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Tesis Doctoral

ANÁLISIS Y GENERACIÓN DE EVIDENCIAS EN REPRODUCCIÓN MÉDICAMENTE ASISTIDA

Doctoranda

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"Lo que importa verdaderamente en la vida no son los objetivos que nos marcamos, sino los caminos que seguimos para lograrlos"

Peter Bamm

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1. RESUMEN

Antecedentes: El acceso a la mejor evidencia científica de reproducción médicamente asistida (RMA) en la práctica clínica es fundamental, ya que la RMA es responsable del 0,2 al 4,3% de nacimientos por año en el mundo, y existe un uso de terminologías y elección de procedimientos controvertidos en este campo.

Objetivo: Analizar y generar evidencias sobre diversos aspectos fundamentales de la reproducción médicamente asistida.

Métodos: Se realizaron tres trabajos de investigación: 1) un estudio de evaluación de la calidad de las guías de práctica clínica sobre técnicas de reproducción asistida con el instrumento *Appraisal of Guidelines Research and Evaluation* (AGREE II); 2) una revisión sistemática Cochrane sobre los vasodilatadores en mujeres sometidas a tratamientos de fertilidad y 3) un estudio caso control de los defectos congénitos en mujeres sometidas a RMA. Además, se llevó a cabo un cuarto estudio de búsqueda manual de ensayos clínicos en revistas españolas de Obstetricia y Ginecología con un análisis de los ensayos de RMA identificados.

Resultados: 1) La calidad de las guías de práctica clínica publicadas en inglés sobre técnicas de reproducción asistida no fue óptima; solo tres de las 14 evaluadas fueron recomendables para su uso. Las calificaciones obtenidas en cada dominio del instrumento AGREE II oscilaron entre el 37% y 80%. El acuerdo entre los revisores fue catalogado como muy bueno. 2) La revisión sistemática sobre los vasodilatadores incluyó 10 ensayos clínicos con un total de 797 mujeres.

La mayoría de los estudios fueron considerados como de riesgo de sesgo incierto. Evidencias de baja calidad demostraron un incremento de la tasa de embarazo clínico, pero no se disponía de suficientes para evaluar ni la tasa de recién nacidos vivos ni la incidencia de efectos secundarios de los vasodilatadores. 3) El estudio caso control reveló un aumento de los defectos congénitos en mujeres sometidas a RMA. En el análisis estratificado se observó que las técnicas de reproducción asistida estaban asociadas a defectos congénitos de diferentes órganos y sistemas; por otra parte, la inseminación lo está solamente al incremento de los defectos del aparato genitourinario, y la inducción de la ovulación únicamente a los de la pared y del aparato gastrointestinal. 4) En la búsqueda manual se encontraron 235 ensayos clínicos publicados, de los cuales 29 (12.34%) trataban de la RMA. En estos últimos el riesgo de sesgo era predominantemente alto.

Conclusiones: Existen diferentes intervenciones en RMA, las cuales están parcialmente estandarizadas en guías de práctica clínica. En la evaluación de las guías sobre técnicas de reproducción asistida se concluyó que la calidad de estas no era óptima. Entre las intervenciones no estandarizadas se detectaron ensayos clínicos sobre vasodilatadores en mujeres sometidas a RMA, cuya calidad era de moderada a baja. En la revisión sistemática de estos ensayos se hallaron evidencias que sugerían que los vasodilatadores aumentaban la tasa de embarazo clínico, pero no se encontraron suficientes evidencias para evaluar ni la tasa de nacidos vivos ni la de efectos secundarios. En relación con los efectos secundarios derivados de la RMA, se observó un aumento de los defectos congénitos en todos los aparatos o sistemas como resultado del uso de técnicas

de reproducción asistida, a excepción de la inseminación y la inducción de la ovulación que elevan los defectos de un solo aparato. La búsqueda manual de ensayos clínicos publicados en revistas españolas de Ginecología y Obstetricia permitió detectar un gran número de evidencias. Se sugiere mejorar la calidad de las guías sobre técnicas de reproducción asistida, desarrollar ensayos clínicos de calidad en este campo y ahondar en la investigación de los defectos congénitos en mujeres sometidas a RMA buscando los factores que subyacen.

2. ABSTRACT

Background: Access to the best scientific evidence on the clinical practice of medically assisted reproduction (MAR) is crucial, considering that the RMA accounts for 0.2 to 4.3% of the births per year worldwide and that there is a very varied and controversial use of the terminology and selection procedures within this field.

Objective: To analyze and generate evidence on medically assisted reproduction.

Methods: Three research projects were conducted: 1) an evaluation study of the quality of the clinical practice guidelines for assisted reproduction techniques with the *Appraisal of Guidelines Research and Evaluation* (AGREE II); 2) a Cochrane systematic review of clinical trials of vasodilators in women on fertility treatments, and 3) a case control study on birth defects in women undergoing RMA. Additionally, a manual search study on clinical trials in Spanish magazines of Gynecology-Obstetrics and an analysis of the identified clinical trials of RMA were carried through.

Results: 1) The quality of the clinical practice guidelines about assisted reproductive technology published in English was not optimal; only three of the 14 evaluated guides were recommended for use. The scores for each domain ranged between 37% and 80%. The agreement between reviewers was very good. 2) The systematic review of vasodilators included 10 clinical trials involving a total of 797 women. Most of the studies were considered of uncertain risk of bias. Low quality

evidence showed an increased rate of clinical pregnancy. However, there was insufficient evidence to assess the rate of live births, and the incidence of side effects of vasodilators. 3) The case control study revealed an increment in birth defects in women undergoing RMA. It was found in the stratified analysis that assisted reproduction techniques are associated with birth defects of different organs and systems, whereas insemination and ovulation induction are only associated with an increase in defects of the genitourinary system and the wall and gastrointestinal tract respectively. 4) In a further manual search study a total of 235 trials were identified, 29 of which (12.34%) were about RMA and showed a predominantly high risk of bias.

Conclusions: There are different interventions in medically assisted reproduction partially standardized in clinical practice guidelines. The quality of the guidelines for assisted reproduction techniques was not optimal, according to the assessment study carried out; only three of them were rated as "recommendable". Clinical trials of vasodilators in women undergoing RMA of moderate to low quality were identified in not standardized interventions. The systematic review of these trials showed that there was enough evidence that suggested that vasodilators raised the clinical pregnancy rate, but there was insufficient evidence to assess both the live births or of side effects rate. Regarding the side effects whereas an increase in birth defects of all body organs and systems due to using assisted reproductive techniques was observed, insemination and ovulation induction caused an increase in the defects of only one particular system each. The manual search of clinical trials in Spanish journals about Gynecology and Obstetrics allowed for

finding a large number of evidence. However, the number of trials per year was low and those about RMA revealed a high risk of bias. It would therefore be suggested to improve the quality of both the guidelines, and clinical trials and do more research into the underlying causes of birth defects in women subjected to RMA.

3. INTRODUCCIÓN

Actualmente, existen controversias en la elección de procedimientos y uso de términos no estandarizado en el ámbito de la reproducción médicamente asistida (RMA),[1] aun cuando gracias a esta se ha logrado la concepción de millones de niños a nivel mundial. En este contexto, es fundamental analizar y generar evidencias de calidad en la práctica clínica.

3.1. Análisis y generación de evidencias

Tanto el análisis como la generación de información científica requieren la aplicación de los conceptos y las herramientas de la medicina basada en evidencias (MBE), la cual es definida como:

> *"El uso consciente, explícito y juicioso de la mejor evidencia disponible para tomar decisiones sobre el cuidado de los pacientes individuales".* [2]

La MBE es un término acuñado por Guyatt y definido por Sackett en la década de los 90; desde entonces ha tenido defensores y detractores.[2-5] Con todo, como dice Fiona Godlee, a pesar de todos sus defectos, carencias y perversiones, aún sigue siendo el instrumento menos malo de los sistemas existentes para tomar decisiones clínicas.[6] Asimismo, Spence afirma que "la MBE alimenta el sobrediagnóstico y el sobretratamiento", y que "esta práctica puede mejorar si la investigación se centra en lo que no sabemos y se estudia la historia natural de la enfermedad, las intervenciones basadas en la investigación no farmacológica, entre otras, promoviendo el escepticismo intelectual".[6] Llama la atención que

Spencer use como fundamento de su análisis un artículo que en ningún momento menciona que la MBE sea responsable del sobrediagnóstico o del sobretratamiento.[7]

La MBE promueve una mejor interpretación del conocimiento disponible y facilita la comunicación entre investigadores y clínicos, lo que, en última instancia, se traduce en una mejor atención al paciente. Por lo tanto, los profesionales de la salud necesitan tener acceso a la mejor evidencia disponible para el diagnóstico y el tratamiento de los pacientes. Estas evidencias deben presentarse en forma de guías de práctica clínica, las cuales deben ser actualizadas y fiables.[8] Las guías en las que podemos confiar deben hacer recomendaciones basadas en revisiones sistemáticas de la literatura e incluir una evaluación relevante y equilibrada de las ventajas y desventajas de las opciones de tratamiento.[9-11] Por otro lado, la calidad de estas revisiones depende de los estudios primarios que deben ser debidamente valorados e identificados.[12,13] Además, se debe considerar que las evidencias tanto de los ensayos clínicos como de los estudios observacionales pueden ser de calidad alta, moderada o baja, según sus características.[10] En suma, los tres grandes instrumentos de la MBE son: las guías de práctica clínica, las revisiones sistemáticas y los estudios primarios de calidad.[8-13]

3.1.1 Guías de práctica clínica

Se dice que todos, y en particular los responsables de los sistemas de atención sanitaria, deben promover la integración de la investigación en la práctica clínica

diaria.[14] Por ello, se plantea la investigación traslacional para reducir la brecha entre ambos elementos en el cuidado de la salud, y mejorar así la prestación de la atención a los pacientes.[15] En este sentido, las guías sirven de instrumento para reducir dicha brecha.

Las guías de práctica clínica (GPC) están constituidas por un conjunto de recomendaciones desarrolladas de forma sistemática para ayudar a profesionales y pacientes en la toma de decisiones sobre la atención sanitaria más apropiada, y en la selección de las opciones diagnósticas o terapéuticas más adecuadas para un problema de salud o una enfermedad clínica específica.[16] Las GPC pueden acortar la brecha entre la investigación y la práctica clínica, y facilitar a los profesionales sanitarios y pacientes la selección de evidencias para encontrar la atención más adecuada, segura y rentable.[17-20]

Sin embargo, la calidad es muy variable; hay desde guías recomendables, como las elaboradas por instituciones claves a nivel internacional por su amplia experiencia en el desarrollo de GPC basadas en la evidencia científica como son el *Scottish Intercollegiate Guidelines Network* SIGN) o el *National Institute for Clinical Excellence* (NICE) del Reino Unido, hasta guías que no satisfacen estándares básicos.[19-22]

El Instrumento AGREE (*Appraisal of Guidelines Research and Evaluation)* para la evaluación de las GPC se desarrolló para examinar la variabilidad en la calidad de las guías. Esta herramienta evalúa el rigor metodológico y la transparencia con la cual se elabora una guía mediante 23 criterios o ítems repartidos en seis áreas o

dominios. Además, presenta los requisitos esenciales que ha de cumplir una GPC. La primera versión del Instrumento, el AGREE I, se publicó en el año 2003 y la segunda, el AGREE II en el 2009, la cual contiene cambios respecto del original con el objeto de mejorarlo y refinarlo. El *AGREE Next Steps Consortium* es el grupo investigador responsable de esta nueva versión.[24,25]

3.1.2 Revisiones sistemáticas

Las Revisiones Sistemáticas (RS) tienen por objeto realizar la identificación sistemática, la evaluación crítica y el resumen de todos los estudios relevantes sobre un tema específico.[26,27] Las RS y/o metanálisis de ensayos controlados aleatorios son cada vez más utilizados por los profesionales sanitarios, los políticos, los responsables de presupuestos y los pacientes para informar acerca de la efectividad de las intervenciones, así como para apoyar o facilitar la toma decisiones terapéuticas. Es decir, la RS aportan la información esencial para la toma de decisiones terapéuticas.[27] La credibilidad de las RS depende de si la revisión abordó una cuestión clínica sensible, si incluyó una búsqueda bibliográfica exhaustiva, de la reproducibilidad de la selección y evaluación de los estudios, y de si presenta los resultados de una manera útil. Para que las revisiones sean lo suficientemente verosímiles, los médicos deben decidir sobre el grado de confianza de las estimaciones de las pruebas (calidad de la evidencia), la cual depende del riesgo de sesgo en el cuerpo de la evidencia, de la precisión y la consistencia de los resultados, de si los resultados se aplican directamente al paciente de interés, y de la probabilidad de sesgo en la información.[26-28]

La toma de decisiones requiere la comprensión de las estimaciones sobre la magnitud de los efectos beneficiosos y perjudiciales, y la confianza en estas estimaciones. El objetivo y el enfoque metodológico transparente de las RS están orientados a minimizar el sesgo.[28] La revisión de mayor rendimiento es la cuantitativa, que analiza datos mensurables (metanálisis). Consiste en la aplicación de métodos estadísticos para resumir los resultados de estudios independientes.[29] A diferencia del caso de los estudios individuales incluidos en una revisión, el metanálisis combina la información de todos los estudios relevantes y puede obtener estimaciones más precisas de los efectos sobre la atención sanitaria. Además, permite examinar las diferencias entre los estudios.[30-32] La Colaboración Cochrane, una de las entidades que produce más RS de calidad, enfatiza que:

> "*Las revisiones sistemáticas tienen como objetivo reunir toda evidencia que se corresponda con unos criterios de elegibilidad establecidos previamente, con el fin de orientar un tema específico de investigación".*
>
> *"El propósito de las revisiones sistemáticas es minimizar sesgos mediante la aplicación de métodos sistemáticos y explícitos".*[13]

Asimismo, sugiere que para poder interpretar, comprender y evaluar una RS, los lectores deben ser capaces de contestar ocho preguntas importantes durante la lectura:[30]

1. ¿Se centró la revisión en una pregunta clínica?

2. ¿Es posible que se hayan omitido estudios importantes o relevantes?

3. ¿Se usaron los criterios de inclusión para seleccionar los artículos apropiados?

4. ¿Se evaluó la validez de los estudios incluidos?

5. ¿Fueron reproducibles los cálculos de los estudios?

6. ¿Fueron parecidos los resultados de un estudio al otro?

7. ¿Cuáles fueron los resultados globales de los estudios y cuán precisos fueron?

8. ¿Ayudarán los resultados al cuidado de los pacientes?

Actualmente, las revisiones Cochrane son elaboradas por más de 31 000 participantes en más de 100 países[33] y se han convertido en valiosas herramientas para la RMA, ya que permiten a los médicos y pacientes tomar decisiones en el ámbito de la atención sanitaria basadas en los estudios disponibles de mayor calidad, fundamentalmente ensayos clínicos aleatorizados.[34]

3.1.3 Estudios primarios

Los estudios primarios se clasifican en observacionales y experimentales. A estos últimos se les conoce comúnmente como Ensayo Clínico Aleatorizado (ECA) y proporcionan evidencia del más alto nivel para responder preguntas terapéuticas.[35] Por otro lado, los estudios observacionales son imprescindibles para responder preguntas etiológicas.

Ensayos clínicos aleatorizados

La validez interna del ECA deriva, en gran parte, de los efectos de la aleatorización y de un entorno de investigación cuidadosamente controlado.[36] Los investigadores siguen estrictos protocolos para la administración del tratamiento, el seguimiento de los pacientes y la medición de resultados de salud. Con la asignación al azar de los pacientes a los grupos de tratamiento, los ensayos clínicos aleatorizados minimizan las diferencias sistemáticas entre los grupos de estudio. Tales diferencias podrían incluir factores conocidos y desconocidos que pueden influir en los resultados. Con la ayuda de protocolos estrictos, los investigadores aseguran que el cambio en los resultados de salud puede ser atribuido al tratamiento.[36-38]

Estudios observacionales

Los estudios observacionales son fundamentales para responder cuestiones de etiología. Sin embargo, presentan serias limitaciones para responder preguntas de intervención porque no asignan al azar a los pacientes en los grupos de tratamientos, lo que genera resultados poco fiables en diferentes campos.[39-40] Sus principales diseños son los siguientes:

Diseño de cohortes: Se realiza un seguimiento en el tiempo de los grupos de personas en riesgo para observar resultados de interés. Son recomendables para patologías de corta duración y frecuentes. Sirven, principalmente, para determinar el pronóstico del paciente.[10; 35]

Diseño de casos y controles: Se observa un grupo de individuos con una condición (casos) y se compara con los que no la tienen (controles). Sirve para determinar si una exposición o un evento pasado son más frecuentes en un grupo que en otro. Pueden utilizarse como casos las personas a las que se le haya diagnosticado la enfermedad en cuestión recientemente (casos incidentes) o personas que la hayan padecido durante algún tiempo (casos prevalentes). Estos estudios sirven, principalmente, para definir la causalidad o etiología y son muy útiles en el caso de patologías poco frecuentes, como los defectos congénitos; para medir el riesgo de ocurrencia de estas; y para patologías de larga latencia, como las neoplasias.[10,35]

Diseño transversal: En este caso, la prevalencia de la exposición y el resultado de la enfermedad se determinan al mismo tiempo. A menudo no es posible establecer una relación temporal entre la exposición y la aparición de la enfermedad o del evento.[10,35]

3.2 Reproducción médicamente asistida

Actualmente existen cada vez más parejas que recurren a la RMA, la cual es responsable de entre 0,2 y el 4,3% de los nacimientos a nivel mundial.[41-45] Este aumento se debe no solo a cambios demográficos de la población (por ejemplo, parejas de mayor edad que tienen dificultades para concebir) sino principalmente a la infertilidad causada por diferentes patologías neoplásicas, infecciosas y

endocrinológicas, entre otras. Desde 1978, fecha en que Patrick Steptoe y Robert Edwuards lograron el nacimiento del primer bebé probeta, las técnicas de reproducción asistida (TRA) se han ido perfeccionando. Sin embargo, el éxito de embarazos a término ha variado poco y se siguen observando complicaciones perinatales como resultado del uso de estas técnicas.[42-46] Las más observadas son: los embarazos y/o nacimientos múltiples, el parto prematuro, la hiperestimulación ovárica, el bajo peso al nacer y el probable incremento de defectos congénitos.[46-49] Según la Organización Mundial de la Salud (OMS), pese a las dificultades y controversias que rodean la prestación y el acceso a la RMA, se están encontrando soluciones innovadoras en el manejo de la infertilidad.

La infertilidad afecta a entre el 3,5% y el 16,7% de las parejas en edad reproductiva en los países desarrollados, y a entre el 6,9% y el 12,6% en los países en desarrollo, con una prevalencia global media del 9%.[50-55] El estudio de infertilidad se realiza considerando factores femeninos (ovárico, uterino, tubárico, cervical y genético) y masculinos. Además, existen la infertilidad de pareja, que puede ser genética o inmunológica, y la denominada infertilidad de origen desconocido.[56-60] Frente a estos diferentes factores se dispone de una variedad de procedimientos de RMA que, a lo largo del tiempo, han recibido distintas denominaciones según el lugar y el momento. En consecuencia la OMS inició en el año 2008 un proceso de estandarización de los términos que aún no ha terminado.[1]

3.2.1 Procedimientos

Según la OMS, los procedimientos considerados en RMA son los siguientes: la inducción de ovulación, la estimulación ovárica controlada, el desencadenamiento de la ovulación, la inseminación (intrauterina, intracervical o intravaginal; con semen del esposo o pareja, o un donante) y las técnicas de reproducción asistida (TRA).[1]

3.2.1.1 Inducción de la ovulación

Se sabe que la ovulación ocurre entre las 34 y 36 horas posteriores al inicio del pico de hormona luteinizante (LH), de 17 a 26 horas después de la detección de LH en la orina y con un diámetro folicular de 20 a 27 mm. Estos marcadores para la ovulación sirven de guía para planificar las relaciones sexuales o la inseminación. Según la OMS, la Inducción de Ovulación es definida como:

> *"El tratamiento farmacológico de mujeres con anovulación u oligo-ovulación con la intención de inducir ciclos ovulatorios normales".*[1]

La ovulación se induce mediante fármacos que actúan en el hipotálamo, en la hipófisis o en otros órganos blancos como el ovario. Además de restaurar la ovulación, también son utilizados para la estimulación ovárica controlada cuando se complementan con otros medicamentos que desencadenan la ovulación.

3.2.1.2 Desencadenamiento de la ovulación

El desencadenamiento de la ovulación es un procedimiento de rotura del folículo mediante fármacos, como la gonadotrofina coriónica o, últimamente, los agonistas de la hormona liberadora de gonadotrofina.[61]

3.2.1.3 Estimulación ovárica controlada

La estimulación ovárica controlada persigue dos objetivos principales: la supresión de la actividad hipofisaria y la estimulación del crecimiento de múltiples folículos. La estimulación ovárica controlada es definida por la OMS en dos contextos:

> *"Estimulación ovárica controlada para ciclos sin TRA: tratamiento farmacológico en el cual las mujeres son estimuladas para inducir el desarrollo de más de un ovocito".*[1]

> *"Estimulación ovárica controlada para ciclos de TRA: tratamiento farmacológico en el cual las mujeres son estimuladas para inducir el desarrollo de múltiples folículos ováricos para obtener múltiples ovocitos en la aspiración folicular".*[1]

Los fármacos más usados para la inducción de la ovulación y la estimulación ovárica controlada son los siguientes:

a) Citrato de clomifeno: Este medicamento fue sintetizado por primera vez en 1956 y tiene las propiedades del estrógeno agonista y antagonista.[62-65] Sus

efectos secundarios son sofocos transitorios, cambios de humor, dolor de pecho, náuseas, hiperestimulación ovárica leve.[63-68] Las GPC la recomiendan en la RMA durante no más de seis meses y con control ecográfico.[69,70] Sin embargo, hay evidencias que dan lugar a controversia en otros temas como el cáncer ginecológico, el riesgo global de defectos de nacimiento o de cualquier otra anomalía en particular.[71-73]

b) Letrozol: Esta molécula es un inhibidor selectivo y reversible de la aromatasa que disminuye el estrógeno que estimula el aumento de la producción y liberación de la hormona folículo estimulante (FSH) que, a su vez, promueve el crecimiento folicular. Otro mecanismo de acción posible del letrozol es el aumento de la sensibilidad a la FSH[74,75] si bien las GPC no lo consideran como alternativa en la RMA[69,70], aunque existen estudios que prueban su eficacia en este campo.[74,75] Además, no hay datos que sugieran que el letrozol utilizado antes de la ovulación tenga efectos teratogénicos.[76,77]

c) Hormona liberadora de gonadotropina (GnRH): Esta molécula fue identificada y sintetizada en 1971. Es un decapéptido de secreción pulsátil con una vida media muy corta, detectable solo a nivel de la circulación portal. Se produce en el hipotálamo y actúa sobre las células gonadotropas de la hipófisis.[78] Existen dos tipos: agonistas y antagonistas.

Agonistas de la GnRH (GnRHa)

Los GnRHa pulsátiles exógenos se utilizan con éxito para la estimulación ovárica controlada desde 1980. Se administra por vía intravenosa o subcutánea,[78,79] para lo cual pueden seguirse tres protocolos diferentes: el corto, ultracorto y largo.[79,80] Sus efectos secundarios son: sofocos, dolor de cabeza, sangrado e hiperestimulación ovárica.[81,82]

Antagonistas de GnRH (GnRHant)

Los GnRHant son inhibidores competitivos de los receptores de GnRH e inducen un bloqueo de los receptores de la adenohipófisis de forma rápida, reversible y dosis dependiente. Estos fármacos bloquean la producción de gonadotropinas de forma directa. Existen dos protocolos para su uso dependiendo de la dosis que se administre.[82-85] Además, los GnRHant son usados como rescate en pacientes que se encuentran en riesgo de padecer el síndrome de hiperestimulación ovárica durante la estimulación con GnRHa.[83,86]

d) Gonadotrofinas: Las gonadotrofinas exógenas son agentes fundamentales utilizados para la estimulación ovárica controlada. Según su mecanismo de acción son de dos tipos: las gonadotrofinas que estimulan el folículo, como las urofolitropinas; y las gonadotropinas que estimulan el cuerpo lúteo, tales como la gonadotropina coriónica humana (hCG). La primera gonadotrofina utilizada fue la gonadotrofina humana menopáusica (hMG); Posteriormente, aparecieron las gonadotropinas urinarias purificadas y altamente purificadas; y, finalmente, la hormona folículo estimulante recombinante (r-FSH) y la hormona luteinizante recombinate (r-LH).[87] Las gonadotropinas han constituido una

piedra angular para la RMA durante medio siglo. Inicialmente, las tasas de éxito parecían aceptables, pero actualmente se ponen en duda.[88]

Gonadotrofina menopáusica humana: Fue descubierta en 1960. Contiene pequeñas cantidades variables de gonadotrofina coriónica. Las hMG contemporáneas están más purificadas y pueden administrarse por vía subcutánea.[87,88]

Urofolitrofina: Se encuentra disponible desde mediados de los 80; está exenta de LH, pero todavía contiene proteínas urinarias. Actualmente, los productos están más purificados y contienen menos de 0,001 UI de LH y niveles mucho más bajos de proteínas en orina. Se pueden administrar por vía subcutánea.[87,88]

FSH recombinante: Se sintetizó en 1988 a través de la ingeniería genética. Hay dos tipos: alfa y beta.[88]

Gonadotrofina coriónica humana: Es una hormona glicoproteica producida durante el embarazo por el embrión en desarrollo después de la fecundación y, posteriormente, por el sincitiotrofoblasto. También se sintetiza en la hipófisis de los hombres y mujeres de todas las edades. La hCG se usa en gran parte como desencadenante de la ovulación y se administra por vía parenteral.[89,90] En muchos estudios se ha determinado un intervalo de 36 horas para la administración de hCG y la recuperación de los ovocitos para conseguir la mayor eficacia en la tasa de fecundación y de embarazo.[91,92]

La GPC del NICE, publicada en el 2013, ofrece recomendaciones en el manejo de la infertilidad. Tras su evaluación con el AGREE II, se le consideró como recomendable. Esta guía recomienda usar cualquiera de las gonadotrofinas urinarias o recombinantes para la estimulación ovárica en el marco del tratamiento de fertilización in vitro (FIV). Pone énfasis en el uso de una dosis inicial de gonadotrofina folículo estimulante individualizada, teniendo en cuenta los factores que predicen el éxito de FIV (el Índice de Masa Corporal, la presencia de ovarios poliquísticos, la reserva ovárica), pero sin exceder los 450 UI/día. Además, puede complementarse con GnRHa de baja regulación o GnRHant como parte de los ciclos de tratamiento. Se puede ofrecer GnRHa solo a las mujeres que presenten un bajo riesgo de padecer el síndrome de hiperestimulación ovárica; y cuando se utilice como parte de un tratamiento de FIV se aconseja utilizar el protocolo largo de baja regulación. En relación con el desencadenamiento de la ovulación, se recomienda la administración de gonadotrofina coriónica humana (hCG) urinaria o recombinante y la monitorización de la respuesta ovárica mediante una ecografía como parte integral del ciclo de tratamiento. Además, la guía afirma que las clínicas donde se realizan estos tratamientos deben contar con protocolos de prevención, diagnóstico y tratamiento del síndrome de hiperestimulación ovárica. [70]

Asimismo, se ha encontrado sendas revisiones sistemáticas que respaldan las recomendaciones de la guía. Una RS detectó ensayos clínicos aleatorios que mostraban claramente que, en la FIV y transferencia de embriones, la combinación de gonadotrofina exógena con una GnRHa se asocia a un aumento de la tasa de embarazo, en comparación con el uso de gonadotrofinas sola.[82] A

esto se le añade que una RS Cochrane consideró que el protocolo largo de GnRHa de baja regulación lograba la mejor tasa de embarazo clínico si se usaba hasta la supresión de ovario durante aproximadamente 14 días. También mostró que no existían diferencias significativas entre la inyección de dosis única frente a la inyección de dosis diaria de GnRHa. [80] En otra RS, que incluyó 902 ciclos, se observó que la adición de LH recombinante a los ciclos de TRA podía mejorar la tasa de implantación y embarazo clínico en pacientes de edad avanzada.[85] Además, una RS Cochrane señaló que la adición de citrato de clomifeno asociado o no a gonadotropinas reducía la carga de las inyecciones y la incidencia del síndrome de hiperestimulación ovárica. Con todo, la tasa de nacidos vivos y la de embarazo no diferían significativamente.[93] En otro metanálisis de 16 estudios con 4040 pacientes, se observó que el tratamiento con hMG resultó en un menor número de folículos en comparación con FSHr. Sin embargo, si se consideran solamente las transferencias frescas, las tasas de embarazo entonces son similares.[94] Otra RS Cochrane mostró que el uso de una dosis media de la FSH de acción prolongada era una opción de tratamiento segura e igualmente eficaz en comparación con la FSH de uso diario.[95] Otra RS Cochrane informó que no se disponía de pruebas que indicaran una diferencia en la tasa de nacidos vivos en el caso de los GnRHant en comparación con los protocolos largos de GnRHa. Sin embargo, los GnRHant se asociaron a una reducción significativa en los casos de síndrome de hiperestimulación ovárica en comparación con los protocolos de GnRHa.[83] En otra RS del Cochrane se examinaron diferentes protocolos de GnRHa para la supresión pituitaria en ciclos

de TRA (largo, corto, ultra-corto). No hubo evidencia indicativa de una diferencia en la tasa de nacidos vivos, si bien la tasa de embarazo clínico fue mayor en el protocolo largo que en el corto. No se observaron diferencias en los resultados de fertilidad entre la variedad de protocolos largos, y tampoco se redujo la tasa de embarazo al detener o reducir la GnRHa en el inicio de la estimulación.[96] Otra RS Cochrane no encontró pruebas de la existencia de una diferencia estadísticamente significativa en la tasa de nacidos vivos al comparar FSHr con cualquiera de las otras gonadotropinas, indistintamente del protocolo de baja regulación utilizado. Los autores de la revisión concluyeron que la elección clínica de gonadotropina debía depender de la disponibilidad, conveniencia y costos.[97]

En relación con el desencadenamiento de la ovulación, se encontraron dos revisiones. Una mostró evidencia de una menor tasa de nacidos vivos y embarazo en curso, y una mayor tasa de aborto involuntario en las mujeres que recibieron GnRHant para desencadenar la ovulación, en comparación con las mujeres que recibieron hCG. Sin embargo, la incidencia del síndrome de hiperestimulación ovárica fue menor en el grupo de GnRHant.[98] La otra RS informó de que no había pruebas de una diferencia estadísticamente significativa entre la hCG o LH recombinante y la hCG urinaria para desencadenar la ovulación, con respecto a la tasa de embarazo y la incidencia de síndrome de hiperestimulación ovárica.[99] Los autores concluyeron que la hCG urinaria seguía siendo la mejor opción para esta etapa de la FIV e ICSI debido a la disponibilidad y el costo.[99,100] En otra RS se observó que el uso de la hCG en la

fase folicular tanto temprana como tardía presentaba la ventaja de disminuir las dosis de FSH.[101]

Cabe resaltar que existen estudios primarios que demuestran que el régimen secuencial de alta regulación (Step-up) con baja regulación (Step-Down) de gonadotrofinas es superior a los regímenes de baja regulación en pacientes con síndrome de ovario poliquístico que se someten a FIV.[102,103]

Con relación a los efectos negativos, la GPC del NICE recomienda continuar investigando las complicaciones a largo plazo y tomar precauciones para la hiperestimulación ovárica en mujeres con poliquistosis ovárica que reciben gonadotrofinas.[70] Respecto a los efectos inmediatos, una revisión narrativa refiere que el uso de gonadotrofinas se asocia a un aumento de la tasa de nacimientos múltiples y del síndrome de hiperestimulación ovárica.[88] Además, una RS Cochrane establece que no hay suficiente evidencia para demostrar que dosis bajas de hCG en reemplazo de FSH mejoren los resultados en hiperestimulación ovárica.[104]

En cuanto a los efectos a largo plazo, una RS narrativa no encontró estudios que demostrasen una correlación general entre la estimulación ovárica y el aumento del riesgo de cáncer de ovario o del aparato reproductor femenino.[73] Por otro lado, estudios primarios publicados posteriormente indican que el uso de gonadotrofinas se asocia a una tasa alta de nacimientos múltiples y del síndrome de hiperestimulación ovárica.[105,106]

3.2.1.4 Inseminación artificial

La inseminación artificial (IA) es un procedimiento por el cual se recogen gametos masculinos y se introducen de forma artificial en el útero, en el orificio cervical o en la vagina. De estas tres, la inseminación intrauterina (IU) ha demostrado ser superior[107] y es la más usada, porque permite reducir los efectos negativos de la acidez vaginal, la hostilidad del moco cervical y colocar el esperma más cerca del gameto femenino.[107-108] La IU debe coincidir con el momento de la ovulación espontánea o inducida y considerar que el esperma normal puede sobrevivir en el tracto reproductivo femenino y conservar su capacidad de fertilizar un óvulo durante por lo menos tres días. Sin embargo, un óvulo puede ser fecundado con éxito solo de 12 a 24 horas después de la ovulación.[108]

La GPC del NICE recomienda usar la IU como una opción de tratamiento en los siguientes grupos: personas que no puedan o les resulte muy difícil mantener relaciones sexuales vaginales debido a una discapacidad física o un problema psicosexual; quienes requieran una consideración especial en cuanto a varón VIH positivo; quienes mantengan relaciones con parejas del mismo sexo; y personas con infertilidad inexplicada, endometriosis leve o "leve factor de la infertilidad masculina", que mantengan relaciones sexuales sin protección regular. Esta guía menciona que más del 50% de las mujeres menores de 40 años que recurren a la IU conciben dentro de los seis ciclos de inseminación. La mitad de las que no conciben dentro de los 6 ciclos de IU, lo hará con otros 6 ciclos (tasa de embarazo acumulada superior al 75%). También refiere que el uso de semen fresco se

asocia a tasas de concepción más altas que con los congelados-descongelados. Agrega que las personas que utilizan la inseminación artificial para concebir deben recibir la inseminación alrededor de la ovulación. Además, señala que la IU, aun con espermatozoides congelados-descongelados, se asocia a tasas de concepción más altas que la inseminación intracervical. Esta GPC recomienda que a las mujeres que ovulan regularmente se les deba ofrecer un mínimo de 6 ciclos de inseminación artificial para reducir el riesgo de embarazo múltiple y sus consecuencias. Además, considera que los principales factores predictivos son una mujer menor de 30 años de edad con infertilidad de causa cervical o anovulatoria y un hombre con más de cinco millones de espermatozoides.[70]

Estas recomendaciones son respaldadas por diferentes RS. Una revisión Cochrane concluye que, en el caso de infertilidad inexplicada, la IU y la estimulación ovárica incrementan la tasa de embarazo frente a IU sola.[109] Por otro lado, un metanálisis en infertilidad masculina, que incluyó cinco ensayos con 1125 ciclos de IU, mostró un incremento de la tasa de embarazo clínico después de dos IU con esperma homólogo en un mismo ciclo en comparación con una sola IU.[110] Asimismo, otra RS concluye que la IU parece ser eficaz y segura en parejas serodiscordantes.[111] Por otro lado, otra RS Cochrane señala que no hay pruebas suficientes para determinar si existe alguna diferencia entre la seguridad y la eficacia de los diferentes métodos de sincronización de la ovulación y la inseminación; por este motivo se necesitan más investigaciones.[112] Además, una RS del año 2006 indica que se requieren más estudios para generar conclusiones en relación con las ventajas del tipo de catéter.[113] No obstante, es importante

resaltar que un estudio primario publicado posteriormente demostró que los mejores resultados se obtenían cuando la IU se realizaba usando un catéter suave en comparación con un catéter rígido.[114] Otro estudio concluye que la IU y el uso de preservativos permiten a los pacientes HIV positivos reproducirse de forma segura.[115] En cuanto a los efectos colaterales, se menciona el incremento de embarazos múltiples y de hiperestimulación ovárica, que están más asociados a la estimulación ovárica que acompaña a la IU. Aunque aparentemente no existe un aumento de los defectos congénitos[116] no hay suficientes estudios al respecto.

3.2.1.5 Técnicas de reproducción asistida

Cuando hablamos de TRA, nos referimos a todos los procedimientos de la definición establecida por la OMS:

> *"Todos los tratamientos o procedimientos que incluyen la manipulación tanto de ovocitos como de espermatozoides o embriones humanos para el establecimiento de un embarazo. Esto incluye, pero no está limitado sólo, a la fecundación in vitro y la transferencia de embriones, la transferencia intratubárica de gametos, la transferencia intratubárica de zigotos, la transferencia intratubárica de embriones, la criopreservación de ovocitos y embriones, la donación de ovocitos y embriones, y el útero subrogado. Las técnicas de reproducción asistida no incluye inseminación asistida (inseminación artificial) usando espermatozoides ni de la pareja ni de un* donante".[1]

3.2.1.5.A Fertilización in vitro y transferencia de embriones

La FIV fue la primera forma de TRA y es todavía la más común. La OMS la define como:

> *"Técnica de Reproducción Asistida que involucra fecundación extracorpórea".*[1]

Los factores predictores de embarazo en este caso son la edad, los ciclos previos fallidos, los antecedentes de embrazo, el índice de masa corporal y el estilo de vida (consumo de alcohol, tabaco o café).[70,117] Asimismo, el número total de ovocitos recuperados es uno de los factores pronósticos más importantes en la TRA.[118]

En relación con las indicaciones de FIV la guía de practica clínica del NICE recomienda ofrecer tres ciclos completos de FIV con/sin inyección intracitoplasmática de espermatozoides (ICSI) a las mujeres menores de 40 años que no hayan concebido después de dos años de relaciones sexuales sin protección normal o tras 12 ciclos de inseminación artificial (de los cuales 6 o más fueron con IU); o que hayan llegado a la edad de 40 años durante el tratamiento. También recomienda completar el ciclo actual si no se han completado ya otros; ofrecer un ciclo completo de FIV, con o sin ICSI, a las mujeres de entre 40 a 42 años que no hayan concebido después de dos años de relaciones sexuales sin protección normal o tras 12 ciclos de IU (de los cuales 6 o más fueron con IU), siempre y cuando no hayan tenido previamente un tratamiento de FIV, no haya

evidencia de baja reserva ovárica y haya habido una discusión sobre las implicancias adicionales de la FIV y del embarazo a esta edad. En todos los casos se debe informar a la paciente de que, normalmente, un ciclo completo de tratamiento de FIV, con o sin ICSI, debe incluir un episodio de estimulación ovárica y transferencia de embriones frescos o congelados.[70]

Además, esta guía recomienda un pre-tratamiento para mujeres que no se sometan a los protocolos largos de baja regulación con el fin de programar la intervención de FIV, e informar de que el uso de pre-tratamiento (ya sea con la píldora anticonceptiva oral o un progestágeno) como parte de la FIV no influye sobre las probabilidades de tener un nacido vivo.[70] De las tres revisiones que abordan el tema, ninguno de los ensayos incluidos trató adecuadamente los eventos adversos.[100]

Se deben seguir una serie de pasos coordinados en la FIV. Si uno de estos no se aplica de forma correcta, la concepción puede no ocurrir. Por tanto, es importante que cada paso esté apoyado por evidencias de estudios bien diseñados.[100]

1. *Estimulación ovárica controlada:* Usar una combinación de fármacos considerando las evidencias antes descritas.
2. *Desencadenamiento de la ovulación:* Ofrecer la hCG urinaria según las evidencias ya expuestas.
3. *Recuperación de ovocitos:* Se realiza mediante la aspiración transvaginal de ovocitos guiada por ultrasonido bajo sedación intravenosa.[119-120]

La GPC del NICE recomienda administrar sedación consciente, ya que es un método analgésico seguro y aceptable. En relación con la recuperación quirúrgica de espermatozoides antes de ICSI, sostiene que se puede realizar utilizando diferentes técnicas según la patología y los deseos del hombre. En cualquier caso, las instalaciones para la crio- conservación de espermatozoides deben estar disponibles. La incubación asistida (AH) no es recomendable, ya que no se ha demostrado que mejore las tasas de embarazo.[70]

Respecto a las revisiones, una RS Cochrane de 21 ECA no encontró ningún protocolo en particular sobre la sedación consciente y la analgesia efectiva para aliviar el dolor durante y después de la recuperación de los ovocitos. Otra RS informó de que no se encontraron pruebas que demostraran que la aspiración folicular y el lavado de ovocitos se asociaban ni a mejores tasas de embarazo clínico o en curso ni a un aumento del rendimiento de los ovocitos.[122] Otra RS indicó que no había pruebas suficientes para recomendar una técnica de recuperación de espermatozoides específica en hombres azoospérmicos sometidos a ICSI. Ante la falta de pruebas para apoyar los métodos más invasivos o más difíciles, los revisores recomiendan la técnica disponible menos invasiva y más simple.[123] En cuanto a la incubación asistida, una RS concluye que no hay evidencia de que esta mejore la tasa de nacidos vivos.[124]

Un estudio primario señaló que la profilaxis antibiótica podría reducir las infecciones.[125] Otros, también primarios, concluyen que una aguja desechable

especialmente diseñada de calibre 17 o 20 con presión de vacío (100 a 200 mm Hg) es la idónea para recuperar ovocitos.[126,127]

Las complicaciones más frecuentes de este procedimiento son los riesgos tromboembólicos, carcinogénicos, de hemorragia y de infección por punción. Además, la presencia de un endometrioma puede generar un aumento en el riesgo de infección.[128,129]

4. *Fertilización o fecundación:* Esta puede conseguirse por microinseminación convencional o por ICSI cuando hay un factor masculino conocido o sospechoso. Cuando es logísticamente factible, el esperma recuperado puede ser utilizado inmediatamente para ICSI o puede ser crio-preservado para su posterior uso.[130] La OMS define ICSI como:

   > *"Procedimiento mediante el cual un solo espermatozoide es inyectado en el citoplasma de un ovocito"*.[1]

Las GPC confiables recomiendan indicar ICSI en varones con semen de baja calidad y en casos de azoospermia obstructiva y no obstructiva. Además indican, que el tratamiento de ICSI se debe considerar para parejas en las cuales un ciclo de tratamiento de FIV anterior ha dado lugar a fertilización fallida o muy pobre.[70,131]

Asimismo, se han introducido dos modalidades de ICSI, la llamada inyección intracitoplasmática de espermatozoides morfológicamente seleccionados (IMSI) y

la de ICSI fisiológico (PICSI). Esta última técnica se basa en elegir los espermatozoides capaces de unirse al ácido hialurónico (biopolímero que envuelve los óvulos de manera natural). Una RS Cochrane informó de que no se encontraron pruebas de la existencia de diferencias en las tasas de nacidos vivos o abortos espontáneos entre ICSI e IMSI, ni de que IMSI aumentara las anomalías congénitas.[132] Otra RS Cochrane señala que no hay evidencias de que PICSI mejore la tasa de nacidos vivos.[133]

Las diferentes modalidades de FIV se basan en el mismo principio: el esperma y el ovocito se incuban durante la noche. Encontramos una RS Cochrane al respecto que menciona que una breve coincubación de los espermatozoides y ovocitos puede mejorar el embarazo en curso y las tasas de embarazo clínico en mujeres infértiles sometidas a ciclos de FIV, en comparación con el protocolo estándar de la inseminación durante la noche.[134]

La incubación se realiza en medios de cultivo y la composición de estos trata de imitar el medio natural. En relación con la composición de los cultivos, una RS Cochrane informó de que no había pruebas de un aumento en la tasa de nacidos vivos, ni en la de embarazo en curso o clínico. Tampoco las había de un incremento de los eventos adversos (embarazo múltiple, aborto involuntario) cuando la concentración de oxígeno en el cultivo de embriones era baja (-5%) en comparación con los cultivos que usaban oxígeno en concentración atmosférica (- 20%).[135]

Además, detectamos estudios primarios que resaltaban que los cultivos permiten el desarrollo de los cigotos a la etapa de blastocisto para poder seleccionar mejor los embriones mediante tamizaje genético.[136-143]

El tamizaje genético preimplantacional (*PGS: preimplantation genetic screening)* es de diferentes tipos y es definido por la OMS como:

> *"Análisis de cuerpos polares, blastómeras o trofoectodermo de ovocitos, zigotos o embriones para la detección de aneuploidías, mutaciones y/o rearreglos del ADN".*[1]

No encontramos recomendaciones sobre el PGS en la guía del NICE.[70] En este aspecto, una revisión Cochrane sobre el PGS informó de que no había diferencias significativas en las tasas de embarazo en curso entre las mujeres sometidas a FIV con y sin PGS.[144] Otras revisiones hallaron resultados similares.[145-149] Sin embargo, siguen apareciendo nuevas técnicas de PGS, tales como los SNIP (single nucleotide polymorphism arrays) que podrían tener algún beneficio.[150-152]

5. *Transferencia de embriones (TE).* Aun cuando los embriones pueden transferirse con éxito en cualquier etapa de desarrollo temprano, lo más frecuente es que se lleve a cabo tres días después de la fertilización. Según la OMS, la transferencia de embriones posee dos acepciones:

   > *"Transferencia de embriones: Procedimiento mediante el cual uno o más embriones son colocados en el útero o en la trompa de Falopio".*[1]

> *"Transferencia electiva de embriones: Transferencia de uno o más embriones, seleccionados a partir de una cohorte más grande de embriones".*[1]

Las GPC recomiendan ofrecer la transferencia de uno o dos embriones según la edad, el pronóstico de la mujer sometida a FIV, el número del ciclo y la calidad de los embriones disponibles. También aconseja realizar la transferencia guiada por ultrasonido porque mejora las tasas de embarazo, informar de que en un endometrio menor de 5 mm es poco probable que ocurra un embarazo y que el reposo en cama por más de 20 minutos después de la transferencia no mejora los resultados.[70,153,154] Asimismo, la guía del NICE recomienda evaluar la calidad del embrión de acuerdo con la *Association of Clinical Embryologists* (ACE) and *UK National External Quality Assessment Service* (UK NEQAS); transferir no más de dos embriones durante un ciclo de tratamiento de FIV y uno solo cuando un blastocisto de alta calidad esté disponible; aconsejar a la gente sobre los riesgos del embarazo múltiple asociados a esta técnica y, por último, ofrecer criopreservación para almacenar los embriones de buena calidad que queden después de la transferencia de embriones.[70]

Existen diversas revisiones Cochrane que fundamentan estas recomendaciones. Una de ellas señaló que el uso del ultrasonido para guiar el catéter, en comparación con el tacto, durante la transferencia incrementa de forma significativa la tasa de embarazo.[155] Otra RS demostró que la tasa de nacidos vivos acumulada de la transferencia de un solo embrión seguida de la

transferencia de un solo embrión, era comparable con la transferencia de dos embriones en un ciclo. Además, la transferencia electiva de un solo embrión resultó en un menor número de embarazos múltiples que la transferencia de dos embriones.[156] Otra RS de técnicas de preparación previa a la transferencia como la eliminación del moco cervical o el lavado del conducto cervical o de la cavidad endometrial no mostró beneficio.[157] Otra revisión sobre la administración de amoxicilina y ácido clavulánico antes de la transferencia de embriones no reveló ninguna mejora de la tasa de embarazo clínico.[158] Una RS que comparó la transferencia en la etapa de blastocisto (de 4 a 5 días) con la transferencia en la fase de segmentación (de 2 a 3 días) observó un aumento de la tasa de nacidos vivos con blastocisto.[159] Otra RS no encontró beneficios del reposo después de la transferencia.[160] Por otro lado, en una RS de reciente publicación se registró un incremento de la tasa de nacidos vivos, la de embarazos clínicos y la de embarazo múltiple como resultado del uso de ácido hialurónico,[161] hallazgo aún no considerado en las GPC.

En cuanto a temas no mencionados ni en las GPC ni en las revisiones se detectaron estudios primarios. Estos mostraban que la introducción de un catéter hasta el orificio cervical interno en la transferencia de embriones no estimulaba las contracciones uterinas. Sin embargo, se observaron contracciones del útero que, al parecer, participan en el proceso de implantación del embrión.[162-164] Respecto a la elección del embrión, todavía no se dispone de técnicas aplicables de forma rutinaria o de dispositivos analíticos para seleccionarlo, a pesar de la llegada de

nuevas tecnologías que pueden mejorar la evaluación no invasiva de los embriones humanos en FIV.[165]

La transferencia de embriones puede complementarse con la Eclosión Asistida, más conocida como *Assisted Hatching* (AH); Además, se sabe que el incumplimiento de la implantación y la concepción puede ser consecuencia de una incapacidad del blastocisto para liberarse de su capa externa, la zona pelúcida Sin embargo, según una RS, la rotura artificial de esta envoltura no parece mejorar las tasas de embarazo.[124]

En relación con los efectos colaterales de FIV, las GPC recomiendan realizar más investigaciones científicas epidemiológicas y básicas para ayudar a determinar la etiología y el alcance del mayor riesgo de anomalías congénitas y de las complicaciones a largo plazo.[70,166] En la misma línea, cuatro RS de estudios observacionales recomiendan realizar grandes estudios poblacionales, aunque señalen que existe un incremento de defectos congénitos con TRA.[42-44,167] De forma similar, otra RS de estudios observacionales muestra que la transferencia de embriones en el estadio de blastocisto se asocia a un mayor riesgo de parto muy prematuro en comparación con la etapa de escisión.[79] Además, un metanálisis asoció FIV a un pequeño aumento del riesgo de retraso mental estadísticamente significativo.[168] Otro metanálisis de ocho estudios de cohorte con 746 455 participantes no encontró asociación entre el tratamiento de FIV y el cáncer (de ovario, mama, cuello uterino).[169] Por otro lado, una RS refiere que la transferencia selectiva de un solo embrión se asocia a la disminución del parto

prematuro y del bajo peso al nacer en comparación con la transferencia de dos embriones.[170] Respecto al embarazo ectópico, una revisión concluye que el riesgo aumenta según el número de embriones transferidos.[171] Dos revisiones apuntan que, al parecer, existe un mayor riesgo de defectos de impronta y epigenéticos en los bebés concebidos vía FIV.[172,173] Finalmente, el incremento de la hiperestimulación ovárica está más relacionada con la estimulación ovárica que acompaña a la FIV.[105,106]

3.2.1.5. B Transferencia intratubárica de zigotos y de gametos

La transferencia intratubárica de gametos (GIFT) y la transferencia intratubárica de zigotos (ZIFT) son técnicas consideradas para mujeres con al menos una trompa de Falopio sana.[174] Sin embargo, la FIV se usa cada vez más en reemplazo de la GIFT y la ZIFT.[46] La OMS define la GIFT y la ZIFT de la siguiente forma:

> *"Transferencia intratubárica de gametos (GIFT): Es un procedimiento de TRA en el cual ambos gametos (ovocitos y espermatozoides) son transferidos a la trompa de Falopio".*[1]

> *"Transferencia intratubárica de zigoto (ZIFT): Se trata de un procedimiento mediante el cual uno o más zigotos son transferidos a la trompa de Falopio".*[1]

La GPC del NICE refiere que no hay constancia de pruebas suficientes para recomendar el uso de la GIFT o la ZIFT en lugar de la FIV en parejas con problemas de fertilidad sin explicación o por factor masculino.[70] No se han

encontrado RS relevantes acerca de la GIFT o la ZIFT. Sin embargo, sí se detectaron estudios primarios: uno con 280 ciclos de ZIFT que mostraba 96 embarazos clínicos por intento (34,3%) y 72 nacimientos (25,7%) y donde la tasa de nacidos vivos por paciente fue de 39,8%. También concluye que esta técnica sigue siendo una herramienta poderosa en el manejo clínico de pacientes seleccionados para FIV en los que la implantación ha fallado de manera repetida.[175] Otro estudio, en casos de FIV difícil y repetida en pacientes con adherencias cervicales o malformaciones genitales, apuntó que la ZIFT laparoscópica era una alternativa interesante.[176] De forma similar, un estudio primario comparó la IU con la GIFT y llegó a la conclusión de que cuando se utilizaba el mismo protocolo de estimulación en las primeras etapas de la endometriosis, unos pocos ciclos de IU podían lograr resultados similares a los obtenidos con la GIFT; por lo que se considera que la IU debería ser de primera línea.[177] Además, la evaluación comparativa entre la transferencia unilateral y bilateral de ovocitos microinyectados en transferencia intratubárica no revela diferencias. Por consiguiente, la transferencia unilateral es el método preferido dentro de esta técnica.[178]

3.2.1.5. C Criopreservación de embriones y óvulos

Aun cuando la transferencia embrionaria fresca es la norma en FIV, en ciertas circunstancias se requiere conservar los gametos o embriones. A este respecto, la GPC del NICE recomienda ofrecer la criopreservación de ovocitos o de embriones a las mujeres en edad reproductiva que se estén preparando para recibir

tratamiento contra el cáncer que pueda volverlas infértiles si están lo suficientemente bien como para someterse a la estimulación ovárica y a la recolección de óvulos, y si esto no va a afectar a su condición y al tiempo disponible antes del inicio del tratamiento. Asimismo, en la criopreservación de ovocitos y embriones, aconseja utilizar la vitrificación en lugar de la congelación a velocidad controlada si se dispone del equipo y la experiencia necesaria. También recomienda conservar el material criopreservado durante un período inicial de 10 años y ofertar la conservación de los espermatozoides criopreservados durante más de 10 años a los hombres que permanezcan en riesgo de infertilidad. Finalmente, propone el uso de la congelación en vapor de nitrógeno líquido como técnica de criopreservación de esperma.[70]

Por otro lado, dos RS muestran que no existe evidencia de que los resultados de la FIV puedan mejorar con la transferencia de embriones criopreservados en comparación con la transferencia de embriones frescos.[179,180] Sin embargo, en una RS reciente se concluye que la vitrificación de ovocitos, en comparación con la congelación lenta, aumenta la tasa de embarazo clínico mediante la FIV. No obstante, la imprecisión es alta, lo que limita la aplicabilidad.[181]

Diferentes estudios primarios muestran que este tratamiento no es demandado solo por pacientes con cáncer, sino también por mujeres sanas que optan por posponer la maternidad y desean conservar su fertilidad.[182,183] Asimismo, mencionan que simplifica los programas de donación de óvulos y que es un adyuvante útil en situaciones donde inesperadamente se necesita esperma

disponible para el momento de la extracción de los óvulos y para las parejas que, por razones morales o éticas, no desean criopreservar los embriones sobrantes creados a partir de la fecundación in vitro.[183-185]

En lo referente a los efectos colaterales, una RS concluye que los embarazos fruto de la transferencia de embriones congelados en la FIV parecen tener mejores resultados obstétricos y perinatales que en el caso de la transferencia de embriones frescos.[96.]

3.2.1.5. D Donación de ovocitos y embriones

Actualmente se utilizan ovocitos recuperados de donantes jóvenes sanas después de la estimulación ovárica controlada y el esperma de la pareja de la receptora para luego transferir los embriones resultantes al útero de la misma.

Con relación a este procedimiento, la GPC recomienda proporcionar información sobre los posibles riesgos de la estimulación ovárica y de la recolección de ovocitos a las donantes. De igual manera, alguien independiente de la unidad de tratamiento debe comunicar a las receptoras y donantes las consecuencias físicas y psicológicas del tratamiento tanto para ellas como para sus hijos genéticos, incluidos los niños que resulten de ovocitos donados. Además, todas las personas que opten por este procedimiento deben recibir asesoramiento acerca de las implicaciones en su caso en particular. Finalmente, la GPC considera que el uso de óvulos donados es eficaz en la insuficiencia ovárica prematura, la disgenesia gonadal (que incluye el síndrome de Turner), la ooforectomía bilateral, el fallo

ovárico después de la quimioterapia o radioterapia y en ciertos casos de fracaso del tratamiento de FIV.[70]

Por otro lado, la donación de embriones es aún objeto de controversia desde el punto de vista ético.[186] Si bien priman las opiniones favorables al respecto, claramente aún no se ha fijado el término idóneo, Sin embargo, no debe usarse la expresión “adopción de embriones”.[187]

Un estudio ha mostrado una tasa de implantación más baja en pacientes receptoras de ovocitos donados por mujeres con endometriosis frente a los de donantes sin endometriosis.[188]

3.2.1.5. E Maternidad subrogada

La subrogación es la transferencia de embriones mediante la cual una mujer acepta quedar embarazada con el fin de gestar y dar a luz a un niño para otros. En algunas jurisdicciones, la posibilidad de subrogación se ha permitido y los futuros padres pueden ser reconocidos como los padres legales desde el nacimiento. En países como la India, la comercialización de la subrogación o "vientre en alquiler", es un negocio en crecimiento.[189]

La subrogación es una opción para las parejas cuando la mujer no tiene útero o este está irreparablemente dañado o padece una enfermedad médica por la que el embarazo supone un riesgo mortal. La subrogación internacional es un fenómeno cada vez más común y un importante problema de salud mundial, en el cual las normas jurídicas son un factor clave para las partes interesadas.[190]

3.2.1 Receptividad uterina

Las hormonas del ovario, como el estrógeno y la progesterona, regulan la implantación del blastocito en el útero. Estas hormonas actúan a través de sus receptores nucleares para dirigir la actividad transcripcional de los compartimentos del endometrio, y crear un período de tiempo definido en el que el útero permite la implantación del embrión. A este periodo se le denomina la "ventana de receptividad".[191] Se sabe que la implantación exitosa requiere un embrión vital y un diálogo molecular con un endometrio receptivo eficaz. Sin embargo, apenas se conoce qué constituye precisamente un endometrio humano receptivo. Se habla de fracaso recurrente de la implantación cuando en una mujer menor de 40 años existe imposibilidad de lograr un embarazo clínico después de la transferencia de por lo menos cuatro embriones de buena calidad en un mínimo de tres ciclos. Este problema es una causa importante del fracaso de la FIV repetida y se estima que aproximadamente el 10% de las mujeres que buscan tratamiento con FIV lo experimentará.[192] Asimismo, existen patologías uterinas congénitas, como el útero bidelfo, y patologías adquiridas que influyen en el éxito de la TRA.[193] Además, la receptividad puede verse afectada por diferentes factores como la estimulación ovárica controlada, el endometrio delgado o las contracciones uterinas.

La estimulación ovárica controlada y las altas concentraciones de estradiol elevan la progesterona que obstaculiza la implantación. No obstante se puede evitar mediante la congelación y la transferencia de embriones en ciclos naturales o por protocolos de estimulación más leves. Estos cambios en el perfil endocrino

durante la estimulación ovárica y las condiciones médicas de la madre podrían dar lugar a un endometrio no receptivo.[194]

Un reciente metanálisis de más de 60 000 ciclos afirma que el aumento prematuro de la progesterona reduce las tasas de embarazo a partir de valores de 0.8 ng/ml[195] y otros varios estudios han demostrado que el perfil de expresión de la proteína en las secreciones endometriales sufre cambios cíclicos. Además, se han observado diferencias significativas entre el ciclo natural y el estimulado. Estos hallazgos sugieren que el análisis de la secreción endometrial proporciona una nueva vía de estudio de los efectos de la estimulación ovárica en el ambiente intrauterino en el momento de la transferencia de embriones, hecho que puede contribuir al desarrollo de protocolos de estimulación ovárica menos perjudiciales para la FIV en el futuro.[196]

Se ha analizado el flujo sanguíneo subendometrial en ciclos estimulados para la inseminación intrauterina con la ecografía doppler como un indicador de la receptividad endometrial. Diferentes estudios apuntan que la presencia de flujo sanguineo subendometrial se asocia al éxito de la inseminación intrauterina.[197,198] Existen diferentes medicamentos, como los vasodilatadores, que podrían mejorar el flujo sanguíneo subendometrial, así como el grosor y el volumen del endometrio y, por ende, optimizar la receptividad endometrial y el éxito de los procedimientos de la RMA.[199]

La GPC del NICE no recomienda transferir embriones con un endometrio de menos de 5mm de espesor, porque es poco probable que resulte en un

embarazo.[70] Un metanálisis concluye que puede haber una relación entre el grosor endometrial y el embarazo, pero el potencial de implantación es probablemente más complejo que una sola medición de ultrasonido.[200] Otros estudios demuestran que en pacientes con endometrios más delgados se observan fallos de implantación recurrentes. Aunque no exista un grosor endometrial idóneo para realizar la transferencia embrionaria, parece ser que un mínimo de 8 mm podría mejorar la implantación.[201,202]

Asimismo, se han encontrado embriones fuera de la cavidad uterina después de la transferencia de embriones como consecuencia de las contracciones uterinas que expulsan estos embriones. Un estudio apuntó que el peristaltismo uterino ejerce control sobre la migración del embrión y, si la frecuencia de onda fuera demasiado alta, podría afectar negativamente a las posibilidades de embarazo. Por lo tanto, el peristaltismo también podría ser utilizado como un predictor de la irritabilidad uterina antes de la transferencia de embriones.[163]

Se ha evaluado diferentes intervenciones que mejorarían la receptividad uterina endometrial. Así, una RS Cochrane señala que la lesión endometrial realizada en el mes previo a la inducción de la ovulación para ART parece aumentar tanto la tasa de nacidos vivos como la de embarazo clínico en comparación con ninguna lesión endometrial.[203] En otras tres RS Cochrane sobre antioxidantes aspirina y acupuntura respectivamente, no se hallaron pruebas suficientes que indicasen una mejora en la tasa de embarazo ni tasa de nacidos vivos con estas intervenciones.[100] Asimismo, una RS Cochrane, que evaluó la preparación

endometrial más eficaz en las mujeres sometidas a la transferencia de embriones con respecto a la tasa de nacidos vivos, sugiere que no hay pruebas suficientes para recomendar un protocolo en particular para la preparación del endometrio.[204] Sin embargo, los estudios primarios sobre vasodilatadores, sugieren que solos o asociados podrían mejorar la receptividad uterina mediante diferentes mecanismos.[205-210]

3.3. Justificación de la tesis

Es fundamental que los profesionales de la salud tengan acceso a la mejor evidencia disponible al elegir procedimientos de reproducción médicamente asistida (RMA). En este contexto, se ha observado un uso de los términos y elección de procedimientos de RMA poco estandarizado y que los resultados de eficacia y seguridad de estas intervenciones son controvertidos, aun cuando su demanda se ha incrementado tanto en países desarrollados como en países en desarrollo. Estos antecedentes motivaron la realización de un trabajo de tesis de análisis y generación de evidencias respecto a la RMA. La estandarización de los manejos en diferentes campos debe efectuarse mediante guías de práctica clínica (GPC). Estas GPC deben estar basadas en revisiones sistemáticas (RS) para ser de calidad y tener ciertas características metodológicas. Las RS deben ser elaboradas sobre la base de todos los estudios primarios para evitar el sesgo de selección y deben llevarse a cabo siguiendo metodologías estrictas para evitar otros tipos de sesgo. Los estudios primarios más fiables para evaluar las intervenciones son los ensayos clínicos; por otro lado, los observacionales son

necesarios para valorar las complicaciones. Así, para realizar este trabajo de tesis se ha considerado las siguientes preguntas de investigación.

¿Cuál es la calidad de las guías de práctica clínica sobre técnicas de reproducción asistida? Para responder esta pregunta se planteó realizar una evaluación sistemática de las GPC, porque existían guías en este campo que no habían sido valoradas en los últimos cinco años, y porque ahora se cuenta con el AGREE II, un instrumento validado internacionalmente y usado por instituciones de prestigio.[23,211]

En las guías evaluadas no se encontraron recomendaciones para algunas intervenciones, como el uso de vasodilatadores en la RMA, aunque sí hallamos ensayos clinicos sobre vasodilatadores en este campo.[207, 209, 210] Por este motivo, se planteó la siguiente pregunta: **¿Cuál es la seguridad y eficacia de los vasodilatadores en mujeres sometidas a reproducción médicamente asistida?** En ese caso se propuso llevar a cabo una revisión sistemática Cochrane que evaluara esta intervención en mujeres sometidas a tratamientos de infertilidad. Esta revisión era viable porque existían ensayos clínicos sobre vasodilatadores en mujeres sometidas a RMA aún no metanalizados, y es relevante porque las revisiones que evaluaron intervenciones que mejoran receptividad uterina no consideraron a los vasodilatadores.

Asimismo, en la evaluación de las GPC se encontraron recomendaciones para continuar investigando las complicaciones a largo plazo derivadas de la RMA, y su relación con los defectos congénitos.[70] Además, al revisar la literatura detectamos

revisiones que abordaban el tema de los defectos congénitos solo en usuarias de técnicas de reproducción asistida, pero no de otros procedimientos.[213,214] En consecuencia, se planteó la siguiente cuestión: **¿Existe o no un incremento de los defectos congénitos en embarazos logrados con el uso de reproducción médicamente asistida frente a embarazos espontáneos?** Para responder esta pregunta, se desarrolló un estudio caso control que fuera viable y factible por varias razones. En primer lugar, porque los expertos recomiendan realizar estudios poblacionales dadas las controversias.[212-214] En segundo lugar, porque la ciudad de Barcelona cuenta con una base de datos de defectos congénitos adscrita a EUROCAT.[215,216] En tercer lugar, porque los estudios similares se centran en la evaluación de los defectos congénitos en usuarias de TRA y no consideran otras formas de reproducción asistida como la inseminación o la inducción de la ovulación.

A esto se le añade que en la elaboración de la revisión se encontraron pocos ensayos clínicos sobre vasodilatadores en mujeres sometidas a la RMA. Este hallazgo dio pie a complementar la búsqueda electrónica con una manual en las revistas españolas no indexadas, en atención a lo cual se formuló la pregunta que sigue: **¿Cuáles son los ensayos clínicos controlados publicados en revistas españolas y cuál es la calidad de los ensayos clínicos de reproducción médicamente asistida publicados en estas revistas?** El estudio es relevante, puesto que al no estar estos ensayos publicados en revistas indexadas, no se podrían incluir en una revisión sistemática sobre alguno de los temas relacionados, hecho que podría conducir a exagerar las estimaciones de la

eficacia de la intervención.[217] Por ello, era fundamental encontrar estos estudios e incorporarlos al Registro Central Cochrane de Ensayos Controlados. Este estudio se ha desarrollado a pesar de que no formaba parte de la propuesta del trabajo de tesis, y actualmente se encuentra en proceso de publicación (ver anexo).

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4. OBJETIVOS

4.1 Objetivo general:

- Analizar y generar evidencias en reproducción médicamente asistida.

4.2 Objetivos específicos:

Objetivo 1: Desarrollar una evaluación sistemática de las guías de práctica clínica sobre el uso de las técnicas de reproducción asistida.

Objetivo 2: Realizar una revisión sistemática Cochrane para evaluar la eficacia y seguridad de los vasodilatadores en las mujeres sometidas a reproducción médicamente asistida.

Objetivo 3: Ejecutar un estudio caso control para evaluar la asociación entre el uso de la reproducción médicamente asistida y los defectos congénitos en recién nacidos e interrupciones voluntarias del embarazo en las gestantes registradas en Barcelona en el período 1992-2007.

Objetivo 4: Llevar a cabo un estudio de identificación de ensayos clínicos controlados en revistas españolas de Ginecología y Obstetricia y analizar la calidad de los ensayos clínicos de reproducción médicamente asistida.

5. METODOLOGÍA

Esta tesis es el compendio de tres publicaciones en revistas de amplia difusión que intentan analizar y generar evidencias sobre la reproducción médicamente asistida (RMA). La propuesta de tesis fue presentada a la Universidad Autónoma de Barcelona en diciembre del 2011.

La metodología seguida se explica en cada publicación porque cada uno tiene un diseño diferente. A continuación, se resumen los pasos seguidos en el desarrollo de los trabajos.

Primer trabajo: Evaluación sistemática de la calidad de las guías de práctica clínica sobre el uso de técnicas de reproducción asistida.

Periodo 2011 - 2012.

- Elaboración y aprobación del protocolo de investigación.
- Búsqueda y selección de GPC publicadas entre 2005 y 2011.
- Evaluación de las GPC seleccionadas utilizando el instrumento AGREE II.
- Análisis de la calidad de las GPC y del acuerdo entre los evaluadores.
- Elaboración y envío del informe final a la revista *Human Reproduction.*

Periodo 2012 - 2013

- Preparación y envío del artículo al *American Journal Ginecology and Obstetrics.*

- Redacción y envío del artículo al *International Journal of Gynecology & Obstetrics.*

- Redacción y envío del artículo a la revista *Human Fertility.*

Periodo 2013 - 2014

- Publicación del artículo en la revista *Human Fertility* en marzo del 2014 con el título de *"Systematic evaluation of the quality of clinical practice guidelines on the use of assisted reproductive techniques*".

Segundo trabajo: Revisión sistemática del efecto de los vasodilatadores en mujeres sometidas a reproducción médicamente asistida

Periodo 2011 - 2012

- Propuesta de títulos a diferentes grupos Cochrane

- Aprobación del título "*Vasodilator for women undergoing assisted reproduction*" por el *Menstrual Disorders and Subfertility Group*.

- Elaboración y aprobación del protocolo por el *Menstrual Disorders and Subfertility Group*.
- Publicación del protocolo el 13 de julio del 2012.

Periodo 2012 – 2013

- Búsqueda sistemática de ensayos clínicos controlados.

- Selección, evaluación y análisis de los ensayos clínicos seleccionados.
- Redacción y envío de la revisión sistemática a la revisión por pares.

Periodo 2013 - 2014

- Actualización de la búsqueda y ajustes en la redacción de la revisión.
- Cambio del título a "*Vasodilator for women undergoing fertyl treatment*" y ajustes en la revisión.
- Envío para su publicación. El 15 de agosto el grupo aprobó la revisión y la envió a la editora.

Periodo 2013 - 2014

- Publicación de la revisión sistemática "*Vasodilator for women undergoing fertyl treatment*" en octubre del 2014.

Tercer trabajo: Estudio caso control de la asociación de defectos congénitos y reproducción médicamente asistida.

Periodo 2011 - 2012

- Elaboración, aprobación y ejecución del proyecto de investigación.
- Presentación de los resultados a las instituciones involucradas en el estudio.

- Redacción del informe final como artículo para el congreso del ISPD *"16th International Conference on Prenatal Diagnosis and Therapy"* en la ciudad de Miami, Florida (EE.UU.) con el título de *"Birth defects in medically assisted pregnancies of the city of Barcelona*".

Periodo 2012 – 2013

- Preparación y envío del artículo para el *British Medical Journal*, aceptado para su publicación en *BMJ open.*
- Preparación y envío del artículo al *American Journal Obstetrics and Ginecology.*
- Preparación y envío del artículo al *Prenatal Diagnosis.*

Periodo 2013 – 2014

- Publicación del artículo "*Birth defects in medically assisted reproduction pregnancies in the city of Barcelona"* en febrero del 2014.
- La revista eliminó la afiliación a la UAB, motivo por el cual se inició un trámite para que la universidad aceptara esta publicación como parte de la tesis, objetivo que finalmente se alcanzó.

Además, se ha desarrollado un estudio de identificación manual de ensayos clínicos controlados en revistas españolas de Ginecología y Obstetricia, y se ha evaluado la calidad de los ensayos clínicos sobre reproducción médicamente asistida hallados en estas revistas. Este

estudio se incluye como anexo a este trabajo de tesis, ya que todavía se encuentra en proceso de publicación.

Periodo 2013 – 2014

- Búsqueda manual y electrónica de ensayos clínicos controlados publicados en revistas españolas de Ginecología y Obstetricia, en la cual participaron siete investigadores.
- Selección y recolección de datos de todos los ensayos clínicos controlados publicados en revistas españolas.
- Incorporación de todos los ensayos clínicos controlados publicados en revistas españolas al Registro Central Cochrane.
- Evaluación de la calidad de los ensayos clínicos controlados sobre reproducción asistida.

Redacción del artículo en español y su traducción al inglés para la revista *European Journal of Obstetrics* (anexo 1).

6. RESULTADOS

6.1 Publicación I: Evaluación sistemática de la calidad de las guías de práctica clínica que indican técnicas de reproducción asistida.

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SHORT REVIEW

Systematic evaluation of the quality of clinical practice guidelines on the use of assisted reproductive techniques

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Abstract

Objective: To conduct a systematic evaluation of clinical practice guidelines (CPGs) on the use of assisted reproductive technologies. *Methods:* We searched Medline, the Turning Research into Practice database, and guidelines-specific databases from December 2006 to November 2011. Three reviewers independently assessed each Guideline using the Appraisal of Guidelines for Research Evaluation (AGREE) II instrument. A standardized score was calculated separately for each of the six domains. *Results:* Fourteen Guidelines were included. Overall, the quality of these was suboptimal. The scores for each AGREE II domain ranged between 37% and 80%. Three (22%) were deemed "Recommended"; nine (64%),"Recommended with modifications"; and two (14%), "Not recommended". Agreement among reviewers was very good (Intraclass Correlation Coefficient: 0.915 [95% CI 0.807–0.970]). *Conclusions:* The overall quality of the CPGs on Assisted Reproduction Techniques published during the last 5 years is suboptimal. Most Guidelines present significant shortcomings in important domains such as "stakeholder involvement", "rigor of development", and "applicability". Instruments such as the AGREE II and "the Grading of Recommendation Assessment Development and Evaluation" system could prove useful to improve CPGs in this field. Guideline users could benefit from the present results when choosing which guidelines to implement.

Keywords: *Assisted reproduction techniques, clinical practice guidelines, in vitro fertilization, assisted embryo transfer, AGREE II instrument, quality assessment*

Introduction

Assisted Reproduction Technologies (ARTs) provide potential treatments for infertility, which has a reported prevalence ranging from 3.5% to 16.7% in developed countries and from 6.9% to 9.3% in developing countries (Boivin et al., 2007). ART is an overarching term for all treatments or procedures that include in vitro handling of both human oocytes and sperm or of embryos for the purpose of establishing a pregnancy (Zegers-Hochschild et al., 2009). Worldwide, ART has facilitated the conception of around 246,000 babies in a given year, (ICMART et al., 2009) and there has been an increase in the number of ART cycles performed during the last few years. However, the clinical pregnancy rates of ART are low (28.6–33%) (Nygren et al., 2011; de Mouzon et al., 2012), and vary depending on a variety of factors (McLernon et al., 2010; Pinborg et al., 2011; Maheshwari et al., 2011; Marinakis and Nikolaou 2011).

Clinical Practice Guidelines (CPGs) are defined as "systematically developed statements to assist practitioner and patient decisions about appropriate health care for specific clinical circumstances" (Field & Lohr, 1990). Developing CPGs may be an effective approach to improve the results of ART. "CPGs can bridge the gap between the growing stream of scientific evidence and clinical practice by facilitating the choices of health care professionals and patients for the most appropriate, safe, and cost-effective care" (Nelen et al., 2008). CPGs can minimize risk and improve performance of ART. However, they need to be of high quality, requiring not only knowledge of the clinical question, but also expertise in their development (Fervers et al., 2003).

The Appraisal of Guidelines for Research Evaluation (AGREE) is a validated instrument used to evaluate the quality of CPGs (The AGREE Collaboration, 2003). The original AGREE instrument, developed in 2005,

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was updated recently and renamed AGREE II (Burls, 2010; Brouwers et al., 2010; Browers et al., 2010a). A recent systematic review showed that, despite an improvement over time, the quality of CPGs has remained moderate to low during the last 2 decades (Alonso-Coello et al., 2010).

The quality of ART guidelines has not been systematically appraised using the AGREE II instrument in the last 5 years (Nelen et al., 2008). In order to address this gap in knowledge, we conducted a systematic evaluation of CPGs on ART published in the period 2006–2011.

Methods

Searching for clinical practice guidelines

We searched MEDLINE (accessed by means of PubMed) using a search strategy with free terms and their corresponding MeSH terms ("Reproductive Techniques", "Fertilization in Vitro", "Sperm Injections Intracytoplasmic", "Embryo Transfer", "Zygote Intrafallopian Transfer", "Cryopreservation", and "Oocyte Donation") limiting the results with a filter. We also searched the TRIP database, websites of professional societies, and guideline-specific databases (Guideline International Network database, National Guidelines Clearinghouse, Guidelines Finder, CMA InfoBase, and National Health and Medical Research Council) using a simple search strategy, and performed complementary searches in Google.

Eligibility criteria

CPGs on ARTs written in English and published between December 2006 and November 2011 were included. ARTs, according to the WHO, include (i) in vitro fertilization and embryo transfer, (ii) gamete intrafallopian transfer, (iii) zygote intrafallopian transfer, (iv) tubal embryo transfer, (v) gamete and embryo cryopreservation, (vii) oocyte and embryo donation, and (viii) gestational surrogacy (Zegers-Hochschild et al., 2009). Systematic reviews, documents explicitly classified as expert consensus, and those that are not available in full text were excluded. Additionally, we excluded GPCs that address laboratory-specific issues of organization or those do not directly address indications of ART. We considered only the latest version of each CPG.

Two reviewers (R.GV. and L.BN.) reviewed the titles and abstracts of the references retrieved in order to verify eligibility. They also independently examined the full-text versions to discover if they could be included. If needed, disagreements were resolved by discussion with a third reviewer (P.AC.).

Appraisal of methodological quality of the selected CPGs

Three reviewers (R.GV, L.BN, and A.A.) appraised the methodological quality of the selected guidelines independently using the AGREE II instrument the purpose of which is to provide a validated framework to assess the quality of guidelines. This instrument contains 23 key items organized in six domains, followed by two overall item score ("global assessment"). Each domain comprises a single quality dimension of the guide. Each item of the AGREE II and the two global assessment items are graded using a 7-point scale (from 1 "strongly disagree" to 7 "strongly agree"). Domains are (1) scope and purpose, (2) stakeholder involvement, (3) rigour of development, (4) clarity and presentation, (5) applicability, and (6) editorial independence (Table I).

When evaluating each item within domains, a score of 1 is assigned when there is no information relevant to the AGREE II item or if the concept is very poor. A score of 7 is given if the quality of reporting is exceptional and if the full criteria and considerations articulated in the User's Manual have been met. A score between 2 and 6 is assigned when the reporting of the AGREE II item does not meet the full criteria or addresses each consideration. For the overall judgement, a 3-point scale is used to rank the CPG as "recommended" (above 60%), "recommended with modifications" (30–60%), or "not recommended" (below 30%). Domain scores were calculated by adding the scores of all individual items in a domain and by standardizing the total as a percentage of the maximum possible score for that domain (thus ranging from 0 to 100%) (Burls, 2010).

$$\frac{\text{Obtained score} - \text{Minimum possible score}}{\text{Maximum possible score} - \text{Minimum possible score}} \times 100$$

Statistical analyses

We performed a descriptive statistical analysis for each domain. Descriptive values included mean, standard deviation, and minimum and maximum values with 95% confidence intervals (CI). Categorical variables were calculated using the number of cases and the corresponding percentages. Agreement between the three reviewers was determined using the Intraclass Correlation Coefficient (ICC) with a 95% CI. A standardized score was calculated separately for each of the six domains. On the scale proposed by Landis and Koch, the degree of agreement of the ICC is classified as < 0: Poor, 0.01–0.20: Mild, 0.21–0.40: Right, 0.41–0.60: Moderate, 0.61–0.80: Substantial, and 0.81–1.00: Very good (Kramer & Feinstein, 1981; Landis & Koch, 1977). Data were analysed using SPSS (19.0) for Windows (Statistical Package for the Social Sciences, Chicago, IL, USA).

Results

Search and selection

From an initial sample of 574 potentially relevant references, 14 CPGs were included (Figure 1). These documents referred to in vitro fertilization (8, 57%),

Table I. The AGREE instrument II.

DOMAIN 1. SCOPE AND PURPOSE
1. The overall objective(s) of the guideline is (are) specifically described.
2. The health question(s) covered by the guideline is (are) specifically described.
3. The population (patients, public, etc.) to whom the guideline is meant to apply is specifically described.
DOMAIN 2. STAKEHOLDER INVOLVEMENT
4. The guideline development group includes individuals from all relevant professional groups.
5. The views and preferences of the target population (patients, public, etc.) have been sought.
6. The target users of the guideline are clearly defined.
DOMAIN 3. RIGOUR OF DEVELOPMENT
7. Systematic methods were used to search for evidence.
8. The criteria for selecting the evidence are clearly described.
9. The strengths and limitations of the body of evidence are clearly described.
10. The methods for formulating the recommendations are clearly described.
11. The health benefits, side effects, and risks have been considered in formulating the recommendations.
12. There is an explicit link between the recommendations and the supporting evidence.
13. The guideline has been externally reviewed by experts prior to its publication.
14. A procedure for updating the guideline is provided.
DOMAIN 4. CLARITY OF PRESENTATION
15. The recommendations are specific and unambiguous.
16. The different options for management of the condition or health issue are clearly presented.
17. Key recommendations are easily identifiable.
DOMAIN 5. APPLICABILITY
18. The guideline describes facilitators and barriers to its application.
19. The guideline provides advice and/or tools on how the recommendations can be put into practice.
20. The potential resource implications of applying the recommendations have been considered.
21. The guideline presents monitoring and/or auditing criteria.
DOMAIN 6. EDITORIAL INDEPENDENCE
22. The views of the funding body have not influenced the content of the guideline.
23. Competing interests of guideline development group members have been recorded and addressed.

embryo transfer (4, 29%), embryo cryopreservation (1, 7%), and embryo donation (1, 7%). They were developed in Canada (6, 43%), United States (4, 30%), United Kingdom (2, 14%), Australia (1, 7%), and in several European countries (1, 7%). Nine (64%) were developed by scientific societies, three (22%) by medical associations, and two (14%) by government agencies (Table II).

Appraisal of the methodological quality of CPGs

The overall and per domain agreement among reviewers for the evaluation with the AGREE II instrument was very good (ICC: 0.915, 95% CI 0.807–0.970).

Scope and purpose (Domain 1)

The first domain refers to the overall purpose of the guide, specific health issues, and the target population (items 1–3). The mean score for this domain was 78% (range: 46–98%). The majority of guidelines 12 (86%) had scores above 60% (range: 67–98%). Two guidelines (14%), scored below 60% (range: 46–56%) (Table III).

Stakeholder involvement (Domain 2)

This domain pertains to whether the CPG development groups included individuals from all relevant stakeholders, if the patients' views and preferences were taken into consideration, and if target users are clearly defined (items 4–6). The mean score for this domain was 45% (range: 17–94%). Most documents (78%) scored below 60% (range: 17–54%). Patients or patient representatives were involved in the development of three of the guidelines (21%) (Lee et al., 2006; NICE 2009; Teede et al., 2011) (Table III).

Rigour of development (Domain 3)

This domain is crucial as it concerns the process used to gather and synthesize the evidence with which to develop the CPG, the methods used to formulate the recommendations, the effects of the recommendations, the explicit link between the recommendations and the supporting evidence, and the methods followed for reviewing the CPG externally and for updating it (items 7–14). The mean score in this domain was 53% (range: 8–99%). Eight guidelines (57%) scored above 60% (range: 63–99%). Six (42%) scored below 60% (range: 8–58%) (Table III).

Clarity of presentation (Domain 4)

This domain assesses whether recommendations were specific and unambiguous, if they covered the corresponding options for disease management according to its scope, and if key recommendations are easily identifiable (items 15–17). With a mean score of 80% (range: 48–98), it was the domain that showed the least variability. Twelve guidelines (86%) scored above 60% (range: 65–98%). Only two (14%) guidelines scored below 60% (range: 48%–59%) (Table III).

Applicability (Domain 5)

This domain assesses whether a CPG describes facilitators and barriers to its application, provides advice on, or tools for, how the recommendations can be put into

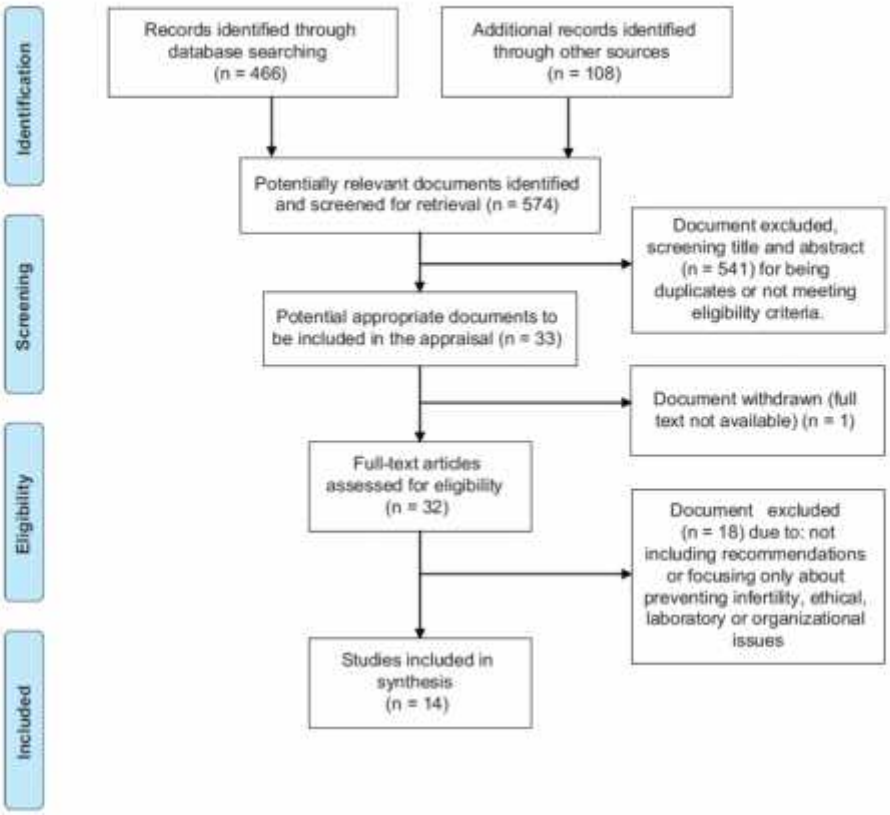

Figure 1. Search and selection results of guidelines on assisted reproduction techniques.

practice, if it presents monitoring or auditing criteria, and the cost of implementing the corresponding recommendations (items 18–21). Scores for this domain were the lowest, with a mean score of 37% (range: 7–89%). Two guidelines (15%) scored above 60% (range: 86–89%) (Table III).

Editorial independence (Domain 6)

This domain refers to the sources of external funding and the possible conflicts of interest among members of the development group (items 22–23). The mean score was 63%, although with great variability (3–100%). Five CPGs (35%) scored below 60% (3–53%), whereas nine (64%) scored above 60% (range: 64–100%) (Table III).

Overall assessment

Our results showed that the highest-quality guidelines were those developed by the European Association of Urology, Polycystic Ovary Syndrome Association of Australia, and National Institute for Health and Care Excellence (NICE). Three CPGs (21%) were deemed "recommended" (NCCWCH/NICE, 2009; Dohle et al., 2010; Teede et al., 2011), nine (64%), "recommended with modification" (JOINT SOGC-CFAS, 2008; Lee et al., 2006; Jarvi et al., 2010; Cutting et al., 2008; American Urological Association (AUA), 2010; Min et al., 2010; Vause et al., 2010; Leyland et al., 2010; Liu & Case, 2011), and 2 (14%), "not recommended" (Practice Committee of the American Society for Reproductive Medicine (ASRM), 2008, 2009) (Table III).

Discussion

Our review shows that the methodological quality of ART CPGs is suboptimal and most are either not recommended or recommended with modifications. Several of the AGREE domains, including "stakeholder involvement", "rigour of development", and "applicability" had poor scores.

The score in "Rigour of development" (53%) was worryingly low. This domain is considered crucial from a methodological point of view and includes, amongst other things, how recommendations are developed and the criteria used to rate the quality of the evidence and the strength of recommendations. The World Health Organization, like many other organizations around the world (including the Cochrane Collaboration, NICE, Scottish Intercollegiate Guidelines Network), has

Table II. Details of selected guidelines on assisted reproduction techniques.

Title	Country	Organization	Author	Publication year
American Society of Clinical Oncology recommendations on fertility preservation in cancer patients.	USA	American Society of Clinical Oncology.	Lee et al.	2006
Elective single embryo transfer, guidelines for practice British Fertility Society and Association of Clinical Embryologists.	United Kingdom	British Fertility Society and Association of Clinical Embryologists.	Cutting et al.	2008
Guidelines for the number of embryos to transfer following in vitro fertilization.	Canada	Society of Obstetricians and Gynaecologists of Canada and the Board of the Canadian Fertility and Andrology Society.	JOINT SOGC-CFAS	2008
Guidelines for gamete and embryo donation: a Practice Committee report.	USA	American Society for Reproductive Medicine; Practice Committee of Society for Assisted Reproductive Technology.	ASRM	2008
Fertility: assessment and treatment for people with fertility problems.	United Kingdom	National Collaborating Centre for Women's and Children's Health Commissioned by the National Institute for Clinical Excellence.	NICE	2009 (2004)
Guidelines on number of embryos transferred. Principio del formulario Final del formulario	USA	American Society for Reproductive Medicine and the Practice Committee of the Society for Assisted Reproductive Technology.	ASRM	2009
The Management of obstructive azoospermia: AUA Best Practice Statement.	USA	American Urological Association Education and Research, Inc.	AUA	2010
Elective single embryo transfer following in vitro fertilization.	Canada	Society of Obstetricians and Gynaecologists of Canada and the Board of the Canadian Fertility and Andrology Society.	Min et al.	2010
Ovulation induction in polycystic ovary syndrome.	Canada	Society of Obstetricians and Gynaecologists of Canada and the Board of the Canadian Fertility and Andrology Society.	Vause et al.	2010
Endometriosis: diagnosis and management.	Canada	Journal of Obstetrics and Gynaecology Canada.	Leyland et al.	2010
CUA Guideline: The workup of azoospermic males.	Canada	Canadian Urological Association.	Jarvi et al.	2010
Guidelines on male Infertility.	European	European Association of Urology.	Dohle et al.	2010
Advanced reproductive age and fertility.	Canada	Society of Obstetricians and Gynaecologists of Canada and the Board of the Canadian Fertility and Andrology Society.	Liu et al.	2011
Assessment and management of polycystic ovary syndrome: summary of an evidence-based guideline.	Australia	PCOS Australian Alliance (Polycystic ovary syndrome).	Teed et al.	2011

JOINT SOGC-CFAS, Joint Society of Obstetricians and Gynaecologist of Canada – Canadian Fertility and Andrology Society; ASRM, American Society for Reproductive Medicine; NICE, National Institute for Clinical Excellence; AUA, American Urological Association.

Table III. Mean standardized score per domain and overall assessment results for each Clinical Practice Guideline.

	Mean standardized score, %						
Guidelines Authors	Scope and purpose	Stakeholder involvement	Rigour of Development	Clarity and presentation	Applicability	Editorial independence	Overall recommendation
Lee et al.	89	89	70	48	32	92	Recommended with modifications
Cutting et al.	87	39	47	81	42	08	Recommended with modifications
JOIN SOGC-CFAS	83	30	66	89	31	33	Recommended with modifications
ASRM	67	17	10	72	07	03	Not recommended
NICE	89	94	90	91	89	83	Recommended
ASRM	72	24	08	67	10	53	Not recommended
AUA	56	28	32	65	32	81	Recommended with modifications
Min et al.	72	35	67	87	38	64	Recommended with modifications
Vause et al.	91	31	65	87	38	64	Recommended with modifications
Leyland et al.	69	31	58	94	33	50	Recommended with modifications
Jarvi et al.	46	30	17	59	19	83	Recommended with modifications
Dohle et al.	91	54	63	96	50	100	Recommended
Liu et al.	85	46	63	91	17	69	Recommended with modifications
Teed et al.	98	94	99	98	86	100	Recommended

recognized the need to use more rigorous processes to ensure that health care recommendations are based on the best available research evidence (Oxman et al., 2006; Fretheim et al., 2006). The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) can help guideline authors improve their performance in this domain, since it provides a system for rating quality of evidence and strength of recommendations that is explicit, comprehensive, and structured (Guyatt et al., 2008; Schünemann, 2009; Brożek et al., 2011; Guyatt et al., 2011).

"Stakeholder Involvement" also had a low score (45%), probably due to patients or patient representatives not being involved in the development of guidelines. Guideline developers should include important stakeholders such as consumers, relevant health professionals who work within the relevant area, and managers or policy makers. They should also have access to the advice of experts or individuals with the necessary technical skills in the field of the CPG. In addition, draft recommendations should be reviewed by consumers, who should be asked explicitly to consider the values that were used (Fretheim et al., 2006; Schunemann et al., 2006; Cavazos et al., 2008).

Finally, the low score in the domain "applicability" (37%) might indicate that CPG developing groups lack training on strategies to implement CPGs and to determine the resources needed for that end. Previous research has identified physician barriers as the main reason for non-adherence to CPGs. These barriers include lack of familiarity with guidelines, perceived limited validity of guidelines, limited applicability of guidelines among specific patients, clinical inertia, influence of prior anecdotal experiences, medical heuristics, and guideline factors such as unclear or ambiguous guideline recommendations (Cavazos et al., 2008; Lugtenberg et al., 2009). Another study showed that "multiple barriers impede physicians' adherence to subfertility guidelines, mainly physicians' lack of self-efficacy and low outcome expectancy" (Haagen et al., 2005). The identification of these factors, which influence the applicability of recommendations, could help developers, and ultimately the implementation and use of recommendations. The ART procedures are not considered by all health insurances but some have suggested that insurance mandates might influence ART utilization. (Sunderam et al., 2012; Butts et al. 2013). National Health systems should be aware of the more trustworthy CPGs if they decide on funding ART.

On the other hand, "Scope and purpose", "Clarity of presentation", and "Editorial independence" had reasonably good scores. The CPGs assessed received high scores on how they defined their "scope and purpose", which indicates that the questions in these CPGs were generally well defined. The score for "Clarity of presentation" domain was above 60% and showed the least variability. The "Editorial independence" domain also had good scores, though with high variability, implying that authors of CPGs tend to provide information about potential conflicts of interest.

These results are broadly consistent with previous evaluations in the field of assisted reproduction (Appleyard et al., 2006; Haagen et al., 2006; Nelen et al., 2008). In a previous review, both the "Scope and purpose" and "Clarity of presentation" domains had the highest scores. Similarly, the scores for "Rigour of development", "Stakeholder involvement", and "Applicability" were far from optimal score (Alonso-Coello et al., 2010). In contrast, the highest score in our review was given to "Editorial independence".

Our evaluation has strengths and limitations. Its strongest point is that three independent evaluators achieved a high degree of agreement when they assessed the CPGs selected. In addition, we independently evaluated the inclusion criteria and implemented a systematic appraisal of the methodological quality of ART guidelines with the recently developed and internationally validated AGREE II instrument. Regarding limitations, we focussed on CPGs that were published in the period 2006–2011 and contained treatment recommendations

about the use of ART. We therefore may have excluded other high-quality guidelines about other issues around ART (such as ethical, laboratory, and organizational issues, or preventing infertility) or CPGs that had been published earlier. However, the vast majority of CPGs on this and other fields are updated periodically. One intrinsic limitation of the AGREE instrument is that it does not differentiate between "not performed" and "Not noted". To the extent that guideline groups did some of the things that they do not report we would be underestimating the true quality of the included guidelines. However, we think that this is unlikely. Finally, expert consensus documents were not included as they lack the basics of evidence-based recommendations (e.g. an explicit search strategy and the classification of the quality of the evidence). These documents are likely to be of lower quality than the ones included in our review. To that end, results may overestimate the quality of CPGs in the field of ART, strengthening our conclusions.

In summary, the overall quality of CPGs on ART published in English during the period 2006–2011 is suboptimal. The low score in the "Rigour of development" domain is particularly remarkable, since this domain is considered an important surrogate of methodological quality. It appears that a great deal remains to be done to reach excellence in CPGs in general, and specifically those on ART. Initiatives like GRADE and AGREE II could prove beneficial in improving CPGs' quality in future. Guideline users could benefit from the present results when choosing which guideline to implement. Similarly, professional organizations that develop CPGs, might take into account these results to improve the domains "stakeholder involvement" and "applicability".

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Appendix 1: Full electronic search strategy for MEDLINE

((((("Reproductive Techniques"[Mesh] OR "Fertilization in Vitro"[Mesh]) OR "Zygote Intrafallopian Transfer"[Mesh]) OR "Gamete Intrafallopian Transfer"[Mesh]) OR "Embryo Transfer"[Mesh]) OR "Cryopreservation"[Mesh]) OR "Surrogate Mothers"[Mesh]) OR "Oocyte Donation"[Mesh] OR "Reproductive Techniques, Assisted"[Mesh] AND Practice Guideline[ptyp] AND English[lang]) AND ("humans"[MeSH Terms] AND ("2006/12/1"[PDAT]: "2011/11/30"[PDAT]))

((((("Reproductive Techniques"[Mesh] OR "Fertilization in Vitro"[Mesh]) OR "Zygote Intrafallopian Transfer"[Mesh]) OR "Gamete Intrafallopian Transfer"[Mesh]) OR "Embryo Transfer"[Mesh]) OR "Cryopreservation"[Mesh]) OR "Surrogate Mothers"[Mesh]) OR "Oocyte Donation"[Mesh] OR "Reproductive Techniques, Assisted"[Mesh] AND Guideline[ptyp] AND English[lang]) AND ("humans"[MeSH Terms] AND ("2006/12/1"[PDAT]: "2011/11/30"[PDAT]))

"Reproductive Techniques, Assisted"[Mesh] AND "Guidelines as Topic"[Mesh] AND #"humans"[MeSH Terms] AND English[lang]# AND ("humans"[MeSH Terms] AND English[lang] AND ("2006/12/1"[PDAT]: "2011/11/30"[PDAT]))

"Reproductive Techniques, Assisted"[Mesh] AND "Practice Guideline"[Publication Type] AND "humans"[All Fields] AND ("humans"[MeSH Terms] AND English[lang] AND ("2006/12/1"[PDAT]: "2011/11/30"[PDAT]))

"Fertilization in Vitro"[Mesh] AND "Guidelines as Topic"[Mesh] AND ("humans"[MeSH Terms] AND English[lang]) AND ("2006/12/1"[PDAT]: "2011/11/31"[PDAT])

"Embryo Transfer"[Mesh] AND "Guidelines as Topic"[Mesh] AND ("humans"[MeSH Terms] AND English[lang]) AND ("2006/12/1"[PDAT]: "2011/11/31"[PDAT])

("ZygoteIntrafallopian Transfer"[Mesh]) AND "Guidelines as Topic"[Mesh] AND ("humans"[MeSH Terms] AND English[lang]) AND ("2006/12/1"[PDAT]: "2011/11/31"[PDAT])

"Gamete Intrafallopian Transfer"[Mesh] AND "Guidelines as Topic"[Mesh] AND ("humans"[MeSH Terms] AND English[lang]) AND ("2006/12/1"[PDAT]: "2011/11/31"[PDAT])

("Cryopreservation"[Mesh] AND "Embryo Transfer"[Mesh]) AND "Guidelines as Topic"[Mesh] AND ("humans"[MeSH Terms] AND English[lang]) AND ("humans"[MeSH Terms] AND English[lang] AND ("2006/12/1"[PDAT]: "2011/11/31"[PDAT]))

("Cryopreservation"[Mesh] AND "Oocytes"[Mesh]) AND "Guidelines as Topic"[Mesh] AND ("humans"[MeSH Terms] AND English[lang]) AND ("humans"[MeSH Terms] AND English[lang] AND ("2006/12/1"[PDAT]: "2011/11/31"[PDAT]))

"Directed Tissue Donation"[Mesh] AND "Guidelines as Topic"[Mesh] AND ("humans"[MeSH Terms] AND English[lang]) AND ("humans"[MeSH Terms] AND English[lang] AND ("2006/12/1"[PDAT]: "2011/11/31"[PDAT]))

(("Directed Tissue Donation"[Mesh] AND "Guidelines as Topic"[Mesh]) AND "Oocytes"[Mesh]) AND "Oocyte Donation"[Mesh] AND ("humans"[MeSH Terms] AND English[lang]) AND ("humans"[MeSH Terms] AND English[lang] AND ("2006/12/1"[PDAT]: "2011/11/31"[PDAT]))

("Embryonic Structures"[Mesh] AND "Directed Tissue Donation"[Mesh]) AND "Guidelines as Topic"[Mesh] AND ("humans"[MeSH Terms] AND English[lang]) AND ("2006/12/1"[PDAT]: "2011/11/31"[PDAT]))

"Surrogate Mothers"[Mesh] AND "Guidelines as Topic"[Mesh] AND ("humans"[MeSH Terms] AND English[lang]) AND ("2006/12/1"[PDAT]: "2011/11/31"[PDAT]))

6.2 Publicación II: Revisión sistemática del efecto de los vasodilatadores en mujeres sometidas a reproducción médicamente asistida.

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Vasodilators for women undergoing fertility treatment (Review)

Gutarra-Vilchez RB, Urrútia G, Glujovsky D, Coscia A, Bonfill Cosp X

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[Intervention Review]

Vasodilators for women undergoing fertility treatment

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ABSTRACT

Background

Since 1978, when Patrick Steptoe and Robert Edwards achieved the birth of the first test tube baby, assisted reproductive techniques have been refined and improved. However, the rate of successful pregnancies brought to term has barely increased. Therefore closer evaluation of the interventions is needed along with working towards improving uterus receptivity. Vasodilators have been proposed to increase endometrial receptivity, thicken the endometrium and favour uterine relaxation, all of which could improve uterine receptivity and enhance the chances for successful assisted pregnancies.

Objectives

To evaluate the effectiveness and safety of vasodilators in women undergoing fertility treatment.

Search methods

We searched the following electronic databases, trial registers and websites: the Cochrane Central Register of Controlled Trials (CENTRAL), EMBASE, MEDLINE, the Menstrual Disorders and Subfertility Group (MDSG) Specialised Register of controlled trials, PsycINFO, the Cumulative Index to Nursing and Allied Health Literature (CINAHL), *The Cochrane Library*, Web of Knowledge, the Open System for Information on Grey Literature in Europe (OpenSIGLE), the Latin American and Caribbean Health Science Information Database (LILACS) and ClinicalTrials.gov. The search was conducted in February 2014. No language restrictions were applied.

Selection criteria

Randomised controlled trials (RCTs) of vasodilators alone or in combination with other treatments compared with placebo or with other agents in women undergoing fertility treatment.

Data collection and analysis

Two review authors independently selected the studies, assessed the risk of bias and extracted data. Risk ratios (RRs) were calculated using the numbers of events in the control and intervention groups of each study. Study data were combined using a random-effects model, and evidence quality was assessed using Grades of Recommendation, Assessment, Development and Evaluation Working Group (GRADE) methods.

Main results

Ten studies with a total of 797 women were included in this review. Most of the studies were judged as having an unclear risk of bias. Three studies reported live births, two reported vasodilator-related side effects, 10 reported clinical pregnancies (diagnosed by differing criteria) and four reported other side effects (multiple gestation, miscarriage, ectopic pregnancy).

Overall, no evidence suggested that treatment with vasodilators increased live birth rates compared with placebo or no treatment (RR 1.18, 95% confidence interval (CI) 0.82 to 1.69, P value 0.37, three RCTs, 350 women, I^2 = 0%, moderate-quality evidence). This indicates that among women undergoing fertility treatment who have a 24% chance of live birth without the use of vasodilators, between 19% and 40% will achieve live birth with the use of vasodilators.

No evidence was found of a difference between vasodilators and placebo or no treatment in the incidence of treatment side effects (RR 1.63, 95% CI 0.33 to 7.93, P value 0.55, two RCTs, 258 women, I^2 = 32%, low-quality evidence). Nor did any evidence show a difference between them in terms of multiple gestation, spontaneous abortion/miscarriage or ectopic pregnancy rates. However few relevant data were available.

Overall, treatment with vasodilators was associated with an increased clinical pregnancy rate compared with placebo or no treatment (RR 1.38, 95% CI 1.00 to 1.92, P value 0.05, eight RCTs, 717 women, I^2 = 0%, low-quality evidence). However, confidence intervals do not rule out no effect of the intervention, and when studies of vasodilators combined with another medication (vitamin E or oestrogen) were excluded, the effects of treatment with vasodilators alone on clinical pregnancy rates were more uncertain.

The evidence was of low or moderate quality, and the main limitations were imprecision and lack of clarity about study methods. Risk of publication bias could not be assessed because of the low number of identified studies.

Authors' conclusions

Evidence was insufficient to show that vasodilators increased the live birth rate in women undergoing fertility treatment. However, low-quality evidence suggests that vasodilators may increase clinical pregnancy rates in comparison with placebo or no treatment. Evidence was insufficient to show whether any particular vasodilator, administered alone or in combination with other active medications, was superior, and evidence was insufficient to allow the review authors to reach any conclusions regarding adverse effects. Adequately powered studies are needed so that each treatment can be evaluated more accurately.

PLAIN LANGUAGE SUMMARY

Vasodilators in women undergoing fertility treatment

Review question: Cochrane review authors investigated the effectiveness and safety of vasodilators (drugs used to widen blood vessels) in women undergoing fertility treatment.

Background: In women undergoing fertility treatment for different causes, interventions aimed at improving the receptivity of the uterus are of utmost importance. Many different drugs have been evaluated, with the aim of increasing rates of implantation and live birth. These include vasodilating agents, which are used to dilate blood vessels to improve endometrial receptivity, thicken the endometrium and favour uterine relaxation, among other effects.

Study characteristics: Ten randomised controlled trials with a total of 797 women were included in this review. Investigators compared the use of vasodilators versus placebo or no treatment in women undergoing fertility treatment. The evidence is current to February 2014.

Key results: Only three of the included studies reported live birth. Overall, no evidence suggests that treatment with vasodilators increased live birth rates compared with placebo or no treatment. Moderate-quality evidence suggests that among women undergoing fertility treatment who have a 24% chance of live birth without the use of vasodilators, between 19% and 40% will achieve live birth with the use of vasodilators. However, low-quality evidence suggests that vasodilators may increase the chance of becoming pregnant. Evidence was insufficient to permit any conclusions regarding adverse effects, as only two studies reported this outcome.

Quality of the evidence: The evidence was of low or moderate quality, and the main limitations were imprecision and lack of clarity about study methods. Risk of publication bias could not be assessed because of the low number of included studies.

SUMMARY OF FINDINGS FOR THE MAIN COMPARISON *[Explanation]*

Vasodilator compared with placebo/no treatment for women undergoing fertility treatment

Patient or population: women undergoing fertility treatment
Settings: secondary care
Intervention: vasodilator[a]
Comparison: placebo/no treatment

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No. of participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Placebo	Vasodilator				
Live birth	Medium-risk population[b]		RR 1.18 (0.82 to 1.69)	350 (3)	⊕⊕⊕◯ moderate[c]	Most information comes from studies with low or unclear risk of bias. These studies have low precision, consistent direction of effect (directionality) and lack unexplained heterogeneity. Studies are insufficient to permit assessment of risk of publication bias
	236 per 1000	278 per 1000 (193 to 398)				
Vasodilator side effects	Medium-risk population[b]		RR 1.63 (0.33 to 7.93)	258 (2)	⊕⊕◯◯ low[c,d]	Proportion of information from studies with high risk of bias is sufficient to affect interpretation of results. In addition, these results have low precision and unexplained heterogeneity. However, they have directionality. Stud-

						ies are insufficient to allow assessment of risk of publication bias
	63 per 1000	102 per 1000 (21 to 496)				
Clinical pregnancy	**Medium-risk population[b]**		**RR** 1.38 (1.00 to 1.92)	717 (8)	ΦΦ○○[d] **low**	Information from studies with low or unclear risk of bias. These studies have very low precision but have directionality and lack unexplained heterogeneity. Studies are insufficient to allow assessment of risk of publication bias
	274 per 1000	340 per 1000 (274 to 526)				
Multiple gestation	**Medium-risk population[b]**		**RR** 0.89 (0.39 to 2.03)	250 (2)	ΦΦΦ○ **moderate**[e]	Information from studies with low or unclear risk of bias. This study has low precision but has directionality and lacks unexplained heterogeneity. Studies are insufficient to allow assessment of risk of publication bias
	80 per 1000	79 per 1000 (35 to 180)				
Spontaneous miscarriage	**Medium-risk population[b]**		**RR** 0.84 (0.37 to 1.91)	350 (3)	ΦΦΦ○ **moderate**[e]	Information from studies with low or unclear risk of bias. This study has low precision but has directionality and lacks unexplained heterogeneity. Studies are insufficient to allow assessment of risk of publication bias

	69 per 1000	58 per 1000 (26 to 132)				
Ectopic pregnancy	**Medium risk population**[a]		**RR** 1.47 [0.24, 8.86]	250 (2)	⊕⊕⊕◯ **moderate**[c]	Information from studies with low or unclear risk of bias. Studies have low precision but have directionality and lack unexplained heterogeneity. Studies are insufficient to allow assessment of risk of publication bias
	16 per 1000	24 per 1000 (4 to 143)				

*The basis for the **assumed risk** (e.g. median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI). **CI:** Confidence interval; **RR:** Risk ratio.

GRADE Working Group grades of evidence.
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[a] The **vasodilators** considered in this review are nitric oxide donors (NTG, ISMN and NTG), PTX, sildenafil and 2 vasodilators associated with other drugs compared with placebo or no treatment. Dose, route of administration, treatment time and frequency varied across treatments.

[b] All trials recruited **participants** with differing risk: participants with history of implantation failure who underwent ICSI/IVI (Oh 2002); participants who underwent ICSI because of female and male infertility when both were present or for unknown reasons (Farzi 2005; Mostafa 2003); infertile women undergoing standardised controlled ovarian hyperstimulation for ICSI-ZIFT (Aleyasin 2009); participants with an antecedent of poor endometrial response and frozen embryos (Firouzabadi 2013); participants with tubal infertility who had had at least 2 unsuccessful IVF and embryo transfer attempts when transferred embryos were of high quality (Alleva 2012); participants undergoing IVF and embryo transfer (Shaker 1993) and participants with a thin endometrium undergoing IVF cycles (Kim 2010).

[c] The confidence interval does not rule out benefit, harm or no effect from the intervention.

[d] Only 2 studies report adverse effects, which are subjective variables.

[e] The confidence interval does not rule out no effect from the intervention; some studies did not clearly describe methods used.

BACKGROUND

Description of the condition

Between 0.2% and 4.3% of babies born in developed countries are conceived through assisted reproduction techniques (Bouillon 2013; Sunderam 2012). A total of 237,809 babies were reported to have been born worldwide in 2004 (Sullivan 2013). In the 21 European countries that report the number of assisted reproduction procedures, 399,020 assisted reproduction technique cycles were performed in a population of 373.8 million (1067 cycles per million). In these countries, the clinical pregnancy rates for in vitro fertilization (IVF) per aspiration and per transfer were 28.9% and 32.9%, respectively. Those for intracytoplasmic sperm injection (ICSI) were 28.7% and 32.0% (Ferraretti 2013). Stats are very similar in recent years (Ferraretti 2012; de Mouzon 2012).

According to the World Health Organization, medically assisted reproduction is reproduction brought about through ovulation induction, controlled ovarian stimulation, ovulation triggering, insemination and assisted reproduction techniques (ART) (Zegers-Hochschild 2009). ART refers to "all treatments or procedures that include the in vitro handling of both human oocytes and sperm or of embryos for the purpose of establishing a pregnancy. This includes, but is not limited to, in vitro fertilization and embryo transfer, gamete intrafallopian transfer, zygote intrafallopian transfer, tubal embryo transfer, gamete and embryo cryopreservation, oocyte and embryo donation, and gestational surrogacy" (Zegers-Hochschild 2009). The success of assisted reproduction varies depending on several factors, such as maternal age (Marinakis 2011; Schmidt 2012), maternal weight (Pinborg 2011), the number of embryos transferred (McLernon 2010), the use of gonadotropins (Maheshwari 2011), inadequate endometrial thickness, uterine contractions and others.

A thin endometrium (measured at less than 8 mm by ultrasound scan) has a negative impact on the success of assisted reproduction (Check 2011); live births are possible despite thin endometria, but the pregnancy rate among these women is poor (Dix 2010). Investigators have expressed a marked interest in studying the role that the endometrium plays in the success of assisted reproduction (Casper 2011; Senturk 2008).

Uterine contractions influence embryo implantation, possibly through mechanical displacement of the embryo. Decreases in pregnancy rates and implantation rates were found as the frequency of uterine contractions increased. Approaches aimed at inhibiting uterine contractions could improve pregnancy rates for assisted reproduction (Aguilar 2010; Bulletti 2006; Fanchin 2001; Fanchin 2009; Lesny 1998).

Description of the intervention

Different vasodilating agents have been proposed to thicken the endometrium and to favour uterine relaxation. Agents used in assisted reproduction include sildenafil, glyceryltrinitrate (GTN), nifedipine, nimodipine, pentoxifylline and isosorbide monohydrate. Sildenafil (Viagra) is a phosphodiesterase-5-specific inhibitor that increases the vasodilatory effects of nitric oxide on vascular smooth muscle by preventing the degradation of cyclic guanosine monophosphate (cGMP). Studies report that vaginally administered sildenafil could lead to an improvement in uterine blood flow (Sher 2002; Takasaki 2010). Nitric oxide donors such as isosorbide monohydrate and glyceryltrinitrate are used in assisted reproduction. Glyceryltrinitrate is also used medically as a vasodilator: in 2002 it was discovered that these effects occur because glyceryltrinitrate is converted in the body to nitric oxide by mitochondrial aldehyde dehydrogenase. Glyceryltrinitrate, which is available in the form of tablets, sprays and patches, is used in assisted reproduction in an effort to improve pregnancy rates (Chen 2005). Pentoxifylline plus vitamin E was used in women undergoing assisted reproduction (Acharya 2009; Letur-Konirsch 2003). Reports have described successful conception and pregnancy with nifedipine given in doses of 30 mg/d after secondary infertility (Wilson 1990).

How the intervention might work

Endometrial thickness varies with the vascularity of the endometrium and the subendometrium, regardless of the concentration of oestradiol or progesterone (Raine-Fenning 2004). It is well known that some vasodilators, such as vaginal sildenafil citrate, produce selective endometrial vasodilation in women with Asherman's syndrome (a condition characterised by the presence of adhesions or fibrosis, or both, within the uterine cavity), which results in endometrial thickening (Zinger 2006). Vasodilators also increase radial artery flow, improving the quality of the endometrium in women with a thin endometrium (Takasaki 2010). It has been observed in animal studies that sildenafil plays a role in both implantation and decidualisation (cellular changes in the endometrium in preparation for implantation of the embryo caused by the effects of progesterone) by affecting $\beta(3)$ integrins (which are cell membrane proteins) and vascular endothelial growth factor (VEGF) expression during the implantation period (Biyiksiz 2011).

In addition, we know that markers of endometrial receptivity are reduced in stimulated cycles compared with natural cycles (Chen 2008; Evans 2012; Revel 2012), and that vasodilators have an effect on amelioration of endometrial receptivity when used in combination with an ovarian hyperstimulation protocol (Biyiksiz 2011). A limited number of studies have reported enhanced endometrial development, implantation rates and ongoing pregnancy rates after administration of vasodilators (Sher 2002; Takasaki 2010; Zinger 2006). Glyceryltrinitrate given in very low doses showed a significant inhibitory effect on human

myometrium in vitro (Orth 2011; Wetzka 2001). Pentoxifylline may be beneficial in reducing hydrogen peroxide-induced embryo damage and in improving outcomes of in vitro fertilisation (Zhang 2004). It also appears to improve the pregnancy rate in patients with a thin endometrium when combined with vitamin E (Acharya 2009; Letur-Könirsch 2002; Lédée-Bataille 2002). Nimodipine, which is a vasodilator calcium channel blocker, is currently under study, as it may prevent or delay the luteinising hormone (LH) surge during controlled ovarian stimulation cycles when clomiphene citrate is used in subfertile patients undergoing assisted reproduction by intrauterine insemination (Penzias 2012).

Why it is important to do this review

Several randomised controlled trials (RCTs) have studied the efficacy of different treatments (gonadotrophin-releasing hormone (GnRH) agonist, progesterone, aspirin, steroids, human chorionic gonadotrophin (hCG), vitamin E, cytokines, and vasodilators) in endometrial preparation for women undergoing assisted reproduction (Aleyasin 2009; Glujovsky 2010; Kim 2010; Ohl 2002; Shaker 1993). However, evidence is insufficient to allow investigators to endorse a particular protocol for endometrial preparation. The effect of vasodilators on endometrial preparation in fertility treatment has been studied only partially. Their role in implantation, decidualisation and uterine relaxation, among others, has not been evaluated. A previous systematic review assessed different treatments for endometrial preparation in embryo transfer (Glujovsky 2010) but excluded the comparison of vasodilators versus other treatments. Instead, the effectiveness of these treatments remains unproven, which could potentially incrementally increase costs or side effects in assisted reproduction. Studies are needed to identify and assess the efficacy and safety of vasodilators with or without other agents, or compared with placebo or other agents, in women undergoing fertility treatment.

OBJECTIVES

To evaluate the effectiveness and safety of vasodilators in women undergoing fertility treatment.

METHODS

Criteria for considering studies for this review

Types of studies

Only RCTs were eligible for inclusion. Cross-over trials were also excluded given that their design is not valid in this context. Quasi-randomised trials were excluded.

Types of participants

Women undergoing fertility treatment were considered, regardless of the thickness of the endometrium. No restrictions on age or comorbidities were applied.

For the purposes of this review, fertility treatment means medically assisted reproduction, such as ovulation induction; controlled ovarian stimulation; ovulation triggering; assisted reproduction technique procedures; and intrauterine, intracervical and intravaginal insemination with the semen of husband, partner or donor (Zegers-Hochschild 2009).

Types of interventions

Vasodilators (nifedipine, nimodipine, pentoxifylline; nitric oxide donors such as GTN and isosorbide mononitrate; and sildenafil, among others) administered via any route, with or without other agents (oestrogen or tocopherol vitamin E) compared with placebo or no treatment or any other active intervention (progesterone, oestrogen or other).

Types of outcome measures

Primary outcomes

1. Live birth or ongoing pregnancy.
2. Vasodilator side effects: hypotension, headache, tachycardia or other effects related to vasodilators, as defined by primary study authors.

Secondary outcomes

3. Clinical pregnancy.
4. Thickened endometrium.
5. Other adverse events: multiple gestation or birth, spontaneous miscarriage, ectopic pregnancy.

Definitions of terms

Live birth: the complete expulsion or extraction of a product of fertilisation from the mother, irrespective of the duration of pregnancy, which after such separation breathes or shows any other evidence of life, such as heartbeat, umbilical cord pulsation or definitive movement of voluntary muscles, irrespective of whether the umbilical cord has been cut or the placenta is attached. A minimum gestational age is not included in this definition.

Ongoing pregnancy: any pregnancy after 12 weeks. This can be combined with live birth.

Clinical pregnancy: a pregnancy diagnosed by ultrasonographic visualisation of one or more gestational sacs or definitive clinical signs of pregnancy. It includes ectopic pregnancies.

Thickened endometrium: an endometrium that measures 8 mm or greater, as determined by ultrasound scan.

Multiple gestation or birth: a pregnancy or delivery with more than one fetus or neonate.

Spontaneous abortion or miscarriage: the spontaneous loss of a clinical pregnancy before 20 completed weeks of gestational age (18 weeks after fertilisation) or, if gestational age is unknown, loss of an embryo or fetus weighing less than 400 g.

Ectopic pregnancy: a pregnancy in which implantation takes place outside the uterine cavity.

Search methods for identification of studies

We searched all published and unpublished RCTs of vasodilators in fertility treatment, without language restriction. Search strategies were designed in consultation with the Menstrual Disorders and Subfertility Group (MDSG) Trials Search Co-ordinator.

Electronic searches

We searched the following electronic databases, trial register and websites: Cochrane Central Register of Controlled Trials (CENTRAL) (Appendix 1), EMBASE (Appendix 2), MEDLINE (Appendix 3), Menstrual Disorders and Subfertility Group (MDSG) Specialised Register of controlled trials (Appendix 4), PsycINFO (Appendix 5) and Cumulative Index to Nursing and Allied Health Literature (CINAHL) (Appendix 6). All databases were date limited from inception to February 25, 2014, except for EMBASE, which was date limited from January 1, 2010 to February 25, 2014, because CENTRAL contains all EMBASE records previous to this date. Other electronic sources of trials were included.

1. ClinicalTrials.gov: http://www.clinicaltrials.gov
2. *The Cochrane Library*: http://www.cochrane.org/index.htm
3. Conference abstracts in the Web of Knowledge: http://wokinfo.com/
4. OpenSigle for Grey Literature from Europe: http://opensigle.inist.fr/
5. Latin American and Caribbean Health Science Information Database (LILACS) database: http://regional.bvsalud.org/php/index.php?lang=en

Searching other resources

The reference lists of articles retrieved by the aforementioned search were reviewed. We contacted experts in the field to obtain additional data. We handsearched conference abstracts of the International Federation of Gynaecology and Obstetrics (FIGO) World Congress from 1985, 1988, 1991, 1994, 1997, 2000, 2003, 2006, 2009 and 2012, and checked the references of relevant identified systematic reviews.

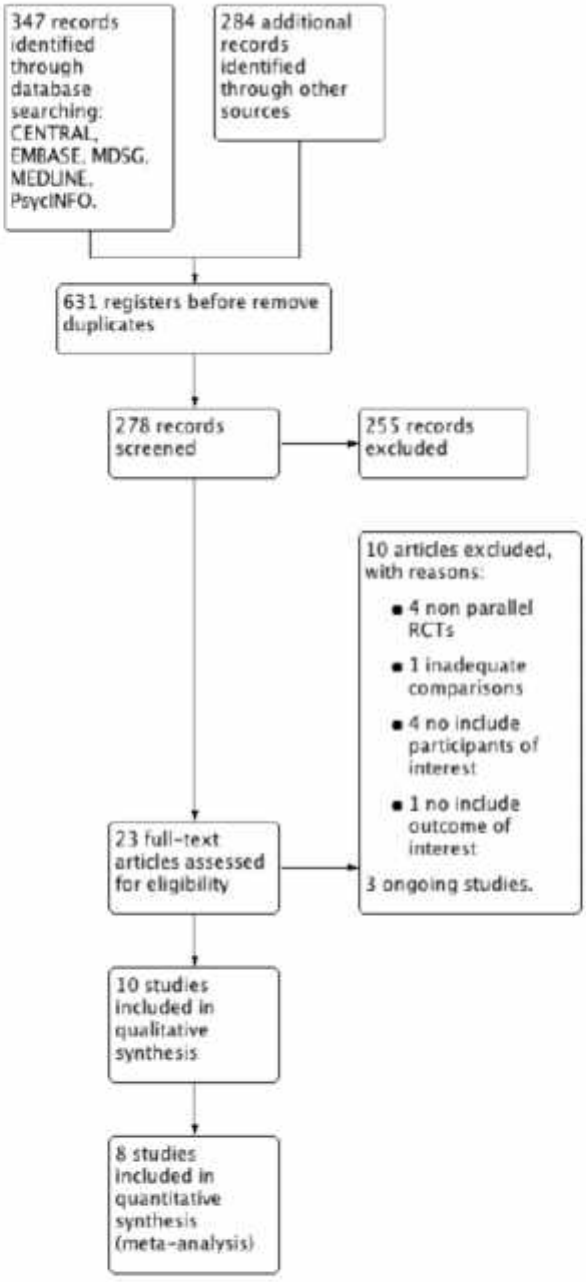
Data collection and analysis

Selection of studies

The pertinent statistical analysis was performed in accordance with the guidelines for statistical analysis developed by The Cochrane Collaboration.

Two review authors (RG and DG or AC) independently examined titles and abstracts retrieved through the search and determined whether studies met the inclusion criteria. For studies with potential or unclear eligibility, the full text of the article was obtained for independent assessment. If needed, study investigators were contacted to clarify study eligibility. Disagreements were resolved by discussion and consensus with a third review author (GU or XB). The selection process was documented on a PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow chart (Figure 1).

Figure 1. Flow of information through different phases of the systematic review.



Data extraction and management

Two review authors (RG and DG or AC) independently extracted data from eligible studies using a data extraction form designed and pilot-tested by the review authors. Disagreements were solved by discussion and consensus with a third review author (GU or XB). Data extracted included study characteristics, methods and outcome data. When a study had multiple publications, the main trial report was used for reference purposes, and additional details were derived from secondary papers. The original study authors were contacted if further information was required. In multiarm studies, data from arms that do not meet eligibility criteria will be excluded.

Assessment of risk of bias in included studies

Two review authors (RG and GU) independently assessed the included studies for risk of bias using the risk of bias assessment tool of The Cochrane Collaboration (Higgins 2011). We assessed allocation (random sequence generation and allocation concealment), blinding of participants and personnel, incomplete outcome data, selective reporting and other biases. Disagreements were resolved by discussion and consensus with a third review author. We fully described all judgements and presented conclusions in the 'Risk of bias' table (Figure 2; Figure 3), which was incorporated into the interpretation of review findings by means of sensitivity analyses (see below).

We assessed whether evidence suggested within-trial selective reporting, including failure to report obvious outcomes or insufficient reporting of outcomes. We searched published protocols to compare outcomes versus those of the corresponding published studies. When a study failed to report live birth but did report interim outcomes such as pregnancy, an informal assessment was undertaken to determine whether interim values (e.g. clinical pregnancy) were similar to those reported in studies that also reported live birth.

Figure 2. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

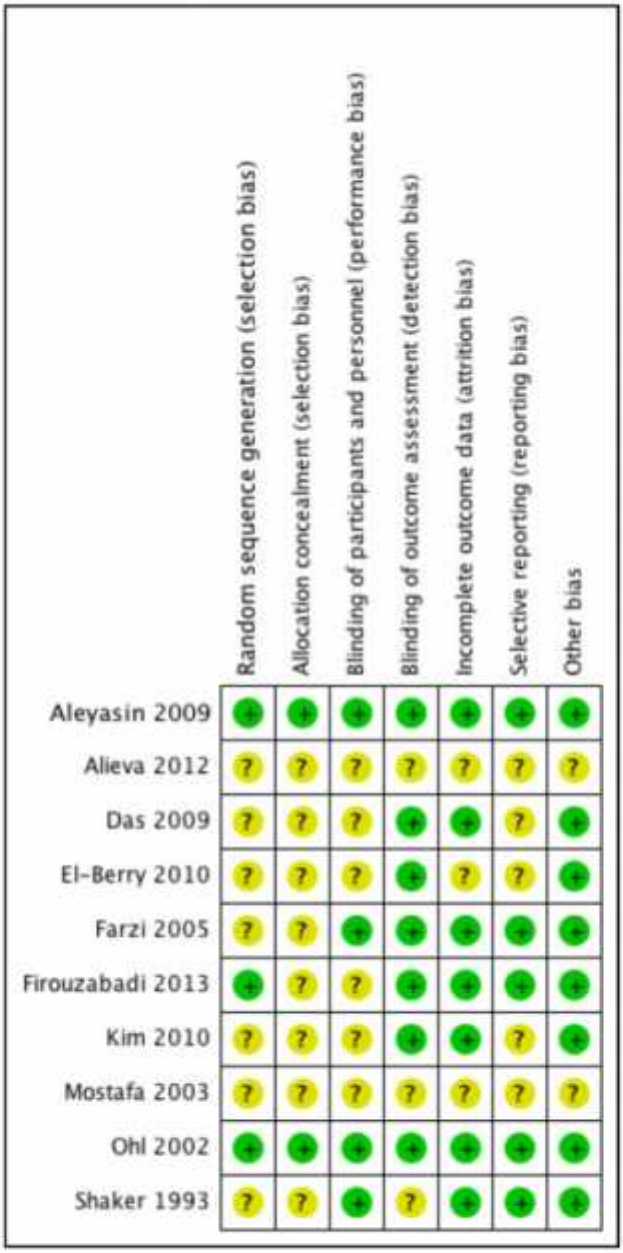
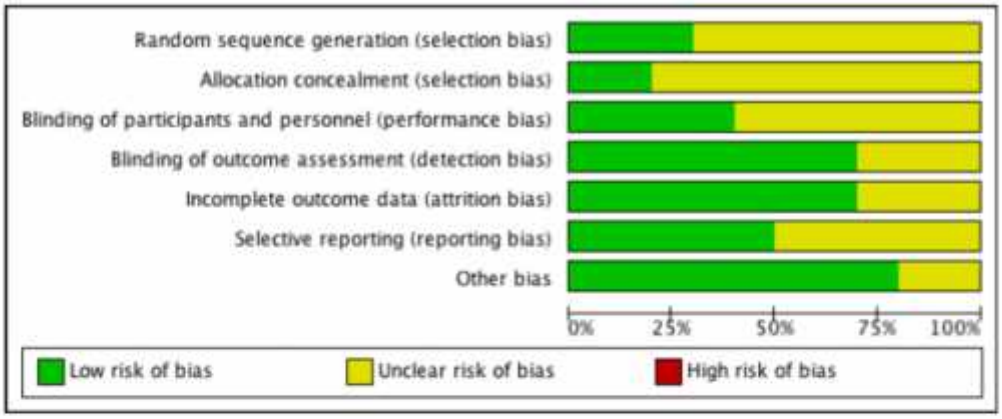


Figure 3. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.



Measures of treatment effect

For dichotomous data (e.g. live birth), we calculated risk ratios (RRs) using the numbers of events in the control and intervention groups of each study. We presented 95% confidence intervals (CIs) for all outcomes. When data were not available to calculate RRs, we used the most detailed available numerical data that can be used to complete similar analysis (e.g. test statics, P value). We compared the magnitude and direction of effect reported by studies against the way in which they are presented in the review, while taking account of legitimate differences.

Unit of analysis issues

All analyses were carried out per woman randomly assigned. When data did not allow valid analyses (e.g. "per cycle" data), study authors were contacted to request "per woman" data. If available data could not be analysed, we planned to summarise the data briefly in an additional table without meta-analysis. Multiple live births (e.g. twins, triplets) were counted as a single live birth event.

Dealing with missing data

Data were analysed on an intention-to-treat basis. Attempts were made to obtain missing data from the original trialists. Because information was not missing for any of the primary outcomes, imputation of individual values was not undertaken. It was not necessary to assume that a live birth did not occur in participants for whom no outcome was reported. For other outcomes, only available data were analysed.

Assessment of heterogeneity

It was determined whether clinical and methodological characteristics of included studies were sufficiently similar for meta-analysis to provide a clinically meaningful summary. Statistical heterogeneity was assessed through the I^2 statistical measure. An I^2 value greater than 50% was considered to show evidence of substantial heterogeneity (Higgins 2003). When we detected substantial heterogeneity, we explored possible explanations in the corresponding analyses. We took statistical heterogeneity into account when interpreting the results.

Assessment of reporting biases

If all eligible studies are not retrieved, the review may be biased. The review authors have tried to minimise the potential impact of publication and other reporting biases by ensuring a comprehensive search for eligible studies and by remaining alert to data duplication. If 10 or more studies had been included in an analysis, we would have used a funnel plot to explore the possibility of small-study effects (a tendency for estimates of the intervention effect to be more beneficial in smaller studies). Because the

included studies were fewer than 10, a funnel plot to explore the possibility of small-study effects was not presented.

Data synthesis

Because the studies were judged to be sufficiently similar, we combined their data using random-effects models in the following comparisons.

1. Vasodilator alone versus placebo or no treatment stratified by mode of administration (oral, vaginal and other) and by vasodilator type (sildenafil, glyceryltrinitrate (GTN) and other).
a. Glyceryltrinitrate (GTN)
b. Isosorbide mononitrate (ISMN)
c. Sildenafil
2. Vasodilator alone versus alternative active therapy, stratified by alternative (oestrogens, progesterone, other) and by vasodilator type.
3. Vasodilator combined with other agent versus placebo or no treatment, stratified by agent (oestrogens, progesterone, other) and by vasodilator type.
a. Pentoxifylline (PTX) and vitamin E.
b. Sildenafil and oestradiol.
4. Vasodilator combined with other agent versus alternative active therapy, stratified by alternative and by vasodilator type.

Some analyses initially proposed as stratified were not conducted because no suitable studies were found.

Subgroup analysis and investigation of heterogeneity

If data had been available, we would have conducted subgroup analyses to determine separate evidence within the following subgroups.

1. Studies in women with thin endometrium (< 8 mm) undergoing fertility treatment.
2. Studies in women with normal endometrial thickness undergoing fertility treatment.

Subgroup analysis was not undertaken according to type of control/other treatments. However, we have added a post hoc subgroup analysis for the secondary outcome of clinical pregnancy to evaluate studies that used only vasodilators with no co-intervention.

Sensitivity analysis

Sensitivity analyses for the primary outcomes were conducted to determine whether conclusions were robust enough to withstand arbitrary decisions regarding eligibility and analysis of included studies.

These analyses included considerations of whether the review conclusions would have differed if:

1. a fixed-effect model had been adopted.
2. treatment effect had been measured by using odds ratios (ORs).

Summary of findings table

We presented a summary of findings table to evaluate the overall quality of the body of evidence for main review outcomes using GRADE (Grades of Recommendation, Assessment, Development and Evaluation) criteria (i.e. study limitations (e.g. risk of bias), consistency of effect, imprecision, indirectness and publication bias). Judgements about evidence quality (high, moderate, low or very low) were justified, documented and incorporated into reporting of results for each outcome.

RESULTS

Description of studies

Results of the search

The search retrieved 631 articles. A total of 23 studies were potentially eligible and were retrieved in full text. Ten studies (Aleyasin 2009; Alieva 2012; Das 2009; El-Berry 2010; Farzi 2005; Firouzabadi 2013; Kim 2010; Mostafa 2003; Ohl 2002; Shaker 1993) met the inclusion criteria of this review. Ten studies (Alborzi 2007; Balasch 1997; Check 2004; Creus 2008; Kamencic 2008; Malinova 2013; Raine-Fenning 2009; Rosen 1987; Sher 2000; Shin 2002) were excluded, and three (Ben-Meir 2014; Casper 2013; Penzias 2012) are ongoing.

For further information, see the following tables: Characteristics of included studies; Characteristics of excluded studies; Characteristics of ongoing studies.

See Figure 1 (PRISMA study screening and selection flow chart) for details of this process.

Included studies

See Characteristics of included studies.

Study design and setting

Ten randomised controlled trials (RCTs) with a parallel design (Aleyasin 2009; Alieva 2012; Das 2009; El-Berry 2010; Farzi 2005; Firouzabadi 2013; Kim 2010; Mostafa 2003; Ohl 2002; Shaker 1993) were included in this review. Publication dates of the included studies ranged from 1993 to 2013.

Participants

Ten studies with a total of 797 women were included in this review. The studies included 396 women in the intervention groups and 401 in the control groups. Mean participant age was 31.39 years (± 4.43). Four trials included women with a "poor prognosis" (i.e. infertile women with a thin endometrium or an antecedent

of poor endometrial response or with a history of two or more previous implantation failures) (Das 2009; Firouzabadi 2013; Kim 2010; Ohl 2002). Six trials included women with a "good prognosis" (i.e. women without a previous history of failure of zygote intrafallopian transfer (ZIFT) or in vitro fertilisation (IVF)) (Aleyasin 2009; Alieva 2012; El-Berry 2010; Farzi 2005; Mostafa 2003; Shaker 1993). Eight of the 10 studies were performed in women undergoing assisted reproduction techniques (Aleyasin 2009; Alieva 2012; Farzi 2005; Firouzabadi 2013; Kim 2010; Mostafa 2003; Ohl 2002; Shaker 1993), one was performed in women undergoing artificial insemination (Das 2009) and one involved ovulation induction (El-Berry 2010).

Interventions

The vasodilators used in the studies include pentoxifylline 400 mg/BD oral dose + tocopherol vitamin E 400 mg/BD oral dose (Aleyasin 2009); nitric oxide donors (isosorbide mononitrate 20 mg vaginal) (El-Berry 2010) and glyceryltrinitrate 0.4 mg oral dose (Farzi 2005); sildenafil 50 mg oral dose (Firouzabadi 2013); sildenafil 25 mg vaginally four times a day (Das 2009); sildenafil 25 mg/d vaginally + oestradiol valerate 4 mg/d oral (Kim 2010); glyceryltrinitrate patch 5 mg (Ohl 2002); glyceryltrinitrate 400 µg/spray (Shaker 1993); sildenafil citrate (Alieva 2012) and glyceryltrinitrate skin patches 5 mg daily (Mostafa 2003).

1. Eight of 10 studies compared vasodilator alone versus placebo or no treatment (Alieva 2012; El-Berry 2010; Das 2009; Farzi 2005; Mostafa 2003; Ohl 2002; Shaker 1993; Firouzabadi 2013).
2. Two of 10 studies compared vasodilator plus other agent versus placebo or no treatment (Aleyasin 2009; Kim 2010).
3. No study used an active comparator.

Outcomes

1. Three of 10 studies reported live birth (Aleyasin 2009; Farzi 2005; Ohl 2002).
2. Two of 10 studies reported side effects (Ohl 2002; Shaker 1993).
3. All 10 studies reported clinical pregnancy (Aleyasin 2009; Alieva 2012; Das 2009; El-Berry 2010; Farzi 2005; Firouzabadi 2013; Kim 2010; Mostafa 2003; Ohl 2002; Shaker 1993). However, two studies reported biochemical pregnancy (Das 2009; El-Berry 2010), and the method used to diagnose pregnancy was not reported by two studies (Alieva 2012; Shaker 1993).
4. Four of 10 studies reported other adverse events (Aleyasin 2009; Alieva 2012; Farzi 2005; Ohl 2002). In one study, the miscarriage rate in the control group looks unusually high (Alieva 2012).

No study provided data on the number of participants with thickened endometrium. Therefore, it was not possible to analyse this outcome. Only two studies (Das 2009; Kim 2010) mentioned that all women had a thin endometrium before treatment.

Excluded studies

Ten studies were excluded from the review for the following reasons.

1. Four of 10 were not parallel RCTs (Check 2004; Raine-Fenning 2009; Sher 2000; Shin 2002).
2. Four of 10 did not include participants of interest for this review (Alborzi 2007; Balasch 1997; Creus 2008; Kamencic 2008).
3. One of 10 did not include comparisons of interest for this review (Rosen 1987).
4. One of 10 did not include outcomes of interest for this review (Malinova 2013).

In addition, three studies are ongoing (Ben-Meir 2014; Casper 2013; Penzias 2012).

Risk of bias in included studies

The judgements of review authors regarding each risk of bias item for each included study are shown and summarised in Figure 2 and Figure 3.

Allocation

Random sequence generation

Two studies (Aleyasin 2009; Ohl 2002) had low risk of selection bias related to sequence generation. The other eight studies (Alieva 2012; Das 2009; El-Berry 2010; Farzi 2005; Firouzabadi 2013; Kim 2010; Mostafa 2003; Shaker 1993) did not describe the method of randomisation, and were ranked as at unclear risk of bias.

Allocation concealment

Two studies (Aleyasin 2009; Ohl 2002) had low risk of bias related to allocation concealment. The other eight studies (Alieva 2012; Das 2009; El-Berry 2010; Farzi 2005; Firouzabadi 2013; Kim 2010; Mostafa 2003; Shaker 1993) did not describe the method used to conceal the sequence and were ranked as having unclear risk of bias.

Blinding

Four of 10 studies had low risk of detection bias (Aleyasin 2009; Farzi 2005; Ohl 2002; Shaker 1993). Three of these (Farzi 2005; Ohl 2002; Shaker 1993) were double-blind and used placebo as a control, and one (Aleyasin 2009) was single-blind (surgeons who conducted the operations were blinded). Two studies did not provide a description of blinding (Alieva 2012; Mostafa 2003). Four of 10 studies (Das 2009; El-Berry 2010; Firouzabadi 2013; Kim 2010) did not mention blinding and were judged as having unclear risk of detection bias. Blinding was not considered as likely to influence the outcome of live birth or clinical pregnancy. The same was not true for adverse events, for which lack of blinding could potentially affect findings.

Incomplete outcome data

Seven of 10 studies (Aleyasin 2009; Das 2009; Farzi 2005; Firouzabadi 2013; Kim 2010; Ohl 2002; Shaker 1993) analysed all or most (> 95%) of the women randomly assigned and had low risk of attrition bias. Only one study (El-Berry 2010) used the number of cycles instead of the number of participants in analysis, and two studies did not describe attrition (Alieva 2012; Mostafa 2003). These studies had an unclear risk of attrition bias.

Selective reporting

Five of 10 studies (Aleyasin 2009; Farzi 2005; Firouzabadi 2013; Ohl 2002; Shaker 1993) reported outcomes that were clearly pre-specified in the methods section and were classified as having low risk of selective reporting bias. Primary outcomes were reported in four of these studies (Aleyasin 2009; Farzi 2005; Ohl 2002; Shaker 1993): Three studies reported live birth (Aleyasin 2009; Farzi 2005; Ohl 2002), and two studies reported adverse effects (Ohl 2002; Shaker 1993). However, the protocol was available for only one study (Firouzabadi 2013).

Other potential sources of bias

Eight of 10 studies (Aleyasin 2009; Das 2009; El-Berry 2010; Farzi 2005; Firouzabadi 2013; Kim 2010; Ohl 2002; Shaker 1993) reported baseline balance between groups in terms of age and duration of infertility. In addition, four studies reported baseline comparability regarding type of infertility, cause of infertility and body mass index. These studies were classified as having low risk of bias. No other potential sources of bias were identified. However, two studies did not report baseline features and were judged to have unclear risk of detection bias (Alieva 2012; Mostafa 2003).

Effects of interventions

See: **Summary of findings for the main comparison** Vasodilator compared with placebo/no treatment for women undergoing fertility treatment

Primary outcomes

1 Vasodilator (with or without an additional intervention) versus placebo or no treatment

1.1 Live birth or ongoing pregnancy

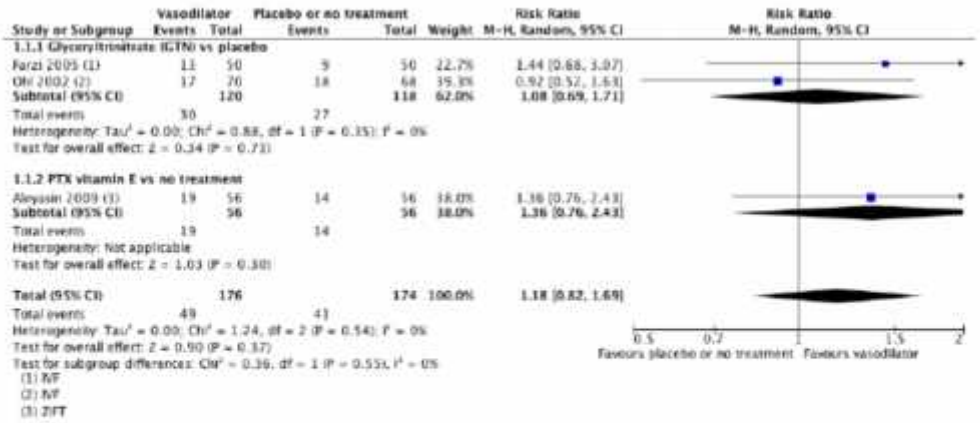
(Analysis 1.1)

Three studies reported this outcome. All reported live birth.

1. Glyceryltrinitrate (GTN) was compared with placebo (Farzi 2005; Ohl 2002).
2. Pentoxifylline + tocopherol vitamin E was compared with no treatment (Aleyasin 2009).

No evidence indicated that treatment with a vasodilator (alone or with another agent) influenced live birth rate compared with placebo or no treatment (RR 1.18, 95% CI 0.82 to 1.69, P value 0.37, three RCTs, 350 women, I^2 = 0%, moderate-quality evidence) (Analysis 1.1; Figure 4). This suggests that among women undergoing fertility treatment who have a 24% chance of live birth without use of vasodilators, between 19% and 40% will achieve live birth with use of vasodilators. No evidence showed an effect in the NTG subgroup (RR 1.08, 95% CI 0.69 to 1.71, P value 0.73, two RCTs, 238 women, I^2 = 0%, moderate-quality evidence) nor in the PTX + vitamin E subgroup (RR 1.36, 95% CI 0.76 to 2.43, P value 0.30, one RCT, 112 women, I^2 = 0%, moderate-quality evidence).

Figure 4. Forest plot of comparison: 1 Vasodilator vs Placebo/No treatment/Other treatment, outcome: 1.1 Live birth.



Sensitivity analyses using a fixed-effect model (RR 1.18, 95% CI 0.83 to 1.69, P value 0.35, three RCTs, 350 women, I^2 = 0%) or an odds ratio effect measure (OR 1.25, 95% CI 0.77 to 2.03, P value 0.36, three RCTs, 350 women, I^2 = 0%) did not affect the statistical significance of the main analysis for this outcome.

1.2 Vasodilator side effects

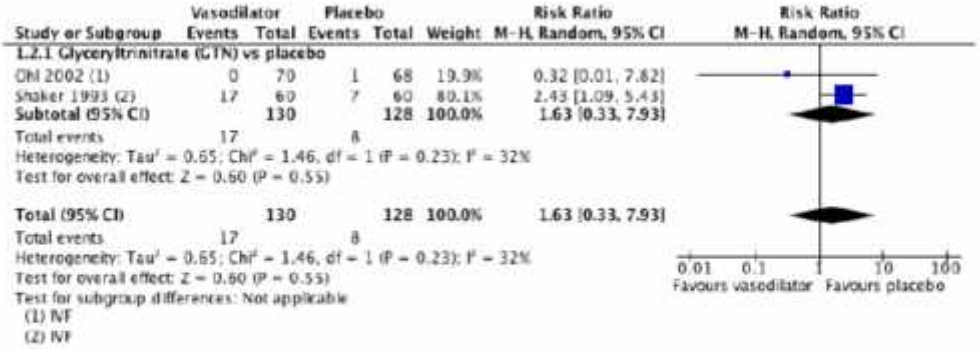
(Analysis 1.2)

Two studies reported this outcome.

The most commonly reported adverse events (AEs) in the vasodilator group were nervousness, insomnia, constipation and a feeling of weakness.

Glyceryltrinitrate (GTN) was compared with placebo (Ohl 2002; Shaker 1993). No evidence suggested that treatment with vasodilators increased the rate of side effects compared with placebo or no treatment (RR 1.63, 95% CI 0.33 to 7.93, P value 0.55, two RCTs, 258 women, I^2 = 0.32%, low-quality evidence) (Analysis 1.2; Figure 5).

Figure 5. Forest plot of comparison: 1 Vasodilator vs Placebo/No treatment/Other treatment, outcome: 1.2 Vasodilator side effects.

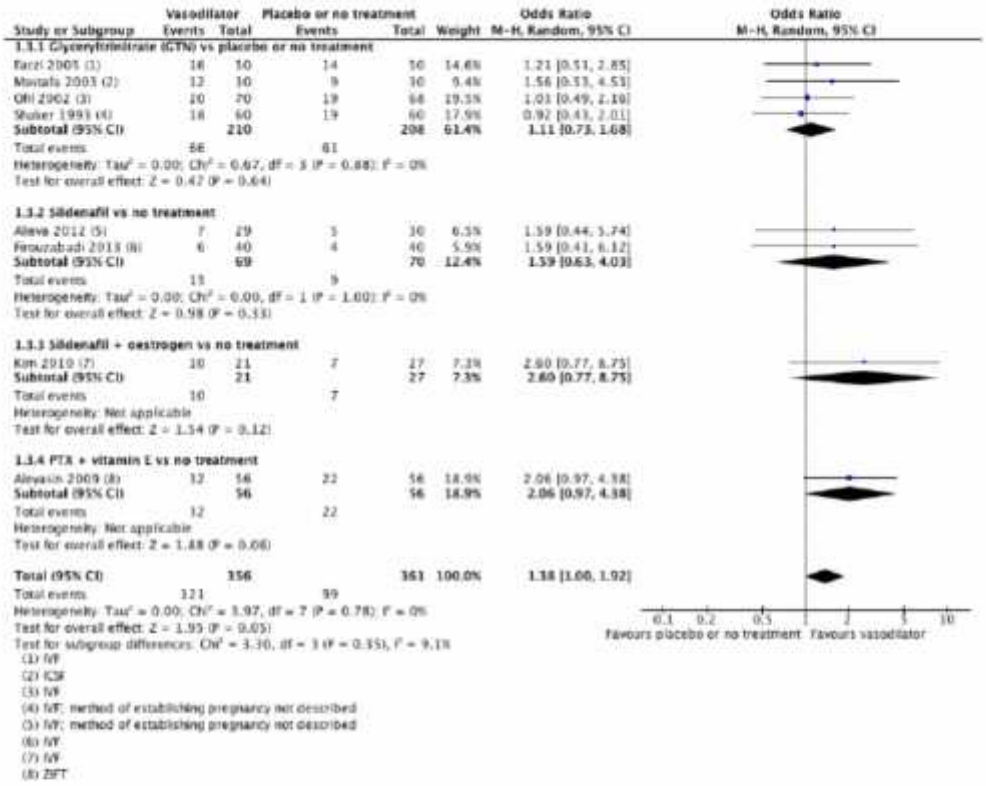


Secondary outcomes

1.3 Clinical pregnancy

(Analysis 1.3) (Figure 6)

Figure 6. Forest plot of comparison: 1 Experimental vs Control, outcome: 1.3 Clinical pregnancy.



Ten studies reported clinical pregnancy.

However, two studies reported biochemical pregnancy (El-Berry 2010; Das 2009). The method used to diagnose pregnancy was not reported by two studies (Alieva 2012; Shaker 1993). We included them in the analyses with this limitation stated in footnotes. We analysed eight studies (Aleyasin 2009; Alieva 2012; Farzi 2005; Firouzabadi 2013; Kim 2010; Mostafa 2003; Ohl 2002; Shaker 1993).

1. Glyceryltrinitrate (GTN) was compared with placebo (Farzi 2005; Mostafa 2003; Ohl 2002; Shaker 1993).
2. Sildenafil was compared with no treatment (Alieva 2012; Firouzabadi 2013).
3. Sildenafil plus oestrogen was compared with no treatment (Kim 2010).
4. Pentoxifylline + tocopherol vitamin E was compared with no treatment (Aleyasin 2009).

Overall, treatment with vasodilators was associated with an increased clinical pregnancy rate compared with placebo or no treatment (RR 1.38, 95% CI 1.00 to 1.92, eight RCTs, 717 women, I^2 = 0%, low-quality evidence). However, if studies of vasodilators associated with other medications (vitamin E, oestrogen) were excluded, treatment with vasodilators alone was not associated with an increased clinical pregnancy rate (RR 1.17, 95% CI 0.80 to

1.72, P value 0.41, six RCTs, 577 women, I^2 = 0%, low-quality evidence).

1.4 Other adverse events

(Analysis 1.4)

1.4.1 Multiple gestation or birth

Two studies reported this outcome.

1. Glyceryltrinitrate (GTN) was compared with placebo (Ohl 2002).
2. Pentoxifylline + tocopherol vitamin E was compared with no treatment (Aleyasin 2009).

No evidence showed that treatment with vasodilators influenced multiple gestation rate or birth rate compared with placebo or no treatment (RR 0.89, 95% CI 0.39 to 2.03, P value 0.79, two RCTs, 250 women, I^2 = 0%, moderate-quality evidence).

1.4.2 Spontaneous miscarriage

Four studies reported this outcome.

1. Glyceryltrinitrate (GTN) was compared with placebo (Farzi 2005; Ohl 2002).
2. Pentoxifylline + tocopherol vitamin E was compared with no treatment (Aleyasin 2009).
3. Sildenafil (NTG) was compared with no treatment (Alieva 2012).

In one study, the miscarriage rate in the control group looked unusually high (Alieva 2012). So, we analysed only three studies (Aleyasin 2009; Farzi 2005; Ohl 2002). No evidence suggested that treatment with vasodilators influenced spontaneous abortion/miscarriage rate compared with placebo or no treatment (RR 0.84, 95% CI 0.37 to 1.91, P value 0.99, three RCTs, 350 women, I^2 = 0%, moderate-quality evidence).

1.4.3 Ectopic pregnancy

Two studies reported this outcome.

1. Glyceryltrinitrate (GTN) was compared with placebo (Ohl 2002).
2. Pentoxifylline + tocopherol vitamin E was compared with no treatment (Aleyasin 2009).

No evidence indicated that treatment with vasodilators influenced the ectopic pregnancy rate compared with placebo or no treatment (RR 1.47, 95% CI 0.24 to 8.86, P value 0.67, two RCTs, 250 women, I^2 = 0%, moderate-quality evidence).

Subgroup analyses

As the included studies did not provide data on the number of women with endometrium measured as greater or less than 8 mm, planned subgroup analyses could not be performed. Only two studies (Das 2009; Kim 2010) mentioned that all women had a thin endometrium before interventions were provided.

In these studies, treatment with a vasodilator with an influence on clinical pregnancy rate was compared with placebo or no treatment (RR 1.17, 95% CI 0.80 to 1.72, P value 0.41, six RCTs, 557 women, I^2 = 0%, low-quality evidence).

DISCUSSION

Summary of main results

The results of this systematic review suggest that evidence is insufficient to show that vasodilators influence the live birth rate in women undergoing fertility treatment. (Aleyasin 2009; Farzi 2005; Ohl 2002).

Evidence was insufficient to permit any conclusions regarding adverse effects, as only two studies reported this outcome (Ohl 2002; Shaker 1993).

Low-quality evidence showed that vasodilators alone or in combination with other treatments (vitamin E, oestradiol) increased clinical pregnancy rate compared with placebo or no treatment (Aleyasin 2009; Farzi 2005; Firouzabadi 2013; Kim 2010; Mostafa 2003; Ohl 2002). Evidence was insufficient to show whether any particular vasodilator, alone or administered in combination with other active medications, was superior. Adequately powered studies are needed to evaluate each treatment more accurately.

Last, no evidence was found to suggest differences between groups for other adverse effects such as multiple gestation or birth (Aleyasin 2009; Ohl 2002), spontaneous abortion/miscarriage (Aleyasin 2009; Farzi 2005; Ohl 2002) and ectopic pregnancy (Aleyasin 2009; Ohl 2002); few relevant data were available.

No evidence showed statistical heterogeneity in this review, suggesting that factors that may have differed between studies had little effect on the overall findings. However, confidence intervals overlapped in individual trials and easily changed in the sensitivity analysis. Therefore, the results of this review should be interpreted with caution.

Overall completeness and applicability of evidence

All studies reported pregnancy as an outcome (Aleyasin 2009; Alieva 2012; Das 2009; El-Berry 2010; Farzi 2005; Firouzabadi 2013; Kim 2010; Mostafa 2003; Ohl 2002; Shaker 1993). However, for women and for clinicians, live birth rate and side effects are the most important outcomes of fertility treatment. As these outcomes were reported in only three studies (Aleyasin 2009; Farzi 2005; Ohl 2002) and in two studies (Ohl 2002; Shaker 1993),

respectively, this review might not address the main concerns surrounding fertility treatment. This, in turn, serves as evidence that more studies should assess these important outcomes.

Age restrictions for women in the inclusion and exclusion criteria of studies were similar across eight included studies. However, some trials included women with a "bad prognosis" (i.e. infertile women with a thin endometrium or with a history of two or more previous implantation failures), while other trials included women with a "good prognosis" (i.e. women without a previous history of failure of ZIFT or IVF). Even though no evidence of statistical heterogeneity among trials was found, the effects of clinical heterogeneity on results could not be ruled out.

Quality of the evidence

Evidence for live birth, clinical pregnancy, multiple gestation, miscarriage and ectopic pregnancy was rated as moderate quality, with the main limitation of low precision. Evidence for vasodilator side effects was of low quality, as it showed low precision and unexplained heterogeneity. Risk of publication bias could not be assessed because of the small number of identified studies (see Summary of findings for the main comparison).

Allocation (selection bias) was unclear in six studies. Among the 10 studies included, one trial used computer-generated randomisation (Aleyasin 2009), in another allocation was randomly permuted by four blocks and stratified by centre (Ohl 2002) and one use randomised tables (Firouzabadi 2013). Concealment of allocation was adequate and was explicitly described in two trials (Aleyasin 2009; Ohl 2002).

Three studies (Farzi 2005; Ohl 2002; Shaker 1993) were placebo-controlled but did not specify the use of blinding. Other studies were not blinded or failed to mention blinding. However, as most assessed outcomes were not subjective (live birth, clinical pregnancy, multiple gestation, ectopic pregnancy, miscarriage), lack of blinding did not imply an increase in risk of bias.

Seven studies (Aleyasin 2009; Das 2009; Farzi 2005; Firouzabadi 2013; Kim 2010; Ohl 2002; Shaker 1993) had low risk of attrition bias. These studies were analysed with intention to treat. Three studies (Alieva 2012; El-Berry 2010; Mostafa 2003) had unclear risk of attrition bias. One study (El-Berry 2010) used cycle numbers in the analysis.

Risk of selective reporting was unclear. Live birth rate was reported in a minority of cases, and only two studies reported adverse events as an outcome (El-Berry 2010; Kim 2010). However, four studies reported clinical pregnancy rates (Aleyasin 2009; Farzi 2005; Kim 2010; Ohl 2002), two studies reported implantation rates and six studies reported pregnancy.

Baseline equality between groups was acceptable in eight studies. Two studies (Alieva 2012; Mostafa 2003) had an unclear risk of bias. No other potential sources of bias were identified.

Therefore, clinically significant differences in treatment effects might be hidden. Additional RCTs with adequate power are required if investigators are to determine whether any of the vasodilators assessed in our review do enhance the live birth rate among women undergoing fertility treatment.

Potential biases in the review process

The process of identifying all potentially eligible studies was thorough and meticulous, even yielding three studies published only in abstract form. The authors of these works were contacted, but only one of them replied (Das 2009). Regarding all other procedures related to this review, we used the updated version of the *Cochrane Handbook for Systematic Reviews of Interventions*, and, as far as possible, protocol methodology was adhered to, so potential biases could be limited. Also, it was not possible to evaluate potential biases in all studies for lack of data. These studies were considered to have unclear risk of bias. We contacted authors of these studies, but only two of them replied (Farzi 2005; Kim 2010).

Agreements and disagreements with other studies or reviews

Other reviews in women undergoing assisted fertility treatment with vasodilators have not been identified. However, other relevant observational studies were identified (Sher 2000; Sher 2002; Takasaki 2010). One of the most important was a cohort study of the effect of vaginal sildenafil on the outcome of in vitro fertilisation after multiple IVF failures attributed to poor endometrial development found high ongoing pregnancy rates (Sher 2002).

AUTHORS' CONCLUSIONS

Implications for practice

Evidence was insufficient to show that vasodilators increased the live birth rate in women undergoing fertility treatment. However, low-quality evidence suggests that vasodilators increase clinical pregnancy rates in comparison with placebo or no treatment. Evidence is insufficient to show whether any particular vasodilator, administered alone or in combination with other active medications, is superior. Evidence is insufficient to permit any conclusions regarding adverse effects.

Implications for research

Although this review suggests that vasodilators increase clinical pregnancy rates compared with placebo or no treatment, future studies on vasodilators should report live birth rates, side effects and other important outcomes to enable consumers and healthcare providers to make well-informed decisions on the best treatment options. Based on the results of this review, the following recommendations are made.

1. RCTs with larger sample sizes are needed to evaluate whether any vasodilator is associated with an increase in live birth rate or pregnancy rate.

2. Future research should help to determine the optimal route of administration and dosage of different vasodilators.

3. Future research probably should focus mainly on sildenafil and should include assessment of the optimal route of administration and dosage.

4. Future research should evaluate relevant outcomes such as live birth, taking baby home and side effects.

5. Future research should investigate whether women with a thin endometrium may benefit from this medication.

6. Improved description of methods and adherence to CONSORT (Consolidated Standards of Reporting Trials) recommendations are needed for all RCTs.

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* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Aleyasin 2009

Methods	Randomised clinical trial. Not blinded
Participants	112 infertile women (56 intervention, 56 control) planned for ZIFT (zygote intrafallopian transfer) They were younger than 39 years of age without a previous history of ZIFT or IVF failure Exclusion criteria were hypothalamic amenorrhoea, drug reactions or complications, endometriosis and fibroids
Interventions	**Intervention:** pentoxifylline 400 mg/BD + tocopherol vitamin E 400 mg/BD 2 cycles before starting ZIFT cycle until the β-hCG became positive or the cycle was cancelled **Control:** Participants did not receive the aforementioned drugs
Outcomes	**Primary outcome:** clinical pregnancy **Other outcomes:** term delivery (equivalent "live birth"), multiple gestation or birth, spontaneous abortion/miscarriage, ectopic pregnancy and preterm labor
Notes	**Both groups** received gonadotrophin-releasing hormone (GnRH) agonist 500 mg SC started at day 22 of previous cycle + hMG (human menopausal gonadotrophins) 150-225 IU/d commenced on day 3 of the next cycle (dose determined for each participant on the basis of age and response to previous treatments) + hCG (human chorionic gonadotrophin) 10,000 IU IM (when < 2 follicles with diameter 17 mm observed) + ICSI and ZIFT (laparoscopic). Luteal phase support was started the day of ovum pick-up via administration of a progesterone suppository of 800 mg/d, and 25 mg progesterone in oil a week later (until fetal heart rate detection)

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Study authors described the method used to generate the allocation sequence in sufficient detail to allow an assessment of whether it should produce comparable groups: Computer-generated random number table was used for randomisation
Allocation concealment (selection bias)	Low risk	Study authors described the method used to conceal the allocation sequence in sufficient detail to reveal whether intervention allocations could have been determined in advance of, or during, enrolment: Group assignments were placed in sealed, opaque, sequentially numbered envelopes

Aleyasin 2009 *(Continued)*

Blinding of participants and personnel (performance bias) All outcomes	Low risk	The intervention was not blinded, but surgeons who performed the operations were blinded to participant groups. However, this does not seem to have affected study results
Blinding of outcome assessment (detection bias) All outcomes	Low risk	No blinding of outcome assessment was described, but the review authors judge that outcome measurement is not likely to be influenced by lack of blinding
Incomplete outcome data (attrition bias) All outcomes	Low risk	No participant dropout was reported (all participants were followed up)
Selective reporting (reporting bias)	Low risk	Study protocol not available, but it is clear that published reports include all expected outcomes, including those that were prespecified
Other bias	Low risk	Groups were comparable for the variables described (age, duration of infertility, type of infertility, cause of infertility, endometrial thickness, retrieved oocytes, metaphase II oocytes)

Alieva 2012

Methods	Randomised controlled trial
Participants	91 participants with tubal infertility, who had undergone at least 2 unsuccessful IVF and embryo transfer attempts when transferred embryos were of high quality and disturbances in uterine haemodynamics were present
Interventions	**Intervention** **Group I:** 32 women for whom impact on rates of blood flow and endometrial condition was assessed using intense low-frequency magnetic therapy in the cycle previous to IVF **Group II:** 29 women treated by sildenafil citrate in the IVF cycle **Control:** Group III (control): 30 women not given additional treatment
Outcomes	**Primary:** evidence of increasing end-diastolic flow velocity, decrease in vascular resistance and increased blood flow to uterine vessels **Secondary:** thickness of the endometrium after intervention and pregnancy rate (we do not know the method used to establish pregnancy) **Other:** spontaneous abortion/miscarriage
Notes	Published currently only as an abstract

Risk of bias

Bias	Authors' judgement	Support for judgement

Alieva 2012 *(Continued)*

Random sequence generation (selection bias)	Unclear risk	Methods were not adequately described
Allocation concealment (selection bias)	Unclear risk	Methods were not adequately described
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Methods were not adequately described
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Methods were not adequately described
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Methods were not adequately described
Selective reporting (reporting bias)	Unclear risk	Methods were not adequately described
Other bias	Unclear risk	Methods were not adequately described

Das 2009

Methods	Randomised controlled trial
Participants	50 infertile women (25 intervention, 25 control) with a thin endometrium (< 9 mm) undergoing Intrauterine Insemination on 2 occasions
Interventions	**Intervention:** sildenafil 25 mg vaginally 4 times a day from day 5 of cycle until day of hCG administration **Control:** no sildenafil
Outcomes	**Primary outcome:** pregnancy or conception rates (positive urine pregnancy test) **Other outcome**: endometrial thickness and uterine artery PI on day of hCG administration
Notes	**Both groups:** Ovulation induction was achieved with clomiphene citrate 100 mg from days 2 through 6. Follicular monitoring was conducted until the follicle reached 18-20 mm, at which time 5000 IU hCG injection was given and IUI was done on 2 occasions: after 24 hours and after 48 hours. Before IUI, couples were advised abstinence for 3-4 days. 200 mg micronised progesterone was given orally as luteal phase support twice daily for 14 days after 2nd IUI

Risk of bias

Bias	Authors' judgement	Support for judgement

Das 2009 *(Continued)*

Random sequence generation (selection bias)	Unclear risk	Methods were not adequately described (alternate participants were taken as case and control. Cases received sildenafil 25 mg vaginal suppositories; controls received no treatment)
Allocation concealment (selection bias)	Unclear risk	Methods were not adequately described (all cases were given tab sildenafil 25 mg vaginally 4 times a day from day 5 of cycle until day of hCG administration)
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Study did not use placebo
Blinding of outcome assessment (detection bias) All outcomes	Low risk	No blinding of outcome assessment was described, but review authors judged that measurement of pregnancy outcome is not likely to be influenced by lack of blinding
Incomplete outcome data (attrition bias) All outcomes	Low risk	Investigators evaluated all randomly assigned women
Selective reporting (reporting bias)	Unclear risk	Study protocol not available, and published reports do not include all expected outcomes-only those that were prespecified
Other bias	Low risk	No statistically significant differences in age or BMI were noted between the 2 groups

El-Berry 2010

Methods	Randomised controlled study
Participants	30 polycystic ovary infertile women diagnosed according to American Society of Reproductive Medicine and European Society of Human Reproductive and Embryology (15 women in intervention group, 15 women in control group) underwent ovulation induction
Interventions	**Intervention:** nitric oxide donors (isosorbide mononitrate (ISMN)) 20 mg vaginally until diagnosis of ovulation and pregnancy **Control:** did not receive this drug
Outcomes	**Primary outcome:** ovulation and pregnancy rates (diagnosed by serum β-hCG) **Other outcome:** number of mature follicles, cervical mucus score and endometrial thickness
Notes	**Both groups** received 100 mg clomiphene citrate on fifth, sixth, seventh, eighth and ninth CD. Treatment in both groups continued for 3 cycles
Risk of bias	

El-Berry 2010 *(Continued)*

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Insufficient information about the sequence generation process to allow judgement
Allocation concealment (selection bias)	Unclear risk	Method of concealment not described to allow a definitive judgement
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Methods used in this study not adequately described
Blinding of outcome assessment (detection bias) All outcomes	Low risk	No blinding of outcome assessment, but review authors judge that measurement of pregnancy outcome is not likely to be influenced by lack of blinding
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	No losses of participants, but unclear how many cycles each participant received and reasons for interrupting treatment. 37 cycles in the intervention group and 40 in the control group, but we used number of women (15 in each group)
Selective reporting (reporting bias)	Unclear risk	Study protocol is not available, and published reports do not include all expected outcomes-only those that were prespecified
Other bias	Low risk	Groups were comparable for variables described (age, body mass index, FSH, LH)

Farzi 2005

Methods	Prospective randomised double-blinded placebo-controlled clinical trial
Participants	100 participants in fresh ICSI-ET (50 participants in the intervention group, 50 in the control group) Participants underwent ICSI regardless of male or female infertility when both were present or when causes were unknown
Interventions	**Intervention:** glyceryltrinitrate (GTN) 0.4 mg oral dose 15 minutes before fresh ET **Control:** placebo
Outcomes	**Primary outcome:** implantation rate and clinical pregnancy rate **Other outcome:** taking baby home (equivalent "live birth"), spontaneous abortion/ miscarriage and biochemical pregnancy
Notes	**Both groups** were initially stimulated with a long protocol. Then, on the third day of of the next menstrual cycle, hMG 150-225 IU was injected and was adjusted with follicular

Farzi 2005 *(Continued)*

	development monitoring by vaginal ultrasound. In addition, 10,000 IU hCG was given IM when at least 3 follicular diameters of 18 mm 38 hours later led to ovarian puncture **Additional information from study authors:** 100 participants entered and completed this study; 1 cycle was performed for each participant

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Information about the sequence generation process was insufficient to allow judgement
Allocation concealment (selection bias)	Unclear risk	Method of concealment not described
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Double-blinded with use of placebo
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Not described, but main outcome not subjective. Outcome measurement not likely to be influenced by lack of blinding
Incomplete outcome data (attrition bias) All outcomes	Low risk	Study described 100 randomly assigned cycles ***Additional information from study authors:*** 100 participants entered and completed this randomisation study; 1 cycle performed for each participant
Selective reporting (reporting bias)	Low risk	Study protocol is not available, but it is clear that published reports include all expected outcomes, including those that were prespecified
Other bias	Low risk	Groups were comparable for variables described: age of the father and age of the mother, duration of infertility, oocyte retrieved, oocyte injected, 2 pronuclei, cleaved embryos, embryos transferred, causes of infertility, embryo quality

Firouzabadi 2013

Methods	Randomised clinical controlled trial, not blinded
Participants	Total of 80 participants with an antecedent of poor endometrial response and frozen embryos were included in this study. Inclusion criteria required participants to be younger than 40 years of age and to have high-quality frozen embryos. Exclusion criteria included

Firouzabadi 2013 *(Continued)*

	history of endocrine disease; history of hysteroscopic surgery; cardiovascular, renal and liver disease; hypotension (blood pressure < 90/50 mmHg) and history of stroke or myocardial infarction
Interventions	**Intervention:** sildenafil citrate tablets (50 mg) daily (from first day of cycle until day progesterone was started) **Control:** no sildenafil
Outcomes	**Primary outcome:** endometrial thickness **Other outcome:** implantation rate and chemical pregnancy rate (we used implantation rate as clinical pregnancy rate)
Notes	**Both groups** On 13th day of menstrual cycle, endometrial thickness was measured by transvaginal ultrasonography. If endometrial thickness > 8 mm, 100 mg progesterone was injected IM Oral oestradiol valerate (first to fourth days of menstrual cycle, 2 mg oestradiol valerate tablets; fifth to eighth day of menstrual cycle, 4 mg oestradiol valerate tablets; ninth to 12th day of menstrual cycle, 6 mg oestradiol valerate tablets) was given daily Administering oestradiol valerate and progesterone continued until 2 weeks after embryos were transferred

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Participants were divided into 2 groups on the basis of randomised tables
Allocation concealment (selection bias)	Unclear risk	Information was insufficient to permit judgement of 'low risk' or 'high risk': Allocation was not described
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Study did not use placebo
Blinding of outcome assessment (detection bias) All outcomes	Low risk	No blinding of outcome assessment, but review authors judge that measurement of pregnancy outcome is not likely to be influenced by lack of blinding
Incomplete outcome data (attrition bias) All outcomes	Low risk	Investigators evaluated all randomly assigned women
Selective reporting (reporting bias)	Low risk	Study protocol is available, and published reports include those that were prespecified. Investigators do not include all expected outcomes

Firouzabadi 2013 (*Continued*)

Other bias	Low risk	Groups were comparable for the variables described (duration of infertility, age, basal FSH, basal LH, basal oestrogen, basal progesterone, basal FSH/LH)

Kim 2010

Methods	Randomised
Participants	48 women (21 intervention, 27 control) among 170 patients with a thin endometrium (< 8 mm: range, 5-7.9 mm) at the time of ET undergoing IVF
Interventions	**Intervention:** vaginal sildenafil 25 mg/d + oral oestradiol valerate 4 mg/d from day of embryo transfer until pregnancy test (11 days) **Control:** did not receive the above drugs
Outcomes	**Primary outcome:** clinical pregnancy **Other outcome:** fertilisation rate
Notes	**Both groups** received recombinant FSH beginning on 3 CD + multiple-dose protocol of GnRH antagonist + 250 μg recombinant hCG (when dominant follicles averaged 19 mm in diameter to trigger ovulation) In all participants, luteal phase was supported by vaginal micronised progesterone 600 mg/d, starting on the day of oocyte retrieval and continued for another 6-8 weeks in cases in which pregnancy was achieved

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Insufficient information about sequence generation process to allow judgement
Allocation concealment (selection bias)	Unclear risk	Method of concealment not described to allow a definitive judgement
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Study did not use placebo
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Not described, but outcome measurement is not likely to be influenced by lack of blinding
Incomplete outcome data (attrition bias) All outcomes	Low risk	Investigators evaluated all randomly assigned women

Kim 2010 *(Continued)*

Selective reporting (reporting bias)	Unclear risk	Study protocol is not available, and no published reports describe all expected outcomes
Other bias	Low risk	Groups were comparable for the variables described (female age, duration of infertility, cause of infertility, total dose of gonadotrophin, day of triggering, endometrial thickness at triggering, number of ICSI cycles, number of embryos transferred)

Mostafa 2003

Methods	Randomised controlled trial	
Participants	Women who underwent IVF/ICSI indicated for infertility associated with a male factor. Ages ranged from 25 to 35 years	
Interventions	**Intervention:** glyceryltrinitrate skin patches 5 mg daily for 2 weeks **Control:** Participants did not receive the aforementioned drug	
Outcomes	**Primary outcome:** Pregnancy (we do not know the method used to establish pregnancy) and implantation rate (number of implantations and pregnancies is equal, so we used this as clinical pregnancy rate) **Secondary outcome:** pulsatility index	
Notes	Published currently only as an abstract	
Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Methods not adequately described
Allocation concealment (selection bias)	Unclear risk	Methods not adequately described
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Methods not adequately described
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Methods not adequately described
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Methods not adequately described
Selective reporting (reporting bias)	Unclear risk	Methods not adequately described

Mostafa 2003 *(Continued)*

Other bias	Unclear risk	Methods not adequately described

Ohl 2002

Methods	Randomised multi-centre double-blinded placebo-controlled trial
Participants	138 participants (70 in intervention group, 68 in control group) with a history of 2 or more previous implantation failures. Exclusion criteria were hypersensitivity to nitric oxide donors, heart failure, severe anaemia, high intracranial blood pressure and high intraocular blood pressure
Interventions	**Intervention:** 5 mg glyceryltrinitrate (GTN) patch applied once daily, beginning the morning of the day before transfer, just after transvaginal ultrasonography and colour doppler were performed **Control:** placebo
Outcomes	**Primary outcome:** clinical pregnancy **Secondary outcomes:** newborn (equivalent "live birth"), multiple gestation or birth, spontaneous abortion/miscarriage, ectopic pregnancy, vasodilator side effects
Notes	**Both groups** received GnRH agonist long protocol daily SC (continued up to the day when hCG was administered) + recombinant FSH + 5000 IU hCG + ICSI or conventional in vitro fertilisation + embryo transfer (embryos were transferred 2 or 3 days after oocyte retrieval)

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Investigators describe a random component in the sequence generation process. Randomisation was performed by using 4 randomly permuted blocks and was stratified by centre
Allocation concealment (selection bias)	Low risk	Participants and investigators enrolling participants could not foresee assignment because central allocation was used to conceal allocation
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Double-blinded with the use of placebo.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Not described, but main outcome measurement is not likely to be influenced by lack of blinding

Ohl 2002 *(Continued)*

Incomplete outcome data (attrition bias) All outcomes	Low risk	Intention-to-treat analysis was performed in this study, but study authors report losses for transvaginal ultrasonography
Selective reporting (reporting bias)	Low risk	Study protocol is not available, but it is clear that published reports include all expected outcomes, including those that were pre-specified
Other bias	Low risk	Groups were comparable for the variables described (age, body mass index, years of infertility, causes of infertility, number of previous pregnancy failures, basal FSH level, number of ICSI cycles, duration of stimulation, oestradiol level on day of hCG, endometrial thickness, secretory change between day before and day of embryo transfer, pulsatility index)

Shaker 1993

Methods	Double-blind study with random allocation
Participants	120 participants on embryo transfer (intervention 60, placebo 60)
Interventions	**Intervention:** 2 sublingual spray emissions of GTN 400 μg/spray or placebo spray **Control:** placebo
Outcomes	**Primary outcome:** pregnancy rate (outcome definition is not clear) **Secundary outcome:** side effect
Notes	All participants received in vitro fertilisation after combined long-course gonadotrophin-releasing hormone analogue and human menopausal gonadotrophin therapy Study authors did not define pregnancy

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Insufficient information about sequence generation process to permit judgement of 'low risk' or 'high risk'
Allocation concealment (selection bias)	Unclear risk	Insufficient information to permit judgement of 'low risk' or 'high risk': allocation not described

Shaker 1993 *(Continued)*

Blinding of participants and personnel (performance bias) All outcomes	Low risk	Double-blinded with use of placebo
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No blinding of outcome assessment, but review authors judge that measurement of pregnancy outcome is not likely to be influenced by lack of blinding
Incomplete outcome data (attrition bias) All outcomes	Low risk	Investigators evaluated women randomly assigned
Selective reporting (reporting bias)	Low risk	Study protocol is not available, but it is clear that published reports include all prespecified outcomes and some expected outcomes
Other bias	Low risk	2 participant groups were comparable with respect to age, duration of infertility and parity

Abbreviations:
BD: twice daily or bi-daily
CD: cycle day
FSH: follicle-stimulating hormone
GnRH: gonadotrophin-releasing hormone
GTN: glyceryltrinitrate
hCG: human chorionic gonadotrophin
hMG: human menopausal gonadotrophin
ICSI: intracytoplasmic sperm injection
ICSI-ET: intracytoplasmic sperm injection - embryo transfer
IM: intramuscular
ISMN: isosorbide mononitrate
IUI: intrauterine insemination
IVF: in vitro fertilisation
LH: luteinising hormone
PI: pulsatility index
SC: subcutaneous
ZIFT: zygote intrafallopian transfer

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Alborzi 2007	Study used pentoxifylline as an immunomodulator for controlling endometriosis. As participants did not undergo AR, they **were not of interest** for this review
Balasch 1997	This study did **not report participants of interest** for this review. Not all underwent AR (the study description is that only 13/29 corrected additional infertility factors in PTX group and 11/27 corrected additional infertility factors in placebo group) and used pentoxifylline as an immunomodulator for controlling endometriosis
Check 2004	Eliminated because it is not a parallel randomised controlled trial. In this study some participants do **cross over**. Nine women were randomly assigned to vaginal sildenafil vs protocol in their first cycle, and seven to vaginal oestradiol. Only 3 women in the vaginal sildenafil group completed both study arms
Creus 2008	This study did **not report participants of interest** for this review. Only some participants underwent insemination or ovulation induction, and investigators used pentoxifylline as an immunomodulator to control endometriosis
Kamencic 2008	Study did **not report participants or outcomes of interest** for this review
Malinova 2013	Study did **not report outcomes of interest** for this review. Time frame was too short for investigator to evaluate them
Raine-Fenning 2009	Eliminated because it is not a parallel randomised controlled trial, but rather is a **cross-over** study
Rosen 1987	Study reported no comparisons of interest for this review. Study compared 0.7% isoflurane + nitrous oxide vs 1.4% isoflurane + nitrous oxide
Sher 2000	Eliminated because this is not a parallel randomised controlled trial, but rather is an**observational** study in 4 participants
Shin 2002	Eliminated because this is**not a parallel randomised** controlled trial, but rather is a controlled clinical trial

AR: assisted reproduction
PTX: pentoxifylline

Characteristics of ongoing studies *[ordered by study ID]*

Ben-Meir 2014

Trial name or title	Nifedipine treatment on uterine contractility in in vitro fertilisation
Methods	Randomised parallel double blind controlled trial
Participants	Women 18-45 years of age undergoing frozen embryo transfer
Interventions	**Experimental group:** nifedipine 5 mg single dose **Control group:** placebo
Outcomes	**Primary outcome measures:** uterine contractility after treatment (time frame 30 minutes after treatment) (designated as safety issue: no) **Secondary outcome measures:** implantation and pregnancy rates (time frame 4 weeks) (designated as safety issue: no)
Starting date	February 24, 2014
Contact information	Assaf Ben-Meir, MD; 972-2-6776425; assaf.benmeir@gmail.com
Notes	Study is not yet open for participant recruitment

Casper 2013

Trial name or title	Use of a calcium channel blocker to prevent premature luteinizing hormone surges in infertility patients (nimodipine)
Methods	Randomised parallel double-blind controlled trial
Participants	Women 25-40 years of age with intact normal ovaries, early follicular phase (day 2-4), serum follicle-stimulating hormone (FSH) level < 20 mIU/mL and diagnosis of infertility, with recommended treatment of ovarian stimulation and intrauterine insemination (IUI)
Interventions	**Experimental group:** Nimodipine 30 mg tablets will be self-administered by participants every 6 hours, starting on the day that the ultrasound criterion for hCG triggering is met. Tablets will be taken for 2 days or until an LH surge is detected, whichever comes first. If no LH surge occurs by 2 days, the hCG trigger (250 micrograms recombinant hCG) will be given, followed by IUI in 40 hours. If luteinising hormone (LH) surge is detected, human chorionic gonadotrophin (hCG) will be given immediately and 2 IUIs will be performed 24 hours apart **Control group:** same as for nimodipine but an identical placebo will be self-administered
Outcomes	**Primary outcome measures:** delay in LH surge by at least 2 days **Secondary outcome measures:** side effect profile of nimodipine or placebo
Starting date	July 2012
Contact information	Robert F Casper: 416-972-0777; casper@lunenfeld.ca

Casper 2013 *(Continued)*

Notes	Study is not yet open for participant recruitment

Penzias 2012

Trial name or title	Using nimodipine, a calcium channel blocker, to prevent LH surge in women undergoing controlled ovarian stimulation and intrauterine insemination: a double-blinded, randomized controlled study
Methods	Randomised parallel assignment, double-blind controlled trial
Participants	Women 25-40 years of age at time of enrolment, with both ovaries intact by history and ultrasound assessment, early follicular phase (day 2-4) serum FSH level < 20 mIU/mL, diagnosis of subfertility with recommended treatment of controlled ovarian hyperstimulation (COH) and IUI Women with unexplained infertility, polycystic ovarian syndrome and ovulatory dysfunction (absence of or irregular ovulation with unknown cause)
Interventions	Nimodipine 30 mg liquid orally 4 times a day for 8 total doses in prefilled syringes
Outcomes	**Primary outcome measure:** LH surge **Secondary outcome measure:** side effect profile **Other outcome measures:** gonadotrophin levels and clinical pregnancy (positive pregnancy test and ultrasound evidence of fetal heart rate)
Starting date	September 2012
Contact information	Khanh-Ha D Nguyen, MD, MPH: knguyen@bostonivf.com Alan S Penzias, MD: apenzias@bostonivf.com
Notes	This study is currently recruiting participants

DATA AND ANALYSES

Comparison 1. Vasodilator vs placebo or no treatment

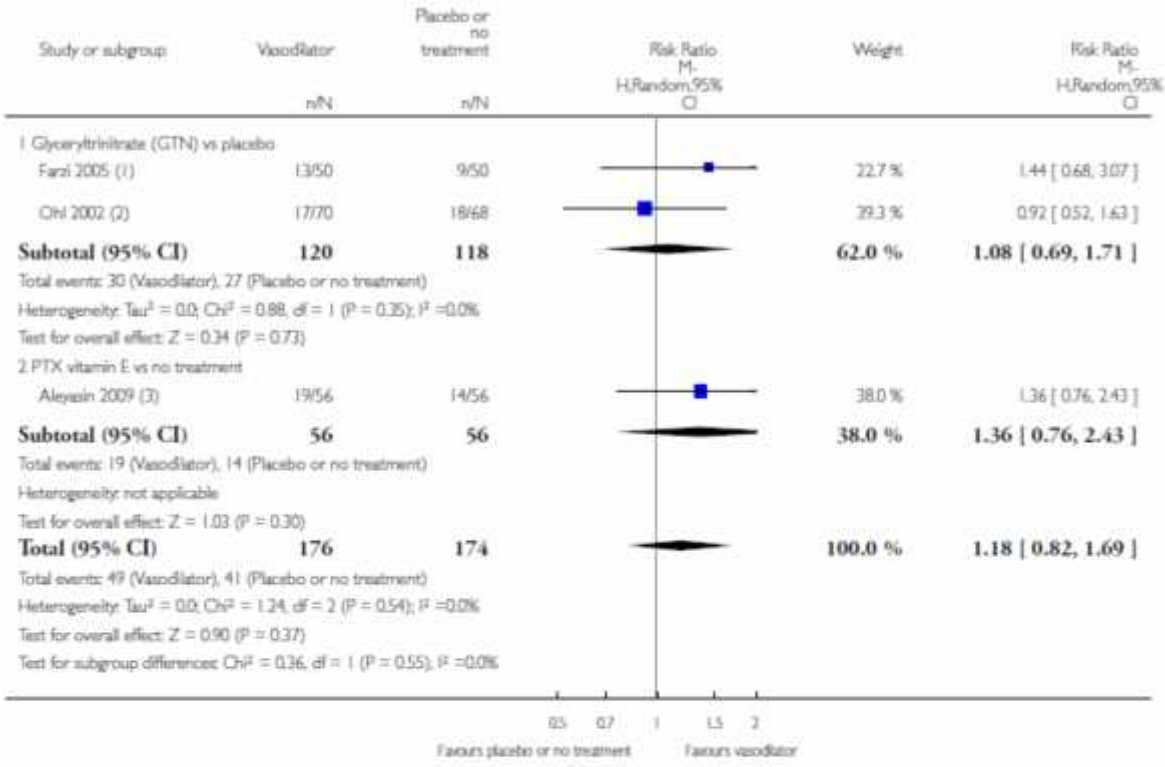
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Live birth	3	350	Risk Ratio (M-H, Random, 95% CI)	1.18 [0.82, 1.69]
1.1 Glyceryltrinitrate (GTN) vs placebo	2	238	Risk Ratio (M-H, Random, 95% CI)	1.08 [0.69, 1.71]
1.2 PTX vitamin E vs no treatment	1	112	Risk Ratio (M-H, Random, 95% CI)	1.36 [0.76, 2.43]
2 Vasodilator side effects	2	258	Risk Ratio (M-H, Random, 95% CI)	1.63 [0.33, 7.93]
2.1 Glyceryltrinitrate (GTN) vs placebo	2	258	Risk Ratio (M-H, Random, 95% CI)	1.63 [0.33, 7.93]
3 Clinical pregnancy	8	717	Odds Ratio (M-H, Random, 95% CI)	1.38 [1.00, 1.92]
3.1 Glyceryltrinitrate (GTN) vs placebo or no treatment	4	418	Odds Ratio (M-H, Random, 95% CI)	1.11 [0.73, 1.68]
3.2 Sildenafil vs no treatment	2	139	Odds Ratio (M-H, Random, 95% CI)	1.59 [0.63, 4.03]
3.3 Sildenafil + oestrogen vs no treatment	1	48	Odds Ratio (M-H, Random, 95% CI)	2.60 [0.77, 8.75]
3.4 PTX + vitamin E vs no treatment	1	112	Odds Ratio (M-H, Random, 95% CI)	2.06 [0.97, 4.38]
4 Other adverse effects	3		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
4.1 Multiple gestation or birth: NTG vs placebo and PTX + tocopherol vs no treatment	2	250	Risk Ratio (M-H, Random, 95% CI)	0.89 [0.39, 2.03]
4.2 Spontaneous abortion/miscarriage NTG vs placebo and PTX + tocopherol vs no treatment	3	350	Risk Ratio (M-H, Random, 95% CI)	0.84 [0.37, 1.91]
4.3 Ectopic pregnancy: NTG vs placebo and PTX + tocopherol vs no treatment	2	250	Risk Ratio (M-H, Random, 95% CI)	1.47 [0.24, 8.86]

Analysis 1.1. Comparison 1 Vasodilator vs placebo or no treatment, Outcome 1 Live birth.

Review: Vasodilators for women undergoing fertility treatment

Comparison: 1 Vasodilator vs placebo or no treatment

Outcome: 1 Live birth



(1) IVF

(2) IVF

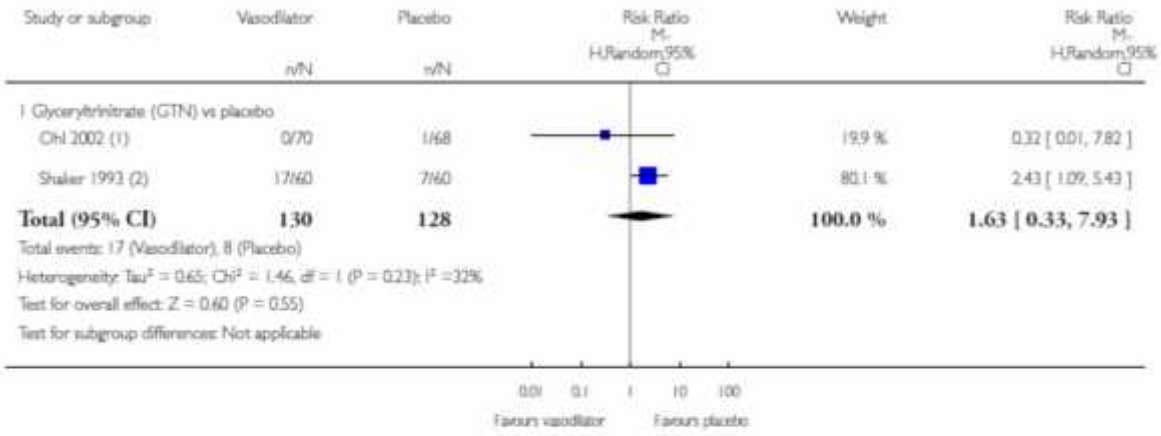
(3) ZIFT

Analysis 1.2. Comparison 1 Vasodilator vs placebo or no treatment, Outcome 2 Vasodilator side effects.

Review: Vasodilators for women undergoing fertility treatment

Comparison: 1 Vasodilator vs placebo or no treatment

Outcome: 2 Vasodilator side effects



(1) IVF

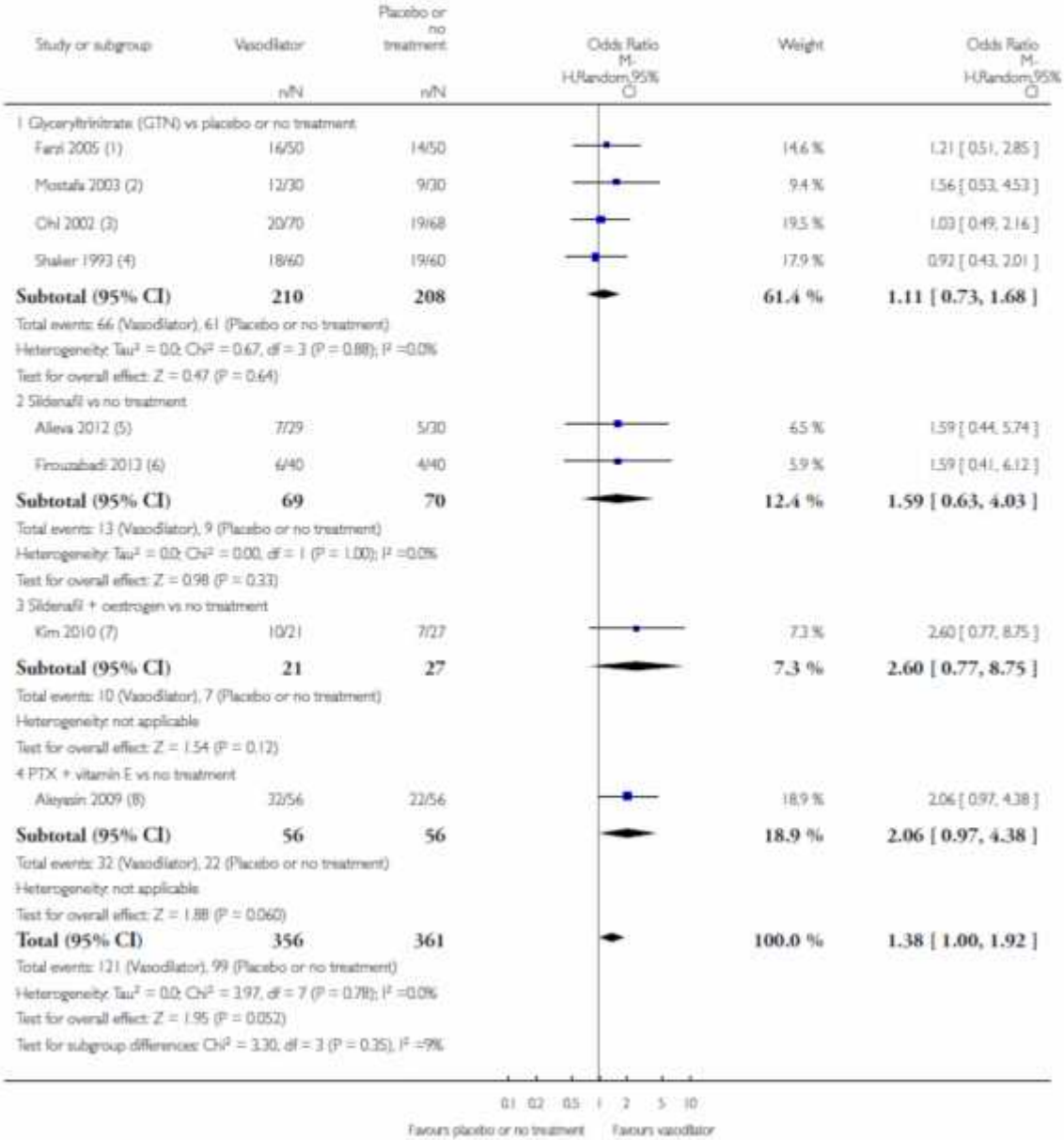
(2) IVF

Analysis 1.3. Comparison 1 Vasodilator vs placebo or no treatment, Outcome 3 Clinical pregnancy.

Review: Vasodilators for women undergoing fertility treatment

Comparison: 1 Vasodilator vs placebo or no treatment

Outcome: 3 Clinical pregnancy



(1) IVF

(2) ICSI

(3) IVF

(4) IVF; method of establishing pregnancy not described

(5) IVF; method of establishing pregnancy not described

(6) IVF

(7) IVF

(8) ZIFT

Analysis 1.4. Comparison 1 Vasodilator vs placebo or no treatment, Outcome 4 Other adverse effects.

Review: Vasodilators for women undergoing fertility treatment

Comparison: 1 Vasodilator vs placebo or no treatment

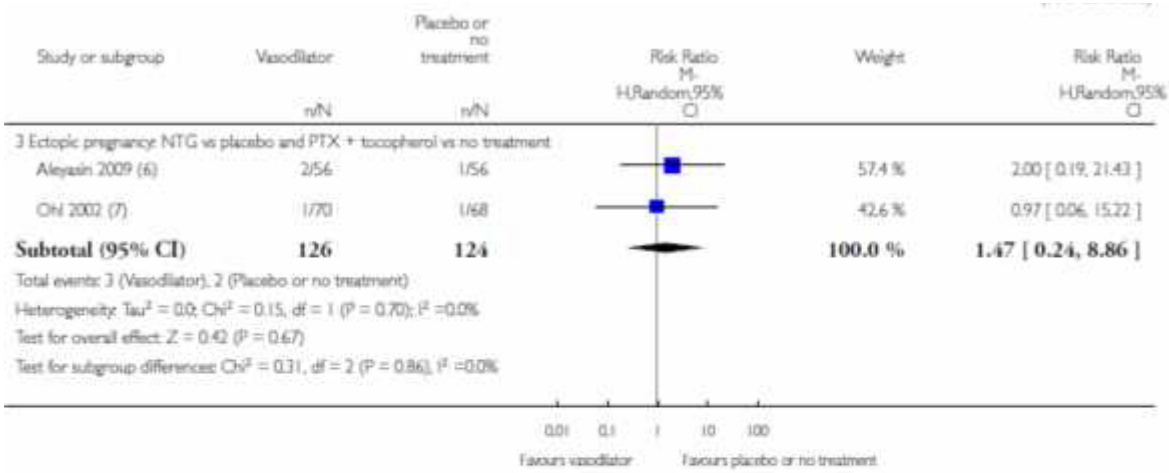
Outcome: 4 Other adverse effects

Study or subgroup	Vasodilator n/N	Placebo or no treatment n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
1 Multiple gestation or birth: NTG vs placebo and PTX + tocopherol vs no treatment					
Aleyasin 2009 (1)	4/56	5/56		42.4 %	0.80 [0.23, 2.82]
Ohl 2002 (2)	6/70	6/68		57.6 %	0.97 [0.33, 2.86]
Subtotal (95% CI)	**126**	**124**		**100.0 %**	**0.89 [0.39, 2.03]**
Total events: 10 (Vasodilator), 11 (Placebo or no treatment)					
Heterogeneity: $Tau^2 = 0.0$; $Chi^2 = 0.05$, df = 1 (P = 0.82); $I^2 = 0.0\%$					
Test for overall effect: Z = 0.27 (P = 0.79)					
2 Spontaneous abortion/miscarriage NTG vs placebo and PTX + tocopherol vs no treatment					
Aleyasin 2009 (3)	6/56	5/56		52.7 %	1.20 [0.39, 3.71]
Farzi 2005 (4)	3/50	5/50		35.4 %	0.60 [0.15, 2.38]
Ohl 2002 (5)	1/70	2/68		11.9 %	0.49 [0.05, 5.23]
Subtotal (95% CI)	**176**	**174**		**100.0 %**	**0.84 [0.37, 1.91]**
Total events: 10 (Vasodilator), 12 (Placebo or no treatment)					
Heterogeneity: $Tau^2 = 0.0$; $Chi^2 = 0.82$, df = 2 (P = 0.66); $I^2 = 0.0\%$					
Test for overall effect: Z = 0.41 (P = 0.68)					

0.01 0.1 1 10 100

Favours vasodilator Favours placebo or no treatment

(Continued . . .)



(1) IVF

(2) IVF

(3) IVF

(4) IVF

(5) IVF

(6) IVF

(7) IVF

APPENDICES

Appendix 1. CENTRAL search strategy

Database: EBM Reviews-Cochrane CENTRAL <August 2012>
Search strategy:

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1 exp embryo transfer/ or exp fertilization in vitro/ or exp sperm injections, intracytoplasmic/ (1573)
2 embryo transfer$.tw. (878)
3 vitro fertili?ation.tw. (1312)
4 ivf-et.tw. (253)
5 ivf.tw. (1872)
6 icsi.tw. (647)
7 intracytoplasmic sperm injection$.tw. (405)
8 (blastocyst adj2 transfer$).tw. (64)
9 exp reproductive techniques, assisted/ or exp insemination, artificial/ or exp ovulation induction/ (2194)
10 assisted reproduct$.tw. (386)

11 artificial insemination.tw. (53)
12 iui.tw. (284)
13 intrauterine insemination$.tw. (386)
14 ovulation induc$.tw. (437)
15 (ovari$ adj2 stimulat$).tw. (725)
16 superovulat$.tw. (129)
17 ovarian hyperstimulation.tw. (541)
18 COH.tw. (121)
19 infertil$.tw. (1762)
20 subfertil$.tw. (128)
21 (ovari$ adj2 induction).tw. (26)
22 endometrium.tw. (764)
23 endometrial.tw. (1933)
24 or/1-23 (6930)
25 exp vasodilator agents/ or exp nifedipine/ or exp nitroglycerin/ or exp endothelium-dependent relaxing factors/ or exp nitric oxide/ (18729)
26 vasodilator$.tw. (2318)
27 nifedipine.tw. (2758)
28 glyceryl trinitrate.tw. (526)
29 nitroglycerin.tw. (1734)
30 nitric oxide.tw. (2726)
31 sildenafil.tw. (655)
32 Viagra.tw. (120)
33 or/25-32 (23294)
34 24 and 33 (55)

Appendix 2. EMBASE search strategy

Database: Embase <1980 to 2012 Week 34>
Search Strategy:

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1 exp embryo transfer/ or exp fertilization in vitro/ or exp intracytoplasmic sperm injection/ (46666)
2 embryo$ transfer$.tw. (10692)
3 in vitro fertili?ation.tw. (18597)
4 icsi.tw. (7785)
5 intracytoplasmic sperm injection$.tw. (5539)
6 (blastocyst adj2 transfer$).tw. (794)
7 ivf.tw. (20546)
8 exp infertility therapy/ or exp artificial insemination/ or exp intrauterine insemination/ or exp ovulation induction/ (69349)
9 assisted reproduct$.tw. (10901)
10 artificial insemination.tw. (4395)
11 iui.tw. (1570)
12 intrauterine insemination$.tw. (2139)
13 ovulation induc$.tw. (4013)
14 (ovari$ adj2 stimulat$).tw. (5917)
15 superovulat$.tw. (2837)
16 ovarian hyperstimulation.tw. (4563)
17 COH.tw. (1213)
18 infertil$.tw. (47649)
19 subfertil$.tw. (3855)
20 (ovari$ adj2 induction).tw. (250)
21 or/1-20 (115602)

22 exp vasodilator agent/ (366977)
23 exp nifedipine/ (42598)
24 exp glyceryl trinitrate/ (31270)
25 nitroglycerin.tw. (10514)
26 exp nitric oxide/ (103890)
27 exp endothelium derived relaxing factor/ (3759)
28 vasodilator$.tw. (34821)
29 nifedipine.tw. (21434)
30 glyceryl trinitrate.tw. (2384)
31 nitric oxide.tw. (119671)
32 sildenafil.tw. (5694)
33 Viagra.tw. (3741)
34 exp sildenafil/ (13110)
35 or/22-34 (511916)
36 21 and 35 (1295)
37 Clinical Trial/ (870370)
38 Randomized Controlled Trial/ (327721)
39 exp randomization/ (59164)
40 Single Blind Procedure/ (16301)
41 Double Blind Procedure/ (110472)
42 Crossover Procedure/ (34756)
43 Placebo/ (203536)
44 Randomi?ed controlled trial$.tw. (77962)
45 Rct.tw. (9835)
46 random allocation.tw. (1171)
47 randomly allocated.tw. (17528)
48 allocated randomly.tw. (1826)
49 (allocated adj2 random).tw. (710)
50 Single blind$.tw. (12457)
51 Double blind$.tw. (129919)
52 ((treble or triple) adj blind$).tw. (277)
53 placebo$.tw. (178186)
54 prospective study/ (211853)
55 or/37-54 (1268861)
56 case study/ (16724)
57 case report.tw. (229488)
58 abstract report/ or letter/ (841849)
59 or/56-58 (1083375)
60 55 not 59 (1233599)
61 36 and 60 (236)
62 (2010$ or 2011$ or 2012$).em. (2835423)
63 61 and 62 (40)

Appendix 3. MEDLINE search strategy

Database: Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Present>
Search Strategy:

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1 exp embryo transfer/ or exp fertilization in vitro/ or exp sperm injections, intracytoplasmic/ (30226)
2 embryo transfer$.tw. (7452)
3 vitro fertili?ation.tw. (15444)
4 ivf-et.tw. (1722)
5 ivf.tw. (14578)
6 icsi.tw. (4836)
7 intracytoplasmic sperm injection$.tw. (4455)
8 (blastocyst adj2 transfer$).tw. (470)
9 exp reproductive techniques, assisted/ or exp insemination, artificial/ or exp ovulation induction/ (48822)
10 assisted reproduct$.tw. (7780)
11 artificial insemination.tw. (4543)
12 iui.tw. (1055)
13 intrauterine insemination$.tw. (1642)
14 ovulation induc$.tw. (3185)
15 (ovari$ adj2 stimulat$).tw. (4372)
16 superovulat$.tw. (2781)
17 ovarian hyperstimulation.tw. (3423)
18 COH.tw. (950)
19 infertil$.tw. (38594)
20 subfertil$.tw. (3181)
21 (ovari$ adj2 induction).tw. (210)
22 endometrium.tw. (20658)
23 endometrial.tw. (38491)
24 or/1-23 (134563)
25 exp vasodilator agents/ or exp nifedipine/ or exp nitroglycerin/ or exp endothelium-dependent relaxing factors/ or exp nitric oxide/ (343609)
26 vasodilator$.tw. (29687)
27 nifedipine.tw. (17611)
28 glyceryl trinitrate.tw. (1992)
29 nitroglycerin.tw. (8836)
30 nitric oxide.tw. (101850)
31 sildenafil.tw. (4111)
32 Viagra.tw. (917)
33 or/25-32 (405820)
34 24 and 33 (935)
35 randomized controlled trial.pt. (335409)
36 controlled clinical trial.pt. (84960)
37 randomized.ab. (250530)
38 placebo.tw. (142828)
39 clinical trials as topic.sh. (162041)
40 randomly.ab. (183445)
41 trial.ti. (107769)
42 (crossover or cross-over or cross over).tw. (54459)
43 or/35-42 (821588)
44 exp animals/ not humans.sh. (3773404)
45 43 not 44 (757928)
46 34 and 45 (73)

Appendix 4. Menstrual Disorders and Subfertility Group database search strategy

Menstrual disorders and subfertility database (MDSG) search for RGB1760 02.04.12
Keywords CONTAINS "ART" or "assisted reproduction" or "assisted reproduction techniques" or "IVF" or "ICSI" or "in vitro fertilisation" or "in-vitro fertilisation techniques" or "in vitro fertilization" or "in vitro maturation" or "intracytoplasmic sperm injection" or "subfertility" or "Infertility" or "IUI" or "Intrauterine Insemination" or "*Embryo Transfer" or "ET" or Title CONTAINS "ART" or "assisted reproduction" or "assisted reproduction techniques" or "IVF" or "ICSI" or "in vitro fertilisation" or "in-vitro fertilisation techniques" or "in vitro fertilization" or "in vitro maturation" or "intracytoplasmic sperm injection" or "subfertility" or "Infertility" or "IUI" or "Intrauterine Insemination" or "*Embryo Transfer" or "ET"
AND
Keywords CONTAINS "vasodilation" or "Vasodilator Agents" or"Nifedipine" or"*Nitric Oxide"or "nitroglyceril"or "nitroglycerin"or "nitrous oxide"or "glycerine trinitrate"or "glyceryl trinitrate" or"Sildenafil"or "viagra" or Title CONTAINS "vasodilation" or "Vasodilator Agents" or"Nifedipine" or"*Nitric Oxide"or "nitroglyceril"or "nitroglycerin"or "nitrous oxide"or "glycerine trinitrate"or "glyceryl trinitrate" or"Sildenafil"or "viagra"

Appendix 5. PsycINFO search strategy

Database: PsycINFO <1806 to August Week 3 2012>
Search Strategy:

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1 exp reproductive technology/ (1164)
2 in vitro fertili?ation.tw. (466)
3 ivf-et.tw. (16)
4 (ivf or et).tw. (84558)
5 icsi.tw. (38)
6 intracytoplasmic sperm injection$.tw. (33)
7 (blastocyst adj2 transfer$).tw. (2)
8 assisted reproduct$.tw. (432)
9 artificial insemination.tw. (214)
10 iui.tw. (19)
11 intrauterine insemination$.tw. (13)
12 ovulation induc$.tw. (16)
13 (ovari$ adj2 stimulat$).tw. (44)
14 ovarian hyperstimulation.tw. (8)
15 COH.tw. (54)
16 superovulat$.tw. (5)
17 infertil$.tw. (2267)
18 subfertil$.tw. (54)
19 (ovari$ adj2 induction).tw. (4)
20 or/1-19 (87609)
21 exp vasodilator drugs/ (469)
22 nifedipine.tw. (327)
23 nitroglycerin.tw. (123)
24 exp Nitric Oxide/ (2039)
25 nitric oxide.tw. (3305)
26 vasodilator$.tw. (404)
27 glyceryl trinitrate.tw. (65)
28 exp Sildenafil/ (227)
29 sildenafil.tw. (426)
30 Viagra.tw. (198)
31 or/21-30 (4954)
32 20 and 31 (157)
33 random.tw. (35907)

34 control.tw. (279142)
35 double-blind.tw. (16217)
36 clinical trials/ (6239)
37 placebo/ (3260)
38 exp Treatment/ (523707)
39 or/33-38 (794477)
40 32 and 39 (61)

Appendix 6. CINAHL search strategy

#	Query	Results
S55	S40 AND S54	28
S54	S41 OR S42 or S43 or S44 OR S45 OR S46 OR S47 OR S48 OR S49 OR S50 OR S51 OR S52 OR S53	881,657
S53	TX allocat* random*	3,855
S52	(MH "Quantitative Studies")	11,768
S51	(MH "Placebos")	8,690
S50	TX placebo*	31,310
S49	TX random* allocat*	3,855
S48	(MH "Random Assignment")	36,961
S47	TX randomi* control* trial*	70,979
S46	TX ((singl* n1 blind*) or (singl* n1 mask*)) or TX ((doubl* n1 blind*) or (doubl* n1 mask*)) or TX ((tripl* n1 blind*) or (tripl* n1 mask*)) or TX ((trebl* n1 blind*) or (trebl* n1 mask*))	708,770
S45	TX ((trebl* n1 blind*) or (trebl* n1 mask*))	104
S44	TX ((trebl* n1 blind*) or (trebl* n1 mask*))	0
S43	TX clinic* n1 trial*	162,145
S42	PT Clinical trial	75,707
S41	(MH "Clinical Trials+")	173,244
S40	S27 AND S39	63

(*Continued*)

S39	S28 OR S29 OR S30 OR S31 OR S32 OR S33 OR S34 OR S35 OR S36 OR S37 OR S38	14,436
S38	TX Viagra	355
S37	TX sildenafil	1,420
S36	TX nitric oxide	8,058
S35	TX nitroglycerin	1,288
S34	TX glyceryl trinitrate	166
S33	TX nifedipine	805
S32	TX vasodilator*	4,087
S31	(MM "Nitric Oxide")	2,408
S30	(MM "Nitroglycerin")	463
S29	(MM "Nifedipine")	302
S28	(MM "Vasodilator Agents")	1,446
S27	S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10 OR S11 OR S12 OR S13 OR S14 OR S15 OR S16 OR S17 OR S18 OR S19 OR S20 OR S21 OR S22 OR S23 OR S24 OR S25 OR S26	13,996
S26	TX endometrial	3,966
S25	TX endometrium	1,437
S24	TX (ovari* N2 induction)	12
S23	TX subfertil*	394
S22	TX infertil*	6,812
S21	TX COH	59
S20	TX ovarian hyperstimulation	296
S19	TX superovulat*	20
S18	TX (ovari* N2 stimulat*)	194

(*Continued*)

S17	TX ovulation induc*	500
S16	TX intrauterine insemination	132
S15	TX IUI	69
S14	TX artificial insemination	427
S13	TX assisted reproduct*	1,172
S12	TX ovulation induction	470
S11	(MM "Ovulation Induction")	200
S10	(MM "Insemination, Artificial")	226
S9	(MM "Reproduction Techniques")	1,611
S8	TX intracytoplasmic sperm injection*	218
S7	TX (blastocyst* adj2 transfer*)	1
S6	TX icsi	231
S5	TX ivf	1,034
S4	TX ivf-et	35
S3	TX embryo transfer*	620
S2	(MM "Embryo Transfer")	227
S1	(MM "Fertilization in Vitro")	1,330

CONTRIBUTIONS OF AUTHORS

RG conceived of and designed the study; co-ordinated the whole review process; and participated in the search and in selection and assessment of studies. She completed data extraction activities; conducted the analysis; wrote the review; and approved the final version of the review.

GU provided general advice on study design and other related aspects of the review; participated in the assessment of potentially eligible studies; solved discrepancies; supervised data analysis; collaborated in the writing process of the review; and approved the final version of the review.

DG provided general advice on study design; coordinated the search to identify potentially eligible studies; participated in selection, assessment and extraction of data; and approved the final version of the review.

AC participated in selection, assessment and extraction of data; and approved the final version of the review.

XB conceived of the study; co-ordinated the whole review process; provided general advice on all processes; solved discrepancies; and approved of the final version of the review.

DECLARATIONS OF INTEREST

The review authors declare that they have no conflicts of interest to report.

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Internal sources

- None, Not specified.

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DIFFERENCES BETWEEN PROTOCOL AND REVIEW

The word "safety" was added to the objectives, and the sentence was edited for brevity.

We have added a post hoc subgroup analysis for the secondary outcome of clinical pregnancy to evaluate studies that used only vasodilators.

INDEX TERMS

Medical Subject Headings (MeSH)

*Pregnancy Rate; Embryo Implantation [*drug effects]; Infertility, Female [*therapy]; Live Birth; Randomized Controlled Trials as Topic; Vasodilator Agents [*therapeutic use]

MeSH check words

Female; Humans; Pregnancy

6.3 Publicación III: Estudio caso control de la asociación de defectos congénitos y reproducción médicamente asistida.

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Factor de impacto: 3,268

DOI: 10.1002/pd.4286 PRENATAL DIAGNOSIS

ORIGINAL ARTICLE

Birth defects in medically assisted reproduction pregnancies in the city of Barcelona

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ABSTRACT

Objective To assess the association between the use of medically assisted reproduction (MAR) and birth defects (BD) in newborns and terminations of pregnancy in pregnant women registered in Barcelona in the period 1992 to 2007.

Methods We studied 1905 cases and 2722 controls in a retrospective population-based case–control study. Cases comprised any newborn presenting at least one major BD, as well as any pregnancy terminated because of BD. Controls were newborns without BD. Exposure was MAR. Adjusted odds ratios (aOR) with 95% confidence intervals (CI) were calculated by means of logistic regression to assess the association.

Results The MAR was demonstrated to be associated with BD, after adjusting for probable confounders (aOR = 1.8; 95% CI = 1.4 to 2.5). Regarding MAR modalities, this association was statistically significant only for assisted reproduction techniques (ART) (aOR = 2.7; 95% CI = 1.8 to 4.1). In the stratified analysis by structural BD categories, frequencies for all categories were increased after ART with the exception of head-face-neck-eye defects, none after artificial insemination, and digestive-abdominal wall defects in ovulation induction.

Conclusions This study demonstrated a strong association between ART and BD, with an almost threefold increased risk for overall BD after ART, as compared with natural conception. Increased associations were also observed for almost all structural BD categories. © 2013 John Wiley & Sons, Ltd.

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Conflicts of interest: None declared

INTRODUCTION

No clear estimates are available for the number of children born with birth defects (BD) worldwide.[1] The EUROCAT, the European population-based BD registries network, reported a 2.38% prevalence of BD for the period 2000 to 2004,[2] similar to the 2% observed by the BD Registry of the city of Barcelona (REDCB) during the period 1992 to 2007.[3] There is a growing evidence for an association between an increased BD prevalence and the use of medically assisted reproduction (MAR), although large population-based studies are scarce.[4,5] And this becomes more evident with the recent huge expansion of ART involving gamete manipulation [assisted reproduction techniques (ART)], accounting in 2002 for the birth of about 233 000 babies worldwide.[6–10] In some developed countries, the resulting higher BD prevalence was found to be 4.2% for ART.[4] Nowadays, concern over the safety of assisted reproduction pregnancies could be an issue, because of interfering iatrogenic factors such as gamete manipulation, ovulation induction, or luteal phase support drugs, not to mention the underlying causes of infertility.[11–13] Furthermore, many studies have found an association between ART and adverse perinatal outcomes in singleton pregnancies, such as preterm delivery, very preterm delivery, small-for-gestation babies, and intrauterine growth retardation, as well as BD.[14–19] However, some studies have not found any association.[20,21]

Systematic reviews on ART [including *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI)] have demonstrated an increased BD risk, as compared with natural conception pregnancies.[22,23] Limitations of the reported studies are limited sample size, lack of an adequate control group, no adjustment for confounding variables (such as maternal age), and no inclusion of MAR modalities other than ART, such as artificial insemination and ovulation induction. Our study was based on the large population-based registry of the city of Barcelona (REDCB), including a considerable number of cases and controls from a 16-year period (1992–2007). The main objective of our study was to assess the overall

and stratified prevalence of BD observed in newborns and terminated pregnancies and their association with different MAR modalities, in Barcelona between 1992 and 2007.

METHODS

This was an individual-based retrospective case–control study using cases and controls already registered in REDCB, the population-based BD Registry of the city of Barcelona, a part of the EUROCAT network. The study population included all pregnant women who delivered at 25 maternity hospitals of Barcelona, 5 public and 20 private, during the 1992 to 2007 period. The REDCB gathered data for more than the 95% of the approximately 12500 annual births. Births among Barcelona residents attended in centers outside the city limits accounted only for 3%, and half of them were also recorded in the REDCB. The three full-time nurses of the REDCB contacted the Obstetric and Pediatric staff in the participating centers to obtain detailed information regarding prenatally as well as postnatally detected BD. In addition, they actively searched for prenatally diagnosed BD in newborns from other sources: Cytogenetic laboratories, Obstetric ultrasound units, and Departments of Pediatrics, Pediatric Cardiology, and Pediatric Surgery. Information from cases and controls was also obtained by a semi-structured questionnaire and through medical history notes.[24,25]

Cases comprised any newborn, delivered alive or dead, beyond 20 weeks' gestation presenting at least one major BD, as well as any pregnancy terminated because of BD. The cases were mostly recruited postnatally until the fifth day of life, just before hospital discharge. After exclusion of genetic syndromes (either chromosomal, microdeletional and single-gene defects), structural BDs were classified into the following seven categories according to the International Classification of Diseases 9: central nervous system, head-face-neck-eye, cardiovascular, respiratory, gastrointestinal and abdominal wall, genitourinary, and skeletal.[23] A yearly random sample of about 2% of newborns without BD, delivered alive or dead in each participating maternity hospital served as the control sample.[24,25] The number of one control for each year was calculated using the previous year data, according to the following formula: $C = mn/N$, where m is the total number of BD cases in the city, n is the number of births in a given hospital, and N is the total number of births from mothers resident in the city

Exposure to MAR was defined as the use of (1) assisted reproductive techniques involving gamete manipulation (ART), consisting in the extra corporeal fertilization procedure known as IVF with or without ICSI, the latter meaning that a single sperm is injected into the cytoplasm of an oocyte, (2) artificial insemination, consisting in the fertilization procedure in which sperm is artificially placed into the uterus, intravaginally or intracervix with partner or donor's sperm, and (3) ovulation induction, which consists in a pharmacological treatment to artificially induce ovulation. Gamete intra-fallopian transfer is the procedure in which both gametes are transferred into the fallopian tubes, but because of its low frequency, it was not analyzed separately.[7]

Several potential confounding variables, either with biological plausibility or described in the literature, were analyzed dichotomously to assess the association between MAR exposure and BD: maternal age (<35 vs ≥35 years); parity (nulliparous vs one or more previous pregnancies); preexisting diabetes mellitus (yes/no); fetal gender (male/female); maternal obesity (body mass index ≥30 vs <30); and toxoplasmosis, rubella, or cytomegalovirus infection in pregnancy (yes/no).

A descriptive analysis was performed, with maternal and pregnancy characteristics being compared between cases and controls and between MAR and natural conception pregnancies. Moreover, a descriptive analysis of structural BD categories was performed and compared between MAR and natural conception pregnancies. Proportions were calculated, and the chi-squared test or the Fisher's exact test was used to determine whether differences were significant. Finally, bivariate and multivariate logistic regression analyses were conducted to assess the associations between the use of any MAR modality (ART, ovulation induction, or artificial insemination) and the status of case, both overall and by structural BD categories (central nervous, head-face-neck-eye, cardiovascular, respiratory, gastrointestinal and abdominal wall, genitourinary, and skeletal). Roughly, in the analysis of each BD category, BDs from other categories were excluded, and all the controls included. We calculated how many MAR and naturally conceived pregnancies were encountered in the BD category and in controls, and the BD rate among MAR and natural conception was subsequently obtained. Associations were assessed with estimated odds ratios (OR), crude, and adjusted, as well as their 95% confidence intervals (95% CI). Variables found to be associated to BD, MAR exposure, or both were used for adjustment.

During the 16-year study period (1992–2007), 4150 cases and 3647 controls were included in our study from the REDCB Registry. The number of BD for one year was used as the number of controls needed the following year. The yearly variation on the BD rate, ranged from 1.6% (222/13 440) in 1992 to 2.6% (319/12 329) in 1998, with a mean BD rate of 2.0% (4150/207 503) through the whole 1992 to 2007 period. In 54% of the cases and 25% of the controls, data on exposure to MAR were missing. Maternal and pregnancy characteristics (maternal age, parity, obesity, preexisting diabetes, fetal gender, and toxoplasmosis/rubella/cytomegalovirus infection in pregnancy) were compared between pregnancies in which MAR exposure data were available and those with missing data to avoid selection bias. Differences were only observed in maternal age and preexisting diabetes. Among women with MAR exposure data, there was a lower proportion of advanced maternal age (≥35 years) (27.2% vs 31.0%; $p < 0.001$) and preexisting diabetes (1.6% vs 3.9%; $p < 0.001$), although the median maternal age was 32 years in both groups. These variables were used for OR adjustments in the final analysis. Therefore, the study sample included 1905 cases and 2722 controls, resulting in a statistical power of 80% to detect an OR of 1.5 with an alpha risk of 0.05.

RESULTS

Cases comprised 1386 (73%) newborns presenting at least one major BD, as well as 519 (27%) pregnancies terminated because of BD, whereas the 2722 infants were selected as controls, paired by center and year. Advanced maternal age, multiparity, preexisting diabetes, obesity, and male fetuses were more common in cases as compared with controls (Table 1). Furthermore, MAR pregnancies were characterized by more advanced maternal age (OR 2.9; 95% CI: 2.3–3.7) and preexisting diabetes (OR 6.3; 95% CI: 2.4–16.6) as compared with naturally conceived pregnancies. Conversely, multiparity

Table 1 Comparison of maternal and pregnancy characteristics between birth defect cases and controls

		BD cases (N= 1905)		Controls (N= 2722)				
		n	%	n	%	p	OR	95% CI
Maternal age (years)	<35	1325	69.6	2046	75.2	<0.001	1	
	≥35	580	30.4	676	24.8		1.3*	[1.2–1.5]
Parity	<1	674	36.1	1117	41.4	<0.001	1	
	≥1	1192	63.9	1583	58.6		1.2*	[1.1–1.4]
	Unknown	39	2.0	22	0.8		2.9*	[1.7–5.0]
Preexisting diabetes mellitus	No	542	97.5	811	99.0	0.025	1	
	Yes	14	2.5	8	1.0		2.6*	[1.1–6.3]
	Unknown	1349	70.8	1903	69.9		1.1	[0.9–1.2]
Obesity (BMI ≥ 30)	No	1045	93.6	2069	95.4	0.028	1	
	Yes	71	6.4	99	4.6		1.4*	[1.0–1.9]
	Unknown	789	41.4	554	20.4		2.8*	[2.5–3.2]
Toxoplasmosis/rubella/cytomegalovirus in pregnancy	No	543	97.7	802	97.9	0.744	1	
	Yes	13	2.3	17	2.1		1.1	[0.5–2.3]
	Unknown	1349	70.8	1903	69.9		1.0	[0.9–1.2]
Fetal gender	Female	792	43.1	1340	49.3	<0.001	1	
	Male	1047	56.9	1380	50.7		1.3*	[1.1–1.4]
	Unknown	66	3.5	2	0.1		–	–

CI, confidence interval; BD, birth defects; OR, odds ratio; BMI, body mass index.
The proportions are valid proportions (excluding missings), except for unknown total (missings included).
*Statistically significant at alpha = 0.05.

(OR 0.8; 95% CI: 0.6–0.9) was less frequent in MAR pregnancies. As mentioned before, these variables were considered as potential confounders in the association between BD and MAR, and were included in the multivariate analysis. Maternal obesity and fetal sex, associated with BD, but not with MAR, were also used for OR adjustment. Given the substantial proportion of missing data on MAR exposure in preexisting diabetes and the different proportions of missing data between cases and controls for parity and obesity, the missing categories for preexisting diabetes, parity, and obesity were included in the models presented in Tables 3 and 4.

Among the 1905 BD cases, there were 576 known genetic syndromes and after exclusion, 1327 structural BD cases remained. In these 1327 fetuses, 1997 defects were observed, resulting in a mean 1.5 structural defects per fetus. Among 1210 naturally conceived pregnancies there were 1801 defects, and among 117 MAR pregnancies there were 196 defects. The distribution of structural BD categories is outlined in Table 2, being the genitourinary (22.8%) and the cardiovascular defects (17.5%) the most commonly observed. No significant differences were found between natural conception and MAR distributions.

Table 2 Distribution of the 1997 structural defects divided by categories, in both naturally conceived and medically assisted reproduction pregnancies

	All structural defects		In naturally conceived pregnancies		In MAR pregnancies	
	n	%	n	%	n	%
Central nervous system	192	9.6	176	9.8	16	8.2
Head/face/neck/eye	169	8.5	157	8.7	12	6.1
Cardiovascular	350	17.5	310	17.2	40	20.4
Respiratory	82	4.1	70	3.9	12	6.1
Digestive and abdominal wall	167	8.4	151	8.4	16	8.2
Genitourinary	456	22.8	416	23.1	40	20.4
Skeletal	273	13.7	244	13.5	29	14.8
Other	308	15.4	277	15.4	31	15.8
Total	1997	100	1801	100	196	100

MAR, medically assisted reproduction.

Table 3 Association between medically assisted reproduction and its modalities with structural birth defects, expressed in crude and adjusted odds ratios

	BD Cases (N= 1327)	Controls (N= 2.722)				
	%(n)	%(n)	OR (95% CI)	p	aOR (95% CI)	p
MAR	8.8 (117)	4.4 (121)	2.1 (1.6-2.7)	<0.001	1.8 (1.4-2.5)	<0.001
MAR modality						
ART (IVF/ICSI)	5.8 (77)	2.1 (55)	3.0 (2.1-4.3)	<0.001	2.7 (1.8-4.1)	<0.001
Artificial insemination	1.8 (24)	1.3 (34)	1.5 (0.9-2.6)	0.121	1.2 (0.6-2.2)	0.604
Ovulation induction	1.1 (15)	1.1 (29)	1.1 (0.6-2.1)	0.740	1.3 (0.6-2.5)	0.507

CI, confidence interval; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; BD, birth defects; OR, odds ratio; aOR, OR adjusted by maternal age, parity, preexisting diabetes mellitus, obesity, and fetal sex; MAR, medically assisted reproduction; ART, assisted reproduction techniques (including conventional IVF/ICSI).

Overall, MAR exposure was observed in 8.8% of the cases and 4.4% of the controls. According to the different MAR modalities, ART was present in 5.8% of cases and 2.1% of controls, artificial insemination in 1.8% of cases and 1.3% of controls, and ovulation induction in 1.1% of cases and 1.1% in controls (Table 3). Gamete intra-fallopian transfer, found in 0.2% of cases and 0.1% of controls, was excluded from subsequent analysis, because of the reduced number of exposed pregnancies.

The MAR and BD were significantly associated (adjusted OR (aOR) 1.8; 95%CI: 1.4–2.5) even after adjustment for confounding variables (advanced maternal age, parity, preexisting diabetes, obesity, toxoplasmosis/rubella/cytomegalovirus maternal infection, and fetal gender) (Table 3). In the stratified analysis by MAR modalities, ART showed a stronger association (OR 3.0; 95% CI: 2.1–4.3), even after adjustment (aOR 2.7; 95%CI: 1.8–4.1). In contrast, no association was found between BD and either artificial insemination or ovulation induction.

Proportions of the different BD categories observed in natural conception and in MAR pregnancies are outlined in Table 4. An association between MAR and BD was demonstrated in all BD categories, for both crude and adjusted analyses, with the exception of head-face-neck-eye defects (Table 5). In the double stratification (by MAR modalities and by BD categories), ART was associated with all BD categories but head-face-neck-eye. Artificial insemination was only associated with an increase in genitourinary BD in the crude analysis, and ovulation induction with digestive and abdominal wall defects in the adjusted analysis (Table 5).

DISCUSSION

The main finding of our study is that assisted reproduction pregnancies and BD were associated and that this association was demonstrated only for ART (including IVF and ICSI) after stratification by MAR modalities. In the stratified analysis by BD categories, this association with ART was observed for all the categories but head-face-neck-eye. Regarding other modalities, significant associations were only found between artificial insemination and genitourinary defects and between ovulation induction and digestive and abdominal wall defects.

Several studies have assessed the association between BD and ART,[16–19] the MAR modality involving gamete manipulation. However, most of them only included relatively few BD cases. Our sample size is comparable with that of larger studies,[13,17] and the results are in agreement with those reported by four meta-analyses.[14,22,23,28] We found that BD were 2.7 times more frequent in pregnancies conceived by ART, as compared with

Table 4 Distribution of structural birth defect categories in natural conception and in medically assisted reproduction pregnancies and its modalities. Denominators change in each category because only controls and BD of each specific category are considered, being other categories excluded from the analysis

	Natural conception % (n)	MAR %(n)	MAR modalities		
			ART %(n)	Artificial insemination %(n)	Ovulation induction %(n)
Central nervous system	6.3 (176/2777)	11.7 (16/137)	16.7 (11/66)	10.5 (4/38)	- (0/29)
Head/face-neck/eye	5.7 (157/2758)	9.0 (12/133)	11.3 (7/62)	5.6 (2/36)	9.4 (3/32)
Cardiovascular	10.6 (310/2911)	24.8 (40/161)	33.7 (28/83)	12.8 (5/39)	17.1 (6/35)
Respiratory	2.6 (70/2671)	9.0 (12/133)	14.1 (9/64)	5.6 (2/36)	3.3 (1/30)
Digestive and abdominal wall	5.5 (151/2752)	11.7 (16/137)	15.4 (10/65)	5.6 (2/36)	12.1 (4/33)
Genitourinary	13.8 (416/3017)	24.8 (40/161)	31.3 (25/80)	24.4 (11/45)	12.1 (4/33)
Skeletal	8.6 (244/2845)	19.3 (29/150)	28.6 (22/77)	8.1 (3/37)	9.4 (3/32)
Other	9.6 (277/2878)	20.4 (31/152)	25.7 (19/74)	17.1 (7/41)	14.7 (5/34)

MAR, Medically Assisted Reproduction; ART, Assisted Reproduction Techniques (including conventional in Vitro Fertilization and Intra Cytoplasm Sperm Injection).

Table 5 Association between medically assisted reproduction modalities and structural birth defects categories, expressed in crude and adjusted odds ratio

	MAR modalities					
	ART		Artificial insemination		Ovulation induction	
	OR (95% CI)	aOR (95% CI)	OR (95% CI)	aOR (95% CI)	OR (95% CI)	aOR (95% CI)
Central nervous system (N=1921)	2.96* (1.52-5.75)	3.58* (1.657.77)	1.74 (0.61-4.95)	1.64 (0.475.68)	-	-
Head/craniofacial (N=1691)	2.11 (0.94-4.71)	1.95 (0.814.66)	0.97 (0.23-4.09)	0.75 (0.173.36)	1.71 (0.525.69)	1.86 (0.55-6.29)
Cardiovascular (N=3501)	4.27** (2.676.83)	2.83* (1.575.08)	1.23 (0.48-3.18)	0.68 (0.232.04)	1.74 (0.724.21)	2.28 (0.895.82)
Respiratory (N=812)	6.08** (2.80-12.79)	5.93** (2.3614.88)	2.19 (0.51-9.28)	1.39 (0.286.94)	1.28 (0.179.54)	2.34 (0.31-17.76)
Digestive and abdominal wall (N=1607)	3.13* (1.57-6.27)	2.94* (1.336.50)	1.01 (0.24-4.26)	0.82 (0.183.81)	2.38 (0.826.85)	3.41* (1.15-10.11)
Genitourinary (N=4504)	2.84** (1.754.61)	2.78* (1.555.00)	2.02* (1.02-4.02)	1.35 (0.603.04)	0.86 (0.302.47)	0.96 (0.29-3.19)
Skeletal (N=2773)	4.26** (2.567.11)	4.21** (2.317.67)	0.94 (0.29-3.08)	0.86 (0.253.00)	1.10 (0.353.65)	1.27 (0.35-4.67)
Other (N=3018)	3.24** (1.905.54)	2.66* (1.405.03)	1.93 (0.85-4.40)	1.41 (0.563.54)	1.62 (0.624.27)	1.50 (0.50-4.44)

CI, confidence interval; OR, odds ratio; aOR, adjusted odds ratio; MAR, medically assisted reproduction; ART, assisted reproduction techniques (including conventional in vitro fertilization and intra cytoplasm sperm injection).
*$p<0.05$.
**$p<0.001$.

those naturally conceived. Our OR is higher than the 2.0 OR (95% CI: 1.5–2.9) reported by Hansen *et al.*[10] and than the 1.24 aOR (95% CI: 1.02-1.50) found by Katalinic *et al.* These differences may be explained by the inclusion of termination of pregnancy in our study (differently to the Hansen study) and by the use of indirect calculation for OR in the Katalinic study.[19]

Not all studies have found an increased BD frequency in MAR. Thus, Ericson and Kallen found a 0.89 aOR (95% CI: 0.74–1.06) for the association between IVF and BD[20]; and Yan *et al.* reported a 1.2% BD frequency in ART pregnancies (similar to the 1.4% frequency in the 2008 annual census).[21] However, both studies were based on small samples, including only 516 and 189 BD, respectively. Unlike ART, an association with BD is not usually detected for other MAR modalities. Thus, our findings regarding artificial insemination are in agreement with Yan *et al.*, who reported 1.1% to 1.3% BD rates, comparable to 1.4% found in the general population.[21] Similarly, the lack of association found between ovulation induction and BD is in agreement with other reports,[27,28] although Zhu *et al.* found an association when hormonal treatment was used.[13]

Regarding BD categories, we found that ART was associated with all of them but head-face-neck-eye, whereas in previous reports, it was demonstrated only for the cardiovascular, skeletal, and urogenital defects.[13,27] Halliday *et al.* explored new BD categories according to the organogenesis step in which the defect is originated and concluded that this was more relevant than the anatomic system involved, given that ART and blastogenesis defects were specifically associated (aOR 2.80; 95%CI: 1.63–4.81).[12] Reefhuis *et al.* found that ART was associated with different BD types, such as heart septal defects (aOR 2.1; 95%CI: 1.1–4.0), facial clefting (aOR 2.4; 95%CI: 1.2–5.1), esophageal atresia (aOR 4.5; 95%CI 1.9–10.5), and anorectal atresia (aOR 3.7; 95%CI: 1.5–9.1).[17] Surprisingly, in our study, the strongest association was observed with respiratory defects, but given its low frequency, the results have to be interpreted with caution. In our study, genitourinary defects were the only BD category showing a trend of increased frequency after artificial insemination, suggesting a real association between male infertility and hypospadias, the most frequent genitourinary defect.[17]

The results of our study suggest that ART pregnancies, contrary to other MAR modalities, present an increased BD frequency. It has been speculated than the cleaning process of the cell cluster surrounding the oocyte, carried out prior to IVF, may produce changes in the progression of meiosis. Particularly in ICSI, not only the spermatozoon pro-nucleus but also the acrosome are introduced into the oocyte, and digestive enzymes could impair cellular homeostasis mechanisms. Furthermore, the methionine content of cultures may represent another iatrogenic factor that could produce changes in the methilation patterns.[10,11] However, this association does not imply a causal relationship, given that the BD excess could also result from the inherent characteristics of the infertile population.[13] A Danish cohort study found that singleton newborns from treated and untreated infertile couples both presented with more BD than newborns from fertile couples, suggesting that population characteristics, rather than the treatment itself, is the cause of BD.[13] Similarly, Davies *et al.* reported that a history of infertility, either with or without assisted conception, was significantly associated with BD.[27] On the other hand, it has been reported that azoospermic men had a high prevalence of chromosomal abnormalities, in contrast to non-azoospermic men also eligible for ICSI.[28]

The major weakness of our study is the high proportion of missing data regarding MAR exposure (54% in the cases and 25% in the controls), although the homogeneity analysis between women with available and unavailable MAR data showed similar results in about 80% of variables, revealing a minimal likelihood of selection bias. In women with missing MAR data, advanced maternal age (≥35 years) and preexisting diabetes were more frequent than in women with data available. Both factors were associated with BD and MAR, and therefore, the study sample could underestimate the true association. Interestingly, the frequencies of missing data were not related to either MAR or to BD. The remarkable amount of missing data on MAR exposure in the registry can be explained by the fact that most women receive MAR in private clinics but deliver in public hospitals. Medical records are not updated, and women may withhold information from their midwives or doctors. When mothers are interviewed by the registry nurses, they are asked about MAR, but this interview is not always feasible. Similarly, data on preexisting diabetes and toxoplasmosis/rubella/cytomegalovirus infection during pregnancy are often missing, equally from cases and controls.

A second major weakness of our study is the presence of potential unbalanced confounders, that is, unbalanced variables between groups that can affect the variable being compared. No matching of controls to cases (on the basis of similar values of confounders or propensity scoring) was carried out in the Registry, so concerns about selection bias could be raised. Omission of even one true confounder from the multivariate adjustment may lead to biased estimates of the true association between MAR and BD. We have identified six maternal/pregnancy-related variables that are suspected of being potential confounders, and we have tested each one for significant association with either MAR or BD; and those that are significantly related to MAR and/or BD were included in multivariate logistic regression to estimate aORs. It is obvious that there is an unlimited number of possible confounders, and only some of them, previously reported in the literature, have been analyzed. However, we assessed nearly 200 000 births, and this could help to balance confounders.

A third limitation of the study is that only BD diagnosed up to fifth day after birth could be registered by the REDCB and therefore included in the study. BD manifested later in life have been overlooked, this limitation being shared by most of the BD registries.[29] Finally, the last limitation is that only fetuses of 20 weeks or more, alive or dead, were included as controls, and the reasons were that in the 1990s, cases diagnosed under 20 weeks were rare, and pathology studies in miscarriages to certify a structural normal fetus were not available.

The main strength of our study is the fact that this is one of the few studies dealing with the association between the different MAR modalities and the different BD categories, something made possible by the large number of BD cases and controls, in contrast to the vast majority of reported studies assessing the association between ART and BD. A second strength is the inclusion of BD cases undergoing termination of pregnancy, which overcomes the detection bias produced by only considering BD in live births. In

addition, all the associations were adjusted for likely confounding variables, such as maternal age, parity, preexisting diabetes, maternal obesity, toxoplasmosis/rubella/cytomegalovirus infection in pregnancy, and fetal gender, unlike some of the published studies.[30]

CONCLUSIONS

Our study shows that there is a strong association between the use of ART and the probability of BD, with an almost threefold increase in comparison to natural conception. In contrast, we did not find an excess risk in pregnancies from artificial insemination or ovulation induction. This association between the use of ART and BD is observed for all BD categories but head-face-neck-eye. Only specific BD categories were found to present an excess risk after artificial insemination (genitourinary defects) and after ovulation induction (digestive and abdominal wall defects). These results should be carefully interpreted because this is primarily descriptive data, and their 'adjusted' values are still quite tentative (because of the unknown effects of missing data and unbalanced groups).

Given that ART accounted for more than 200 000 babies worldwide in 2002,[10] and taking into account our results, the prevalence of BD may increase further.[7–10] Thus, a public health issue is emerging.[2] A surveillance system for ART at the European level should identify mothers at increased risk for BD and the ideal conditions of embryonic development in MAR techniques.

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WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- The association of assisted reproduction techniques with birth defects still requires confirmation via large population-based studies.

WHAT DOES THIS STUDY ADD?

- This study, with a large birth defect sample from a population-based registry, demonstrates an increased birth defects risk after assisted reproductions techniques, not observed after artificial insemination or ovulation induction. This strong association with the use of assisted reproduction techniques was observed in all birth defects categories but head-face-neck-eye defects.

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7. DISCUSIÓN

En este trabajo de tesis se incluyen tres artículos centrados en el análisis y la generación de evidencias sobre diversos aspectos de la reproducción médicamente asistida (RMA). Cada publicación, aparte de generar las conclusiones específicas de cada uno de dichos análisis, ha contribuido tanto a conocer la calidad de las evidencias disponibles en este campo como a crear conocimiento y conocer la necesidad de investigaciones futuras.

La metodología de esta tesis se caracteriza por el uso de diseños apropiados para responder a cada cuestión planteada y minimizar los sesgos para, así, obtener evidencias de alto nivel. A continuación, se hará una reflexión sobre la interpretación de los resultados de cada uno de los estudios, y se pondrá especial énfasis en los principales hallazgos, en los puntos fuertes y las limitaciones de los mismos. Posteriormente, se llevará a cabo una discusión general del trabajo de tesis.

7. 1 Discusión específica derivada de las publicaciones

Evaluación de las Guías de Práctica Clínica en Técnicas de Reproducción Asistida

La evaluación de las guías de práctica clínica que contienen recomendaciones sobre técnicas de reproducción asistida muestra que la calidad de estas no es óptima. Solo tres guías (22%) fueron consideradas "recomendables", [69,70,131] las 11 (78%) restantes se calificaron como "No recomendables" o "recomendables con modificaciones" [154,221-230] Estos resultados son similares a los que se hallaron

en otros estudios en ámbitos diferentes.[19, 22] Por otro lado, los siguientes dominios obtuvieron una baja puntuación: "Participación de las partes interesadas", "Aplicación" y "Rigor en el desarrollo". Este último, que es uno de las más preocupantes al considerarse crucial desde el punto de vista metodológico, explica, entre otras cosas, cómo se desarrollan las recomendaciones y los criterios utilizados para evaluar la calidad de los estudios y el grado o fuerza de dichas recomendaciones. Estos resultados subrayan la necesidad de mejorar la calidad de las guías sobre TRA y, además, son ampliamente consistentes con otras evaluaciones realizadas previamente en este campo.[218-220] Por otro lado, la baja puntuación en el dominio "La participación de las partes interesadas" es también preocupante ya que podría comprometer el futuro seguimiento de las guías por parte de los diferentes actores implicados. Así, al formular directrices, deben incluirse elementos importantes como son los consumidores, los profesionales de la salud que trabajan en este ámbito y los administradores o encargados de formular políticas. Además, expertos externos y consumidores deberían revisar estas guías antes de ser aprobadas.[231-233] Por último, la baja puntuación en el dominio "aplicabilidad" (37%) podría indicar que los grupos que elaboran guías carecen de formación sobre estrategias para su implementación y para determinar los recursos necesarios para llevarla a cabo, o bien no han tenido en cuenta estos importantes aspectos. Según investigaciones anteriores, esto se debe principalmente a la existencia de barreras médicas que incluyen la falta de familiaridad con las directrices, la percepción de validez limitada y la aplicabilidad reducida de las mismas entre los pacientes específicos, y las recomendaciones

poco claras o ambiguas.[232-233] Otro estudio mostró que las principales barreras que impiden que se sigan las guías en RMA fueron la falta de eficacia de las recomendaciones aplicadas y el no cumplimiento de las expectativas de los médicos.[219] La identificación de estos factores, que influyen en la aplicabilidad de las recomendaciones, podría ayudar a la implementación y al uso de las mismas en este campo.

Uno de los puntos fuertes de este trabajo es que se han evaluado por primera vez las guias de este campo publicadas en inglés con ayuda del Instrumento AGREEII. Además, los tres revisores alcanzaron un alto grado de acuerdo cuando analizaron la calidad de las guías seleccionadas de forma independiente. Asimismo, se valoraron por separado los criterios de inclusión de las GPC y se realizó una evaluación sistemática de la calidad metodológica de cada guía utilizando un instrumento recientemente desarrollado, evaluado y validado internacionalmente.

Sin embargo, este estudio también tiene sus limitaciones. La primera es que la evaluación se centra en las GPC publicadas en el período 2006-2011 y que presentaban recomendaciones sobre el uso de TRA. En consecuencia, se pueden haber excluido directrices de alta calidad sobre otros temas relacionados con este campo (como ética, laboratorio y cuestiones de organización o prevención de la infertilidad) o guías publicadas anteriormente; si bien es cierto que la gran mayoría de las GPC se actualizan periódicamente. La segunda limitación es que el instrumento utilizado, el AGREE II, no diferencia entre “procesos no realizados” y “procesos realizados pero no mencionados”. Además, los documentos de

consenso entre expertos no se incluyeron porque carecen de los fundamentos de las recomendaciones basadas en la evidencia (por ejemplo, una estrategia de búsqueda explícita y la clasificación de la calidad de las pruebas); esto hace que tiendan a ser de menor calidad que los incluidos en nuestra revisión y que hubieran subestimado la calidad de las guías en TRA.

Revisión Sistemática de los Vasodilatadores en mujeres sometidas a reproducción médicamente asistida

Esta revisión consolida la mejor evidencia disponible sobre vasodilatadores en mujeres sometidas a reproducción médicamente asistida; cuenta con diez estudios con un total de 797 mujeres.[205-207, 209, 210, 235-239] La mayoría de los estudios fueron considerados como de riesgo de sesgo incierto, fundamentalmente por limitaciones en el reporte. Tres estudios informaron sobre nacidos vivos,[206,210,237] solo dos ECA notificaron efectos secundarios relacionados con los vasodilatadores,[210,239] 10 contenían datos sobre embarazos clínicos y cuatro informaron sobre otros efectos secundarios.[206,210,235,237] Los principales resultados indican que no hay pruebas suficientes para demostrar que los vasodilatadores incrementan la tasa de nacidos vivos en mujeres sometidas a RMA.[206,210,237]. Asimismo, no se encontraron indicios suficientes para llegar a conclusiones con respecto a los efectos adversos, ya que solo dos estudios abordaron este desenlace[2010,239]. Tampoco se encontró ninguna evidencia que sugiriese diferencias entre los grupos respecto a otros efectos adversos, tales como la gestación múltiple,[206,210] el aborto [206,210,235,237] o el embarazo ectópico.[206,210] Sin

embargo, existen evidencias de baja calidad que indican que los vasodilatadores solos o en combinación con otros tratamientos (vitamina E o estradiol) incrementan la tasa de embarazo clínico, en comparación con el placebo o ningún tratamiento.[205,206,209,210,237,238] No obstante, no se hallaron pruebas suficientes que demostrasen que un vasodilatador en particular, administrado solo o en combinación con otros medicamentos activos, fuera eficaz. En consecuencia, se necesitan estudios con una potencia adecuada para evaluar cada tratamiento con mayor precisión. Por último, no se ha detectado heterogeneidad estadística entre los resultados de los estudios de esta revisión, lo que sugiere que los factores que pueden haber diferido entre estudios tuvieron poco efecto en los resultados generales. No obstante, los intervalos de confianza se superponen en los ensayos individuales y fácilmente cambian en el análisis de sensibilidad. De este modo, los resultados de esta revisión deben ser interpretados con cautela.

Un segundo punto fuerte de este estudio es que se desarrolló en el marco de la Colaboración Cochrane, lo cual significó seguir todas las exigencias y contar con todo el apoyo de esta prestigiosa institución durante todo el proceso. Además, es la primera revisión que evalúa la eficacia de los vasodilatadores en RMA. Otra de las claves es que no se han reportado pruebas de heterogeneidad estadística ni estudios con alto riesgo de sesgo entre los ensayos; y la edad de las mujeres evaluadas y los criterios de inclusión y exclusión de los estudios fueron similares entre los estudios contenidos en el metanálisis. La mayoría de las variables de resultado (nacidos vivos, embarazo clínico, gestación múltiple, embarazo ectópico o aborto involuntario) son sólidas y no se ven afectadas por los investigadores. Por

lo tanto, la falta de cegamiento no implicó un aumento en el riesgo de sesgo. Igualmente, siete de los ocho estudios metanalizados[206, 209, 210, 236-239] revelaron un riesgo de sesgo de deserción bajo. La igualdad inicial entre los grupos era aceptable en ocho estudios y no se identificaron otras fuentes de sesgo. Además, el proceso de identificación de todos los estudios potencialmente elegibles fue exhaustivo y meticuloso.

Una limitación importante de este trabajo es que todos los estudios indicaron el embarazo clínico como criterio de valoración principal.[205-207,209,210,235-239] Sin embargo, para las mujeres y para los médicos, la tasa de nacidos vivos y la de efectos secundarios son desenlaces más importantes. Dado que se informó sobre dichos desenlaces solo en tres [206, 210, 237] y dos estudios[210, 239] respectivamente, esta revisión no ha podido responder las dos principales cuestiones, lo cual demuestra que nuevos estudios deberían evaluar estos desenlaces. Además, no fue posible analizar los probables sesgos en todos los estudios por falta de detalles en el reporte de los mismos y se consideró que presentan un riesgo de sesgo poco claro. No obstante, se estableció contacto con los autores de las investigaciones, pero solo dos de ellos respondieron.[209,237]

Estudio caso-control de los defectos congénitos en embarazos de reproducción asistida en la ciudad de Barcelona

El principal resultado de este estudio caso-control, después de la estratificación por modalidades de RMA, es que los concebidos mediante TRA tienen un mayor riesgo de defectos congénitos. Estos resultados concuerdan con lo señalado por

tres metanálisis.[167,213,240] En el análisis estratificado por categorías de defectos congénitos se observó que las técnicas se asocian con todos los tipos de defectos por aparato o sistema. Sin embargo, los resultados no coinciden con otros estudios primarios.[241-243] Con respecto a la inseminación artificial, se encontró que esta se asocia solo a defectos del aparato genitourinario, y la inducción de la ovulación solo a defectos del aparato digestivo y de la pared abdominal. El incremento de defectos congénitos en técnicas de reproducción asistida fue 2,7 veces mayor en los embarazos concebidos por estas técnicas, en comparación con los concebidos naturalmente. Algo parecido al estudio de Hansen que obtuvo un OR 2,0 (IC del 95%: 1,5 a 2,9) y al de Katalinic que estimó un RR 1.24 (IC del 95% CI: 1.02 a 1.50). Las diferencias pueden explicarse por la inclusión en nuestro estudio de interrupciones voluntarias del embarazo por defectos congénitos. Sin embargo, estos resultados difieren de los obtenidos por Ericsson y Kallen, quienes no señalan ningún incremento en los defectos congénitos en mujeres sometidas a FIV (OR 0,89; IC del 95% 0,74 a 1,06).[244] Estos resultados tampoco coinciden con Yan, quien reportó una prevalencia del 1,2% de defectos en embarazos concebidos con técnicas de reproducción asistida, similar a la del 1,4% del censo anual 2008.[245] Es importante destacar que ambos estudios se basaron en muestras pequeñas, que incluyen sólo 516 y 189 casos, respectivamente. En relación con la inseminación artificial, los resultados de este estudio concuerdan con los de Yan, quien presentó tasas de 1,01% a 1,03%, comparables al 1,4% de la población general.[245] De la misma manera, la falta de relación entre la inducción de la ovulación y los defectos congénitos coincide con dos estudios realizados en

otros países.[116,246] Por otro lado, la correlación que se estableció en este estudio entre los defectos congénitos de cada uno de los órganos con técnicas de reproducción asistida no se encontró en otros estudios. Otros dos encontraron una asociación solo respecto a los defectos del sistema cardiovascular, esquelético y aparato urogenital.[116,247] Además, el estudio de Halliday indicó una relación solo en cuanto a los defectos congénitos originados durante la blastogénesis (ORa 2,80; IC del 95%: 1,63 a 4,81)[248] y Refhuis en cuanto a los defectos cardíacos septales (ORa 2,1; IC del 95%: 1,1 a 4,0), la hendidura facial (ORa 2,4; IC del 95%: 1,2 a 5,1), la atresia esofágica (ORa 4,5; IC del 95% 1,9 a 10,5) y la atresia anorrectal (ORa 3,7; IC del 95% 1,5 a 9,1).[47] Sorprendentemente, en nuestro estudio se observó que la asociación más fuerte era con los defectos del aparato respiratorio (ORa 6,08; IC 2,89 a 12.79) pero, dada su baja frecuencia, los resultados deben ser interpretados con precaución. En esta investigación, los defectos genitourinarios fueron los únicos asociados a la inseminación artificial, lo que sugiere una relación real entre la infertilidad masculina y los defectos de este aparato. Con estos resultados se ha especulado que algunos procedimientos como la limpieza del grupo de células que rodea el ovocito y la introducción del pro-núcleo del espermatozoide, así como del acrosoma en el ovocito en técnicas de reproducción asistida, podrían perjudicar los mecanismos de homeostasis celular. Además, el contenido de metionina de los cultivos puede representar otro factor iatrogénico que podría alterar el proceso de metilación.[249] Sin embargo, esta asociación no implica una relación causal, dado que el exceso de defectos congénitos podría también ser el resultado de las características inherentes de la

infertilidad de esta población. Un estudio de cohorte danesa mostró que los recién nacidos de parejas infértiles tratadas y no tratadas presentan más defectos que los recién nacidos de parejas fértiles, lo cual se atribuye a las características de la población, más que al tratamiento en sí.[247] Del mismo modo, Davies señaló que la infertilidad, ya sea con o sin la concepción asistida, se asocia significativamente a defectos congénitos esqueléticos.[116] En la misma línea, se ha demostrado que los hombres con azoospermia tienen una alta prevalencia de anomalías cromosómicas, en comparación con los hombres no azoospérmicos aptos para la ICSI.[250,251] En este contexto, nuestros resultados sugieren que los defectos congénitos genitourinarios están ligados a la infertilidad de fondo, mientras que los hallados en TRA lo están al procedimiento.

Como decíamos anteriormente, uno de los puntos fuertes de este estudio es que se trata de uno de los pocos trabajos que, gracias al gran número de casos y controles encontrados, investiga la interrelación entre las diferentes modalidades de RMA y las distintas categorías de defectos congénitos; a diferencia de la gran mayoría de los estudios previos que solo evaluaron la asociación con las técnicas de reproducción asistida.[241-243] Un punto fuerte adicional es la inclusión de los casos sometidos a interrupción voluntaria por defectos congénitos, que superan el sesgo de detección establecido por los estudios que consideran solo defectos en los nacidos vivos.[241-243] Asimismo, todas las asociaciones fueron ajustadas por probables variables de confusión, como la edad materna, paridad, diabetes preexistente, la obesidad materna, la toxoplasmosis / rubéola / infección por citomegalovirus en el embarazo y el sexo del feto, a diferencia de otros estudios

publicados que no efectuaron los ajustes correspondientes.[252] Finalmente, el gran tamaño de la muestra de este estudio es comparable solo a dos estudios.[47,247]

La alta proporción de datos que faltan sobre la exposición a la RMA (54% en los casos y 25% en los controles) supone un límite para este estudio, si bien el análisis de homogeneidad entre las mujeres y los datos sobre exposición a RMA disponibles y no disponibles mostró resultados similares en alrededor del 80% de variables, lo que revela un riesgo de sesgo de selección mínimo. Otra limitación importante de este estudio es la presencia de posibles factores de confusión no equilibrados. Se han identificado seis variables maternas relacionadas con el embarazo que podrían ser posibles factores de confusión; también se ha buscado una asociación significativa de cada una con la exposición a RMA o la presencia de defecto congénito. Las que presentan una relación significativa se incluyeron en el modelo de regresión logística multivariada para estimar la OR ajustada. Es obvio que hay un número ilimitado de posibles factores de confusión, y sólo algunos de ellos se han notificado previamente en la literatura que se ha analizado. Sin embargo, la evaluación de casi 200 000 nacimientos realizada permitió detectar las diferencias significativas en ambos grupos. Nos encontramos, pues, con una limitación más relacionada con los defectos congénitos, los cuales se diagnosticaron solo hasta el quinto día después del nacimiento. Actualmente, gracias a los avances de la ecografía, gran parte de estos se diagnostican intraútero. Finalmente, solo los fetos de 20 semanas o más se incluyeron como controles; pero, los casos de menos de 20 semanas fueron infrecuentes.

Ensayos clínicos controlados aleatorizados de reproducción médicamente asistida publicados en revistas españolas de ginecología y obstetricia.

Para reducir el sesgo de selección es recomendable hacer una búsqueda manual de todos los ensayos clínicos publicados en revistas no indexadas, con la finalidad de que estos ensayos se incluyan en la elaboración de revisiones. Esta búsqueda se realizó en 16 revistas españolas de Ginecología y Obstetricia hasta el 31 de diciembre de 2013. Además, se han analizado 27 ensayos clínicos de RMA de un total de 224 identificados en estas revistas. Estos últimos 224 estudios fueron publicados entre 1967 y 2013, lo que supone un promedio de 4,82 por año. La revista líder en publicación fue *Progresos de Obstetricia y Ginecología*, la cual concentra el 24,1% de ensayos clínicos detectados. En los 224 estudios identificados se observó que los problemas de salud más investigados fueron las patologías de la gestación, tales como la rotura prematura de la membrana, la restricción del crecimiento intrauterino, la amenaza de parto prematuro, la diabetes gestacional y la anemia, entre otras. Sin embargo, se esperaba que el problema más estudiado fuera la RMA, ya que España es uno de los países de la Comunidad Europea con menor tasa de fecundidad (1,48/mujer), junto con Grecia, Italia y Alemania.[253] La mayoría de los ensayos clinicos eran unicéntricos (210; 89,4%) y se desarrollaron en un entorno de atención hospitalaria (216; 91,9%), lo que significa que se llevaron a cabo mayoritariamente en pacientes con patologías. La edad media de las participantes fue de 34,04 años (DS 9,318), pero esta variable no fue notificada en 104 ensayos, esto es, el 45,8% de los estudios

identificados. Estas deficiencias coinciden, con ligeras variaciones, con otros estudios similares realizados en otras especialidades.[254, 255] Además, se detectó que solo 11 estudios (4,9%) describieron quién era el promotor del estudio, frente a 224 (95,8%) que no lo señalaron. Así, el 99,6% de los ensayos clinicos no indica si hubo conflicto de interés. Estos hallazgos son parecidos a los de otros estudios similares.[255-257]

El análisis de los 27 ensayos clínicos de RMA reveló que se publicó menos de uno por año. La revista líder en publicaciones de esta temática fue la *Revista Iberoamericana de fertilidad*, con el 85,2% de los estudios. En la evaluación de la calidad metodológica de estos estudios se observó que la mayoría presenta un riesgo de sesgo alto. En el 64% se desconocen las características de la aleatorización y en más del 60% no se oculta la secuencia aleatoria. Además, solo el 17,2% indica algún método para el enmascaramiento de las intervenciones. Estos probables sesgos pueden exagerar los resultados.[258] No obstante, más del 70% de los ECA realizó un análisis por intención a tratar, un 82,8% informó de todas las variables clínicas y más del 75% tenía grupos comparables al inicio del tratamiento. Estos datos son alentadores porque contribuyen a disminuir sesgos de notificación y desgaste.[30]

Un punto a favor de este estudio es que se destaca que los ensayos clinicos detectados en la búsqueda manual no se habrían identificado a través de una búsqueda electrónica en MEDLINE (PubMed). Esto es una prueba más de las restricciones de las búsquedas exclusivamente electrónicas y del papel invaluable

de la búsqueda manual en la identificación de ECA, especialmente de aquellos caracterizados como resúmenes, cartas al director o los escritos en idiomas diferentes al inglés.[255-257] También cabe mencionar la gran cantidad de artículos revisados en un total de 15 revistas. La búsqueda manual, siempre de acuerdo con los criterios de la Colaboración Cochrane, fue sistemática y exhaustiva para todos los volúmenes y suplementos.

Para este estudio la búsqueda se realizó únicamente en revistas españolas y no se registraron estudios realizados en España pero publicados en revistas extranjeras o que no fueran específicamente de Ginecología y Obstetricia. De este modo, este trabajo se enfoca exclusivamente en estudios ejecutados en España, y queda, así, pendiente un estudio similar en países latinoamericanos, el cual se encuentra actualmente en desarrollo. Asimismo, la revisión de revistas se efectuó de manera individual, lo que pudo resultar en la exclusión de algunos ensayos clinicos que fueran aptos. Por otro lado, se minimizó la posibilidad de falsos negativos porque cada investigador hizo una prueba piloto para estandarizar la revisión de cada revista. Se intentó reducir también la posibilidad de falsos positivos mediante la verificación de los 235 estudios identificados por parte de al menos dos de los autores.

7.2 Discusión de los aspectos generales

Desde su inicio, la reproducción médicamente asistida ha ido acompañada de controversias no solo éticas, legales y sociales, sino también en cuanto a la elección de los procedimientos y al manejo de los términos. Actualmente, la tasa

de fracaso de RMA es de 70% o más por ciclo.[42-46] Además, existe un incremento de complicaciones obstétricas y de riesgos pediátricos.[46-49] En este contexto la estandarización del uso de estos procedimientos es fundamental y se ha intentado desde 1978, fecha en que el programa Especial del Grupo Asesor de la OMS estuvo de acuerdo en crear un equipo de Investigación, Desarrollo y Formación de Investigadores sobre Reproducción Humana. Sin embargo, este grupo estableció pautas solo para algunos procedimientos de RMA y 30 años después otro grupo de la OMS empezó a estandarizar los términos más usados en reprodución médicamente asistida, proceso que aún no ha terminado.[1] Entre tanto, otras instituciones de prestigio conscientes de la necesidad de seguir trabajando para elevar la calidad asistencial en este campo, emprendió esta labor mediante la elaboración de guías de práctica clínica que proporcionan recomendaciones, fundamentadas en bases científicas sólidas.[19-22,70,131]

La abundante cantidad de información científica generada en todo el mundo en RMA hace imposible que el clínico se mantenga actualizado en este campo. Por otro lado, existe una gran variedad de sistemas de clasificación del nivel de evidencia que aporta la información científica disponible. Frecuentemente estos sistemas se basan en metodologías poco claras y poco prácticas. Sin embargo, el grupo de estudio GRADE (Grading of Recommendations, Assessment, Development and Evaluation) ha desarrollado un sistema que es un método riguroso y transparente para la clasificación de la calidad de la evidencia y la fuerza de la recomendación.[10] Así mismo, el instrumento AGREE da pautas para el desarrollo y evaluación de guías. Estos instrumentos han mejorado la calidad de

las guías que las utilizan.[23-25,111] Por lo tanto, en el ámbito de la RMA existe una gran cantidad de directrices cuya calidad varía.[19-22] Considerando que la OMS, al igual que muchas otras organizaciones de todo el mundo, reconocen la necesidad de emplear procesos más rigurosos para lograr que la asistencia sanitaria cuente con recomendaciones de la mejor investigación disponible. Este trabajo de tesis ha evaluado la calidad de las guías de práctica clínica que indican TRA, pues el conocer la calidad de estas permite promover el uso y la aplicabilidad de recomendaciones de alta calidad en este campo.[12; 231; 233; 259]

La rápida evolución de los procedimientos de RMA requiere de una reevaluación y de un análisis sistemático periódico de las nuevas evidencias. Además, la elaboración de recomendaciones de intervenciones se debe dar en base a revisiones sistemáticas y ensayos clínicos aleatorizados.[17-20] Estos últimos deben ser bien valorados en revisiones sistemáticas y/o metanálisis, como las desarrolladas por diferentes grupos, como la Colaboración Cochrane.[13,30,33] En RMA encontramos un gran número de RS que son la base de algunas directrices, que formulan recomendaciones con mayor rigor y transparencia.[69-70] Sin embargo, en algunas intervenciones hay ausencia de revisiones sistemáticas. En estas circunstancias, en este trabajo de tesis se desarrolló una RS Cochrane sobre el uso de vasodilatadores en mujeres sometidas a RMA. La elaboración de esta revisión dentro de este prestigioso grupo cuenta con tres metanálisis de calidad, lo que aportará a los desarrolladores de directrices en este campo recomendaciones de mayor rigor científico. En esta revisión, se han seguido exigentes estándares metodológicos, se ha contado con el apoyo del *Menstrual Disorder and Subfertility*

Group (MDSG), así como del Centro Cochrane Iberoamericano y se han encontrado evidencias de baja calidad que demuestran que los vasodilatadores incrementan la tasa de embarazo clínico, pero no la de nacidos vivos. Este resultado permite abrir líneas de investigación en este ámbito, no solo para evaluar la tasa de embarazo, sino principalmente para valorar otros resultados, como nacidos vivos o efectos colaterales.

Por otro lado, es importante analizar la seguridad de los diferentes procedimientos de RMA. En relación a esto, las guías recomiendan continuar investigando la asociación de estos procedimientos con los defectos congénitos, así como de las complicaciones a largo plazo.[70] Sin embargo, se han encontrado revisiones sistemáticas que concluyen que los defectos congénitos aumentan en usuarias de TRA, [167; 213; 240] dado que los estudios primarios se centran en el estudio de esta y no en inseminación ni inducción de la ovulación.[212,214] En este trabajo de tesis se realizó un estudio caso-control de defectos congénitos. Este tipo de estudio resulta de máxima utilidad para valorar enfermedades de baja frecuencia poblacional, como es el caso de los defectos congénitos.[260] En él se ha analizado la asociación de los defectos congénitos con las tres modalidades de RMA (inseminación, inducción de la ovulación y TRA). Los hallazgos nos permiten plantear la posibilidad de que ciertos defectos congénitos, como los genitourinarios, están más relacionados con los problemas subyacentes de infertilidad masculina, mientras que otros defectos lo estarían más con las TRA. Asimismo, otros como los defectos gastrointestinales y de la pared están más relacionados a los medicamentos usados en la inducción de la ovulación. Si los resultados de este

estudio se pudieran extrapolar al contexto europeo, sería razonable esperar un incremento de defectos congénitos en el futuro. Si consideramos que entre un 0,2 y un 4,3% del total de nacimientos anuales en Europa se debe a RMA, [41-45] el problema, que es considerado un problema de salud pública en Europa, se vería agravado.[216] Por ello, es fundamental implementar un sistema de vigilancia en este campo e investigar los mecanismos del desarrollo embrionario sobre los cuales podrían estar actuando las TRA.

Por último, para evaluar la calidad de evidencias en este ámbito, es importante complementar la identificación electrónica de evidencias con una búsqueda manual que permite identificar los ensayos publicados en revistas no indexadas y, así, reducir el sesgo de selección que amenaza todas las revisiones. Bajo este precepto, la búsqueda manual en revistas españolas nos permitió identificar ensayos clínicos que no podrían haber sido hallados ni analizados, al no estar estas en revistas indexadas. La síntesis de estas evidencias en RMA permitirá al clínico la elección más adecuada de estos procedimientos.

Fortalezas

La principal fortaleza de este trabajo de tesis es que nos permite contar con la síntesis de los conocimientos sobre reproducción medicamente asistida desde la perspectiva de la metodología de la medicina basada en evidencias, cuyos tres grandes instrumentos son las guías de práctica clínica, las revisiones sistemáticas y los estudios primarios de calidad.[3-11] Para generar estas evidencias se ha seguido una metodología estandarizada y exigente. Así, en la

evaluación de la guía se utilizó un instrumento validado internacionalmente: el *Appraisal of Guidelines Research and Evaluation* II (AGREE II*).* La revisión sistemática se desarrolló conforme a la metodología de la Colaboración Cochrane y con el instrumento del *Grading of Recommendation Assessment Development and Evaluation (*GRADE), lo cual significó cumplir con todas las pautas de estos prestigiosos grupos. El estudio caso control se llevó a cabo con una base datos de más de 20 años adscrita al EUROCAT, una reputada institución que se encargó de la monitorización de los diagnósticos de defectos congénitos. Estos tres trabajos de tesis han pasado también por rigurosos procesos de revisión por pares antes de ser publicados. En consecuencia, estos conocimientos contribuirán a mejorar la práctica clínica y a potenciar la toma de decisiones en este campo. Asimismo, han dado pie a otras líneas de investigación que parten de las brechas identificadas.

Debilidades

Sin embargo, existen limitaciones propias de la medicina basada en evidencias que deberían tenerse en cuenta. Estas son: la existencia de evidencias sesgadas, inconclusas, contradictorias y la ausencia de ensayos clínicos con variables irrelevantes en pacientes sometidos a RMA. Otra limitación es que el análisis de algunas evidencias, como el de las guías, se realizó de los artículos publicados en inglés debido al acceso limitado a las bases de datos en otros idiomas. No obstante, gracias al apoyo de colaboración Cochrane y a que se realizó una búsqueda manual complementaria se minimizo este problema en la identificación

de los ensayos clínicos. Sin embargo, esta búsqueda manual se efectuó solo en revistas publicadas en España, lo cual resta valor a la investigación, ya que se pueden haber publicado más estudios fuera de este ámbito. Además, al tratarse de una búsqueda limitada a España no se puede reducir el sesgo de selección por completo, pero sí abrir líneas de investigación que en Latinoamérica ya están en curso. Por último, el estudio primario se elaboró a partir de una base de datos cuyas limitaciones ya se describieron; pero en el caso patologías de tan baja frecuencia, como los defectos congénitos, el gran número de casos detectados reduce esta limitación.

8. CONCLUSIONES

Este trabajo de tesis ha contribuido a la generación de nuevas evidencias en reproducción médicamente asitida (RMA), por medio de cuatro artículos que fueron el principal motor de desarrollo personal en investigación y la oportunidad de trabajar con grupos de prestigiosos investigadores. Asimismo, este trabajo ha identificado brechas de investigación en este campo, con el fin de que sean oportunamente contestadas científicamente.

8.1 Implicaciones para la práctica

- Se requiere mejorar la calidad general de las guías de práctica clínica que contienen recomendaciones sobre técnicas de reproducción asistida. La baja puntuación en dominios como el "rigor en el desarrollo" es particularmente preocupante, ya que este se considera un indicador importante de la calidad metodológica. Las nuevas guías en este campo deberían ser elaboradas utilizando criterios como los del GRADE (*Grading of Recommendation Assessment Development and Evaluation*) y con el rigor metodológico propuesto por el AGREE II (*Appraisal of Guidelines for Research & Evaluation II).*

- Existen evidencias de baja calidad que muestran que los vasodilatadores aumentan la tasa de embarazo clínico en comparación con placebo o ningún tratamiento. Sin embargo, no se han hallado pruebas suficientes para demostrar que los vasodilatadores elevan la tasa de nacidos vivos en mujeres

sometidas a reproducción médicamente asistida. Tampoco hay pruebas suficientes para llegar a conclusiones con respecto a los efectos adversos de los vasodilatadores.

PK Se ha observado que los defectos congénitos han aumentado casi el triple en concepciones mediante técnicas de reproducción asistida en comparación con la concepción natural. Esta asociación entre el uso de estas técnicas y defectos se encuentra en todas las categorías de defectos congénitos. Solo algunas más específicas se asocian a la inseminación artificial (defectos genitourinarios) y a la inducción de la ovulación (defectos del aparato digestivo y la pared abdominal). Estos resultados orientan acerca de los mecanismos que subyacen en el incremento de los defectos congénitos. Además, dado que este problema de salud está emergiendo con fuerza en todo el mundo, es necesario implementar un sistema de vigilancia de estos procedimientos.

QK El número de ensayos clínicos publicados en revistas españolas de Ginecología y Obstetricia es bajo mientras que el riesgo de sesgo de los ensayos de reproducción médicamente asistida detectados es alto. Se aconseja a los futuros autores de los ensayos en este campo cuidar tanto el diseño y la ejecución de los estudios para minimizar posibles sesgos y asegurar su calidad metodológica, como adherirse a la declaración *Consolidated Standards of Reporting Trials* (CONSORT) al publicar los resultados de sus estudios.

8.2 Implicaciones para la investigación

1. Desarrollar guías de práctica clínica que proporcionen recomendaciones sobre el uso de técnicas de reproducción asistida con el debido rigor metodológico.

2. Realizar ensayos clínicos aleatorios con tamaños de muestra más grandes con el fin de evaluar si un vasodilatador específico se asocia con el aumento de la tasa de nacidos vivos en mujeres sometidas a RMA.

3. Determinar la mejor vía de administración y dosificación del vasodilatador en mujeres sometidas a RMA, centrándose en el sildenafil y, al mismo tiempo, valorar los desenlaces pertinentes, tales como recién nacidos vivos y efectos secundarios. Asimismo, deben ayudar a determinar si las mujeres con endometrio delgado pueden o no beneficiarse de estos medicamentos.

4. Realizar estudios analíticos en busca de los mecanismos que subyacen en el incremento de defectos congénitos en los diferentes procedimientos de reproducción médicamente asistida.

5. Realizar estudios que detecten ensayos clínicos en mujeres sometidas a reproducción médicamente asistida mediante una búsqueda manual en revistas no indexadas publicadas en diferentes idiomas para contribuir a la reducción del sesgo de publicación en las revisiones sistemáticas.

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11. ANEXOS

ANEXO 1

Title:

Identification and description of controlled clinical trials published in Spanish Gynaecology and obstetrics journals and risk of bias assessment of trials on assisted reproductive techniques

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Condensation

This article summarizes the evidence available on Gynaecology and Obstetrics CCTs published in Spain, with a special emphasis on assisted reproductive techniques. Although focused on the evidence published in a single country, the methodological rigor used to conduct it may encourage similar projects in other languages, countries, or even disciplines.

Abstract

Objective: To identify and describe controlled clinical trials (CCTs) published in Spanish Gynaecology and Obstetrics journals. In addition, to assess the quality of the CCTs on Assisted Reproduction Techniques (ART) identified in this project.

Materials and methods: In order to identify eligible CCTs, all Spanish Gynaecology and Obstetrics journals were handsearched. Handsearching was conducted following the guidelines provided by the Cochrane Collaboration, which state that each journal article must be carefully reviewed, including original articles and other types of studies, letters to the editor, abstracts, and conference presentations. The results of the handsearching process were compared with an electronic search conducted in MEDLINE (PubMed). A descriptive analysis of the main characteristics of the identified CCTs was performed, as well as a methodological assessment of CCTs on ART.

Results: Sixteen Gynaecology and Obstetrics journals were identified, four of which have been indexed in MEDLINE at some point, although not currently. The journal with the most CCTs was “Progresos de Obstetricia y Ginecología”. A total of 235 CCTs were published in these journals, of which 29 were on ART. Most CCTs (216, 91.9%) were carried out in a hospital setting; 201 (89.4%) were unicentric. Obstetrics was the most studied subspecialty (46.4%). Among CCTs on ART, the risk of bias was predominantly high.

Conclusions: The number of CCTs published in Spanish Gynaecology and Obstetrics journals is limited. CCTs on ART present deficiencies in the report of

results and low methodological quality. It is advised that authors and journals adhere to the CONSORT statement and to the Cochrane Collaboration recommendations to reduce risk of bias when designing and disseminating research projects.

Key Words: Controlled clinical trials, Evidence-based Medicine, Gynaecology and Obstetrics, Assisted Reproduction Techniques.

Introduction

Well-designed and properly executed controlled clinical trials (CCTs) provide the best evidence on the impact of health interventions. Nevertheless, these might result in exaggerated estimates of this effect, if carried out using an inappropriate methodology. [1-3] Therefore, CCTs ought to be properly evaluated before being used in clinical practice. Evidence-Based Medicine (EBM) is defined as “conscientious, explicit, and judicious use of current best evidence in making decisions about the care of individual patients". [4] EBM depends largely on the methodological quality of the CCTs and its exhaustive identification. These features are equally indispensable for conducting systematic reviews (SR) [4, 5] and Clinical Practice Guidelines (CPG).[6, 7] Given these prerequisites, identifying the highest number of CCTs is critical to have SR and CPGs with no publication bias.

It is known that the best strategy to identify clinical trials is to combine electronic and manual (handsearch) search strategies.[8.9] Electronic search strategies, widely used and based on filters and keywords applied to databases, are limited for

different reasons.[10 - 12] First, because only since 2004 the World Health Organization (WHO) recorded CCTs of different languages, which means that many CCTs published in previous years may not have be included in this platform. [12] Second, because even though there are other bibliographic databases accessible online that allow identifying CCTs through electronic searching, the sensitivity of these searches is limited due to issues with classification (indexing) of the CCTs. In addition, the term "controlled clinical trial" was only indexed as such in 1990, and introduced in MEDLINE and EMBASE in 1991 and 1994, respectively. For this reason, it is considerably more difficult to identify older CCTs publications using algorithms and search filters.[10, 1]

Given this problem, the Cochrane Collaboration proposed to complement electronic search strategies with a handsearching strategy. Handsearching involves the progressive review of all articles (conference proceedings, theses, letters, editorials, etc.) published in a journal.[8-13] It requires a rigorous inspection, which allows the identification of CCTs that are not included in electronic databases, as well as CCTs that have not been properly indexed or that simply cannot be retrieved through an electronic search. Handsearching allows identifying 92% to 100% of CCTs available, whereas electronic searches of the main databases, MEDLINE and EMBASE, contribute only 55% and 49% of these, respectively[13]. This difference is due to the fact that the handsearching allows the identification of CCTs published before 1991, those published as abstracts or letters, and those published in languages other than English.[8, 13]

Several CCTs identification studies that combine electronic and handsearching strategies conducted in Pharmacology journals[14], General and Internal Medicine, [15] Ophthalmology [16] and patient safety[17], confirm the limitations of electronic search strategies. Through these projects, it has been found that the sensitivity of electronic searches is about 77%, whereas the accuracy or specificity hoovers around 50%, with cases in which it does not exceed 5%. [15,17]

During recent years, there is a marked increase in scientific production published on Gynaecology and Obstetrics in Spanish language. To our knowledge, no handsearching project has been conducted so far in order to identify these CCTs. In reference to Assisted Reproduction Techniques (ART), and the importance of gathering all available evidence on this field, it is essential to evaluate the quality of work carried out, considering that since 1978, when the first baby conceived through Fertilization In vitro was born, ART techniques have evolved considerably without this being reflected in higher pregnancy rates[20 -23].

This study was conducted in order to identify and describe the main features of CCTs published in Spanish Gynaecology and Obstetrics journals. Additionally, in order to obtain a clearer picture of the strengths and challenges of research in ART, a description of the methodological aspects and potential risk of bias of CCTs identified in this area was made. The studies identified in this article will be incorporated into CRS, the global CCTs registry of the Cochrane Collaboration.

Material and methods

Identification of eligible journals and handsearching of clinical trials

The first step of this study consisted of determining which journals should be handsearched, considering eligible those that publish original research in the field of Gynaecology and Obstetrics. Eligible journals were identified through the Spanish Medical Index (SMI), the National Catalogue of Spanish Publications in Health Sciences Libraries C-17, Latindex, Periodic, LILACS, Scielo, and MEDLINE (PubMed). This search was carried out by a trained investigator who followed a protocol that established the order in which the sources had to be consulted. All relevant information for each journal was collected and their full texts were identified on the Internet, libraries, publishers, corporations, and other sources.

The handsearching of each journal was systematic and performed according to the guidelines of the Cochrane Collaboration, which establish that each journal must be carefully reviewed, not only original articles but also letters to the editor, abstracts and conference presentations. Handsearching consists of four stages: first, reading table of contents; second, location of keywords in the title of each article (randomized, random, fortuitous, blind, etc.); third, reading of the summary (abstract) of each article; and fourth, reading of the materials and methods section. The process must be completed retrospectively, i.e. backwards from the last year of publication. If no CCTs are found in a period of five years, handsearching for the corresponding journal must be stopped.

The process of handsearching involved 12 reviewers. Following the recommendations of the Cochrane Collaboration, each reviewer conducted a pilot test which involved handsearching of a volume journal that had been previously reviewed by personnel with expertise in this field.

Electronic identification of clinical trials

Additionally, an electronic search was conducted on MEDLINE (PubMed access) in order to identify CCTs amongst the eligible journals for this study and compare results with those of the handsearch. The search strategy used can be found in Annex 1. Subsequently, we calculated the sensitivity and specificity of this search with the following definitions:

- Sensitivity: Proportion of all studies identified by the electronic search over those identified by handsearching.
- Specificity: Proportion of truly eligible studies among all those recovered by the electronic search strategy

Inclusion criteria

To be considered a CCT, a study had to fulfil the eligibility criteria of clinical trials proposed by the Cochrane Collaboration:

a) The study compares treatments in humans.

b) The study is prospective (interventions are planned before the study takes place, and assignment of subjects to intervention is decided by the researchers).

c) Two or more treatments or interventions are compared (one can be a control with no treatment or placebo group). Interventions can be of any type: drugs, surgery, diagnostic, educational, rehabilitative, organizational, etc.

d) The allocation to treatments should be randomized or quasi-randomized.
- Random: the authors explained that the compared groups were formed by random assignment, usually describing the allocation method.
- Quasi-randomized: it attempts to produce similar groups to assign each participant intervention. The methods used include allocation according to date of birth of the subject, day or month of the year, even and odd numbers, or medical record number.

The review of journals was conducted individually. Two authors (RG and IA or DB) verified the eligibility of each possible CCT identified. Discrepancies were resolved by consensus or consultation with a third author (HP, XB).

Data Extraction

Once the CCTs are identified and classified according to the previous criteria, in order to make a descriptive analysis of trials, the variables collected were evaluated in a data sheet specifically designed for this study.

Also, an assessment of risk of bias (high / medium / low) of the identified CCTs in ART was conducted using the Cochrane Collaboration tool recommended for this objetive.[9] This tool value several aspects of the methodology of CCTs, including method of generating the allocation sequence, concealing of this sequence, blinding of patients or investigators, intention-to-treat analysis, reasons for missing data (where applicable) and other likely sources of bias.

Analysis

A descriptive analysis of the variables of interest was performed using SPSS version 17 (SPSS, Inc., Chicago, IL, USA). Central measures and dispersion

measures were calculated and the features of quantitative variables were described. In addition, the absolute and relative frequencies of qualitative variables were calculated.

Results

Sixteen Gynaecology and Obstetrics journals were identified, of which 11 published CCTs. A total of 235 CTTs on different subspecialties were retrieved from these publications. The most active journal was *Progresos de Obstetricia y Ginecología* with 54 CCTs (23%), followed by *Clínica e Investigación en Ginecología y Obstetricia* with 46 CCTs (19.6%) and *Acta Ginecológica* with 35 CCTs (14.9%) (Table 1). Of all the CCTs, 29 (12.3%) were of ART.

Table 1: Spanish Gynaecology and Obstetrics Journals

Journal	Posted period	Publication years	Nº CCT	% CCT
1. PROGRESOS DE OBSTETRICIA Y GINECOLOGÍA	1958 a 2013	36	54	23%
2. CLINICA E INVESTIGACION EN GINECOLOGIA Y OBSTETRICIA	1971 a 2013	42	46	19.6
3. ACTA GINECOLÓGICA	1950 a 2010	48	35	14.9%
4. REVISTA ESPAÑOLA DE OBSTETRICIA Y GINECOLOGIA	1962 a 2011	43	27	11.5%
5. REVISTA IBEROAMERICANA DE FERTILIDAD Y REPRODUCCIÓN HUMANA	1990 a 2013	23	26	11.1%
6. TOKO GINECOLOGÍA PRÁCTICA	1971 a 2013*	42	14	6.0%
7. ACTUALIDAD OBSTÉTRICO GINECOLÓGICA	1989 a 2000	9	12	5.1%
8. CIENCIA GINECOLÓGIKA	1996 a 2008	12	7	3.0%
9. ACTA OBSTÉTRICA Y GINECOLÓGICA HISPANO-LUSITANA	1968 a 2013	32	7	3.0%
10. GINECOLOGÍA CATALANA	1998 a 2002	5	4	1.7%
11. AVANCES EN OBSTETRICIA Y GINECOLOGÍA	1976 a 1983	7	3	1.3%
12. CLÍNICA GINECOLÓGICA	1988 a 2000	1	0	0%
13. REVISTA DE SENOLOGÍA Y PATOLOGÍA MAMARIA	1987 a 2013	5	0	0%
14. FOLIA CLÍNICA EN OBSTETRICIA Y GINECOLOGÍA	1997 a 2010	5	0	0%
15. PROGRESOS DE DIAGNÓSTICO PRENATAL	1978 a 2013	5	0	0%
16. GINECOLOGÍA CLÍNICA Y QUIRÚRGICA**	2000 a 2001	2	0	0%
GINECOLOGÍA Y OBSTETRICIA CLÍNICA **	2000 a 2002	2	0	0%
GINEDIPS**	1970 a 1999	5	0	0%
TOTAL	1950 a 1984		235	100%

The first CCT was published in 1967 in *Acta Ginecológica* (Víctor Ruiz Velasco y Gonzalo Río de la Rosa. *La pentazocina en la analgesia obstétrica*. Acta Gin. 1967; 18(6):368-372), while the first CCT on RMA was published in 1987 in the

same journal (J. Balash, et al. *Endometriosis y esterilidad: Tratamiento con acetato de medroxiprogesterona y danazol*. Acta Gin. 1987; 44(2):39-72). The year with the highest number of CCTs was 1982 with a total of 14 (6%), followed by 1990 with 12 (5.1%). The decade with the most identified CCTs was the nineties (Figure 1).

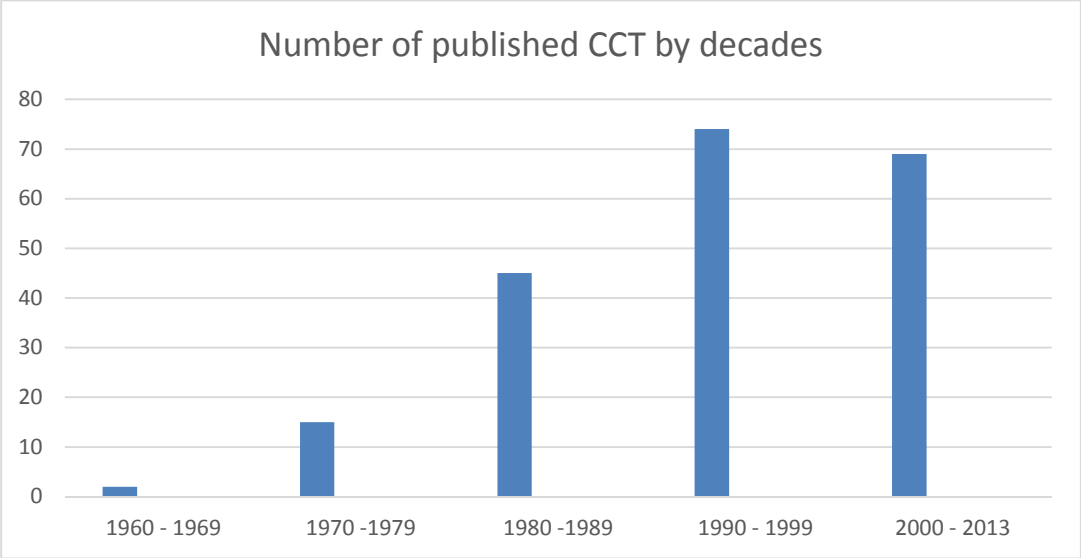

Figure 1- Number of published CCT by decade

A total of 187 (79.6%) of the CCTs identified were classified as randomized clinical trials (RCTs), whereas 48 (20.4%) were quasi-randomized. The most studied subspecialty was Obstetrics with 109 CCTs (46.4%), followed by Gynaecology with 72 (30.6%). The main researched topics were pregnancy and any associated conditions, including premature rupture of membranes, intrauterine growth restriction, preterm labour, gestational diabetes, anaemia, and others (24.7%). Infertility treated with RMA was studied in 12.1% of the studies (Figure 2).

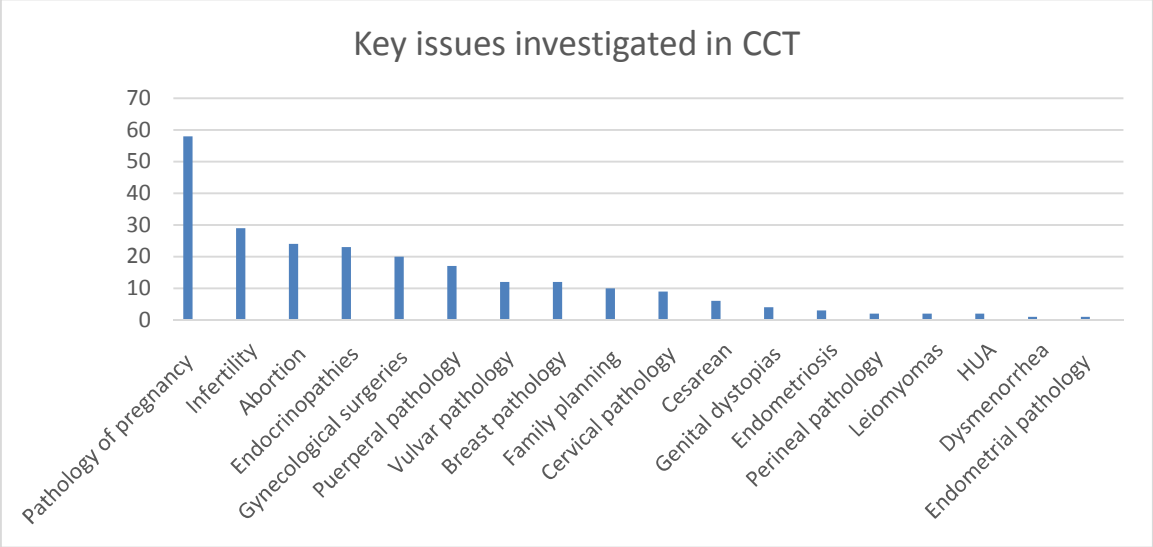

Figure 2: Key issues investigated in CCT

The average age of participants in the identified CCTs was 34.04 years (DS 9.318), with a minimum age of 20 and a maximum of 60. However, age was reported only in 126 CCTs, representing 54.2% of the identified studies. Most studies were conducted among subjects with pathology, and the most common comparison was treatment versus treatment (156, 66.4%). Most CCTs were conducted in hospitals (216, 91.9%), followed by primary care centres (9, 3.8%). A total of 210 (89.4%) of the CCTs were single centre, compared to 9 (3.8%) multicentre (Table 2).

In 224 (95.3%) of the cases, the authors did not specify the sponsor(s) of the study, while nine (3.8%) reported receiving private funds and two (0.9%) public funds. 234 CCTs (99.6%) did not report whether there was a conflict of interest, compared to one (0.4%) that did report it (Table 2).

Table 2: Characteristics of the identified CCTs

Features	n	%
õ **Speciality**		
Gynaecology	72	30.6%
Obstetrics	109	46.4%
Assisted Reproduction Techniques	29	12.3%
Gynaecologic Oncology	7	3.0%
Mastology	3	1.3%
Endocrinology	15	6.4%
• **Centre**		
One centre	210	89.4%
Multicentre	9	3.8%
Not reported	16	6.8%
• **Area**		
Hospital care	216	91.9%
Primary Care	9	3.8%
Others	5	2.1%
Not reported	5	2.1%
Intervention		
Drug vs. Drug	156	66.4%
Drug vs. placebo or no treatment	32	13.6%
Others	47	20.0%
Design		
Randomized CCTs	187	79.6%
Quasi-randomized CCTs	48	20.4%
Type of funding		
Public	2	0.9%
Private	9	3.8%
Not reported	224	95.3%
Conflict of interest		
Reported	1	0.4%
Not reported	234	99.6%

Twenty nine ART studies were identified in six of the elegible journals (*Revista Iberoamericana de Fertilidad y Reproducción Humana, Revista Española Obstétrica Ginecológica, Acta Ginecológica, Progresos de Obstetricia y Ginecología, Tokoginecología Práctica* and *Actualidad Obstétrica Ginecológica*). Regarding their methodological quality, we found that risk of bias was high in 20 CCTs (69%). In most (18, 62.1%), randomization sequence generation features were unidentified, while in nine (31%) randomization was performed properly. Only one CCT (3.4%) implemented a proper strategy to conceal allocation of patients to

treatments or interventions. Double-blind assessment of results was adopted in five CCTs (17.2%). Intention-to-treat analysis was implemented in 21 studies (72.2%); in 24 (82.8%) all relevant data was reported, and groups were comparable at baseline in 22 (75.9%) studies (Table 3).

Table 3: Risk of bias assessment of CCTs on ART

Quality assessment	Number	Percentage
Risk of bias:		
• Low	1	3.4%
• Moderate	8	27.6%
• High	20	69.0%
1. **Selection bias**		
Sequence generation		
• Adequate	9	31.0%
• Unclear	18	62.1%
• Inadequate	2	6.9%
Allocation concealment		
• Adequate	1	3.4%
• Unclear	26	89.7%
• Inadequate	2	6.9%
2. **Performance bias**		
Double blind		
• Adequate	5	17.2%
• Unclear	0	0%
• Inadequate	24	82.8%
3. **Detection bias**		
Blinding of outcome assessors		
• Yes	0	0%
• No	5	17.2%
• Not reported/Unclear	24	82.8%
4. **Attrition bias (incomplete outcome data)**		
Analysis by intention to treat		
• Yes	21	72.4%
• No	2	6.9%
• Not reported/Unclear	6	20.7%
5. Selective reporting of results		
• Clinical	24	82.8%
• Intermediate	2	6.9%
• Not reported/Unclear	3	10.3%
6. **Other sources of bias**		
Comparable at baseline groups:		
• Yes	22	75.9%
• No	1	3.4%
• Not reported/Unclear	6	20.7%

The electronic search conducted in MEDLINE allowed identifying five potential CCTs with no summaries (abstracts) available. These articles were identified in four journals: *Acta Obstétrica Ginecológica Hispana Lusitana, Acta Ginecológica, Toko Ginecología Práctica,* and *Revista Española de Obstetricia y Ginecología.* These journals are indexed in MEDLINE in different periods of time, although not currently. The full-text of the potential CCTs was retrieved; it was then determined that none were CCTs. Therefore, the sensitivity and specificity of the electronic search was 0%.

Table 4: Items identified by both searches

Items identified	Identified		C0nfirmed	
	N°	%	N°	%
Identified by handsearching	257	98,09%	235	100%
Identified by electronic search	5	3.91%	0	0%
Total	262	100	236	100

Discussion

The main objective of this study was to identify and describe the CCTs published in Spanish Gynaecology and Obstetrics journals until December 31, 2013. The number of CCTs identified in these publications is low, specifically 224 published between 1967 and 2013, an average of 4.82 CCTs per year. The journal that published the most CCTs was *Progresos de Obstetricia y Ginecología,* with 24.1% of CCTs.

The most researched health problems were conditions associated with pregnancies, including premature rupture of membranes, intrauterine growth restriction, preterm labour, gestational diabetes, and anaemia, among others. However, it was expected that the most studied problem would be ART, since Spain is one of the countries in the European Union with the lowest fertility rates (1.48 per female), along with Greece, Italy and Germany. [24]

Most CCTs were single-centre (210, 89.4%) and were developed in hospital care (216, 91.9%), which is consistent with the fact that these studies were conducted mainly among patients with pathologies. The average age of participants was 34.04 years (DS9.318), but this variable was not reported in 104 CCTs, which represents 45.8% of the identified studies. These deficiencies coincide with similar studies in other specialties, with slight deviations.[14, 15] It was detected that only 11 CCTs (4.9%) reported who was the promoter of the study, compared with 224 (95.8%) who did not. In the same line, 99.6% of CCTs did not report whether there were conflicts of interest, which is consistent with the results of other similar studies.[15, 17]

A total of 29 studies were on ART, equivalent to less than one per year. The leading journal in publishing on this subject was *Revista Iberoamericana de Fertilidad y Reproducción Humana*, with 85.2% of the total. In relation to the methodological quality of these studies, the majority present a high risk of bias. Randomization sequence generation characteristics are unknown in 62.1% of the cases, and frequently the sequence of allocation was not concealed. Furthermore, only 17.2% reported a method for blinding the interventions. These biases may

overstate results of the corresponding studies.[24] On the other hand, over 70% of the CCTs completed an intention-to-treat analysis, 82.8% reported all clinical variables, and 75% had groups comparable at baseline groups. These data is encouraging because it contributes to reduce reporting and attrition biases.[9]

This study emphasizes that the CCTs identified through handsearching would not have been identified via an electronic search in MEDLINE (PubMed). This is proof of the limitations of exclusively electronic searches and the invaluable role of handsearch in identifying CCTs, especially those reported as abstracts or letters to the editor, or reported in languages other than English.[15-17].Moreover, it is worth-mentioning the large number of publications reviewed: a total of 15 journals. Handsearching, in accordance with the criteria of the Cochrane Collaboration, was systematic and exhaustive for all volumes and supplements.

A possible limitation of this study is that the electronic and handsearching strategies were limited to Spanish journals. This may have left out CCTs published in foreign journals, or in journals that are not specifically on Gynaecology and Obstetrics. In addition, this paper focuses exclusively on studies in Spain: a study on journals from Latin American countries is being carried out at present. Another limitation is that the handsearching of journals were conducted individually, which could have left out some eligible CCTs. However, the possibility of false positives was minimized since the 235 CCTs identified were verified by at least two of the authors. Likewise, the possibilities of false negatives were minimized since each researcher conducted a pilot test to standardize the handsearching process.

In conclusion, the number of CCTs published in Spanish Gynaecology and Obstetrics journals is low. The CCTs identified in this study would not have been retrieved through an electronic search, which highlights the importance of handsearching of journals. Regarding CCTs on ART, the number of articles published is similarly low; they carry a high risk of bias in their methodology. Authors are advised to carefully consider the design and completion of CCTs, in order to minimize potential bias and ensure their methodological quality. They are advised to adhere to the Consolidated Standards of Reporting Trials (CONSORT) statement [27] when publishing the results of their studies.

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The authors have no conflicts of interest to declare.

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ANNEX I

Strategy search in PubMed

(("ActaSuomlaakDuodecim"[Journal] OR "acta"[All Fields] OR "ActaSuomLaakDuodecim"[Journal] OR "acta"[All Fields]) AND ginecol.[All Fields]) AND (Clinical Trial[ptyp] OR Clinical Trial, Phase I[ptyp] OR Clinical Trial, Phase

II[ptyp] OR Randomized Controlled Trial[ptyp] OR Clinical Trial, Phase III[ptyp] OR Clinical Trial, Phase IV[ptyp])

("TokoginecolPract"[Journal] OR ("tokoginecol"[All Fields] AND "pract"[All Fields]) OR "tokoginecolpract"[All Fields]) AND (Clinical Trial[ptyp] OR Clinical Trial, Phase I[ptyp] OR Clinical Trial, Phase II[ptyp] OR Randomized Controlled Trial[ptyp] OR Clinical Trial, Phase III[ptyp] OR Clinical Trial, Phase IV[ptyp])

("Rev EspObstetGinecol"[Journal] OR ("rev"[All Fields] AND "esp"[All Fields] AND "obstet"[All Fields] AND "ginecol"[All Fields]) OR "rev espobstetginecol"[All Fields]) AND (Clinical Trial[ptyp] OR Clinical Trial, Phase I[ptyp] OR Clinical Trial, Phase II[ptyp] OR Randomized Controlled Trial[ptyp] OR Clinical Trial, Phase III[ptyp] OR Clinical Trial, Phase IV[ptyp])

(Rev.[All Fields] AND senol.[All Fields] AND patol.[All Fields] AND mamar.[All Fields]) AND (Clinical Trial[ptyp] OR Clinical Trial, Phase I[ptyp] OR Clinical Trial, Phase II[ptyp] OR Randomized Controlled Trial[ptyp] OR Clinical Trial, Phase III[ptyp] OR Clinical Trial, Phase IV[ptyp])

("ClinGinecol"[Journal] OR ("clin"[All Fields] AND "ginecol"[All Fields]) OR "clinginecol"[All Fields]) AND (Clinical Trial[ptyp] OR Clinical Trial, Phase I[ptyp] OR Clinical Trial, Phase II[ptyp] OR Randomized Controlled Trial[ptyp] OR Clinical Trial, Phase III[ptyp] OR Clinical Trial, Phase IV[ptyp])

(Actual.[All Fields] AND obstet.[All Fields] AND ginecol.[All Fields]) AND Madr[All Fields] AND (Clinical Trial[ptyp] OR Clinical Trial, Phase I[ptyp] OR Clinical Trial, Phase II[ptyp] OR Randomized Controlled Trial[ptyp] OR Clinical Trial, Phase III[ptyp] OR Clinical Trial, Phase IV[ptyp])

(Av[All Fields] AND obstet[All Fields] AND ginecol[All Fields]) AND (Clinical Trial[ptyp] OR Clinical Trial, Phase I[ptyp] OR Clinical Trial, Phase II[ptyp] OR Randomized Controlled Trial[ptyp] OR Clinical Trial, Phase III[ptyp] OR Clinical Trial, Phase IV[ptyp])

(Cienc.[All Fields] AND ginecol.[All Fields]) AND (Clinical Trial[ptyp] OR Clinical Trial, Phase I[ptyp] OR Clinical Trial, Phase II[ptyp] OR Randomized Controlled Trial[ptyp] OR Clinical Trial, Phase III[ptyp] OR Clinical Trial, Phase IV[ptyp])

(Folia[All Fields] AND Clin.[All Fields] AND Obstet.[All Fields] AND Ginecol.[All Fields]) AND (Clinical Trial[ptyp] OR Clinical Trial, Phase I[ptyp] OR Clinical Trial, Phase II[ptyp] OR Randomized Controlled Trial[ptyp] OR Clinical Trial, Phase III[ptyp] OR Clinical Trial, Phase IV[ptyp])

(ginecol.[All Fields] AND catalano[All Fields]) AND (Clinical Trial[ptyp] OR Clinical Trial, Phase I[ptyp] OR Clinical Trial, Phase II[ptyp] OR Randomized Controlled Trial[ptyp] OR Clinical Trial, Phase III[ptyp] OR Clinical Trial, Phase IV[ptyp])

(Ginecol.[All Fields] AND obstet.[All Fields] AND clin.[All Fields]) AND (Clinical Trial[ptyp] OR Clinical Trial, Phase I[ptyp] OR Clinical Trial, Phase II[ptyp] OR Randomized Controlled Trial[ptyp] OR Clinical Trial, Phase III[ptyp] OR Clinical Trial, Phase IV[ptyp])

(Gine[All Fields] AND ("3,5-diisopropylsalicylic acid"[Supplementary Concept] OR "3,5-diisopropylsalicylic acid"[All Fields] OR "dips"[All Fields])) AND (Clinical Trial[ptyp] OR Clinical Trial, Phase I[ptyp] OR Clinical Trial, Phase II[ptyp] OR Randomized Controlled Trial[ptyp] OR Clinical Trial, Phase III[ptyp] OR Clinical Trial, Phase IV[ptyp])

(Prog.[All Fields] AND diagn.[All Fields] AND trat.[All Fields] AND prenat.[All Fields]) AND (Clinical Trial[ptyp] OR Clinical Trial, Phase I[ptyp] OR Clinical Trial, Phase II[ptyp] OR Randomized Controlled Trial[ptyp] OR Clinical Trial, Phase III[ptyp] OR Clinical Trial, Phase IV[ptyp])

(Prog[All Fields] AND Obstet[All Fields] AND Ginecol.[All Fields]) AND (Clinical Trial[ptyp] OR Clinical Trial, Phase I[ptyp] OR Clinical Trial, Phase II[ptyp] OR Randomized Controlled Trial[ptyp] OR Clinical Trial, Phase III[ptyp] OR Clinical Trial, Phase IV[ptyp])

(Rev.[All Fields] AND iberoam.[All Fields] AND fertil.[All Fields] AND reprod.[All Fields] AND hum.[All Fields]) AND (Clinical Trial[ptyp] OR Clinical Trial, Phase I[ptyp] OR Clinical Trial, Phase II[ptyp] OR Randomized Controlled Trial[ptyp] OR Clinical Trial, Phase III[ptyp] OR Clinical Trial, Phase IV[ptyp])

(("GinecolClin"[Journal] OR ("ginecol"[All Fields] AND "clin"[All Fields]) OR "ginecolclin"[All Fields]) AND quir.[All Fields]) AND (Clinical Trial[ptyp] OR Clinical Trial, Phase I[ptyp] OR Clinical Trial, Phase II[ptyp] OR Randomized Controlled Trial[ptyp] OR Clinical Trial, Phase III[ptyp] OR Clinical Trial, Phase IV[ptyp])

("Clin Invest GinecolObstet"[Journal] OR ("clin"[All Fields] AND "invest"[All Fields] AND "ginecol"[All Fields] AND "obstet"[All Fields]) OR "clin invest ginecolobstet"[All Fields]) AND (Clinical Trial[ptyp] OR Clinical Trial, Phase I[ptyp] OR Clinical Trial, Phase II[ptyp] OR Randomized Controlled Trial[ptyp] OR Clinical Trial, Phase III[ptyp] OR Clinical Trial, Phase IV[ptyp])