg)	Postpartum (1 month)	2747.45 (2209.54)	2359.72 (1400.36)	0.87	0.38

Note: *Group 1 = Participants´infants were assessed using the BSID; Group 2 = Participants´infants were not assessed with the BSID.

** T-test was used to quantitative variables and Chi-square test to categorical variables.

Table 2. Intercorrelations between maternal and infants hair cortisol levels.

		Maternal hair cortisol levels				**Neonates´ hair cortisol levels**
		T1	T2	T3	T4	T5
Maternal hair	T1		**.57***	.13	.23	.03
cortisol levels	T2			**.55***	.24	-.11
	T3				.14	-.01
	T4					-.09

Note. T1 = First trimester; T2 = Second trimester; T3 = Third trimester; T4 = Postpartum

*$p < .05$

Pearson´s bivariate correlations between the predictors

As shown in Table 2, Pearson´s bivariate correlations between the predictors (maternal and infants hair cortisol levels) were all < .80, indicating lack of multicollinearity. Thus, maternal and hair cortisol levels were used as predictors in a hierarchical linear regression analyses.

Hierarchical Linear Regression Analyses for maternal hair cortisol levels

The results of the hierarchical regression model for maternal hair cortisol levels throughout pregnancy as predictors, adjusted for potential covariates (maternal school

level, salary and infant birth weight), and infants´ neurodevelopment as predicted variables are shown in Table 3.

Table 3. Hierarchical regressions using maternal hair cortisol levels as predictors of infant's neurodevelopment.

			BSDI scales	**Cognitive**			**Language**					**Motor**				
							Receptive		**Expresive**		**Global**	**Fine**		**Gross**		**Global**
				Raw score	Scaled score	Percentile Rank	Raw score	Scaled score	Raw score	Scaled score	Percentile Rank	Raw score	Scaled score	Raw score	Scaled score	Percentile Rank
Maternal hair cortisol levels	**T1**	R^2		.17	.22	.21	.12	.17	.18	.21	.12	.01	.03	.22	.07	.03
		β		.04	.12	.11	.02	.09	.17	.17	.16	.08	.16	-.17	.11	.12
		F		1.94	2.65	2.46	1.27	1.85	2.06	2.25	1.25	.13	.30	2.63	.74	.31
		p		.12	**.04***	.07	.31	.14	.11	.08	.31	.96	.87	**.05***	.57	.87
	T2	R^2		.17	.22	.21	.12	.16	.15	.17	.09	.01	.01	.22	.07	.01
		β		-.02	.08	.05	-.04	.05	.03	.03	.02	-.09	-.01	-.16	.12	.01
		F		1.93	2.54	2.34	1.28	1.76	1.67	1.86	.96	.14	.06	2.58	.76	.15
		p		.12	.06	.07	.29	.15	.18	.14	.44	.96	.99	**.05***	.55	.96
	T3	R^2		.19	.21	.21	.14	.16	.16	.19	.15	.02	.01	.23	.06	.01
		β		-.13	-.03	-.05	-.14	-.8	-.11	-.16	-.25	-.14	-.07	-.21	-.01	-.05
		F		2.12	2.45	2.35	1.49	1.81	1.79	2.17	1.65	.24	.11	2.79	.61	.17
		p		.09	.06	.07	.22	.15	.15	.09	.18	.91	.97	**.04***	.65	.95
	T4	R^2		.22	.24	.26	.12	.16	.18	.22	.13	.01	.01	.22	.11	.02

β	.22	.18	.23	.01	.05	-.18	-.22	-.21	-.06	-.04	.16	.23	.08
F	2.59	2.9	3.14	1.27	1.77	2.08	2.49	1.41	.09	.08	2.55	1.21	.21
p	**.05***	**.03***	**.02****	.31	.16	.11	.06	.25	.98	.98	**.05***	.32	.92

Note. The model is adjusted for potential covariates (Maternal school level, salary and infant birth weight). *p<0.05; ** p<0.02

T1 = First trimester; T2 = Second trimester; T3 = Third trimester; T4 = Postpartum.

Maternal hair cortisol levels at the first trimester could predict 22% of variance of infants´ cognitive development [R^2 = .22, (F = 2.65, p < .05), ß = 0.12, p < .05] and 22% of variance of infants´ gross motor development [R^2 = .22, (F = 2.63, p < .05), ß = -0.17, p < .05]. At the second trimester, maternal hair cortisol levels accounted for 22% of variance of infants´ gross motor development [R^2 = .22, (F = 2.58, p < .05), ß = -0.16, p < .05]. At the third trimester, maternal hair cortisol levels predicted 23% of variance of infants´ gross motor development [R^2 = .23, (F = 2.79, p < .05), ß = -0.21, p < .05]. During the postpartum, maternal hair cortisol levels accounted for 26% of variance of infants´ cognitive development [R^2 = .26, (F = 3.14, p < .05), ß = 0.23, p < .05], and 22% of variance of infants´ gross motor development [R^2 = .22, (F = 2.55, p < .05), ß = 0.16, p < .05]. Thus, high maternal hair cortisol levels during the first, second and third trimester predicted low motor development. High maternal hair cortisol levels during the first trimester and the postpartum period predicted a higher cognitive development.

Hierarchical Linear Regression Analyses for neonates´ hair cortisol levels

The hierarchical regression model for infants´ hair cortisol levels as predictors, adjusted for potential covariates (maternal school level, salary and infant birth weight), and infants´ neurodevelopment as predicted variables are shown in Table 4. Newborns´ hair cortisol levels predicted 24 % of cognitive development in infants [R^2 = .24, (F = 2.91, p < .05), ß = -0.18, p < .05], 22% of language development in infants [R^2 = .22, (F = 2.57, p < .05), ß = -0.25, p < .05], and accounted for 28% of variance of gross motor development in infants [R^2 = .28, (F = 3.61, p < .05), ß = -0.31, p < .05]. As shown in

Figure 2, high neonates´ hair cortisol levels predicted low cognitive, motor and language development of infants at 6 months of age.

Table 4. Hierarchical regressions using newborns´ hair cortisol levels as predictors of infant's neurodevelopment.

		Cognitive			**Language**				**Motor**			
					Receptive		Expresive	Global	Fine		Gross	
		Raw score	Scaled score	Percentile rank	Raw score	Scaled score	Scaled score	Percentile Rank	Raw score	Scaled score	Raw score	Scaled score
Newborns hair cortisol levels	R^2	.18	.24	.21	.20	.22	.17	.13	.01	.01	.28	.09
	β	-.08	-.18	.06	-.29	-.25	-.03	-.19	-.06	-.03	-.31	-.18
	F	2.01	2.91	2.36	2.29	2.57	1.85	1.35	.11	.07	3.61	.96
	p	.11	**.03***	.07	.08	**.05***	.14	.27	.98	.99	**.01****	.44

Note. The model is adjusted for potential covariates (Maternal school level, salary and infant birth weight).

**p* < .05; ** p<0.02

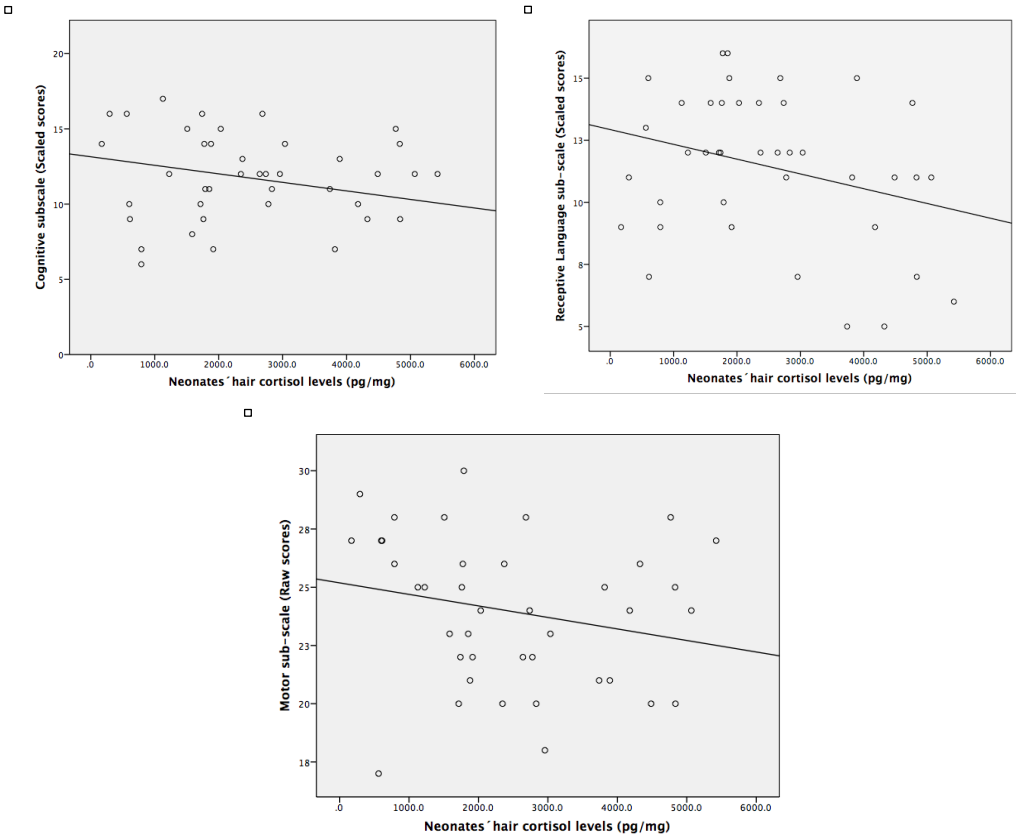


Figure 2. Higher neonate´s hair cortisol levels predicted low infants´ development

Discussion

The aim of this study was to assess maternal hair cortisol levels during the first, second, and third trimester, and neonate´s hair cortisol levels that may predict infants´ neurodevelopment. For this purpose, we followed up a group of pregnant women since the first trimester throughout pregnancy, delivery, and postpartum until their infants were 6 months of age. Due to maternal school level, salary and infant´s birth weight have been informed to be relevant when evaluating infants´ neurodevelopment (Glover, 2015; González-Alzaga et al. 2015; Rodríguez-Barranco et al., 2014), these variables were included as covariates in further analyses. Our results suggested that maternal hair

cortisol levels and neonates´ hair cortisol levels could predict infants´ neurodevelopment at age 6 months.

Assessing hair cortisol levels during pregnancy reflect maternal chronic stress during the last three months (D´Anna-Hernandez et al., 2011: Wosu et al., 2013). Biomonitoring neonates´ stress levels through hair cortisol levels provides an unique opportunity to assess the level of stress the fetus was exposed to while in the womb (Braig et al., 2015; Kapoor et al., 2016). In this respect, some prospective studies have suggested that the timing of antenatal exposure to cortisol during the fetal development has an impact on long-term infants´ neurodevelopment (Davis & Sandman, 2010; Glover, 2015). According to our findings, high maternal hair cortisol levels during the first, second and third trimester predicted a slower motor development. Our study agrees with those reporting an inverse association between high levels of stress throughout pregnancy and low motor development among infants (Buitelaar et al, 2003; Huizink et al., 2003; Moss et al., 2017). Nevertheless, a previous study using salivary cortisol and a prior edition of the Bayley scale could not find that maternal cortisol during pregnancy predicted motor development (Davis & Sandman, 2010). Besides, during the postpartum period, higher maternal hair cortisol levels predicted an accelerated motor development in infants at 6 months of age. Our results support previous studies that stated high prenatal and postnatal stress were harmful for the motor development in infants (Karam et al., 2016). Due to the fact that hair cortisol levels reflect chronic stress levels during the last three months (Kirschbaum, et al., 2009), hair cortisol levels during the postpartum refer to stress levels the fetus was exposed to during the last trimester. Consequently, high levels of stress during the third trimester have long-term consequences on the infants´ development (Stalder et al.,

2012b). Besides, ensuring high cortisol levels after birth may promote an adequate fetal lung function during the extrauterine period (Berhard, 2016; Roberts et al., 2017).

Regarding the cognitive development, our results reflected that high hair cortisol levels at the first trimester predicted higher cognitive development among infants. Hair cortisol levels at the first trimester is a retrospective marker informing of maternal levels of stress before and around the conception period, this is three months before the hair sample was assessed (D'Anna-Hernandez, Ross, Natvig & Laudenslager, 2011). Contrary to our results, previous studies suggested that high cortisol levels in the first trimester resulted in a tendency to lower cognitive development (Bergman et al., 2010; Davis & Sandman, 2010; Graignic-Philippe, Dayan, Chokron, Jacquet, & Tordjman, 2014). A potential explanation to these controversial findings may be that during the first trimester of pregnancy the enzyme 11β-HSD2 protects the developing fetus from high maternal cortisol levels (Dunthie & Reynolds, 2013). Besides, those studies assessed cortisol from amniotic fluid, saliva and urine, reflecting acute levels of stress. Nevertheless, our findings offer an additional value supporting the importance to assess stress levels during the periconception period (Witt, Litzelman, Cheng, Wakeel, & Barker, 2014). In this respect, stress levels during the periconception period can have long-term effects on infants´ development (Ord, Fazeli, & Watt, 2017).

During the postpartum, high hair cortisol levels, reflecting high levels of stress during the third trimester of pregnancy (Braig et al., 2015), predicted higher infants´ cognitive development. Our findings agrees with the fact that during the third trimester of pregnancy, levels of placental 11β-HSD2 decrease (Murphy, Smith, Giles, & Clifton, 2006), resulting of higher exposure of fetuses to cortisol. Increasing levels of cortisol at the end of pregnancy are necessary for fetal organ maturation and development (Howerton & Bale, 2012). Nevertheless, while a gentle increase in cortisol improve

brain development, extreme exposure to cortisol lead to neurological degeneration triggering long-term consequences (Tsiarli et al., 2017). The limbic system has a key role on this association as it is highly sensitive to high levels of cortisol. In this regard, the hippocampus, which plays a central role in cognition and behavior, have cortisol receptors since the second trimester of pregnancy (Noorlander, De Graan, Middeldorp, Van Beers, & Visser, 2006).

Fetal levels of cortisol can be reflected in hair assessed after delivery (Hoffman, D'Anna-Hernandez, Benitez, Ross, & Laudenslager, 2017). Although our study reflected that high maternal hair cortisol levels at certain time-points can improve cognitive and motor development, our results reflect that high neonates´ hair cortisol levels negatively affects their neurodevelopment. According to our findings, high neonates´ hair cortisol levels predicted a lower cognitive, language and motor development in infants´ aged 6 months. Our study agrees with a previous study reporting that early neonatal treatment using a cortisol derived substance (hydrocortisone) may have undesired effects on neurodevelopment in infants (Peltoniemi et al., 2016). Nonetheless, a prior study reported no association between infants´ cortisol levels from blood samples with their neurodevelopment when assessing motor and cognitive development (Voltas et al., 2017). Probably since blood cortisol levels inform of acute stress, and hair cortisol reflect chronic stress (Wosu et al., 2013). Consistent with our study, those neonates exposed to high levels of cortisol during their intrauterine life showed a lower language development (Laplante et al., 2004; Weinstock, 2008). Although hair cortisol has been previously used as a biological marker of chronic stress in neonates (Yamada, Stevens, de Silva, Klein, & Koren, 2003), our study is the first one to use hair cortisol levels from neonates to predict infants´ neurodevelopment.

Strenghts of this study include the longitudinal design that allowed assessing cortisol levels during the first, second and third trimester, and postpartum in pregnant women, along with neonates´ cortisol levels. Additionally, the use of hair to assess cortisol levels through a non-invasive method that provides a retrospective stress status of pregnant women during the periconception period and give the opportunity to estimate fetal cortisol (Hoffman et al., 2017; Ord et al., 2017; Witt, Litzelman, Cheng, Wakeel, & Barker, 2014). Besides, infants´ neurodevelopment was assessed at the early age of 6 months which may allow detecting potential associations between maternal and neonates´ hair cortisol levels and infants´ development at early stages. Previous studies assessing the association between maternal cortisol levels and infant´s neurodevelopment analysed cortisol levels from blood, urine or amniotic fluid samples (Bergman et al., 2010; Davis & Sandman, 2010; Graignic-Philippe, Dayan, Chokron, Jacquet & Tordjman, 2014). This studies only provided data regarding acute levels of stress using invasive techniques affected by circadian rhythms. Nonetheless, our study provides a novel biological assessment of cortisol from hair samples informing of chronic stress (Wosu et al., 2013).

A limitation of the present study comes from its longitudinal design. Hence, a high amount of participants decided not to continue collaborating at different time-points. An additional potential limitation was that the infants´ neurodevelopment was only assessed at a single time point when infants were 6 months of age. Future studies may introduce in their protocols a long-term follow-up that may offer the possibility to assess the implications of prenatal and postnatal maternal and neonates´ cortisol levels on infants´ development. Besides, postpartum depression and psychological variables that may be related with infants´ development was not assessed in our study (Perra, Phillips, Fyfield, Waters, & Hay, 2015).

In summary, our findings revealed that high maternal hair cortisol levels during the first, second and third trimester are associated with an lower motor development. Nevertheless this association is not always negative, depending on the time during pregnancy cortisol levels are assessed and the neurodevelopment assessment tool used (Glover, 2015). Thus, elevated maternal hair cortisol levels at the first trimester predicted a higher cognitive development. During the postpartum period, higher maternal hair cortisol levels stimulate cognitive and motor development.

Regarding neonate´s cortisol, the association is negative. Specifically, upper neonates´ hair cortisol levels predicted a weaken cognitive, motor and language development among infants aged 6 moths.

Due to hair cortisol levels reflect chronic stress levels during the preceding 3 months (Wosu et al., 2013), our findings reflect the importance of the preconception and prenatal time as sensitive periods related to infants´ neurodevelopment, along with cortisol levels the fetus is surrounded while in the womb (Glover, 2015, 2017; Hoffman et al., 2017). In line with our study, changes in the HPA axis during the preconception period may have long-term consequences (Caparros-Gonzalez et al., 2017). In this regard, antenatal effective stress assessment and interventions should be applied widely during women´s reproductive time to improve fetal and infants´ neurodevelopment (Dunkel Schetter & Tanner, 2012). The findings reported in this study are of high value for obstetricians and midwives when taking care of pregnant women and researchers, who may use them to prevent negative outcomes and enhance an adequate development among infants. The perinatal period is a highly vulnerable stage when future mothers should be provided the best maternity care (Alderdice et al., 2013).

CAPÍTULO XII: "DISCUSIÓN GENERAL, CONCLUSIONES Y PERSPECTIVAS FUTURAS"

El objetivo de esta Tesis Doctoral fue estudiar las repercusiones del estrés psicológico materno prenatal sobre la salud materna e infantil durante el embarazo, parto y puerperio así como sus repercusiones en el neurodesarrollo temprano del bebe. Para cumplir este objetivo, se llevaron a cabo seis estudios cuyos objetivos fueron en primer lugar realizar una revisión sistemática sobre evaluación de estrés en embarazo, posteriormente la validación y adaptación a la población española de un cuestionario de preocupaciones prenatales, siguiendo con el diseño del protocolo de evaluación del efecto del estrés en el embarazo. Preparado el protocolo de investigación los siguientes estudios implicaron el estudio de las variables psicológicas y hormononales relacionadas con la depresión postarto, el estrés en la reproducción asistida y consecuencias del estrés del embarazo en el neurodesarrollo del bebé.

La revisión sistemática llevada a cabo en esta Tesis Doctoral, cuyo objetivo fue averiguar cuál de las medidas de estrés durante el embarazo (Test Psicológicos vs. Cortisol), era el mejor predictor de resultados negativos maternos y en la descendencia, concluyó con que los niveles elevados de cortisol materno durante el embarazo están más asociados a bajo peso del recién nacido (Baibazarova y cols., 2013; Bolten y cols., 2010; D'Anna y cols., 2012; Diego y cols., 2006; Field y cols., 2006; Goedhart y cols., 2010; Hompes y cols., 2012; Kivlighan y cols., 2008), una baja edad gestacional (Hoffman y cols., 2016; Kramer y cols., 2009) y un crecimiento intrauterino retardado (Field y cols., 2006; Diego y cols., 2006). Además, se encontró que los niveles de cortisol también están asociados al neurodesarrollo infantil (Bergman y cols., 2010; Buitelar y cols., 2003; Davis y cols., 2010; Huizink y cols., 2003). Por el contrario, la evidencia encontrada en torno a la asociación entre cuestionarios psicológicos y consecuencias negativas no es concluyente. (Diego y cols., 2006; Hoffman y cols., 2016; Kramer y cols., 2009). Por otro lado, los estudios muestran que si existe

asociación entre los cuestionarios psicológicos y el neurodesarrollo infantil (Bergman y cols., 2010; Buitelar y cols., 2003; Davis y cols., 2010; Huizink y cols., 2003). Algo a destacar es la poca evidencia en torno a la asociación entre cuestionarios psicológicos de estrés y niveles de cortisol. Este estudio nos ha ayudado a determinar la importancia de usar tanto medidas psicológicas como biológicas para estudiar las consecuencias del estrés materno prenatal.

En el bloque II de validación, se expone el estudio 2. Dada la ausencia de una medida psicológica para medir estrés específico del embarazo para la población española, se consideró como un objetivo de este Tesis la traducción, adaptación y validación del PDQ (Alderdice y cols., 2013; Yali y Lobel, 1999). Los resultados de este estudio mostraron que el PDQ posee una moderada y aceptable fiabilidad, manteniendo la estructura factorial de los creadores del instrumento con tres factores (Factor 1: Estrés sobre el parto/nacimiento; Factor 2: Estrés sobre las relaciones sociales; Factor 3: Estrés sobre los síntomas físicos) equivalente a su versión original. Además, se presentaron evidencias de validez psicométrica del PDQ, al hallarse correlaciones significativas con la EEP y algunas sub-escalas del SCL-90-R. Todas las embarazadas evaluadas para este estudio presentaron estrés y preocupaciones relacionadas con algún evento específico del embarazo. Lo que menos preocupaba a estas embarazadas era el manejo del recién nacido en el hospital, siendo lo que más estrés específico del embarazo generaba la posibilidad de tener un recién nacido que no estuviese sano, seguido por la ocurrencia de un parto prematuro y por el momento del parto. Dados los resultados psicométricos obtenidos, este estudio permitirá la aplicación del PDQ en embarazadas que utilizan como propio el idioma castellano, para estudios que impliquen evaluación de estrés específico del embarazo. El uso de este instrumento

favorecerá la investigación acerca de estrés materno prenatal en nuestro entorno, y su potencial asociación con resultados negativos maternos e infantiles.

Con las conclusiones obtenidas en la revisión sistemática y tras la validación de un instrumento clave para la evaluación del estrés especifico del embarazo se diseñó el protocolo para la evaluación del estrés durante el embarazo. En dicho protocolo se describen las medidas psicológicas de estrés (estrés específico del embarazo y general) y del cortisol en pelo que se tomarán durante los tres trimestres de embarazo, posparto así como la evaluación del neurodesarrollo infantil. Así, se expuso que en cada trimestre de embarazo, se tomará una muestra de pelo y se aplicarán los cuestionarios psicológicos (PDQ, EEP, SCL-90-R, CD-RISC); en el posparto se tomará muestra de pelo materno e infantil, y se aplicarán los cuestionarios psicológicos (EEP, SCL-90-R, CD-RISC, EPDS). Para la evaluación infantil se usará la escala Bayley de Desarrollo Infantil (Bayley, 2006) y el Cuestionario de Comportamiento Infantil para la evaluación del temperamento (Rothbart, 1981). Este protocolo nos ayudará a encontrar posibles asociaciones entre estrés materno prenatal y su relación en la salud materna e infantil.

El siguiente estudio de esta tesis tuvo como objetivo comprobar las variables sociodemográficas, psicológicas y hormonales predictoras de la depresión posparto. Para ello se evaluó qué variables sociodemográficas, obstétricas, psicológicas, de estrés y niveles de cortisol en pelo que durante el primer, segundo y tercer trimestre eran capaces de predecir síntomas de depresión posparto. Los resultados mostraron que las embarazadas que posteriormente presentarán síntomatología de depresión posparto, tenían mayores niveles de somatización en el primer trimestre, mayores niveles de somatización, depresión y ansiedad en el segundo trimestre, mayores niveles de estrés específico del embarazo en el tercer trimestre que las embarazadas que no desarrollaban una posterior depresión. Por otro lado, las embarazadas que posteriormente presentarán

sintomatología de depresión posparto, tienen niveles mayores de cortisol en el primer y tercer trimestre que las que no tendrán síntomas de depresión posparto. En concreto, los resultados mostraron que los niveles de cortisol en pelo en el primer y tercer trimestre eran capaces de predecir la ocurrencia de depresión posparto. Nuestros hallazgos coinciden con estudios previos que señalaron que altos niveles de cortisol se relacionan con síntomas de depresión posparto (Fan y cols., 2009; Nierop y cols., 2006). Sin embargo no existe consenso a este respecto ya que por el contrario otros estudios encontraron que bajos niveles de cortisol se relacionan con depresión posparto (Scheyer y Urizar, 2016; Seth y cols., 2016). Posiblemente estos resultados contradictorios se deban a que estos estudios se usaron medidas de cortisol agudo, mientras que en nuestro estudio se evaluaron los niveles de estrés crónico mediante cortisol en pelo. No obstante, estos hallazgos ofrecen una información crucial en la detección precoz de la depresión posparto. Mediante una evaluación psicológica y junto al uso de los niveles de cortisol en pelo durante el embarazo, podemos detectar qué embarazadas serán más vulnerables a desarrollar síntomas de depresión posparto lo que contribuiría enormemente a la prevención de la misma.

Por otro lado, y siendo conscientes de las implicaciones del estrés en el embarazo, diseñamos el siguiente estudio, donde se comparó un grupo de mujeres embarazadas con altos niveles de estrés psicológico, como son las mujeres embarazadas mediante técnicas de reproducción asistida (TRA), con un grupo de embarazadas de forma espontánea. Para ello, fueron evaluados los niveles de estrés psicológico y niveles de cortisol en pelo en embarazadas con TRA en comparación con embarazadas de forma natural. Se realizó un seguimiento de las embarazadas durante todo el embarazo y posparto, momento en el que se evaluaron también a sus recién nacidos. Los resultados reflejaron que las embarazadas mediante TRA mostraban mayores niveles de cortisol en

pelo en el primer trimestre, y mayores niveles de estrés percibido en el tercer trimestre, que las embarazadas de forma natural, no se encontraron diferencias entre los dos grupos en las otras variables. Estos resultados concuerdan con estudios previos que han relacionado los tratamientos de TRA con altos niveles de estrés (Bailey, Ellis-Caird y Croft, 2017; Patel, Sharma, Narayan, Binu, Dinesh y Pai, 2016). Sin embargo, una vez más, los estudios realizados no muestran acuerdo en estos resultados como muestra una reciente revisión sistemática que concluyó que no existían diferencias en cuanto a los niveles de estrés percibido entre embarazadas mediante TRA versus de forma natural (Gourounti, 2015). Además, los recién nacidos mediante TRA tenían menores niveles de cortisol en pelo que los recién nacidos concebidos de forma natural. Estos recién nacidos, estuvieron expuestos a altas concentraciones de cortisol durante el primer trimestre, lo que concuerda con los resultados en modelos animales (macaco Rhesus), en los que altos niveles de cortisol en el primer trimestre se relacionó con bajos niveles de cortisol en los recién nacidos (Kapoor y cols., 2016). En concreto, se ha informado que el paso de altas cantidades de cortisol materno a través de la placenta, en las etapas tempranas del desarrollo fetal, podrían alterar la correcta producción de cortisol por parte del feto (Glover, Bergman, Sarkar y O´Connor, 2009). Finalmente, en el grupo de embarazadas por TRA, y también en el grupo de embarazadas de forma natural, mayores niveles de cortisol en pelo materno se relacionaron con una menor longitud fetal. Estos hallazgos concuerdan con estudios previos que han relacionado mayores niveles de cortisol materno con un menor desarrollo neonatal (Alderdice y cols, 2013), bajo peso al nacer, prematuridad y pequeño para la edad gestacional (Glover, 2015; Lobel y Dunkel-Shetter, 2016).

El último estudio llevado a cabo en esta Tesis Doctoral tuvo como objetivo comprobar si existe relación del estrés de la madre con el neurodesarrollo del bebé. Para

ello, se midieron los niveles de cortisol en pelo materno durante los tres trimestres de embarazo y posparto, así como los niveles de cortisol en pelo de los recién nacidos y su neurodesarrollo a los 6 meses.. Los resultados reflejaron que altos niveles de cortisol en pelo materno en el primer trimestre predecían un mejor neurodesarrollo cognitivo, sin embargo, niveles elevados de cortisol en pelo en el primer, segundo, tercer trimestre y posparto predecían un menor neurodesarrollo motor grueso en el bebé a los seis meses. En concordancia con estudios previos (Buitelaar, Huizink, Mulder, de Medina y Visser, 2003; Huizink, Robles de Medina, Mulder, Visser y Buitelaar, 2003), nuestros hallazgos reflejan que ciertos niveles de cortisol al principio del embarazo son beneficiosos al promover un neurodesarrollo cognitivo acelerado. Por otro lado, tal y como reflejan nuestros resultados, y en concordancia con estudios previos, altos niveles de estrés a lo largo del embarazo enlentecen el neurodesarrollo motor (Glover, 2015). Nuestro estudio ha sido el primero que evalúar la relación existente entre niveles de cortisol neonatal e infantil con el posterior neurodesarrollo infantil.

Es importante destacar que, los niveles elevados de estrés materno prenatal tienen consecuencias negativas en la madre y en su descendencia. Sin embargo, es necesario determinar cuáles son los períodos ventana durante el embarazo en los que hay mayor vulnerabilidad, y profundizar en las consecuencias que determinados niveles de estrés tienen cuando ocurren en un momento dado del embarazo (Glover, 2015; La Marca-Ghaemmaghami y Ehlert, 2015).

Los resultados que se han encontrado en esta Tesis Doctoral presentan algunas limitaciones ya que sería interesante haber evaluado a las mujeres antes de que estuvieran embarazadas, en una consulta preconcepcional, y realizar un seguimiento a largo plazo de su descendencia, incluso evaluar los efectos transgeneracionales.

También sería interesante haber contado con una mayor muestra, sin embargo los estudios longitudinales suelen implicar una importante muerte experimental.

12.1 CONCLUSIONES

Las conclusiones principales que se derivan de los estudios que se han realizado en esta Tesis Doctoral son las que aparecen a continuación:

1. El nivel de cortisol durante el embarazo, es mejor predictor que las técnicas de autoinforme de estrés con respecto a las consecuencias adversas en la embarazada y en su descendencia, en relación con la edad gestacional al nacimiento, peso del recién nacido, crecimiento intrauterino y neurodesarrollo infantil.
2. La versión española del PDQ muestra una estructura factorial de tres factores, replicando la estructura factorial encontrada para este instrumento en su versión original en contextos anglosajones. Además reúne unas adecuadas propiedades psicométricas.
3. Las embarazadas que desarrollan síntomas de depresión posparto tienen mayores niveles de estrés, más síntomas psicopatológicos y mayores niveles de cortisol en pelo durante el embarazo, que las embarazadas que no tendrán síntomas de depresión posparto. En concreto, en el primer trimestre, estas diferencias se producen en torno a niveles de cortisol en pelo y Somatización; en el segundo trimestre existen diferencias en Somatización, Depresión, Ansiedad e Índice de Gravedad Global del SCL-90-R; en el tercer trimestre en lo referente a niveles de cortisol en pelo y estrés específico del embarazo.

4. Las mujeres embarazadas mediante técnicas de reproducción asistida (TRA) presentan mayores niveles de cortisol en pelo en el primer trimestre y mayores niveles de estrés percibido en el tercer trimestre, que las mujeres embarazadas de forma espontánea. Además, el patrón de cortisol de las embarazadas por TRA era también de U invertida, en contraposición al aumento progresivo de los niveles de cortisol a medida que progresa la gestación de los embarazos espontáneos.
5. En primer lugar, altos niveles de cortisol en pelo maternos durante el primer trimestre predicen un elevado neurodesarrollo cognitivo a los 6 meses. En segundo lugar, altos niveles de cortisol en pelo maternos durante todo el embarazo y posparto predicen un bajo neurodesarrollo motor a los 6 meses. En tercer lugar, elevados niveles de cortisol en pelo en los recién nacidos predicen un bajo neurodesarrollo cognitivo, lenguaje y motor grueso a los 6 meses.

12.2. PERSPECTIVAS FUTURAS

En torno a las perspectivas futuras de investigación que se derivan de esta tesis doctoral, están las siguientes:

1. Estudiar la implicación de otros mecanismos alternativos al cortisol, de transferencia de estrés psicológico desde la embarazada al feto, como citoquinas, triptófano, reactivos al oxígeno, catecolaminas y microbiota del canal del parto, para una mejor explicación de las vías por las que el estrés materno prenatal afecta a la salud materna e infantil.

2. En un entorno de consulta preconcepcional, realizar un seguimiento de la salud de la mujer, incluyendo una evaluación de los niveles de estrés, para estudiar la influencia que determinados factores que ocurren antes de la concepción, tienen sobre el embarazo y la descendencia.
3. Comprobar si el patrón de cortisol de U invertida se produce también en otras embarazadas de riesgo, como aquellas con abortos de repetición o en víctimas de violencia de género durante el embarazo.
4. Evaluar las diferencias en niveles de estrés en las mismas mujeres en sucesivos embarazos y sus consecuencias en la salud materna e infantil.
5. Desarrollar terapias psicológicas eficaces para el control del estrés en embarazadas, que reduzcan los niveles de estrés percibido, estrés específico del embarazo y los niveles de cortisol y sus posteriores consecuencias en el proceso de parto y neurodesarrollo del bebe a largo plazo.
6. Estudiar la influencia de los niveles de estrés psicológico de las parejas de las embarazadas, sobre las variables maternas, desarrollo fetal y neurodesarrollo infantil.

12.3 IMPLICACIONES CLÍNICAS

Los hallazgos que provienen de esta tesis doctoral deben hacer orientar a aquellos clínicos que se dediquen a la atención sanitaria del embarazo. En este sentido, las implicaciones clínicas que se desprenden son:

1. Debería evaluarse durante el embarazo los niveles de estrés a los que está expuesta la embarazada propiciando atención psicológica a aquella que lo necesite.
2. Toda mujer que ha tenido un bebé debería tener disponible una evaluación de depresión posparto durante los primeros días tras el nacimiento, preferiblemente en los días inmediatos al parto.
3. El uso de cuestionarios psicológicos específicos del embarazo, como el PDQ, mejorarían la evaluación psicológica de los niveles de estrés materno durante la gestación.
4. La evaluación de los niveles de cortisol en pelo materno durante el embarazo y posparto ofrecería información válida y fiable de los niveles de estrés que tiene la embarazada.
5. Mediante la evaluación psicológica y el cortisol en pelo de embarazadas y recién nacidos, los clínicos pueden predecir qué mujeres embarazadas son más vulnerables a desarrollar depresión posparto.
6. Aquellas embarazadas mediante técnicas de reproducción asistida deberían recibir especial atención psicológica, ya que sus niveles de estrés psicológico y cortisol en pelo son más elevados que las embarazadas de forma espontánea.

INTERNATIONAL PhD

SUMMARY

An estimation of 211 million pregnancies occur in the world every year. High levels of stress during pregnancy, as those caused by the death of a relative, exposure to a natural disaster or being the victim of an armed conflict, can have transgenerational consequences on the developing fetus and maternal health. However, the specific consequences of prenatal stress, and the most sensitive periods to the exposure of stressors in the intrauterine environment are still unknown. For these reasons, this Doctoral Thesis was designed to assess the influence of stress during pregnancy on maternal, fetal and infant health and illness.

This Doctoral Thesis consists of twelve chapters structured as follows: a) introduction (chapters I, II, III and IV); b) justification and objectives (chapter V); c) systematic review study (chapter VI); d) validation study (chapter VII); e) empirical studies (chapters VIII, IX, X and XI) and f) general discussion, conclusions and future perspectives (chapters XII). These chapters are organized on two parts, Theoretical Part and The Empirical Part.

The first section consists on the theoretical introduction of this Thesis, divided into four chapters. In chapter I, information regarding the normal process of pregnancy, embryonic and fetal development, as well as the physical and psychological adaptations of women during pregnancy is provided. Chapter II defines psychological stress and its assessment, maternal prenatal stress, activation of the Hypothalamic-Pituitary-Adrenal axis, cortisol as a stress hormone, and types of maternal prenatal stress. In Chapter III, the maternal consequences of stress during pregnancy are shown. Chapter IV of the

introduction describes the consequences of maternal prenatal stress on fetal and infant development.

The second section is made up of chapter V, which includes the justification, and the general and specific objectives of the studies included in this Doctoral Thesis.

The third section consists of six chapters (VI, VII, VIII, IX, X and XI) that include each of the studies forming this Thesis. Chapter VI is a systematic review study that shed light on which of the methods to assess stress during pregnancy (psychological measures or cortisol) is a better predictor of negative consequences in pregnant woman and the offspring. The results showed that cortisol levels were a better predictor of these negative outcomes. Chapter VII includes the study of adaptation and validation of a pregnancy-specific stress measure named Prenatal Distress Questionnaire (PDQ) in Spanish pregnant women. In this study results showed that the PDQ has good psychometric properties and a three factors factorial structure, agreeing with the original version. Chapter VIII is formed by the protocol of the *GESTASTRESS* study on the effects of maternal stress during pregnancy on the health of pregnant women and their newborns. *GESTASTRESS* is the acronym of the study that was granted an *Innovative & Development* project in 2015 with a duration of 3 years. The protocol describes the design of the study aiming to assess stress levels during pregnancy, and their infants´ cortisol levels and neurodevelopment. Chapter IX is a study that aimed to evaluate the psychological, hormonal, obstetric and sociodemographic variables throughout pregnancy that could predict postpartum depression symptoms. The results of this study showed that those pregnant women who develop postpartum depression symptoms have higher hair cortisol levels during pregnancy, and higher levels of psychological stress and psychopathological symptoms in the first and second trimester, in comparison with those pregnant women who do not develop postpartum depression symptoms. In

Chapter X, the aim was to verify whether pregnant women using assisted reproduction techniques (ART) had higher levels of stress than pregnant women that conceived naturally. The findings revealed higher hair cortisol levels in the first trimester in pregnant women conceiving through ART than pregnant women who conceived naturally. In addition, pregnant women using ART had higher levels of psychological stress in the third trimester than the group of pregnant women conceiving naturally. Chapter XI consists of a study aiming to assess whether levels of maternal stress during pregnancy and levels of neonatal stress predict infants´ neurodevelopment. The results showed that maternal cortisol levels in the first trimester and the postpartum, predicted infants´ cognitive neurodevelopment at 6 months of age; maternal cortisol levels in the first, second, and third trimesters predicted gross motor neurodevelopment at 6 months of age. Newborns´ hair cortisol levels predicted infants´ cognitive, language and motor neurodevelopment at age 6 months.

The final section is composed of chapter XII that includes the General Conclusions and Future Perspectives, along with the main findings of the six studies of this Doctoral Thesis.

CONCLUSIONS

The main conclusions that are derived from the studies in this Doctoral Thesis are the following:

1. Cortisol level during pregnancy is the best predictor of negative outcomes (gestational age at birth, birth weight, intrauterine growth and infants´ neurodevelopment) in pregnant women and their offspring.

2. The Spanish version of the PDQ has a three factors factorial structure, replicating the factorial structure found for this instrument in its original version in English-speaking pregnant women. It also has adequate psychometric properties.

3. Pregnant women who develop postpartum depression symptoms have higher levels of stress, more psychopathological symptoms and higher hair cortisol levels during pregnancy, compare with pregnant women who do not have postpartum depression symptoms. Specifically, in the first trimester, these differences are significant regarding hair cortisol levels and the Somatization sub-scale of the SCL-90-R; in the second trimester, pregnant women with postpartum depression symptoms obtained higher scores on the Somatization, Depression, Anxiety, and the Global Severity Index sub-scales of the SCL-90-R; in the third trimester, pregnant women with postpartum depression symptoms had higher hair cortisol levels and higher pregnancy-specific stress than pregnant women with no postpartum depression symptoms. In addition, taking into account the rising cortisol pattern during pregnancy, which consists of a gradual increase from the first to the third trimester, pregnant women who later have postpartum depression symptoms, present an inverted U-shaped cortisol pattern throughout pregnancy.

4. Pregnant women using assisted reproductive techniques (ART) have higher hair cortisol levels in the first trimester and higher levels of perceived stress in the third trimester, than pregnant women who conceived naturally. In addition, the cortisol pattern of pregnant women with ART was also an inverted U-shape.

5. First, higher maternal hair cortisol levels during the first trimester predict a higher cognitive neurodevelopment at 6 months. Second, higher maternal hair cortisol levels during the first, second, third trimester and the postpartum period predict a lower

motor neurodevelopment at 6 months. Third, elevated higher newborn hair cortisol levels predict a lower cognitive, language and gross motor neurodevelopment. Finally, higher infants´ hair cortisol levels at 6 months after birth are related to an accelerated cognitive and language neurodevelopment.

FUTURE PERSPECTIVES

In respect to the future perspectives of research derived from this Doctoral Thesis, are the following:

1. To study the implication of other alternative mechanisms to cortisol, by which psychological stress is transferred from pregnant women to fetuses, such as cytokines, tryptophan, oxygen reagents, catecholamines and microbiota of the birth canal, for a better explanation of the pathways by which maternal prenatal stress affects maternal and child health.

2. At a preconception appointment, monitor the woman's health, including an evaluation of stress levels, in order to study the influence that certain factors that occur prior to conception have on pregnancy and offspring.

3. Check if the inverted U-shape cortisol pattern is replicated in high-risk pregnant women, such as those with repeated abortions or victims of gender violence during pregnancy.

4. Evaluate the differences on stress levels in successive pregnancies and their consequences in maternal and child health.

5. Develop effective psychological therapies for the control of stress in pregnant women to reduce the levels of perceived stress, pregnancy-specific stress and cortisol

levels. Besides, the effects of the psychological therapies should be assessed on delivery and birth, and the long-term consequences on the infants´ neurodevelopment.

6. Study the influence of psychological stress during pregnancy on pregnant women´s couples, assessing its effects on pregnant women´s health, fetal development and infants´ neurodevelopment.

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ANEXO

ANEXO 1

Hair cortisol levels, psychological stress and psychopathological symtoms as predictors of postpartum depression

Caparros-Gonzalez, Romero-Gonzalez, Strivens-Vilchez, Gonzalez-Perez, Martinez-Augustin y Peralta-Ramirez (2017). Hair cortisol levels, psychological stress and psychopathological symptoms as predictors of postpartum depression. *PLoS ONE, 12*(8): e0182817

PLOS ONE

RESEARCH ARTICLE

Hair cortisol levels, psychological stress and psychopathological symptoms as predictors of postpartum depression

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Abstract

Postpartum depression affects a huge number of women and has detrimental consequences. Knowing the factors associated with postpartum depression during pregnancy can help its prevention. Although there is evidence surrounding behavioral or psychological predictors of postpartum depression, there is a lack of evidence of biological forecasters. The aim of this study was to analyze the sociodemographic, obstetric, and psychological variables along with hair cortisol levels during the first, second, and third trimesters of pregnancy that could predict postpartum depression symptoms. A sample of 44 pregnant women was assessed during 3 trimesters of pregnancy and the postpartum period using psychological questionnaires and hair cortisol levels. Participants were divided into 2 groups: a group with postpartum depression symptoms and a group with no postpartum depression symptoms. Results showed significant positive differences between groups in the first trimester regarding the Somatization subscale of the SCL-90-R ($p < .05$). In the second trimester, significant differences were found in the Somatization, Depression, Anxiety, and GSI subscales ($p < .05$). In the third trimester significant differences between both groups were found regarding pregnancy-specific stress. We found significant positive differences between groups regarding hair cortisol levels in the first and the third trimester. Hair cortisol levels could predict 21.7% of the variance of postpartum depression symptoms. In conclusion, our study provided evidence that psychopathological symptoms, pregnancy-specific stress, and hair cortisol levels can predict postpartum depression symptoms at different time-points during pregnancy. These findings can be applied in future studies and improve maternal care in clinical settings.

ea0/?vgnextoid=33881f4368aef110VgnVCM1000001034e20aRCRD. The funder's role was providing funding for data collection and analysis, and preparation of the manuscript.

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Introduction

Although mental health problems during the postpartum period often go unrecognized and untreated, the National Institute for Health and Care Excellence [1] recommends an urgent intervention due to potential detrimental effects on the newborn and the mother's life. Postpartum depression affects from 10% to 15% of women after delivery and consists of emotional liability and sometimes suicidal ideation [2]. An extensive number of studies have shown an association between postpartum depression and poor bonding between the mother and the newborn as well as lower infant neurodevelopment [3, 4]. Early detection of factors associated with postpartum depression can prevent its appearance and negative outcomes (e.g. postpartum psychosis) [5].

It has been previously reported that there is an association between sociodemographic risk factors during pregnancy (e.g. being younger than 25 years old) and postpartum depression symptoms [6, 7]. Furthermore, obstetric risk factors (e.g. previous miscarriages) have been found to be related to postpartum depression [8, 9]. Past histories of psychopathological symptoms or suffering from depression, anxiety, or stress during pregnancy have been reported as psychological variables associated with postpartum depression [10, 11]. Fig 1 shows a summary of risk factors associated with postpartum depression in previous studies.

Another risk factor associated with postpartum depression is the dysregulation of the hypothalamic-pituitary-adrenal axis which results in an increased exposure of pregnant women to cortisol [12, 13]. The hypothalamus synthesizes corticotrophin-releasing hormone (CRH) as part of the biological stress response. CRH stimulates the release of cortisol to prepare the organism to cope with stressful stimuli [14, 15]. Due in part to the presence of the placenta, the hypothalamic-pituitary-adrenal axis is deeply altered during pregnancy. The placenta, a fetal origin endocrine organ, promotes an increased release of cortisol from the adrenal gland through a dramatically increase of placental CRH over pregnancy [12]. Although cortisol negatively regulates the production of CRH from the hypothalamus, cortisol increases the release of placental CRH during pregnancy [12]. Other physical changes during pregnancy include how the pituitary gland doubles its size, and the level of production of cortisol from the adrenal gland increases [14]. Cortisol levels have been generally assessed from urine, saliva, blood, or amniotic fluid samples in pregnant women [16, 17]. Though each matrix offers information about the stress levels the women were experiencing at the time the sample was taken, these methods of assessment require an invasive technique and can be affected by situational variables or circadian rhythms [14, 18].

Alternatively, testing via hair cortisol levels is an innovative technique that offers a retrospectively chronic stress measure of the preceding 3 months, is not invasive, is not affected by the time of the day, and is easy to transport and preserve [15, 19, 20].

The association between postpartum depression and the activation of the hypothalamic-pituitary-adrenal axis during pregnancy remains a challenge. On one hand, an association between postpartum depression and high hair cortisol levels during pregnancy has been reported [21]. On the other hand, urine and blood cortisol levels were not associated with postpartum depression [2, 22]. However, certain associations have been reported between low blood cortisol levels and postpartum depression [23].

A recent review reported future research should improve the accuracy of cortisol measurements over time and use appropriate tools to assess depression [24]. More studies on risk factors associated with postpartum depression may reduce negative pregnancy outcomes [5, 25]. Predicting those variables related to postpartum depression can improve pregnancy and infant health outcomes through tailored interventions during pregnancy [4].

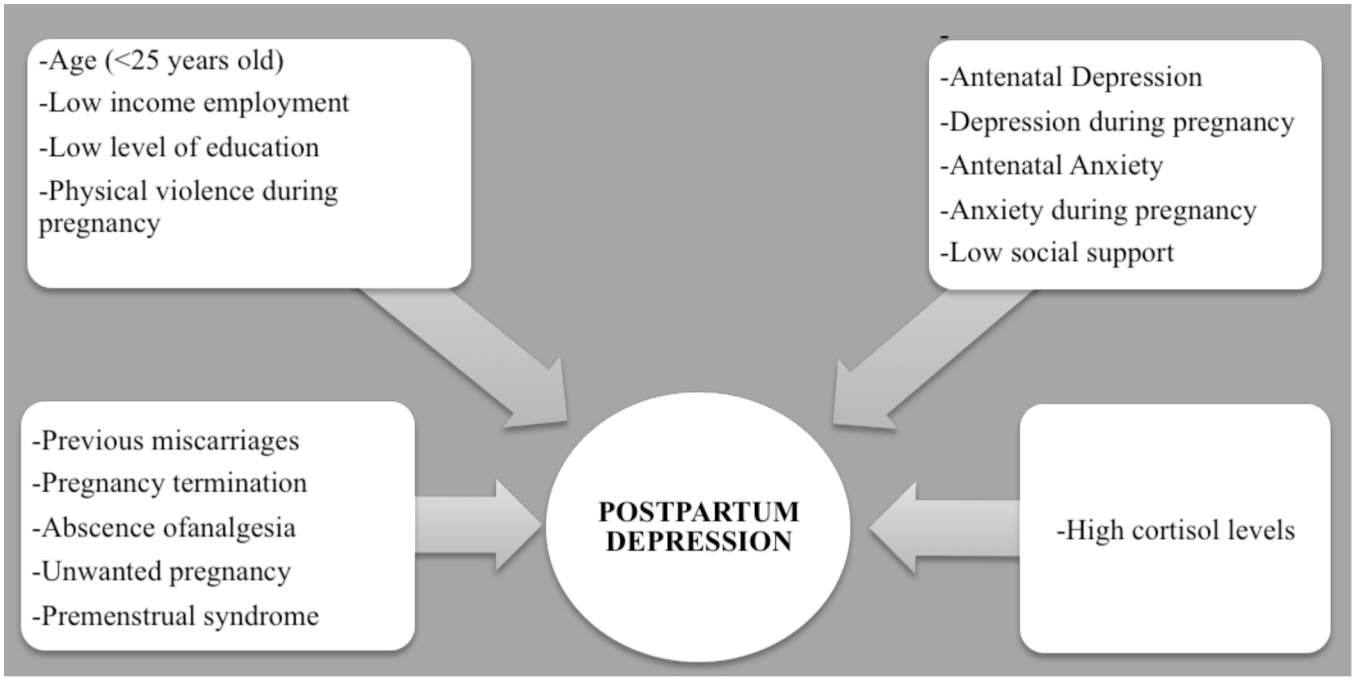

Fig 1. Summary of risk factors during pregnancy associated with postpartum depression [6–13].

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In this respect, the aim of this study was to analyze sociodemographic, obstetric, and psychological variables along with hair cortisol levels during the first, second, and third trimester of pregnancy that could predict postpartum depression symptoms.

Materials and methods

Subjects

Participants were recruited at 3 health centers and a general hospital in the South of Spain, while attending a prenatal appointment. Fifty-seven pregnant women voluntarily agreed to participate in this study. Five women had a spontaneous miscarriage during the first trimester. Seven participants were unable to continue in the study during pregnancy due to lack of time. One participant moved to another city before giving birth. Finally, a total sample of 44 pregnant women was longitudinally assessed during the first trimester (M = 12.36 weeks of gestation; SD = 3.60), the second trimester (M = 25.32 weeks of gestation; SD = 3.24), and the third trimester (M = 34.94 weeks of gestation; SD = 3.34). Assessments took place while participants were attending a prenatal appointment with their midwives (health center) and obstetricians (general hospital). Participants were followed up during a postpartum appointment with their respective health care practitioners (M = 15.79 days after birth; SD = 9.78) and divided into 2 groups: a group of women with postpartum depression symptoms (n = 16), scoring 10 or greater on the Edinburgh Postnatal Depression Scale, and a group of women with no postpartum depression symptoms (n = 28), scoring below 10 on the Edinburgh Postnatal Depression Scale. We used the cut off of 10, as it is the best cut-off score for European Spanish mothers. This cut off was indicative of highly likely to be suffering from postpartum depression [26].

Inclusion criteria was low-risk pregnant women above 18 years old with proficiency in the Spanish language. Participants were excluded if they presented any pathology before or during pregnancy. To minimize the confounding effect of risk variables, pregnancies with Cushing's disease, asthma, steroid medication, diabetes, and other conditions known to affect cortisol levels, were excluded.

This study was approved by the Human Ethics Research Committee of the University of Granada (reference 881), the Biomedical Ethics Research Committee and the Ethics Research Committee of the Health Centers, and the hospital where this study was implemented. Moreover, this study followed the guidelines of the Helsinki Declaration (AMM, 2008) and the Good Clinical Practice Directive (Directive 2005/28/EC) of the European Union. Participation was voluntary and an informed written consent document was read and signed by every participant.

Instruments

Sociodemographic and obstetrics data. Demographic information was collected my means of the Pregnancy Health Document [27] since it is the official record of the health of every pregnant woman and her newborn.

Biological measures. For the purpose of assessing the activation of the hypothalamic-pituitary-adrenal axis, hair cortisol levels were measured through hair samples proximal to the scalp with a length no greater than 3 cm (assuming an average growth rate of 1 cm/month, a 3 cm segment contains cortisol that has been deposited over approximately the last 3 months). Samples consisting of approximately 150 strands of hair were collected from the posterior vertex of the head [28]. The hair samples were wrapped in a piece of aluminum foil to protect them from light and humidity and they were stored in an envelope at room temperature. Afterwards the hair samples were sent for analysis to the Faculty of Pharmacy at the University of Granada. The hair samples were weighed and ground to a fine powder to break up the hair's protein matrix and increase the surface area for extraction using a ball mill. Cortisol from the interior of the hair shaft was extracted into HPLC-grade methanol by incubation of the sample for 72 hours at room temperature in the dark with constant inversion using a rotator. After incubation, the supernatant was evaporated until completely dry using a vacuum evaporator and the extract was reconstituted in 150 ul of phosphate buffered saline at a pH of 8.0. The reconstituted sample was immediately frozen at -20˚C for later analysis [29, 30, 31].

The cortisol in the hair sample was measured using the a salivary ELISA cortisol kit with the reagent provided following the manufacturer's directions. Using a salivary ELISA cortisol kit is a validated method to assess hair cortisol levels and is highly positive correlated with liquid chromatograph–mass spectrometry (LC–MS/MS) [31]. The sensitivity of the cortisol ELISA kit is 1.0 ng/ml as reported by the manufacturer and the cross reactivity is as follows: Prednisolone 13.6%, Corticosterone 7.6%, Deoxycosticosterone 7.2%, Progesterone 7.2%, Cortisone 6.2%, Deoxycortisol 5.6%, Pednisone 5.6% and Dexamethasone 1.6%. No cross-reaction was detected with DHEAS and Tetrahydrocortisone.

The intra- and inter-assay variations were analyzed on internal quality controls used for routine salivary cortisol measurement, measured in duplicate on eight consecutive assays. The intra-assay coefficients of variance (CV) were 2.7% at 10.7 ng/ml and 4.3% at 43.9 ng/ml. The inter-assay CVs were 4.4% and 6.3%, respectively.

Maternal perceived stress. Psychological stress was assessed by means of the 14-item Spanish version of the Perceived Stress Scale (PSS) [32] to evaluate the perception of general stress during the preceding month. Each of the 14 items scores on a 5-point Likert scale

(0 = never, 1 = almost never, 2 = once in a while, 3 = often, 4 = very often). The Cronbach's alpha reliability coefficient of the Spanish version is α = 0.81 [33].

Psychopathological symptoms. In this respect, the Spanish version of the SCL-90-R [34, 35] was used to assess psychopathological symptoms. This 90-item scale is scored using a 5-point Likert scale from 0 (never) to 4 (extremely). This instrument is used to assess 9 dimensions: Somatization, Obsession-compulsion, Interpersonal sensitivity, Depression, Anxiety, Hostility, Phobic anxiety, Paranoid ideation, and Psychoticism. The scale also has 7 extra items distributed among 3 global indexes of distress: the GSI, which measures overall psychological distress; the PSDI, which is used to measure the intensity of symptoms; and Positive Symptom Total, used to measure the number of self-reported symptoms. The Cronbach's alpha reliability coefficients of the Spanish version range are between $.67 < \alpha < .94$ [35].

Pregnancy-specific stress. For this purpose, the Spanish version of the Prenatal Distress Questionnaire (PDQ) [36, 37] was used to assess pregnancy-specific stress. It is a 12-item instrument scored on a 5-point Likert scale from 0 (none at all) to 4 (extremely) to assess specific worries and concerns pregnant women experience regarding medical problems, physical symptoms, body changes, labor, childbirth, relationships, and the baby's health. The Cronbach's alpha reliability coefficient of the Spanish version is $\alpha = .71$ [37].

Measurement of postpartum depression. The Spanish version of the Edinburgh Postnatal Depression Scale [38, 39] was used to assess the risk of postpartum depression. This 10-item instrument is scored on a 4-point Likert scale ranging from 0 (as always) to 3 (absolutely not). The best cut-off score for the Spanish version was 10/11 as highly likely to be suffering from postpartum depression [26]. A cut off of 10 was also reported to be useful to screen for a posterior psyquiatric assessment in Spanish sample. Using a cut-off of 10 identified 100% of women with major depression, resulting in a combined sensitivity of 79%, specificity of 95%. Although some authors recommend a 12/13 cut-off [38, 40, 41] even in Spanish Americans [42], the sensitivity for depression decreased to 62% and 14% of women with a major depression remained undiagnosed in European Spanish women during the postpartum period [26]. A study lead by the Postpartum Depression: Action Towards Causes and Treatment (PACT) Consortium also used a cut-off of 10 to capture a wider range of severity of postpartum depression (minor to severe) [42]. The Cronbach's alpha reliability coefficient of the Spanish version is $\alpha = .79$ [43].

Procedure

Pregnant women attending antenatal appointments at 3 public health centers in Granada and Roquetas de Mar, Spain, and a general hospital in El Ejido, Spain, (September 2015-July 2016) completed a battery of self-report questionnaires during their first, second, and third trimesters of pregnancy, and during the postpartum period. Participants received informative leaflets and stated their intention to participate at the next prenatal appointment. In our context, pregnant women attend an appointment with a General Practitioner (GP) before visiting a midwife. Clinical interviews performed by GPs reflected an absent of any decisional impairment that could affect their capacity to consent [44]. Following the written consent, hair samples were obtained by a specifically trained midwife, according to suitable guidelines and participants completed all 3 previously mentioned questionnaires (PDQ, PSS, SCL-90-R) at home during each trimester, and returned the questionnaires at their next antenatal appointment. The Depression sub-scale of the SCL-90-R was used to assess antenatal depression during pregnancy. Information regarding sociodemographic and obstetric data was obtained at the first antenatal appointment.

After delivery, participants attending a postnatal appointment ($M = 15.79$ days after birth; $SD = 9.78$) with a midwife at a public health center were assessed with the Edinburgh Postnatal Depression Scale.

Statistical analysis

Firstly, participants were divided into 2 groups: a group of women with postpartum depression symptoms (n = 16), scoring 10 or greater in Edinburgh Postnatal Depression Scale, and a group of women with no postpartum depression symptoms (n = 28), scoring below 10 in Edinburgh Postnatal Depression Scale. In order to verify both groups were equivalent in terms of the main sociodemographic, obstetric, and hair characteristics, 2-sample *t*-tests and a chi-square test were used to compare sociodemographic, obstetric, and hair characteristics between groups.

A mixed 2 × 3 analysis of ANOVA was conducted to check for statistically significant differences between both groups. The first factor includes two levels (women with postpartum depression symptoms and women with no postpartum depression symptoms) between the independent groups. The second was a repeated-measures within-subject factor during three trimesters: 1st trimester (hair cortisol, psychopathological symptoms and stress); 2nd trimester (hair cortisol, psychopathological symptoms and stress); 3rd trimester (hair cortisol, psychopathological symptoms and stress). The Greenhouse-Geisser correction was applied in the repeated-measures analyses. When a significant Group x Sampling Time interactions was found, *Bonferroni* analysis was conducted to determine the trimesters there were differences between. *Bonferroni* analysis is a conservative post hoc procedure designed to compare different combinations while controlling the overall Type I error rate (α) [45]. A follow-up Student's *t*-test was conducted to determine whether there were differences in cortisol, psychopathological symptoms and stress levels between both groups Due to the fact that hair cortisol levels did not have a normal distribution, a natural log transformation (natural log; *LN* base e) was done.

Previous studies reported subjects with dyed hair presented lower hair cortisol levels [28, 46, 47]. Participants with dyed hair were not excluded. Consequently, we controlled this factor on analysis. A previous study found that fetal sex could affect maternal cognition [48]. For this reason, a 2-sample *t*-test was conducted to test whether the sex of the fetus could influence cortisol levels.

Finally, with the purpose of testing which hair cortisol levels (first, second or third trimester) best explained the Edinburgh Postnatal Depression Scale scores, we carried out a multiple regression analyses using the introduction method. The independent variables were the hair cortisol levels on the first, second and third trimester. The dependent variables were the Edinburgh Postnatal Depression Scale scores.

Data analyses were performed using Statistical Package for Social Sciences 20.0 Mac version (SPSS, Armonk, NY). Differences were considered significant when $p < .05$.

Results

Descriptive sample characteristics

A total sample of 44 low-risk pregnant women between the ages of 24 and 39 years old ($M = 32.38$; $SD = 3.96$) participated in this study. As shown in Table 1, *t*-tests and a chi-square test indicate no differences between groups in respect to main sociodemographic data, obstetrics, and hair characteristics. Due to significant differences were found between groups on previous miscarriages ($X^2 = 4.71$, $p < .05$) and the sex of the fetus ($X^2 = 6.03$, $p < .05$), we included these variables as covariates on further analysis.

Associations of maternal postpartum depression symptoms with indicators during pregnancy

We examined the associations of maternal postpartum depression symptoms with pregnancy-specific stress, perceived stress, and psychopathological symptoms during the first, second,

Table 1. Differences in sociodemographic, obstetrics variables and depression symptomatology between women with postpartum depression and without postpartum depression.

			No depression X(SD)/%	Depression X(SD)/%	Test a	*p*
Socio-demographic variables						
Age			32.11(4.05)	32.94(3.62)	-.67	*.50*
Nationality	Spanish		24(85.70%)	4(25.0%)	7.86	.37
	Immigrant		4(14.3.60%)	12(75.0%)		
Marital status	Single/divorced/widow		10(35.7%)	8(50%)	.86	.35
	Married/cohabitant		18(64.3%)	8(50%)		
Employment situation	Working		23(82.1%)	11(68.8%)	1.04	.31
	Unemployed		5(17.9%)	5(31.2%)		
Occupation	Health		8(28.6.%)	5(31.2%)	-2.57	.79
	Education		7(25.0%)	2(12.5%)		
	Other		3(46.4%)	9(36.2%)		
Level of education	Secondary school		3(42.85%)	4(57.15%)	3.89	.14
	University		23(69.70%)	10(30.30%)		
Sport	Yes		19(67.9%)	7(43.8%)	2.45	.12
	No		9(32.1%)	9(56.2%)		
Pet	Yes		8(28.6%)	8(50%)	2.02	.15
	No		20(71.4%)	8(50%)		
Hair aspect	Nature		13(46.4%)	6(37.5%)	0.33	.56
	Dyed		15(53.6%)	10(62.5%)		
Obstetric information						
Primiparous	Yes		20(71.4%)	8(50%)	2.02	.15
	No		8(28.6%)	8(50%)		
Wanted pregnancy	Yes		24(85.7%)	13(81.2%)	1.52	.69
	No		4(14.3%)	3(18.8%)		
Pregnancy method	Spontaneous		22(78.6%)	13(81.2%)	0.45	.83
	Fertility treatment		6(21.4%)	3(18.8%)	4.71	.03*
Previous miscarriages	Yes		4(14.3%)	7(43.8%)	2.77	.78
Labor and delivery	No		24(85.7%)	9(56.2%)		
	Eutocic		20(74.1%)	9(56.2%)		
	Dystocic		4(14.8%)	2(15.4%)		
	C-section		3(11.1%)	1(7.7%)		
Pain relief in labor	None		2(8.0%)	3(23.1%)	4.11	.13
	Epidural		18(72.0%)	10(76.9%)		
	Warm bath		5(20.0%)	0(0%)		
Sex of the fetus	Female		7(25%)	10(62.5%)	6.03	.01*
	Male		21(75%)	6(37.5%)		
Depression						
Antenatal depression	Depression subscale clinical scores (> 70)	1st trimester	4(36,36%)	1 (10%)	0.65	.41
		2nd trimester	2(18,18%)	4(40%)	2.75	.09
		3rd trimester	5(45,45%)	5(50%)	1.04	.30
Postnatal depression	EPDS	< 10 scores	28(100%)	3(18.8%)	32.29	.001*
		10–12 scores	0(0%)	5(31.2%)		
		>12 scores	0(0%)	8(50%)		

Note. Significant at the * = p $\leq$, 05.

[a] T-test of students used to quantitative variables and Chi-square test to categorical variables. Sport is presented to inform whether participants practiced or did not practice any regular physical activity during pregnancy.

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Table 2. Mean differences on stress and psychopathological symptoms with interaction effects between groups*trimesters.

Trimester	Questionnaires	Subscalesa	No depression X(SD)	DepressionX(SD)	*t*	*p*
Trimester 1	PDQ		13.96(6.44)	15.81(5.64)	*-1.79*	*.08*
	SCL-90-R	SOMS	57.14(26.29)	70.66(19.33)	*-2.70*	*.01**
		DEP	43.04(23.13)	44.28(18.79)	*-.89*	*.37*
		ANX	54.63(28.19)	60.01(17.95)	*-.74*	*.46*
		GSI	53.66(25.24)	57.59(19.83)	*-.53*	*.59*
Trimester 2	PDQ		12.57(5.54)	14.25(4.41)	*-1.03*	*.31*
	SCL-90-R	SOMS	41.90(17.78)	55.96(21.39)	*-2.34*	*.02**
		DEP	30.04(18.71)	47.33(23.60)	*-2.67*	*.01**
		ANX	40.41(21.06)	62.72(23.78)	*-3.22*	*.002**
		GSI	41.22(21.78)	58.22(25.16)	*-2.38*	*.02**
Trimester 3	PDQ		11.25(4.36)	14.75(3.78)	*-2.67*	*.01**
	SCL-90-R	SOMS	64.09(29.68)	74.42(27.29)	*-2.01*	*.051*
		DEP	44.24(27.15)	59.60(22.79)	*-1.90*	*.06*
		ANX	55.50(26.58)	66.43(25.89)	*-1.32*	*.19*
		GSI	55.63(29.83)	68.44(27.21)	*-1.41*	*.16*

Note.

* Significant at the $p \leq .02$ level

[a]PDQ = Prenatal Distress Questionnaire; SCL-90-R = Symptom CheckList 90 Revised; SOMS = Somatisation; DEP = Depression; ANX = Anxiety; GSI = Global Severity Index.

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and third trimester. The group with postpartum depression symptoms had higher scorers on the Edinburgh Postnatal Depression Scale ($M = 13.50$; range = 10–24) than the group with no postpartum depression symptoms ($M = 4.75$; range = 2–8), $t = 9.92$, $p < .001$. Five participants scored in the range of 10–12 on the Edinburgh Postnatal Depression Scale (see Table 1).

Regarding the between group analysis, an interaction effect was found between women with postpartum depression symptoms and women with no postpartum depression symptoms on pregnancy-specific stress throughout the three trimesters, $F(1, 41) = 4.08$, $p = .05$, which remained significant when including previous miscarriages, sex of the fetus and antenatal depression as covariates, $F(1, 37) = 5.67$, $p < .05$). *Bonferroni post hoc* analysis on the pregnancy-specific stress revealed no significant differences during the first, second or third trimester on the any of the two groups. Although pregnancy-specific stress levels were higher among participants with postpartum depression symptoms at the first, second, and third trimester of pregnancy, significant differences between both groups were found during the third trimester regarding pregnancy-specific stress ($t = -2.67$, $p = .01$) (see Table 2).

In respect to perceived stress, no significant interaction effect was found between both groups.

Regarding psychopathological symptoms, the group with postpartum depression symptoms scored higher in every single SCL-90-R subscales during the first, second, and third trimester of pregnancy. This group had clinical scoring (score above 70) in the Somatization, Phobic anxiety, and Psychoticism subscales during the first trimester; Phobic anxiety sub-scale during the second and third trimester; Somatization, Obsessive-compulsive, Paranoid ideation and Psychoticism subscales at the third trimester (see Fig 2).

The number of participants with clinical scores (above 70) on the Depression subscale of the SCL-90-R for both groups in the first, second and third trimester are displayed in Table 1.

Regarding the psychopathological symptoms subscales, an interaction effect between groups on the Somatization subscale, $F(1, 42) = 6.95$, $p < .05$, which remained significant

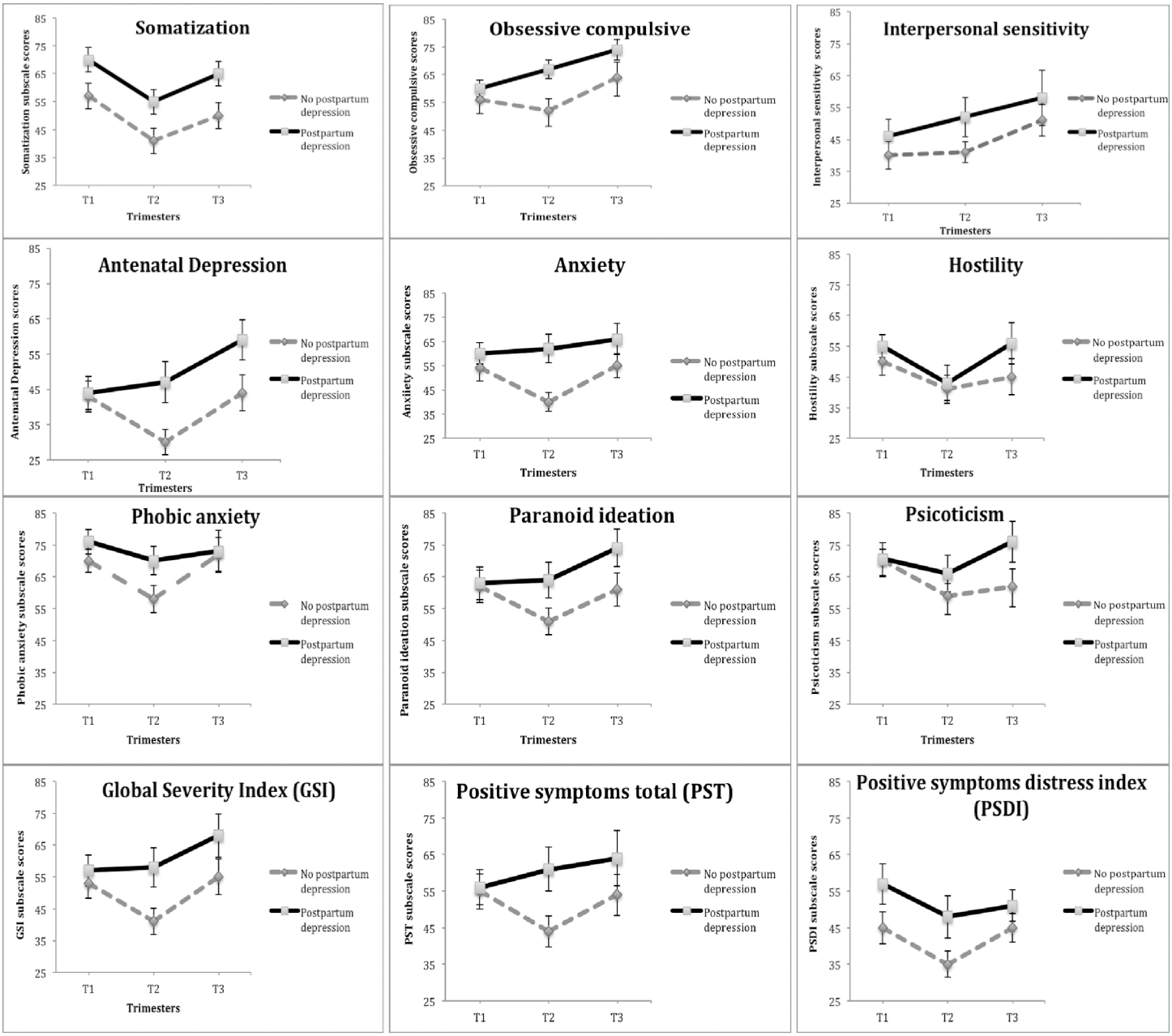

Fig 2. SCL-90-R scores throughout pregnancy in both groups. *Note*. SOMS = Somatization; OBS = Obsessive-compulsive; SEN = Interpersonal sensitivity; DEP = Depression; ANX = Anxiety; HOS = Hostility; PHOB = Phobic anxiety; PAR = Paranoid ideation; PSI = Psychoticism; GSI = Global severity index; PST = Positive symptoms total; PSDI = Positive symptoms distress index; PPD = Postpartum depression; NO PPD = No postpartum depression.

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when controlling for previous miscarriages, sex of the fetus and antenatal depression in analysis, $F(1, 37) = 8.54$, $p < .05$. Several repeated measures ANOVA revealed interaction of group*trimester on the Depression subscale, $F(1, 42) = 3.14$, $p <. 05$, which remained partially significant when including previous miscarriages, sex of the fetus and antenatal depression as covariates, $F(1, 37) = 2.99$, $p = .059$; Anxiety subscale, $F(1, 42) = 4.21$, $p <. 05$, even when controlling for previous miscarriages, sex of the fetus and antenatal depression in analysis, $F(1, 37) = 8.79$, $p <. 05$, and the GSI subscale after including previous miscarriages, sex of the fetus and antenatal

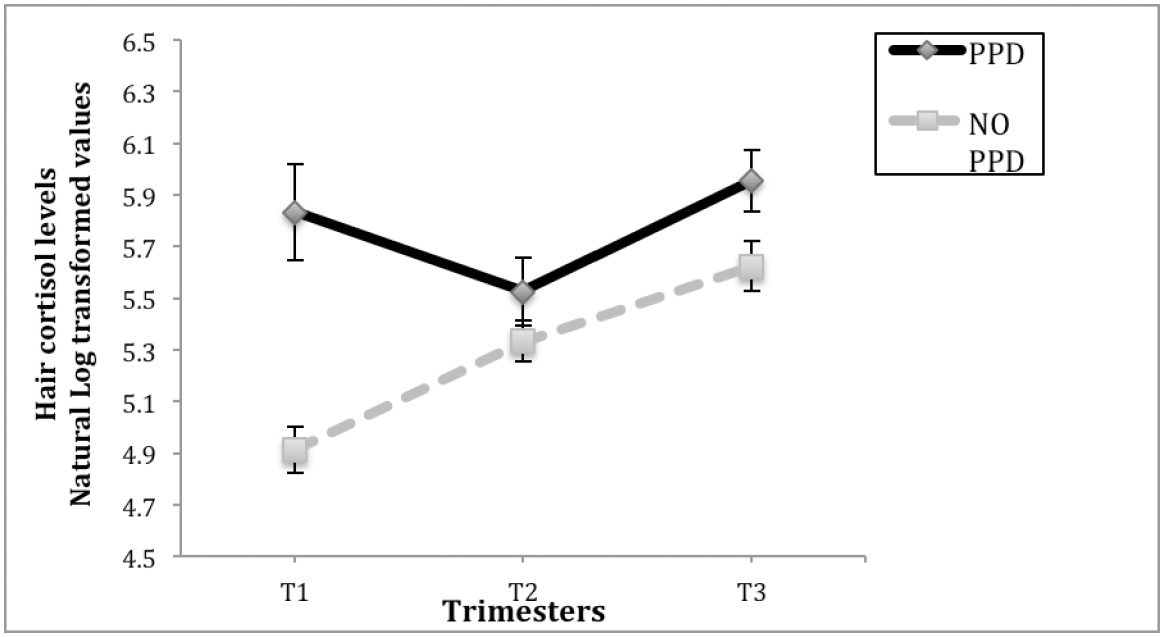

Fig 3. Hair cortisol levels differences (pg/mg) in each trimester between women with and without postpartum depression symptoms. *Note.* *Significant at the $p \leq .05$ level.

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depression as covariates, $F(1, 37) = 5.10$, $p < .05$. Scales showing significant interaction effect group*trimester mean differences on psychopathological symptoms are displayed in Table 2. Regarding the postpartum depression symptoms group, the pairwise comparisons in for the main effect of trimesters using *Bonferroni* analysis showed significant differences on the Somatization subscale between trimester 1 and 2 ($p <. 05$), and trimester 2 and 3 ($p <. 05$); regarding the Depression subscale significant differences between trimester 2 and 3 ($p <. 05$); in respect to the GSI subscale significant differences between the trimester 2 and 3 ($p <. 05$). No significant differences were found in the no postpartum depression group throughout pregnancy.

Significant differences between both groups were found during the first trimester regarding the Somatization subscale ($t = -2.70$, $p = .01$); during the second trimester regarding the Somatization subscale ($t = -2.34$, $p = .02$), the Depression subscale ($t = -2.67$, $p = .01$), the Anxiety subscale ($t = -3.22$, $p = .002$) and the GSI global index ($t = -2.38$, $p = .02$). As shown in Table 2 psychopathological measures are higher within the group with postpartum depression symptoms. No significant differences were found between both groups during the third trimester.

Association between hair cortisol levels with postpartum depression symptoms

We examined the associations between hair cortisol levels during the first, second, and third trimester with postpartum depression symptoms. We found the group with postpartum depression symptoms obtained higher hair cortisol levels during the first, second, and third trimesters. A repeated measures ANOVA revealed a significant interaction between trimester*postpartum depression symptoms group on hair cortisol levels, $F(1, 41) = 7.96$; $p < .001$, which remained significant when including previous miscarriages, antenatal depression, sex of the fetus and dyed hair in the model, $F(1, 36) = 7.78$; $p < .001$.

In the group with postpartum depression symptoms, the pairwise comparisons for the main effect of trimesters using *Bonferroni* analysis showed a significant difference between

Table 3. Maternal hair cortisol levels and sex of the fetus.

Trimesters	Female fetus *X*(*SD*)	Male fetus *X*(*SD*)	*t*	*p*
1st trimester	5.49(.94)	5.08(.56)	-1.81	.07
2nd trimester	5.30(.55)	5.46(.40)	1.11	.27
3rd trimester	5.67(.54)	5.38(.52)	.69	.49

Note. Hair cortisol levels are log transformed values

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trimesters 1 and 3 ($p < .01$), and a significant difference between trimesters 2 and 3 ($p < .001$). No significant differences were found in this respect in the group with no postpartum depression symptoms.

A 2 sample *t*-test revealed significant differences between groups regarding hair cortisol levels in the first trimester ($t = -4.77$; $p < .001$) and the third trimester ($t = -2.06$; $p \leq .045$). No significant differences were found between groups in the second trimester (see Fig 3).

A linear regression was carried out to test whether the mothers' hair cortisol levels could predict postpartum depression symptoms. Results of the regression revealed that hair cortisol levels could predict 21.7% of the variance of postpartum depression symptoms [$R^2 = .21$, ($F = 3.703$, $p < .05$)]. More precisely, hair cortisol at the first trimester ($ß = 0.32$, $p < .05$) and the third trimester ($ß = 0.32$, $p < .05$) significantly predicted the Edinburgh Postpartum Depression Scale scores.

A 2-sample *t*-tests was used to assess whether the sex of the fetus could influence the release of cortisol during pregnancy. The independent variable was the sex of the fetus and the dependent variable were hair cortisol levels during the first, second and third trimester. No significant differences were found ($p > .05$) (see Table 3).

As shown in Fig 3, hair cortisol levels increased from the first to the third trimester in the group with no postpartum depression symptoms, getting the higher hair cortisol levels at the third trimester. Nonetheless, in the group with postpartum depression symptoms, hair cortisol levels decreased from the first to the second trimester, and increased in the third trimester.

Discussion

The aim of this study was to study the sociodemographic, obstetric, psychological, and hormonal variables that may predict postpartum depression symptoms. For this purpose, we compared a group of pregnant women with postpartum depression symptoms with a group of women with no postpartum depression. Sociodemographic variables have been informed to be relevant when assessing the psychological wellbeing of women in the postpartum period [49–51]. Significant differences were found between groups in respect to previous miscarriages and the sex of the fetus. Therefore, these variables were included as covariates in further analysis. The group with postpartum depression symptoms had higher pregnancy specific stress, perceived stress, psychopathological symptoms and hair cortisol levels during the three trimesters of pregnancy.

However, significant differences were found in this study in respect to psychopathological symptoms during the first and second trimester, and in respect to pregnancy-specific stress during the third trimester between both groups. More precisely, during the first trimester, significant differences were found in respect to the Somatization SCL-90-R subscale. During the second trimester, significant differences were found in respect to Somatization, Obsessive-compulsive, Depression, Anxiety, IGS, and Positive Symptom Total subscales. Accordingly, it has been reported high correlations between the Anxiety and Somatization SCL-90-R subscales during the first and second trimester and the Edinburgh Postnatal Depression Scale [52]. In

this respect, recent studies reported the presence of psychopathological symptoms through the SCL-90-R during pregnancy to be related with postpartum depression [53, 54]. More specifically, depression, anxiety, and stress during pregnancy have been related with negative pregnancy outcomes, including postpartum depression [55, 56]. Psychological stress during pregnancy has been related to postpartum depression [57]. We could not find any significant differences between groups regarding perceived stress at any time point. These findings do not agree with those reported by Scheyer and Urizar [58] who reported significant association between perceived stress during the 3 trimesters of gestation and postpartum depression. We hypothesize this could be due to the PSS being a general stress measure and that pregnancy-specific measures may likely improve pregnant women's psychological assessments. In this regard, pregnancy-specific stress was significantly different between groups during the second trimester. The fact that although both stress measures (PDQ and PSS) have been widely used when assessing stress levels during pregnancy, is noteworthy that the PDQ offers the opportunity to assess specific worries and concerns related to pregnancy and therefore is a more consistent pregnancy-specific stress measure and a better predictor of negative pregnancy outcomes [55, 59].

Biochemical measures as those provided through hair cortisol levels inform of chronic stress levels [29]. According to our results, hair cortisol levels in the group with postpartum depression symptoms descended from the first to the second trimester and ascended from the the second to the third trimester, resembling an U shape when plotted on a graph. This is the first study to report hair cortisol levels throughout pregancy in a group of women with postpartum depression sypmtoms. Although an upward course regarding cortisol levels throughout pregnancy has been previously reported [60], these findings were reported in respect to pregnant women with no postpartum depression symptoms.

In the present study, hair cortisol levels were higher in the group with postpartum depression symptoms compared to the group with no postpartum depression symptoms in the 3 trimesters and were significantly different in the first and the third trimester. A previous study reported that cortisol levels during the second trimester, but not in the third trimester, were higher and significantly different in those women with postpartum depression [61]. Our findings in this regard support the fact that high stress levels during pregnancy are related to postpartum depressive symptoms [62, 63]. In fact, hair cortisol levels predicted postpartum depression symptoms in our study. More precisely, hair cortisol at the first trimester and the third trimester could predict postpartum depression symptoms. Our results do not support other studies reporting low cortisol levels during pregnancy with postpartum depression [24, 58]. We hypothesize these differences may be due to the fact that previous studies have used acute stress biological measures (e.g. urine cortisol levels), and in our study hair cortisol samples were used which reflects levels of chronic stress within the last 3 months [15, 19, 20]. Our study has several strenghts. First, the longitudinal design of our study offered an unique possibility of observing the range of psychological symptoms, including prenatal stress and cortisol levels throughout pregnancy in both groups. Second, we have used the PDQ, a pregnancy-specific stress measure which shows a consistent relation with negative pregnancy outcomes [55]. More importanltly, the innovative assessment of stress through hair cortisol levels gives evidence of chronic stress through a single measurement [19]. Several studies have shown the benefits of knowing the risk factors involved in postpartum depression to improve maternal and infant outcomes [2, 4]. Finnally, we considered the influence of a variety of sociodemographic and obstetric variables that have been previously associated with pospartum depression [6–9]. It is important to note that this study focused on assessing the association between sociodemographic and obstetric variables, psychological stress, psychopathological symptoms, and biological stress measured through hair cortisol levels, with postpartum depression

symptoms. The percentage of women in the postpartum depression group (36%) in our study is quite high with respect to previous studies reporting percentages of 10–15% [2]. In our context, it exits a lack of clinical screening and prevention related to postpartum depression, which might lead to greater numbers of women with postpartum depression symptoms. Nevertheless, the prevalence of postpartum depression can vary between studies, due to the different criteria used to define postpartum depression [13].

Although participants were longitudinally and prospectively assessed throughout pregnancy and the postpartum period, a limitation of the present study is the relatively small sample size, which should be considered for the interpretation of the data. A further potential limitation was that postpartum depression symptoms were only assessed at a single time point. A second follow-up after delivery would have offered the possibility to evaluate the participants' long-term psychological wellbeing and study possible associations with health variables during pregnancy.

In summary, according to our findings, high levels of maternal stress during pregnancy are associated with postpartum depression symptoms. Psychopathological symptoms in the first and second trimester, high pregnancy-specific stress in the second trimester, and high hair cortisol levels in the first and the third trimester were associated with postpartum depression symptoms. Since hair cortisol levels reflect stress levels during the previous 3 months preceding the time the sample was taken [20], our findings reflect that the preconception period, and the second trimester of pregnancy, are particularly sensitive periods related to postpartum depression symptoms. Our findings do not agree with a previous study reflecting changes in the HPA related with postpartum depression occur during the postpartum period [64] instead, we found changes in the HPA may begin during the preconception period. In line with our findings, a recent study reported higher cortisol levels during pregnancy were related to postpartum depression symptoms [13]. In this respect, prenatal effective stress screening interventions should be used widely during this time to reduce adverse outcomes [65]. These findings are of clinical and research importance since they show the health variables that can predict postpartum depression symptoms among pregnant women. Assessing psychological health in the perinatal period can aid practitioners in making adequate decisions and provide valuable data on maternity care [66].

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