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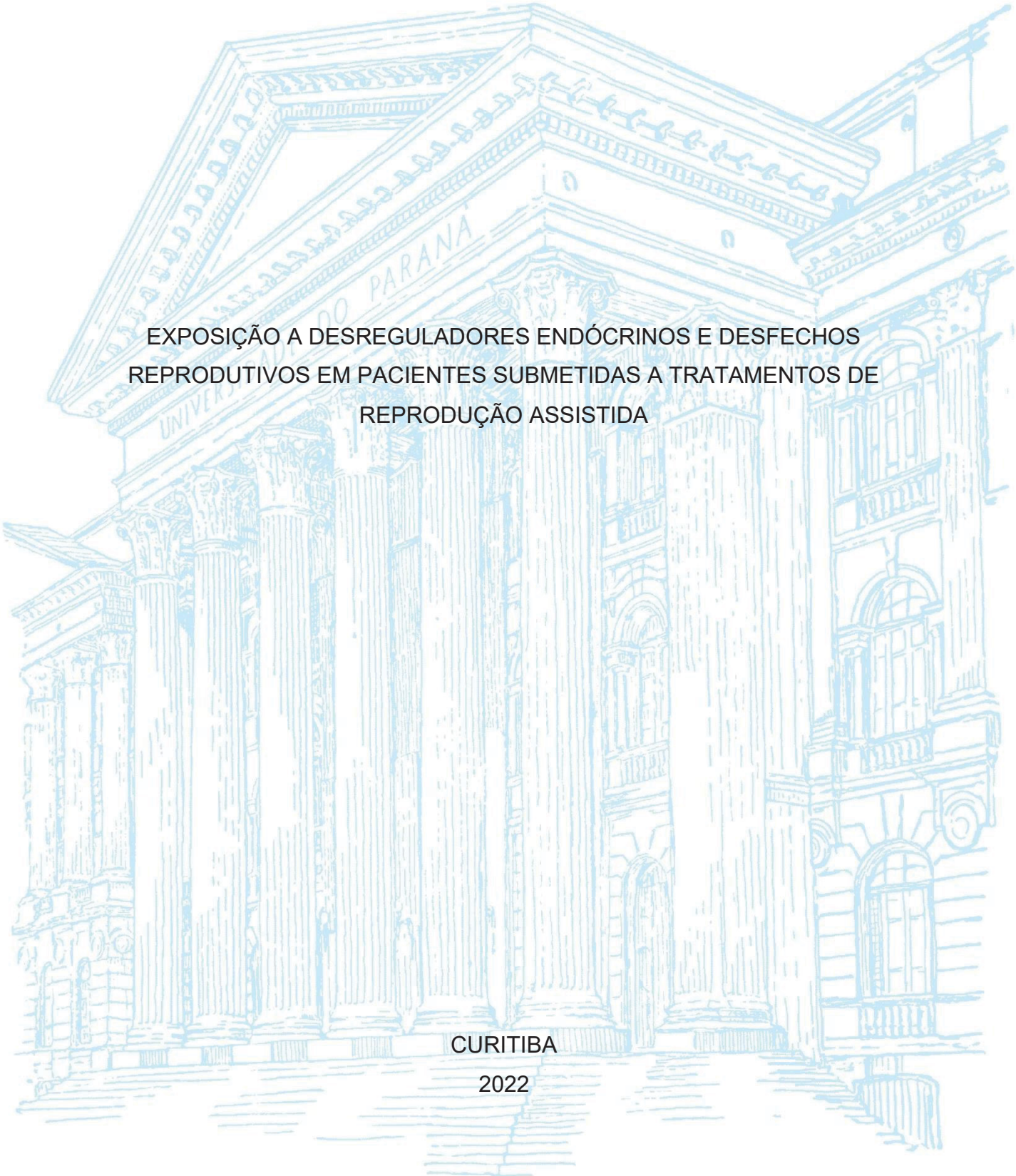
CARLA GIOVANA BASSO



EXPOSIÇÃO A DESREGULADORES ENDÓCRINOS E DESFECHOS REPRODUTIVOS EM PACIENTES SUBMETIDAS A TRATAMENTOS DE REPRODUÇÃO ASSISTIDA

CURITIBA

2022

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Tese apresentada ao Curso de Pós-graduação em Fisiologia, Setor de Ciências Biológicas, da Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Fisiologia.

Orientador: Prof. Dr. Anderson Joel Martino Andrade

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação FISIOLOGIA da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **CARLA GIOVANA BASSO** intitulada: **"Exposição a desreguladores endócrinos e desfechos reprodutivos em pacientes submetidas a tratamentos de reprodução assistida."**, sob orientação do Prof. Dr. ANDERSON JOEL MARTINO ANDRADE, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

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RESUMO

As crescentes taxas de infertilidade na população vêm sendo justificadas na literatura como um reflexo de fatores ambientais e estilo de vida. Dentro desse contexto, ganham destaque os efeitos de compostos desreguladores endócrinos (DEs), como os ftalatos, em parâmetros reprodutivos. A exposição a ftalatos está associada com efeitos adversos no desenvolvimento e funcionamento do sistema reprodutivo feminino. Um dos possíveis mecanismos de ação de compostos DEs está associado a desequilíbrios nas vias de prostaglandinas, chamando atenção para outro grupo com potencial efeito de DEs, os medicamentos analgésicos. Esses compostos também apresentam mecanismo de ação associado as vias de prostaglandinas e dessa forma - tendo em vista o importante papel dessas vias em processos reprodutivos - é biologicamente plausível que analgésicos também possam atuar como DEs. Portanto, nosso objetivo foi revisar dados da literatura sobre o impacto de ftalatos na saúde reprodutiva feminina e avaliar as associações entre a exposição a desreguladores endócrinos, especificamente medicamentos analgésicos de venda livre e ftalatos, com desfechos reprodutivos em mulheres em tratamento de reprodução assistida (ART). Para a realização do estudo, foram recrutadas mulheres com infertilidade e indicação para procedimentos de ART. As pacientes que aceitaram participar do estudo responderam questionários no início e fim do tratamento englobando dados demográficos, hábitos de vida e consumo de medicamentos (questionadas sobre o uso específico dos analgésicos mais utilizados no Brasil, paracetamol, dipirona, ácido acetilsalicílico e ibuprofeno). Ainda, foram coletadas amostras de urina e líquido folicular a fim de avaliar a exposição das pacientes a ftalatos – sendo quantificados 17 metabólitos de ftalatos em urina e líquido folicular, por meio de cromatografia líquida acoplada a espectrometria de massa. Já para os desfechos reprodutivos foram avaliados os dados laboratoriais englobando parâmetros oocitários e de desenvolvimento embrionário e resultados clínicos do tratamento de ART. Os dados foram analisados por modelos lineares generalizados para elucidar a associação entre o uso de medicamentos e desfechos reprodutivos e por modelos lineares generalizados mistos para as associações entre as concentrações de metabólitos de ftalatos e desfechos reprodutivos. Nosso estudo observou associações entre os desfechos reprodutivos e desreguladores endócrinos, especificamente medicamentos analgésicos e ftalatos, em pacientes de ART. Em relação a medicamentos analgésicos, o consumo de paracetamol, ibuprofeno e dipirona apresentou associação com desfechos reprodutivos de parâmetros oocitários e de qualidade embrionária, não sendo observadas associações com desfechos clínicos. Entretanto, os resultados observados variaram de acordo com as covariáveis adicionadas ao modelo - com exceção da associação negativa entre paracetamol e qualidade embrionária que se mostrou significativa em todos os modelos. Em relação à exposição a ftalatos, observamos que maiores concentrações de metabólitos de DEHP, especificamente mEHHP e mEOHP, foram associadas com parâmetros oocitários e de desenvolvimento embrionário – especialmente em urina das pacientes do estudo. Ainda, foram observadas associações em líquido folicular do metabólito mEP e em urina dos metabólitos mBzP e mIPrP com desfechos laboratoriais. Dessa forma, nosso estudo observou que exposição a desreguladores endócrinos pode estar associada a parâmetros iniciais do desenvolvimento embrionário e de desfechos reprodutivos do tratamento de pacientes em ART.

Palavras-Chave: Desreguladores endócrinos; Ftalatos; Medicamentos analgésicos; Infertilidade; Reprodução Assistida.

ABSTRACT

Increasing rates of infertility in the population are justified in the literature as a reflection of environmental factors and lifestyle. Within this context, the effects of endocrine disrupting compounds (EDCs), such as phthalates, on reproductive parameters are highly considered. Exposure to phthalates is associated with deleterious effects on the development and functioning of the female reproductive system. One of the possible mechanisms of action of EDCs is associated with imbalances in prostaglandin pathways, drawing attention to another group with a potential effect of EDCs, mild analgesics. These compounds also present effects through prostaglandin pathways and therefore - in view of the important role of prostaglandin pathways in reproductive processes - it is biologically plausible that mild analgesics can also act as EDCs. Therefore, our goal was to review the literature on the impact of phthalates on female reproductive health and to assess the associations between exposure to EDCs, specifically mild analgesics, and phthalates, with reproductive outcomes in women undergoing assisted reproduction treatment (ART). Hence, women with infertility and indication for ART were recruited. Patients who agreed to participate in the study answered questionnaires at the beginning and end of treatment surrounding demographic data, lifestyle habits and analgesic consumption (asked specifically about the use of the most used analgesics in Brazil, paracetamol, dipyrone, acetylsalicylic acid and ibuprofen). Furthermore, samples of urine and follicular fluid were collected in order to assess the exposure of patients to phthalates – being quantified by liquid chromatography 17 phthalate metabolites in urine and follicular fluid. As for reproductive outcomes, laboratory data were evaluated, covering oocyte and embryo development parameters and clinical results of ART. Data were analyzed by generalized linear models to elucidate the association between analgesic use and reproductive outcomes and by mixed generalized linear models for associations between phthalate metabolite concentrations and reproductive outcomes. Our study observed associations between reproductive outcomes and EDCs exposure, specifically mild analgesics and phthalates, in ART patients. Regarding mild analgesics, the consumption of paracetamol, ibuprofen and dipyrone was associated with reproductive outcomes of oocyte parameters and embryo quality, with no association with clinical outcomes. However, the observed results varied according to the covariates added to the model - with exception of the negative association between paracetamol and embryo quality, which was significant in all models. Regarding phthalate exposure, we observed that higher concentrations of DEHP metabolites, specifically mEHHP and mEOHP, were associated with oocyte and embryo development parameters – especially in the urine of study patients. Furthermore, associations were observed in mEP follicular fluid concentrations and mBzP and mIPrP urine concentrations with laboratory outcomes. Thus, our study observed that exposure to endocrine disruptors may be associated with early parameters of embryo development and reproductive outcomes in the treatment of ART patients.

Keywords: Endocrine disruptors; Phthalates; Mild analgesics; Infertility; Assisted Reproduction.

LISTA DE FIGURAS

ARTIGO CIENTÍFICO 1

ARTIGO CIENTÍFICO 2

ARTIGO CIENTÍFICO 3

LISTA DE TABELAS

LISTA DE ABREVIATURAS

AAS - ácido acetil salicílico

AINEs - anti-inflamatórios não esteroidais

AGD - distância anogenital (do inglês *Anogenital distance*)

ART – Técnicas de reprodução assistida (do inglês Assited Reproduction Techniques)

SOP - Síndrome de ovário policístico.

BBzP - benzilbutil ftalato (do inglês *Benzyl butyl phthalate*)

BPA - Bisfenol A

COX - enzimas ciclooxigenases

DEHP - Di-(2-etil-hexil) ftalato (do inglês *Di(2-ethylhexyl) phthlate*)

DiNP - di-iso-nonil ftalato (do inglês *Diisononyl phthalate*)

DnOP - di-n-Octil ftalato (do inglês *Di-n-octyl phthalate*)

DPhP - di(2-propilheptil) ftalato (do inglês *Di(2-propylheptyl) phthalate*)

DMP - dimethil ftalato (do inglês *Dimethyl phthalate*)

DnBP - di-n-butil ftalato (do inglês *di-n-butyl phthalate*)

DiBP - di-iso-butil ftalato (do inglês *Diisobutyl phthalate*)

DiPeP - di-iso-pentil ftalato (do inglês *Diisopentyl phthalate*)

DE – Desregulador endócrino

DEP - diethil ftalato (do inglês *Diethyl phthalate*)

EDCs - Desreguladores endócrinos (do inglês *Endocrine Disruptors*)

FIV - Fertilização *in vitro*

ICSI - Injeção intracitoplasmática de espermatozoides.

HMWP - Ftalatos de cadeia longa (do inglês *High-Molecular-Weight Phthalates).*

LMWP - ftalatos de cadeia curta (do inglês *low-molecular-weight phthalate*)

MEP - Monoetil ftalato (do inglês *Monoethyl phthalate*)

MEHP - mono-(2-etilhexil) ftalato (do inglês *Mono-2-ethylhexyl phthalate*)

MBzP - monobenzilo (do inglês *Monobenzyl phthalate*)

MEHHP - mono (2-ethil-5-hidroxihexil) ftalato (do inglês *Mono(2-ethyl-5-hydroxyhexyl) phthalate*)

PVC - Policloreto de vinila

SUMÁRIO

1-INTRODUÇÃO

Infertilidade é definida como a incapacidade de um casal obter uma gestação após 12 meses de tentativas. Estima-se que 15% da população apresente algum grau de infertilidade, sendo que as causas de infertilidade são igualmente provenientes de homens e mulheres (Agarwal et al., 2015). Fatores ambientais e estilo de vida apresentam potencial significativo de impactar a saúde geral e qualidade de vida da população. Dentro desse contexto, os fatores ambientais e de estilo tem chamado a atenção nos últimos anos como possíveis fatores que atuam no crescente aumento das taxas de infertilidade. Estudos já observaram que hábitos de vida que implicam em estresse, tabagismo e obesidade afetam a saúde feminina a longo prazo, reduzindo a competência reprodutiva e significativamente diminuindo as chances de gestação (Bala et al., 2021). Entretanto, nos últimos anos a exposição a compostos químicos tem ganhado papel de destaque em trabalhos que avaliam a relação da exposição a esses agentes e seus potenciais efeitos na saúde reprodutiva da população. Dessa forma, diversos estudos têm investigado os efeitos de compostos desreguladores endócrinos (DEs). DEs são compostos definidos como qualquer substância exógena que interfere na ação de hormônios, atuando na síntese, secreção, transporte, ligação a receptores em células alvo e/ou eliminação de hormônios fisiológicos, ocasionando desequilíbrio na homeostase, reprodução e desenvolvimento do organismo exposto (Tang et al., 2020). Diversos químicos encaixam-se nessa categoria como, pesticidas, fertilizantes, plastificantes, entre outros. Entre esses compostos os ftalatos têm ganhado papel de destaque, principalmente pelo alto volume de produção e consequentemente alto grau de exposição da população. Ftalatos são compostos químicos utilizados principalmente como plastificantes, sendo adicionados a misturas de polímeros com o objetivo de conferir maleabilidade aos plásticos (Blount et al., 2020). Ainda, os ftalatos podem ser utilizados como fixadores de aroma na indústria, sendo bastante utilizados em produtos de uso pessoal. Dessa forma, esses compostos podem ser encontrados em diversos produtos, como alimentos, cosméticos, vestimentas, medicamentos e produtos hospitalares (Huang et al., 2017, Martino-Andrade et al., 2009). Dados experimentais e epidemiológicos já demonstraram que a exposição a ftalatos está associada com efeitos adversos no desenvolvimento e funcionamento do sistema reprodutivo feminino. Alterações como puberdade precoce, insuficiência ovariana, endometriose, síndrome de ovário policístico e parto prematuro já foram associadas à exposição a ftalatos em diversas fases do desenvolvimento e

especialmente durante o período gestacional (revisado por Mesquita et al., 2021 e Basso et al., 2022). Estudos epidemiológicos têm relacionado a exposição a ftalatos com piores desfechos reprodutivos em diferentes contextos. Srilanchakon e colegas (2017) observaram que adolescentes com puberdade precoce apresentavam maiores níveis urinários de Monoetil ftalato (MEP), indicando uma associação da exposição a ftalatos com puberdade precoce. Ainda, trabalhos observaram que mulheres adultas com maior exposição a ftalatos apresentam alteração na função ovariana, sendo descritos desequilíbrios na foliculogênese e alterações na qualidade e quantidade da reserva ovariana (Hannon & Flaws, 2015, Messerlian et al., 2016, Du et al., 2018). Especificamente, alguns dados coletados em populações em tratamento para infertilidade também demonstram efeitos deletérios da exposição a ftalatos (Hauser et al., 2016; Al-Saleh et al., 2019). Em estudo prospectivo realizado com casais em clínica de reprodução assistida foi observado que maiores concentrações urinárias de mono-(2-etilhexil) ftalato (MEHP) em mulheres em tratamento foi associado com risco de gestação bioquímica, falha em gestação clínica e menor taxa de nascimento (Al-Saleh et al., 2019). Os dados presentes na literatura demonstram que ftalatos são importantes compostos DEs com significativo impacto na saúde reprodutiva em diferentes fases do desenvolvimento. Entretanto, os estudos avaliando possíveis mecanismos de ação desses compostos ainda são divergentes, sendo necessárias maiores investigações nesse campo.

Uma das possíveis vias de ação dos ftalatos e de compostos DEs, pode estar associada a desequilíbrios nas vias de prostaglandinas, sendo essa uma das possíveis rotas responsáveis pelos efeitos danosos da exposição a ftalatos no sistema reprodutor feminino (Tetz et al., 2015, Tran-Guzman e Culty, 2022). Prostaglandinas estão envolvidas em diversos processos do sistema reprodutivo feminino, como ovulação, fertilização e implantação embrionária (Niringiyumukiza et al., 2018). Dentro desse contexto, esse possível mecanismo de ação dos ftalatos chama atenção para outro grupo de compostos com potencial efeito desregulador endócrino, os medicamentos analgésicos. Medicamentos analgésicos de venda livre, como anti-inflamatórios não esteroidais (AINEs), paracetamol e dipirona, apresentam suas atividades terapêuticas por meio de inibição específica ou não especifica das enzimas ciclooxigenases (COX) e alteração das cascatas de prostaglandinas (Vane e Botting, 1998). Kristensen e colegas (2011) observaram em estudo *in vitro* que desreguladores endócrinos específicos são capazes de inibir a via de prostaglandinas, sendo sugerido que esses compostos podem interferir diretamente na atividade de enzimas COX de maneira similar a analgésicos como ácido

acetil salicílico (AAS), paracetamol e ibuprofeno. Dessa forma, tendo em vista que a inibição de COX e prostaglandinas pode fazer parte do modo de ação de desreguladores endócrinos, especialmente ftalatos, é possível que fármacos que apresentam mecanismo de ação de inibição de prostaglandinas, como os analgésicos de venda livre também possam atuar como DEs.

Trabalhos experimentais e epidemiológicos já demonstram os potenciais efeitos deletérios da exposição a medicamentos analgésicos em períodos específicos do desenvolvimento. Em modelo animal, já foram observadas associações entre o consumo de analgésicos e alterações como redução da distância anogenital (AGD), redução dos níveis de testosterona e alteração espermática (Kristensen et al., 2011, 2012; Thiele et al., 2013). Já em estudos realizados em humanos, foi observado que gestantes que reportaram o uso de paracetamol por mais de 2 semanas durante a gestação apresentaram risco aumentado de terem filho do sexo masculino com criptorquidismo (Kristensen et al., 2011). Ainda, em estudo realizado com mulheres tentando conceber foi observado que pacientes com maior uso de naproxeno, um fármaco do grupo de AINEs, apresentavam taxa de fecundidade menor em relação a mulheres que não faziam uso desse medicamento, sugerindo que o uso de analgésicos pode impactar na capacidade reprodutiva feminina (Mcinerney et al., 2017). Dessa forma, é possível sugerir com base nos dados experimentais e epidemiológicos presentes na literatura que medicamentos analgésicos apresentam potencial de atuar como desreguladores endócrinos. Entretanto, poucos são os dados que avaliam os efeitos desses compostos na população tentante ou infértil, sendo importante considerar os efeitos nessa população especifica uma vez que esse grupo faz uso significativamente maior de medicamentos e suplementação. Assim, é necessário estudos que investiguem os efeitos da exposição a medicamentos analgésicos em população infértil tentando gestar, avaliando os possíveis impactos dessa exposição nos desfechos reprodutivos dessa população.

2- REVISÃO BIBLIOGRÁFICA

2.1 INFERTILIDADE

Na última década tem sido observado um preocupante aumento nos índices de infertilidade, sendo estimado que mundialmente 48 milhões de casais apresentem algum grau de infertilidade (WHO, 2021). A redução da capacidade reprodutiva da população apresenta, além das implicações médicas, um importante impacto psicológico, financeiro

e na qualidade de vida dos casais, tornando-se um significativo problema de saúde pública (Mascarenhas et al., 2012). Infertilidade é definida como a falha na obtenção de uma gestação de sucesso após 12 meses de coito frequente sem uso de métodos contraceptivos, sendo que a etiologia da doença pode ser relacionada com fatores masculinos, femininos, do casal ou ainda, fatores idiopáticos (Carson et al., 2021). Estima-se que 85% dos casais apresentam infertilidade por causa conhecida, sendo as causas mais comuns disfunção ovulatória, infertilidade por fator masculino e alterações anatômicas no sistema reprodutor feminino (WHO, 2021). Os fatores femininos de infertilidade são responsáveis por cerca de 35-40% das causas de infertilidade, englobando alterações hormonais, endometriose, alterações das tubas uterinas, infecções prévias e fatores ambientais (Jurczewska e Szostak-W˛egierek, 2022). Em relação a fatores ambientais, estudos demonstram que, juntamente ao estilo de vida do paciente, o ambiente pode ser o principal responsável de causas idiopáticas de infertilidade – grupo que compreende 15-20% dos casais com subfertilidade, em que não existe causa de infertilidade aparente (Mohamed et al., 2004, No, 2013). O aumento nas taxas de infertilidade tem levado a um aumento na procura por auxílio médico na concepção (Serafin et al., 2022). Atualmente, a principal opção de tratamento para os casais que apresentam infertilidade são as técnicas de reprodução assistida. Dentre os possíveis métodos para tratamento, independentemente do tipo de infertilidade, os tratamentos complexos – englobando fertilização *in vitro* (FIV) e injeção intracitoplasmática de espermatozoides (ICSI) - apresentam as maiores taxas de sucesso. Dessa forma, é possível observar que o aumento das taxas de infertilidade também se reflete na procura por tratamentos de reprodução assistida, sendo reportado no Brasil um aumento de 112% no número de ciclos de tratamentos complexos realizados entre os anos de 2012 e 2019 (Figura 1 – Anvisa, 2021). Entretanto, a causa de infertilidade e fatores ambientais relacionados ao estilo de vida também impactam no sucesso do tratamento com técnicas de reprodução assistida (Tesarik et al., 2022).

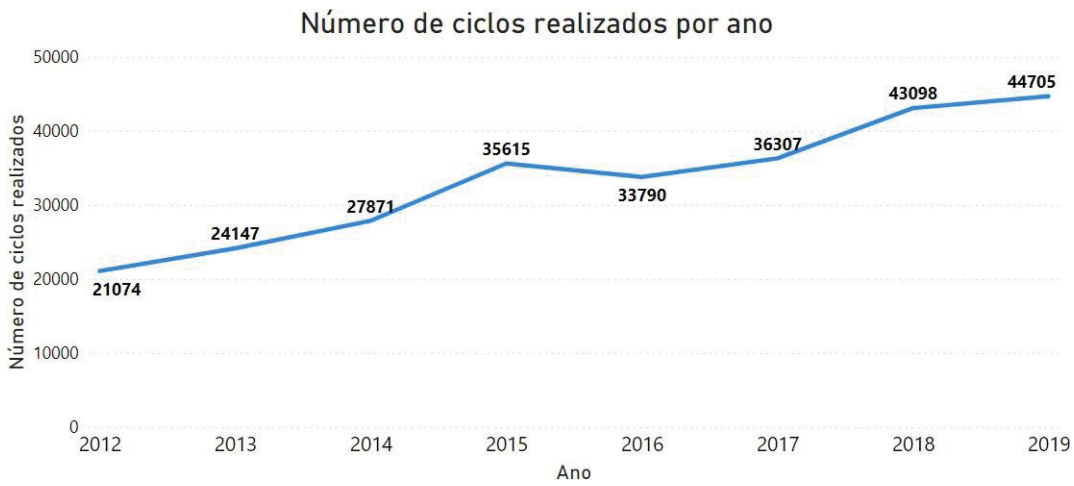

Figura 1: Número de ciclos de reprodução assistida realizados no Brasil anos de 2012, 2013, 2014, 2015, 2016, 2017, 2018 e 2019. (Anvisa, 2021).

O progressivo aumento nas taxas de infertilidade na população é influenciado por diversos fatores, estando entre os principais a postergação da gestação, estilo de vida e fatores ambientais (Karwacka et al., 2019). A postergação da gestação, implica em gestação com idade avançada. A idade feminina apresenta barreiras fisiológicas e genéticas para concepção, sendo essa relacionada com redução da reserva ovariana, alterações na ovulação, aumento de erros meióticos do oocito entre outros (Hart et al., 2016). Já em relação a estilo de vida e fatores ambientais, os fatores de risco aos quais mulheres em idade reprodutiva podem se expor vem aumentando juntamente com o processo de industrialização (Caserta et al., 2011). Fatores de estilo de vida como, tabagismo e obesidade já apresentam papel estabelecido na modulação da fertilidade e fecundidade feminina (Kelly-Weeder e Cox, 2006). Entretanto, nos últimos anos a exposição a compostos químicos presentes no ambiente e na dieta dos pacientes que buscam conceber tem chamado a atenção. Evidências toxicológicas têm indicado o potencial dessas substâncias em prejudicar a capacidade reprodutiva de homens e mulheres tanto direta quanto indiretamente (Foster et al., 2006, Caserta et al., 2008). Em mulheres, a exposição prolongada a tóxicos ambientais foi relacionada com alteração em processos como ovulação e implantação, assim como redução da fertilidade por alteração do equilíbrio endócrino (Caserta et al., 2011, Hart et al., 2016). Ainda, a exposição a compostos químicos também pode ocasionar alterações patofisiológicas no sistema reprodutivo feminino, como síndrome de ovário policístico (SOP) e endometriose, indiretamente levando à redução da capacidade reprodutiva (Kahn et al., 2020). Dentro desse contexto, os DEs compreendem um grupo de compostos químicos que vem

ganhando destaque na literatura devido sua capacidade de ocasionar efeitos deletérios na capacidade reprodutiva e consequentemente nos tratamentos de infertilidade.

2.2 DESREGULADORES ENDÓCRINOS

Desreguladores endócrinos (DEs) são reconhecidos como um problema global de saúde pública, apresentando potencial tóxico ambiental para humanos e animais (Gore et al., 2016). Um composto desregulador endócrino pode ser descrito como uma substância exógena que interfere na ação de hormônios, atuando na síntese, secreção, transporte, ligação a receptores e/ou eliminação de hormônios fisiológicos, consequentemente podendo ocasionar desequilíbrio na homeostase, reprodução e desenvolvimento do organismo exposto (Kavlock et al., 1996, Tang et al., 2020). Atualmente é estimado que existam aproximadamente 1000 substâncias químicas que apresentam propriedades de DEs. Dessa forma, a exposição a esses compostos é largamente distribuída e constante, abrangendo pesticidas, químicos industriais, plastificantes, medicamentos, produtos cosméticos e fitoestrogênios – podendo ser encontrados comercialmente em diversos produtos de uso comum, incluindo embalagens de plástico, enlatados, detergentes, brinquedos, cosméticos e alimentos em geral (Tabela 1 - Yilmaz et al., 2020).

Tabela 1. Desreguladores endócrinos e usos na indústria

Categoria de uso	**Exemplos de EDCs**
Pesticidas	DDT, atrazina, glifosfato,
Produtos infantis	chumbo, ftalatos, cadmio
Embalagens de alimentos	BPA, Ftalatos, fenol
Eletrônicos e material de construção	retardantes de chama bromados, PCBs
Produtos de cuidado pessoal	Ftalatos
Produtos antibacterianos	Triclosan
Têxteis	Perfluorados

Tabela 1:– adaptada de Andrea et al., 2014.

Diversas alterações no sistema reprodutor feminino têm sido observadas como resultado da exposição a desreguladores endócrinos, incluindo maior incidência de síndrome de ovário policístico, endometriose (Coiplet et al., 2022, Jala et al., 2022,) e alterações em ciclo menstrual (Lu et al., 2000).

O aumento no uso de técnicas de reprodução assistida permitiu a avaliação de novas evidências do impacto de fatores ambientais no processo reprodutivo permitindo, por exemplo, a avaliação de repercussões sobre parâmetros iniciais de desenvolvimento, como a qualidade embrionária (Kadhel et al., 2012). Ainda, possibilitou acesso a qualidade oocitária e avaliação de efeitos em líquido folicular, possibilitando identificar mais precisamente em qual momento do processo reprodutivo (ovulação, fertilização ou implantação) tóxicos ambientais possivelmente podem ocasionar efeitos deletérios. Dentro desse contexto, estudos na literatura também indicam o potencial de desreguladores endócrinos apresentarem efeitos deletérios em desfechos reprodutivos de tratamentos de reprodução assistida. Em estudo de Petro e colegas (2012), amostras de líquido folicular de mulheres em tratamento de reprodução assistida foram analisadas com o objetivo de quantificar a presença de diversos compostos considerados DEs como, bisfenol A (BPA), pesticidas organoclorados, poluentes orgânicos persistentes, entre outros. No trabalho, maiores concentrações de DEs no micro-ambiente do líquido folicular foram associadas a menores taxas de fertilização e menores chances de desenvolvimento embrionário (Petro et al., 2012). Em estudos similares também foi observado que maiores concentrações urinárias de BPA em pacientes de reprodução assistida estão associadas com menor contagem de folículos antrais – indicativo de falência ovariana, também sendo observadas associações com menores taxas de implantação, gestação clínica e nascimentos (Chavarro et al., 2016, Souter et al., 2013). Entretanto, dentre os estudos experimentais e epidemiológicos que avaliam efeitos de DEs na capacidade reprodutiva feminina um grupo de compostos chama atenção pelo seu alto grau de exposição. Os ftalatos são compostos de alto volume de produção, sendo praticamente onipresentes na população e com isso vem ganhando papel de destaque na literatura. Estudos epidemiológicos também têm demonstrado que exposição a ftalatos está negativamente associada a desfechos reprodutivos femininos. Maiores concentrações urinárias de metabólitos de ftalatos foram associadas com menores taxas de fertilização, baixa qualidade embrionária e maior tempo para gestação em mulheres em tratamento de reprodução assistida (Burdorf et al., 2011, Machtinger et al., 2018). A ampla aplicação de ftalatos em produtos de consumo e características específicas desses compostos propiciam o alto grau de exposição da população e levantam preocupações em relação a saúde humana e animal. Dessa forma, associado ao grande aumento nas taxas de infertilidade é necessária maior informação sobre os efeitos de ftalatos no sistema reprodutor feminino.

2.2.1 FTALATOS

Os ftalatos são compostos químicos manufaturados por meio da esterificação de ácido ftálico com diferentes tipos de álcool, tendo ampla variedade de aplicação (Blount et al., 2020). Esses compostos são utilizados principalmente como plastificantes, sendo adicionados a misturas de polímeros com o objetivo de conferir maleabilidade aos plásticos, principalmente ao policloreto de vinila (PVC), apresentando também presença significativa em produtos de uso pessoal como fixadores de aroma. Os ftalatos têm se ramificado em diferentes setores, incluindo a indústria de alimentos, cosméticos e farmacêutica, podendo ser usados como aditivos e fixadores em outros produtos, como cosméticos, tintas e componentes de medicamentos (Huang et al., 2017, Martino-Andrade et al., 2009). Dessa forma, a presença de ftalatos pode ser encontrada em diversos produtos de consumo como roupas, embalagem de alimentos, brinquedos, cosméticos, equipamentos médicos, entre outros (Benjamin et al., 2017). Esses compostos podem ser divididos em 2 grupos em função do seu peso molecular: (A) Ftalatos de alto peso molecular ou ftalatos de cadeia longa (HMWP), que são ftalatos utilizados principalmente na produção de plásticos e inclui Di-(2-etil-hexil) ftalato (DEHP), di-iso-nonil ftalato (DiNP), di-iso-decil ftalato (DiDP), di-n-Octil ftalato (DnOP), di(2-propilheptil) ftalato (DPhP), e (B) Ftalatos de baixo peso molecular ou ftalatos de cadeia curta (LMWP), que incluem dimethil ftalato (DMP), diethil ftalato (DEP), benzilbutil ftalato (BBzP), di-n-butil ftalato (DnBP) and di-iso-butil ftalato (DiBP), sendo esses compostos frequentemente utilizados na composição de produtos de uso pessoal, cosméticos, solventes e adesivos (Mesquita et al., 2021, Yen et al., 2011, Petroviˇcová et al., 2014). Recentemente, dados do nosso laboratório demonstraram, em contraste com outras populações mundiais, a exposição ubíqua de gestantes brasileiras ao di-iso-pentil ftalato (DiPeP), um ftalato de baixo peso molecular que apresenta potente atividade antiandrogênica (Bertoncello Souza et al., 2018). A Figura 2 ilustra a estrutura química de alguns dos ftalatos mais utilizados, assim como de seus metabólitos primários (TEDX, 2019).

Os plastificantes ftálicos são compostos semi-volatéis e apresentam a característica de não serem quimicamente ligados às cadeias de polímeros, característica que facilita os processos de lixiviação, migração e vaporização desses compostos (Benjamin et al., 2017, Lucattini et al., 2018). Dessa forma, os ftalatos são facilmente

liberados no meio ambiente resultando em exposição crônica na população (Polanska et al., 2014; Sakhi et al., 2014).

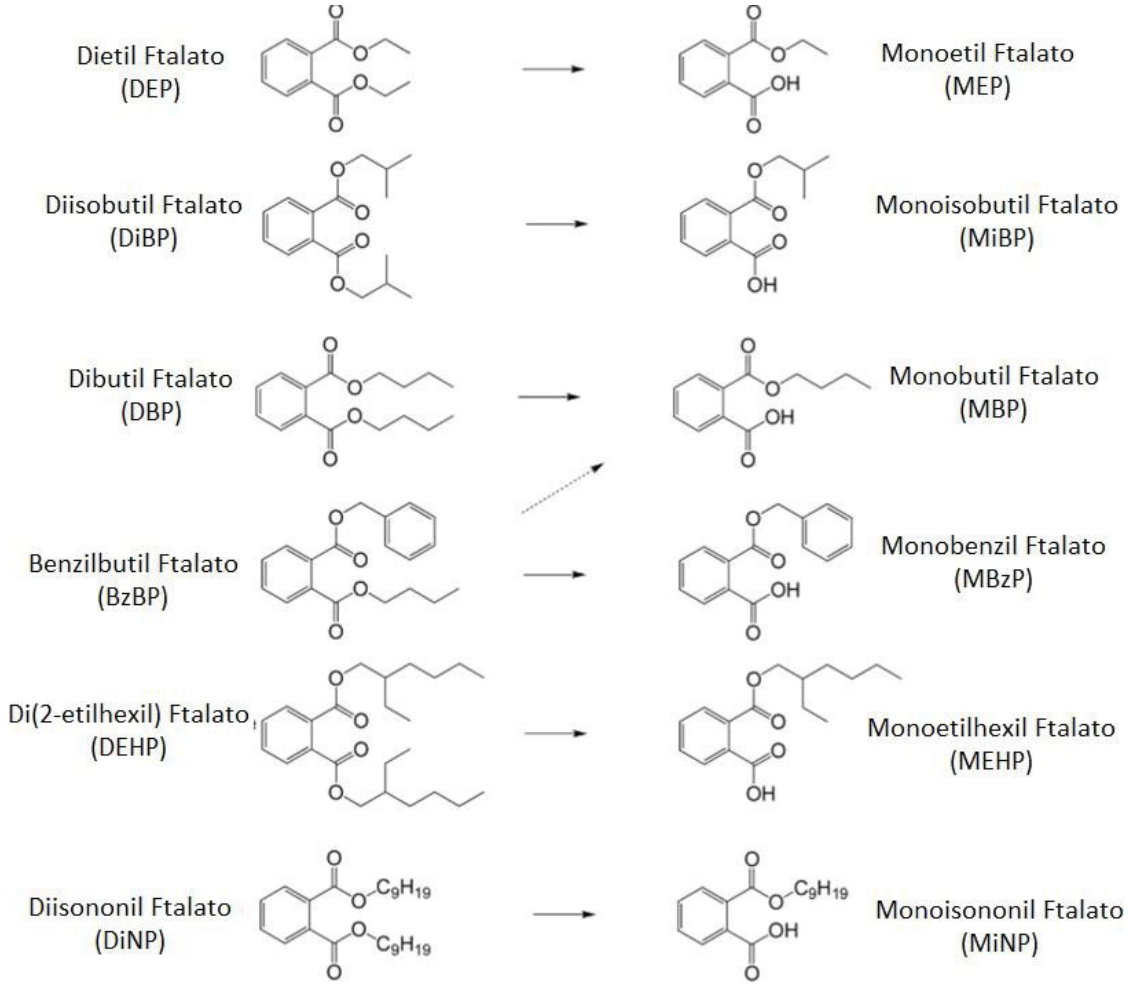

Figura 2: Ftalatos mais comuns, com peso molecular crescente e demonstração dos seus metabólitos primários (Adaptado, Warner et al., 2021).

Estudos mostram que a presença de ftalatos no ambiente é constante, com esses compostos sendo encontrados em amostras de solo, água potável, amostras de ar e poeira (Przybyli ´nska et al., 2016). Consequentemente, humanos e animais podem ser expostos por diferentes vias – alimentar, inalação, através de contato dérmico, entre outros (Yen et al., 2011, Petrovi˘cová et al., 2014). Em humanos, a exposição a ftalatos ocorre principalmente pela via oral, por ingestão de alimentos e água que estiveram em contato com plastificantes, como embalagens plásticas, plástico filme e embalagens de microondas. Entretanto, outras rotas de exposição como inalação e contato dérmico também contribuem para os níveis de contaminação da população atual (Martino-

Andrade et al., 2010). Ainda, a exposição, especialmente em mulheres, também pode ocorrer por meio do uso de produtos de cuidados pessoais, produtos de limpeza, cosméticos e brinquedos infantis (CDC, 2017). O maior uso de cosméticos e produtos de cuidados pessoais por mulheres é um dos possíveis motivos pelos quais estudos epidemiológicos e de biomonitoramento demonstram que mulheres apresentam um perfil de maior exposição a ftalatos, sendo, dessa forma, necessária uma maior atenção aos possíveis efeitos desses compostos na saúde feminina (Li et al., 2020).

Entretanto, apesar do grande grau de exposição a ftalatos, esses compostos apresentam tempo de meia vida curta e, uma vez absorvidos, são rapidamente metabolizados. Diesteres de ftalatos são metabolizados até monoesteres por enzimas que exibem atividade de lipase e esterase. Por meio de rápida hidrólise, eles são convertidos em monoesters bioativos e metabólitos secundários. Esses compostos são posteriormente excretados principalmente pela urina e fezes (Wittassek et al., 2008, Hlisnikova et al., 2020). Consequentemente, essas substâncias químicas podem ser encontradas em diversos fluídos corporais, como sangue, leite materno, saliva, urina, entre outros - sendo que a exposição a diferentes tipos de ftalatos é mais bem avaliada por meio da mensuração dos níveis individuais de metabólitos nesse fluídos corporais (biomonitoramento), especialmente na urina (Lessmann et al., 2017, Eales et al., 2022) (Figura 3).

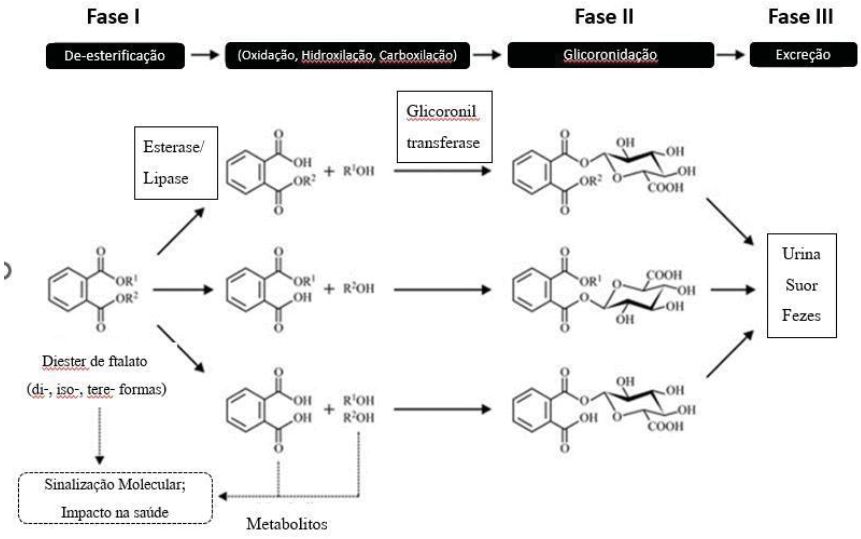

Figura 3. Metabolismo de ftalatos em humanos (Adaptado, Benjamin et al., 2017).

Apesar de serem químicos não persistentes e de rápida metabolização e excreção a exposição a esses compostos é contínua, sendo possível ainda a interação desses com outros agentes químicos levando à sinergia ou efeito aditivo desses xenobióticos

(Repouskou et al., 2019). Dessa forma, diversos estudos avaliam os possíveis efeitos deletérios dos ftalatos, sendo dada grande ênfase para ao potencial tóxico reprodutivo e de desenvolvimento. Ftalatos já foram vastamente documentados na literatura como compostos com atividade anti-androgenica, anti-estrogenica e anti-progestogenica (Lauretta et al., 2019; Morgenstern et al., 2017; Sathyanarayana et al., 2014). Estudos na literatura já demostraram que exposição a ftalatos pode interferir com o desenvolvimento do sistema reprodutor masculino e feminino, assim como influenciar na capacidade reprodutiva tanto em modelo animal como em humanos (Mesquita et al 2021, Eales et al., 2022). Especialmente no sexo masculino, a exposição a ftalatos tem sido associada com uma gama de efeitos endócrinos que engloba diversas anomalias no sistema reprodutivo masculino, incluindo a síndrome de ftalatos – alterações observadas em modelo animal usualmente após exposição a ftalatos durante o período uterino (Gray et al., 2009). O conjunto dessas alterações engloba malformação de genitália externa, criptorquidismo, redução da distância anogenital, alterações histopatológicas no testículo e epidídimo e alterações de capacidade reprodutiva como redução da contagem espermática (Foster et al., 2006, Mesquita et al., 2021). Recentemente, exposição a ftalatos durante a vida adulta também foi associada a efeitos deletérios em parâmetros seminais, sendo demonstrado que a concentração de espermatozoides assim como sua qualidade apresentava relação inversa com os níveis de metabólitos de DEHP na urina dos pacientes avaliados (Mínguez-Alarcón et al., 2018, Caporossi et al., 2020). Esses trabalhos demonstram que ftalatos podem direta ou indiretamente interferir com a fertilidade masculina através de alterações no sistema reprodutivo e na qualidade seminal.

Efeitos similares também são observados com fêmeas em trabalhos experimentais e mulheres em estudos epidemiológicos. Em trabalhos epidemiológicos a exposição a ftalatos já foi relacionada a puberdade precoce, sendo observada uma associação entre níveis urinários de metabólitos de ftalatos em meninas e a presença de marcadores de puberdade precoce (Srilanchakon et al., 2017). Diversos estudos também têm demonstrado o impacto de ftalatos na função ovariana (Hannon & Flaws, 2015), incluindo alterações na foliculogênese e, consequentemente, na qualidade e quantidade da reserva ovariana (Du et al., 2018). Em relação à fertilidade, recentemente estudos demonstram que ftalatos podem desempenhar um papel ou atuar como potenciais agravantes de causas de infertilidade já conhecidas como endometriose e síndrome de ovários policísticos (SOP). A endometriose é definida como a presença de tecido endometrial fora da cavidade uterina, sendo essa condição comumente associada com

infertilidade em mulheres (Khan, 2019). Níveis mais altos de metabólitos urinários de ftalatos, especialmente metabólitos de DEHP, foram observados em mulheres com endometriose quando comparado com pacientes sem a condição (Nazir et al., 2018). De forma similar, em estudo de Pednekar e colegas (2018), amostras de plasma de pacientes inférteis, com diagnóstico de endometriose ou SOP, foram comparadas com amostras de pacientes sem infertilidade. Os autores observaram que as amostras de pacientes inférteis apresentavam concentrações de metabólitos de ftalatos, especificamente ftalato de monobenzilo (MBzP) e mono (2-ethil-5-hidroxihexil) ftalato (MEHHP), maiores do que as de pacientes do grupo controle.

Em relação a pacientes com infertilidade, estudos também já abordaram os efeitos da exposição a ftalatos em casais em tratamento para infertilidade. Casais que passam por tratamentos para infertilidade apresentam maior uso de medicamentos e suplementação, assim como passam por mais tratamentos médicos, podendo apresentar maior exposição a ftalatos (Alur et al., 2015). Em estudo prospectivo realizado em casais subférteis em tratamento foi observado que maior exposição maternal a BPA e ao ftalato DEHP antes da concepção foi associado com parto prematuro (Zhang et al., 2021). Em estudo similar, maiores concentrações urinárias de MEHP em mulheres em tratamento foram associadas com maior risco de gestação bioquímica, falha em gestação clínica e menor taxa de nascimento (Al-Saleh et al., 2019). Os estudos experimentais e epidemiológicos evidenciam resultados que demonstram o potencial efeito deletério que a exposição a ftalatos pode exercer na capacidade reprodutiva feminina, em diversas populações. Esses efeitos refletem-se no potencial de gestação, tempo para gravidez e fertilidade geral dessa população. Tendo em vista que ftalatos são os DEs com maior exposição global é importante que tenhamos conhecimento sobre o potencial desses compostos no sistema reprodutor feminino e em suas funções.

2.3 ANALGÉSICOS COMO DESREGULADORES ENDÓCRINOS

Os recentes dados sobre a exposição a desreguladores endócrinos e os crescentes índices de infertilidade demonstram que diversos fatores biológicos podem apresentar efeito na capacidade reprodutiva da população. Os fatores de estilo de vida e exposição a tóxicos ambientais são sugeridos como contribuintes dos efeitos deletérios sobre a saúde reprodutiva humana e animal. Nesse contexto, a exposição a medicamentos desreguladores endócrinos, como analgésicos, associada a exposição a ftalatos e a outros

DEs, tem sido considerada como um importante fator ambiental relacionado a desfechos reprodutivos negativos.

Estudos na literatura já demonstraram que a exposição a ftalatos pode estar associada a desequilíbrios nas vias de prostaglandinas, sendo essa uma das possíveis vias responsáveis pelos efeitos danosos da exposição a ftalatos no sistema reprodutor feminino (Tran-Guzman e Culty, 2022). O desequilíbrio dos níveis de prostaglandinas já foi identificado na patofisiologia de diversas doenças, como câncer e doenças inflamatórias (FitzGerald 2003). A estrutura singular das prostaglandinas permite que elas apresentem diversos papeis fisiológicos (Seo e Oh, 2017). Prostaglandinas levam a alterações em cascatas celulares que podem ativar ou inibir respostas celulares, dependendo do tipo de prostaglandina e do tipo de cascata celular ativada (Tran-Guzman and Culty, 2022). Especificamente, prostaglandinas estão envolvidos em diversos processos regulatórios no sistema reprodutivo feminino, apresentando papel na cascata de ovulação - atuando na maturação meiótica, na expansão das células do cumulus e no rompimento folicular – na fertilização, otimizando as condições para a penetração do espermatozoide, e no processo de implantação embrionária (Niringiyumukiza et al., 2018). Ainda, a enzima COX, responsável pela síntese de prostaglandinas, é expressa no epitélio uterino durante o processo de implantação, reforçando a influência dessa enzima e das prostaglandinas no processo de implantação e manutenção do blastocisto (Tran-Guzman and Culty, 2022). Dentro desse contexto, já é descrito na literatura que ftalatos podem ocasionar desequilíbrios nas vias de prostaglandinas e, consequentemente, alterar processos reprodutivos (Tetz et al., 2015). Medicamentos analgésicos de venda livre como anti-inflamatórios não esteroidais (AINEs), paracetamol e dipirona, apresentam suas atividades terapêuticas através de inibição específica ou não especifica da enzima COX e alteração das cascatas de prostaglandinas (Vane e Botting, 1998). Em estudo prévio da literatura, Kristensen e colegas (2011), investigaram através de estudo *in vitro* e ensaios organotípicos se a inibição da via de prostaglandinas poderia ser um novo ponto de ação de desreguladores endócrinos. Os autores observaram que desreguladores endócrinos específicos são capazes de inibir a via de prostaglandinas, sendo sugerido que esses compostos podem interferir diretamente na atividade de enzimas COX de maneira similar a analgésicos como o ácido acetilsalicílico (AAS), paracetamol e ibuprofeno. Ademais, foi observado que ftalatos compartilham similaridades estruturais com salicilatos, como o AAS (Tavares and Vine 1985, Kristensen et al., 2011 -Figura 4). Dessa forma, tendo em vista que a inibição de COX e prostaglandinas pode fazer parte do modo de ação de

desreguladores endócrinos, especialmente ftalatos, é possível que fármacos que apresentam mecanismo de ação de inibição de prostaglandinas, como os analgésicos de venda livre também possam atuar como DEs.

Figura 4. Representação estrutural das similaridades entre ftalatos (direita) e salisatos (esquerda) (Adaptado Kristensen et al., 2011).

Medicamentos analgésicos de venda livre são os fármacos mais consumidos mundialmente (Kristensen et al., 2016). O crescimento no consumo desse grupo específico de fármacos pode se dar pela alta taxa de prescrição, assim como pela sua distribuição de venda livre que leva consumidores a não identificarem esses compostos como medicamentos (Kristensen et al., 2011, Hassoun-Barhamji et al., 2015). Analgésicos de venda livre englobam paracetamol, dipirona e alguns AINES – como ipubrofeno e AAS. Esses medicamentos, ingeridos usualmente para alívio de dor, febre e mal-estar, são de fácil acesso e, portanto, amplamente usados pela população. Em estudo de biomonitoramento realizado na Alemanha, foi demonstrada a presença ubíqua de paracetamol na população alemã, sendo observado níveis urinários significativos de paracetamol, inclusive em participantes que não apresentavam consumo recente do medicamento, indicando exposição intencional e não intencional (Modick et al., 2014). O alto consumo desses medicamentos traz preocupações com os possíveis efeitos deletérios que possam ser ocasionados por esses compostos, principalmente levando em consideração que esses fármacos são comumente usados por mulheres em idade reprodutiva e até mesmo durante a gestação e período pre-concepcional (Turunen et al., 2005). Em relatórios prévios já foi indicado que o sistema endócrino e o sistema reprodutor masculino e feminino podem ser vulneráveis a exposição a esses compostos, em especial ao paracetamol (Brune et al., 2015, Roberts et al., 2016).

Trabalhos experimentais e epidemiológicos já demonstram os potenciais efeitos deletérios da exposição a medicamentos analgésicos em períodos específicos do desenvolvimento. Em modelo animal, já foram observadas associações entre o consumo de analgésicos e alterações endócrinas como redução da AGD, redução dos níveis de testosterona e alteração espermática (Kristensen et al., 2011, 2012; Thiele et al., 2013). Já em estudo epidemiológico, foi observado que gestantes que reportaram o uso de paracetamol por mais de 2 semanas durante a gestação apresentaram risco aumentado de terem filho do sexo masculino com criptorquidismo (Kristensen et al., 2011). Ainda, estudos já demonstraram o potencial de AINEs em alterar os mecanismos fisiológicos de rompimento folicular, levando assim a inibição do processo de ovulação, sendo sugerido que uso de AINEs pode apresentar papel importante em disfunções ovulatórias em mulheres (Gaytan et al., 2006). Já em homens, dados epidemiológicos também já demonstraram o potencial de AINEs em alterar os níveis de hormônios esteroides. Halpern e colegas (2020) observaram que homens com uso regular de AINES apresentavam níveis séricos de testosterona 17% menores do que homens sem uso de AINEs. Entretanto, apesar de dados já descreverem que a exposição a medicamentos analgésicos pode influenciar em processos reprodutivos, poucos são os dados que avaliam os efeitos desses compostos na população tentante ou infértil.

2.3.1 MEDICAMENTOS ANALGÉSICOS E INFERTILIDADE

O aumento das taxas de infertilidade na população vem sendo associado na literatura a fatores ambientais, incluindo a exposição a compostos químicos e farmacêuticos (Skakkebaek et al., 2022; Levine et al., 2017). Em acordo com esse aumento, a procura por tratamentos de infertilidade, como técnicas de reprodução assistida, também tem apresentado crescimento nos últimos anos (Skakkebaek et al., 2022). Dessa forma, a preocupação com os efeitos desreguladores endócrinos dos medicamentos analgésicos de venda livre tem ganhado papel de destaque. Medicamentos desse grupo, como ibuprofeno, são altamente eficazes no tratamento de sintomas como dismenorreia, distúrbio regularmente associado com síndrome de ovários policísticos (SOP) e endometriose, condições que são reportadas como as causas mais frequentes de infertilidade feminina e procura por tratamentos de reprodução assistida em mulheres jovens (Esteves et al., 2019). Ainda, alguns estudos epidemiológicos já observaram associação do consumo de analgésicos de venda livre com fecundidade. Smarr e colegas (2016) observaram, em estudo que avaliava casais tentando gestar, uma associação

positiva entre as concentrações urinárias de paracetamol em homens (>73.47 ng/ml) e o tempo para gestação, indicando uma possível influência da exposição ao paracetamol na capacidade reprodutiva.

Diversos medicamentos analgésicos de venda livre apresentam potencial analgésico por inibirem enzimas que sintetizam prostaglandinas (Cashman, 1996). Dessa forma, é possível que esses compostos causem efeito na capacidade reprodutiva feminina uma vez que prostaglandinas são essenciais para o processo de reprodução por atuarem nos processos de ovulação e implantação (Agrawal et al., 2009). A dipirona inclui em seu mecanismo de ação analgésico a inibição da enzima ciclooxigenase (COX) e potencialmente outros mecanismos complementares que poderiam interferir negativamente nos processos de ovulação e implantação (Duffy et al., 2015). Por sua vez, o analgésico de venda livre mais consumido – paracetamol, apresenta ação anti-inflamatória limitada e pouco influencia na síntese de prostaglandinas ou na via da COX em tecidos inflamados (Botting et al., 2000). Entretanto, resultados de estudos experimentais demonstram que paracetamol tem o potencial de atuar como um anti-andrógeno e, portanto, potencialmente inibir a produção de testosterona o que pode ocasionar efeitos danosos no sistema reprodutivo (Ben Maamar et al., 2017; Kristensen et al., 2011, 2012, 2016; van den Driesche et al., 2015). Dessa forma, é possível que analgésicos de venda livre possam potencialmente interferir no equilíbrio do sistema reprodutor feminino e masculino não apenas pelos seus efeitos inibitórios da síntese de prostaglandinas, mas possivelmente também por outros mecanismos de ação. Entretanto, os poucos dados presentes na literatura ainda apresentam conflitos em relação aos efeitos desses compostos na capacidade reprodutiva.

Em um estudo experimental *in utero* foi demonstrado que a exposição de ratas prenhes a doses terapêuticas a paracetamol e ibuprofeno leva a atrasos na entrada e progressão da meiose em células germinativas embrionárias de fêmeas da geração F1. Essa alteração resulta na redução da ativação folicular em ovários pós-natais demonstrando a influência de analgésicos de venda livre na fertilidade (Rossitto et al., 2019). Ainda, o consumo de analgésicos também já foi associado com o metabolismo de estrogênio, sendo observada uma associação inversa entre o consumo frequente de aspirina (AAS) e metabolitos de estrogênio em mulheres na pré-menopausa. Nessa mesma população, também foi observada uma associação positiva entre o consumo de paracetamol e os níveis séricos totais de estrogênio (Fortner et al., 2014, Matyas et al., 2015), demonstrando o potencial desses compostos de influenciar no equilíbrio hormonal

feminino. Ainda, em estudo epidemiológico, o uso de naproxeno, um medicamento da categoria AINE, foi associado com baixa fecundidade em mulheres planejando gestação, sendo também observada uma relação dose-resposta entre o consumo de naproxeno e fecundidade (Mcinerney et al., 2017). Entretanto, o uso de AINES ou paracetamol não demonstrou associação com parâmetros de fertilidade em mulheres tentando conceber em um estudo que avaliou 3 períodos do ciclo menstrual – pré-ovulatório, peri-ovulatório e implantação (Jukic et al., 2020). Ainda, Akande e colegas (2006) descreveram que o consumo diclofenaco (100mg) por pacientes em tratamento para fertilidade no momento da coleta de oocitos, não influenciou as taxas de implantação e gestação.

Dessa forma, apesar do amplo uso regular de analgésicos de venda livre por mulheres em idade reprodutiva, e da plausibilidade biológica desses compostos influenciarem a capacidade reprodutiva, pouco se sabe sobre os efeitos do consumo de analgésicos na fertilidade humana. Tendo em vista os dados divergentes, são necessários estudos adicionais a fim de avaliar o impacto do consumo de AINEs, paracetamol e outros analgésicos de venda livre sobre biomarcadores reprodutivos e a fertilidade da população, principalmente em relação ao potencial desses compostos em influenciar em desfechos reprodutivos de populações subférteis tentando gestar. Em particular, é importante destacar que o padrão de consumo de analgésicos e de exposição a contaminantes químicos ambientais, incluindo ftalatos, pode apresentar grande variabilidade entre diferentes países ou regiões. Nesse sentido, é importante examinar o perfil de exposição de diferentes populações e possíveis desfechos associados a esses diferentes padrões de exposição. No Brasil, são escassos os dados sobre o consumo de analgésicos e avaliações de exposição a químicos ambientais por biomonitoramento em populações vulneráveis, como mulheres em idade reprodutiva e gestantes. Da mesma forma, não há dados sobre a influência desses agentes ambientais sobre desfechos de reprodução assistida.

3-HIPÓTESES

Nossa hipótese de trabalho é de que a exposição a desreguladores endócrinos específicos possa influenciar os desfechos laboratoriais e clínicos de pacientes em reprodução assistida. Dessa forma, nossa proposta foi avaliar as possíveis associações entre a exposição a medicamentos analgésicos de venda livre e ftalatos sobre os desfechos laboratoriais e clínicos de pacientes e tratamento de reprodução assistida. Sendo nossa hipótese verdadeira, esperamos observar que pacientes com maior exposição a

medicamentos analgésicos de venda livre e/ou ftalatos apresentarão piores desfechos reprodutivos em seus ciclos de tratamento.

4-OBJETIVOS

4.1-OBJETIVO GERAL

Revisar dados da literatura sobre o impacto de ftalatos na saúde reprodutiva feminina e avaliar, em um estudo coorte prospectivo, as associações entre a exposição a desreguladores endócrinos, especificamente medicamentos analgésicos, anti-inflamatórios e ftalatos, com desfechos reprodutivos em mulheres submetidas a tratamentos de infertilidade.

4.2-OBJETIVOS ESPECÍFICO

-Avaliar as associações do uso de medicamentos analgésicos e anti-inflamatórios, previamente ao tratamento de infertilidade, e desfechos reprodutivos de mulheres submetidas a procedimentos de reprodução assistida.

-Avaliar as associações do uso de medicamentos analgésicos e anti-inflamatórios, durante o período de tratamento, e desfechos reprodutivos de mulheres submetidas a procedimentos de reprodução assistida

-Avaliar as associações entre a exposição a ftalatos previamente ao tratamento de infertilidade, e desfechos reprodutivos de mulheres submetidas a procedimentos de reprodução assistida.

5- ARTIGOS CIENTÍFICOS

5.1 ARTIGO 1: EXPOSURE TO PHTHALATES AND FEMALE REPRODUCTIVE HEALTH: A LITERATURE REVIEW.

Este trabalho consiste em uma revisão da literatura sobre os efeitos da exposição a ftalatos na saúde reprodutiva feminina. Este artigo foi aceito para publicação no periódico Reproductive toxicology, como requisito para defesa de doutorado.

Exposure to phthalates and female reproductive health: a literature review.

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ABSTRACT

Endocrine-disrupting chemicals (EDCs) are exogenous compounds that have been known for their ability to interfere with the action of hormones and affect endocrine pathways, including the ones involved in the development and function of both male and female reproductive systems. EDCs comprise a wide class of compounds, such as pesticides, bisphenol A, phthalates and, parabens, that are present in the environment and in several daily use products. Phthalate esters, compounds commonly used as plasticizers and additives in many industrial applications, have attracted special attention because of the widespread human exposure and the potential for disruption of androgen-dependent development in males. Although phthalates are rapidly metabolized and excreted, their ubiquitous presence ensures continuous exposures throughout different life stages from conception to adult life, as documented by a number of human biomonitoring studies worldwide. Although most research efforts have been placed on the impact of phthalates on male reproductive development and functions, there is a large body of recent experimental and observational data indicating that phthalates can negatively affect female reproductive health, and in particular alter ovarian and uterine functions, potentially contributing to disorders like polycystic ovarian syndrome, endometriosis, and other common female reproductive problems. This review summarizes the most recent experimental and epidemiologic literature on the potential effects of phthalate exposures on female reproductive health and their impact on female fertility.

Keywords: Endocrine disruptors; Phthalates; Infertility; Female Fertility; Reproductive Toxicology.

1-INTRODUCTION

An escalating amount of scientific evidence has suggested a worldwide trend of reduced human reproductive capacity. Infertility is a significant problem in society, being estimated that the condition affects over 15% of reproductive-aged couples [1]. The increase of infertility rates in the past years is influenced by many factors, and environmental influences upon reproduction are under growing assessment. Several studies have suggested that synthetic and naturally occurring endocrine-disrupting chemicals (EDC) present in the environment and daily products may contribute to impaired fecundity [2, 3, 4]. EDCs are exogenous compounds that are capable to interfere in the action of hormones affecting endocrine pathways, including the ones involved in the development and function of both male and female reproductive systems. Their mechanisms of interference include interaction with hormone receptors, interference with hormone action, and alteration of hormone synthesis, transport, or metabolic processes [5], resulting in altered hormonal activity. EDCs can be divided into two categories according to their environmental persistence and bioaccumulative potential: (A) persistent chemicals that usually present high potential to accumulate in tissues of living organisms and (B) non-persistent compounds that are rapidly metabolized and excreted. There has been growing concern about the endocrine-disrupting effects of non-persistent chemicals such as phthalates and bisphenol A (BPA), since they are used in everyday personal care products and plastics and have been ubiquitously detected in human body fluids [6]. Although they have low potential to bioaccumulate, their ubiquitous presence in the environment ensures continuous exposures throughout different life stages from conception to adult life.

Chronic exposure to phthalates could lead to deleterious effects in the endocrine system and the functioning of multiple organs. Since phthalates can disrupt and impair normal physiological endocrine mechanisms, they can directly or indirectly lead to dysfunctions of several body systems, being associated with human diseases. Experimental and human epidemiological studies have indicated that in addition to reproductive and endocrine organs, phthalates can target other body systems, including the cardiovascular, respiratory, neurological, and immune systems [7, 8, 9, 10]. The immune system is greatly susceptible to endocrine modulation by phthalates and other EDCs, since hormones regulate multiple immunological functions [11, 12]. Epidemiological studies indicate that exposure to certain phthalates is associated with an increased risk of airways, nasal, ocular, and skin allergic diseases, although the associations are usually weak and with inconsistencies regarding the outcomes of different phthalate esters [9, 13, 14, 15, 16]. However, in support of the epidemiological data, animal and cell culture studies indicate that phthalates can

enhance immune and inflammatory responses induced by common allergens [17, 18, 19]. Recent studies also suggest that phthalate exposure may adversely affect the neurological system, especially neuro and cognitive development. In a longitudinal study, children's cognitive function was evaluated at four different time points (2,5, 8 and 11 years) using the Bayley and Wechsler tests scores for assessment of neurocognitive functions and intelligence. The authors observed that children urinary concentrations of di(2-ethylhexyl) phthalate (DEHP) metabolites were associated with decreased cognitive development [7]. The negative impacts of phthalates have also been studied in the cardiovascular system, which can be affected by the ability of phthalates and other EDCs to modulate the actions of estrogenic signaling in this system. Several studies have already related phthalate exposure with cardiovascular health, demonstrating among other outcomes, a relationship with hypertension and atherosclerosis [8,10]. Lastly, possible negative cardiovascular outcomes may be linked to the associations between phthalate exposures and metabolic syndrome. This syndrome involves a cluster of clinical conditions comprising obesity, hypertension, insulin resistance, dyslipidemia, and cardiovascular disease [20] and studies have already shown the association between these clinical conditions and phthalate metabolites [21, 22]. There is particular concern about the link between phthalate exposures and increased risk of childhood insulin resistance and obesity. Trasande and colleagues [23] examined associations between urinary phthalate metabolites and body mass outcomes in a cross-sectional analysis of 2,884 children 6–19 years of age, observing that low molecular weight phthalate metabolites are associated with overweight and obesity.

Overall, a large amount of data has demonstrated that exposure to phthalates can adversely influence the function of multiple processes and body systems. EDCs exposure may cause an imbalance in general health, which in the long-term could also impact on the success of pregnancy [24]. In this context, EDCs have also been associated with direct impact on the reproductive capacity. Some of these compounds have been shown in experimental and clinical studies to negatively affect women's reproductive health, extend the time to pregnancy, increase the chance of developing complications during gestation and enhance the risk of diseases in the offspring later life [25, 26, 27]. Therefore, it is important to understand how non-persistent EDCs can affect the female endocrine and reproductive system. In this context, phthalate esters are of particular concern, considering the numerous biomonitoring and epidemiologic studies showing that women have a special exposure profile to these compounds. In this review, we will briefly summarize the common uses of these non-persistent chemicals and present an overview of the current knowledge concerning the phthalates exposure effects on female reproductive health and their impact on female fertility.

2- PHTHALATES

Phthalates are synthetic chemicals manufactured after the esterification of the phthalic acid with different alcohols and with applications in a wide variety of consumer products. They are used primarily as plasticizers, mainly to soften and increase the flexibility of plastics, most commonly polyvinyl chloride (PVC) products, and as fragrance ingredients used as carriers to allow the scent to endure [28]. The presence of phthalates can be found in several everyday products as clothing, food packing, toys, and cosmetics, but also, in vinyl tiles, detergents, lubricants, medical devices, pharmaceuticals/medications, pesticides, and wood finishes [8, 29, 30]. They are high production volume chemicals with specific chemical characteristics that confer high potential for human exposure. Phthalates are semi-volatile compounds that are not chemically bound to plastic polymers [31], features that enable easy migration and emission of the chemicals to water, air, or other media from production all through to disposal of phthalate-containing products [32]. They can be classified into two groups based on their molecular weight: (A) High molecular weight or long-chain phthalates (HMWP), that are used in the production of plastics and include di(2-ethylhexyl) phthalate (DEHP), di-iso-nonyl phthalate (DiNP), di-iso-decyl phthalate (DiDP), di-n-octyl phthalate (DnOP), di(2-propylheptyl) phthalate (DPhP), and (B) low molecular weight or short-chain phthalates (LMWP), including dimethyl phthalate (DMP), diethyl phthalate (DEP), benzylbutyl phthalate (BBzP), di-n-butyl phthalate (DnBP) and di-iso-butyl phthalate (DiBP), being frequently used in the manufacture of personal care products, cosmetics, solvents, or adhesives [33, 34, 35]. The most used phthalates are BBzP, DnBP, DEHP, and DiNP [36]. In humans, exposure to phthalates occurs mainly by oral ingestion through drinking and eating food that has been in contact with products containing plasticizers, such as plastic containers, plastic film, and microwavable packaging. Furthermore, exposure, especially in women, is also attributed to the use of personal care products, cleaning products, and children's toys [37]. Higher female use of cosmetics is one of the suggested reasons why biomonitoring and epidemiologic studies show that women have a special exposure profile to phthalates, which should also bring more attention to their potential reproductive health hazards [38]. Once ingested, phthalates have a short half-life, undergoing rapid hydrolysis into bioactive monoesters and secondary metabolites which are posteriorly excreted mainly in urine and feces [39]. Thus, the exposure to different types of phthalates is usually and more precisely measured via individual urinary metabolites analyses. Although phthalates are considered non-persistent chemicals that are rapidly metabolized and excreted, there is continuous exposure sustained by daily contact with food, personal care products, beverages, and other media containing traces of different phthalate esters. Because of this continuous exposure they are capable to interact with each other and other chemicals which can lead to synergistic, additive, or antagonistic toxic health effects [40, 41].

Until recently, a greater emphasis was given to the reproductive and developmental toxicity of phthalate esters in males rather than in females. This is possibly related to the ability of certain

phthalates to disrupt male reproductive development by affecting fetal testicular production of hormones such as insulin like factor 3 (insl3) and, in particular, testosterone. *In utero* exposure of laboratory rats to high doses of these active phthalates are associated with a range of reproductive tract abnormalities, characterized by malformations of external genitals, cryptorchidism, reduced anogenital distance, nipple retention, histopathological changes in testis and epididymis, and low sperm counts [42, 43]. These abnormalities comprise the rat phthalate syndrome and are usually observed after in utero exposure to high doses that are not considered human relevant [44]. Yet, several studies performed in animal models, especially rats, demonstrate that the profile of toxicological effects induced by phthalates may vary with the dose levels tested and nonmonotonic dose-response relationships have been reported for several endpoints [45, 46, 47]. Another important point to mention is that animal studies have also demonstrated that mixtures of phthalates and other antiandrogenic chemicals may disrupt male reproductive development in a dose-additive fashion and at doses below the individual No Observed Adverse Effect Levels (NOAELs) of each chemical present in the tested mixtures. More recently, studies have also demonstrated that prenatal exposures to phthalate mixtures or mixtures of multiple EDCs can induce reproductive changes in female offspring of rodents, including alterations in reproductive organs, puberty onset, and infertility up to the second and third generations [48, 49].

Although much of the initial research efforts to understand the reproductive and endocrine disrupting effects of phthalates have focused on males, there is mounting scientific evidence on the impacts of this class of compounds on female reproductive health. In the following sections we will summarize some of the main findings observed in experimental settings as well as in human observational studies showing associations between phthalate exposures and adverse outcomes in women.

3-OVARIAN EFFECTS

The ovary is a crucial component of the female endocrine system, being vital for reproductive health and processes, including folliculogenesis, steroidogenesis, and proper maturation of female gametes [50], but also having an important role on cardiovascular, mood, brain, and skeletal health [51, 52]. The ovarian development and its function are carefully regulated, and any defect or disturbance of these processes can lead to impaired reproductive function and compromised general female health. Phthalates have the potential to target the ovary in several stages of life and disrupt its normal functions [53 ,54] (Figure1). It has already been shown in animal studies that specific phthalates can induce changes in ovarian weights and hormonal levels [55]. Phthalates metabolites have been measured in the amniotic and follicular fluid [56], indicating the potential for disruption of ovarian function from *in utero* development throughout adult life.

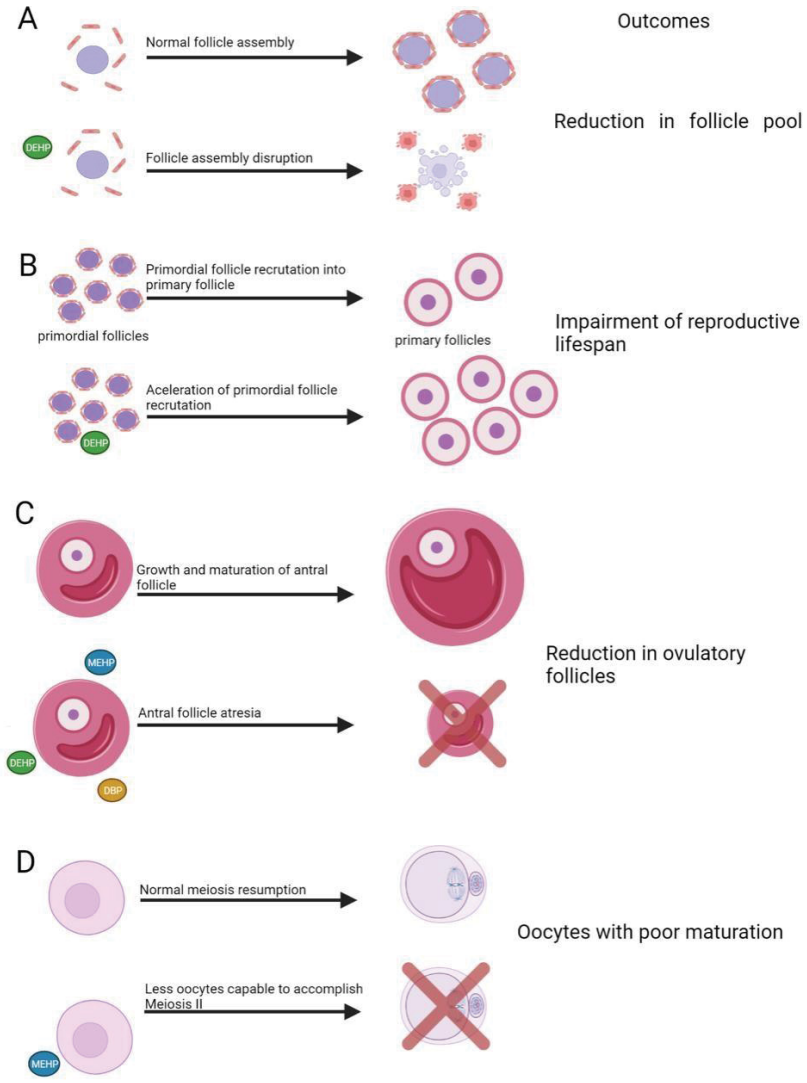

Figure1: Examples of phthalate effects on ovarian development and folliculogenesis - A) Effects of DEHP exposure on follicle assembly; B) Effects of DEHP on primordial follicle recruitment; C) Effects of MEHP, DEHP, and DnBP on oocyte development process; D) Effects of MEHP on oocyte maturation process.

3.1-FOLLICULOGENESIS

The ovarian follicle is the functional unit of the ovary and the process of folliculogenesis allows the maintenance, development, and maturation of the oocyte [50]. The optimum follicular microenvironment is accomplished by the interplay between somatic cells and the oocyte and the action and secretion of several hormones and growth factors. The ovary contains follicles at different stages of development and the effects on reproductive health depend on the type of

follicle/ovarian structure that is impacted. Briefly, toxicity in early follicular stages (primordial and primary) can cause follicular reserve damage and, if exposure is continuous, even infertility. Acute toxicity to preantral and antral follicles can alter steroidogenesis and block ovulation. Lastly, experimental studies show that toxic effects on the *corpus luteum* can also alter steroidogenesis and the ability to maintain early stages of pregnancy [57]. Although few studies investigated the effects of phthalates on folliculogenesis in humans, several experimental pieces of evidence indicate that specific phthalates, especially DEHP and its metabolites, could alter the process of folliculogenesis at all stages of development.

Previous experimental data demonstrated that in rodents, exposure to phthalates could disrupt the development of follicles during primordial [58,59], primary [60], preantral [60,61], antral phases [62,63] and could act at *corpus luteum* [64]. Those experimental studies, usually performed during pre-natal stages, determined that exposure to specific phthalates could disrupt the folliculogenesis process and therefore inhibit oocyte development and decrease their viability. In a study focusing on the effects of diethylhexyl phthalate (DEHP) in primordial follicle assembly, neonatal female mice were injected with DEHP [2.5–10mg/kg body weight/day intraperitoneally for 4 days post-birth. The main outcomes were impaired cyst breakdown associated with the detection of fewer follicles [65]. In pregnant mice treated orally with 40mg DEHP/Kg body weight day from 0.5 to 18.5-day *post coitum*, Li and coworkers [66] reported reduced methylation of CpG sites in two critical imprinting genes of primordial germ cells of the offspring, affecting their oocyte development. This alteration was also observed in oocytes of the F2 offspring suggesting that epigenetic effects from phthalate exposure could be heritable. A similar result was found *in vitro,* demonstrating that DEHP could inhibit primordial follicle assembly. Newborn mouse ovaries cultured with DEHP showed a decrease in primordial follicle numbers and increased apoptosis in oocytes due to decreased mRNA levels and altered DNA methylation of factors associated with oocyte survival and primordial follicle development [67]. In the same context, newborn mouse ovaries exposed *in vitro* to DEHP displayed up-regulation of cyclic nucleotide phosphodiesterase 3A (PDE3A), resulting in decreased cAMP levels that are critical to the maintenance of meiosis arrest. As a consequence, there was a reduction of primordial follicle assembly in the DEHP-exposed ovaries, which may be one of the most important alterations caused by DEHP during early folliculogenesis [68]. Disturbance of folliculogenesis was also found following gestational exposure to DiBP in rats [69] and in adult mice treated with DnBP [70]. Lastly, an *in vitro* study with fetal human ovaries demonstrated that mono-2-ethylhexyl phthalate (MEHP), the primary monoester metabolite of DEHP, could also affect human ovarian development. Fetal human ovaries were exposed to MEHP for 72h and presented dysregulated lipid/cholesterol synthesis [71], however, no oocyte alterations were seen with this exposure. Regarding recruitment of primordial follicles, DEHP exposure could speed up the process. Adult mouse orally exposed to

DEHP demonstrated accelerated primordial follicle recruitment, as demonstrated by a decrease in primordial follicles and an increase in primary follicles [58, 59]. In another similar study, the decrease in primordial follicles was also observed in F1 offspring ovaries of treated mice [59]. A similar result was observed when pregnant female rats were orally exposed to MEHP, with the F1 offspring displaying increased number of preantral and antral follicles and premature reproductive senescence [61]. Since the primordial follicle reserve is non-renewable, the results described in the previous experimental data regarding primordial follicle recruitment can impact reproductive female lifespan.

Reaching the last stages of folliculogenesis, the growth and maturation process also seems to be affected by phthalate exposure as demonstrated by the ability of certain phthalate esters to inhibit antral follicle growth. Experiments with whole antral follicle culture system demonstrated that DnBP exposure can induce cell cycle arrest in follicles following 24 h of culture leading to inhibition of antral follicle growth [62] and consequently increased follicular atresia. DEHP and MEHP exposure also inhibits antral follicle growth *in vitro*, as demonstrated in similar experiments [63, 72]. *In vivo*, a study of our group showed increased number of atretic follicles in adult female offspring of rats exposed *in utero* and during lactation to DEHP [73], although this effect was seen only at the highest maternal dose tested (405 mg/kg/day). In a multi-generation study, maternal exposure to a mixture of plastic-derived chemicals, including DnBP, led to low primordial follicle counts and an increased number of ovarian cysts in the daughters and granddaughters of treated rats [74]. However, *in vitro* studies with an experimental design that considered phthalate exposure comparable to human follicular fluid concentrations, did not observe growth inhibition or follicular death. Only when the cell cultures were exposed to higher concentrations of phthalates inhibition of antral follicle growth and disruptions in cell cycle gene expression were observed [62, 75]. Nevertheless, in study of Meling and colleagues [76] isolated antral follicles of 32-42 days aged female mice were culture with a mixture of phthalate metabolites. The mixture in the study was developed containing the predominant forms of phthalates that reach the human ovary after oral ingestion and in dosage proportions based on molar sums of metabolites found in the urine of pregnant women. After examination, it was observed that the follicles exposed to the phthalates mixture displayed inhibited follicle growth, decreased expression of steroidogenic enzymes, and altered levels of sex steroids relative to control. Collectively, results from experimental studies suggest that either isolated or mixtures of phthalate esters and their metabolites can directly impact follicle health, although the dose levels needed to induce such changes are still a matter of debate. Oocyte maturation can also be affected by phthalate exposure. *In vitro* maturation studies show that denuded rodent and larger mammals (mares and cow) oocytes exposed to MEHP have reduced meiotic resumption linked to increased number of oocytes in the germinal vesicle stage and reduced number of oocytes that progress to

metaphase II [77, 78, 79]. Oral administration of DEHP in mouse markedly reduced the maturation and fertilization of oocytes *in vivo* which was associated with increased reactive oxygen species generation and DNA damage in mouse oocytes [80]. The effects of DnBP in oocyte maturation were also assessed, being observed that DnBP could reduce meiosis competence and mouse oocyte development. The *in vitro* and *in vivo* data reported by Li et al. [81] indicate that DnBP could significantly reduce mice oocyte germinal vesicle breakdown and polar body extrusion rates. Liu and colleagues [82] also investigated the effects of DEHP on meiotic progression. The authors cultured fetal mouse ovarian tissues *in vitro* with DEHP for 6 days (10–100 μM DEHP). The results showed that 10 or 100 μM DEHP exposure inhibited the progression of oocytes throughout meiotic prophase I. Yet, they also found that oocytes exposed to DEHP showed increased DNA damage. On the other hand, pregnant mice treated with DEHP (20 μg/kg body weight/day) + high fat diet from embryonic day 10.5 to litter birth were analyzed for the effect on fetal oogenesis and folliculogenesis. The authors observed that prenatal DEHP exposure alone did not cause an observable delay in meiotic progression of F1 oocytes from DEHP-exposed dams. However, the combination of DEHP exposure with the consumption of high-fat diet (that has been shown to be another potential disruptor of fetal ovarian function) significantly increased synapsis defects in meiosis affecting the F1 generation folliculogenesis [83]. Phthalates appear to inhibit germinal vesicle breakdown and resumption of meiosis in multiple models, and these effects on oocyte maturation may be detrimental to ovulation and normal embryonic development. A recent *in vitro* study reveals a possible effect of phthalate exposure in the proteomic profile of bovine oocytes matured in culture with an environmentally relevant concentration of MEHP. The results show alterations in gene expression of several genes involved in various biological pathways in the exposed oocytes, including 9 genes that had impaired expression in both oocytes exposed to MEHP and blastocysts developed from those oocytes [84]. Lastly, recent data have demonstrated that exposure to phthalates can influence ovulation *in vitro.* In a study that investigated the effects of an environmentally relevant phthalate mixture on ovulation, antral follicles from CD-1 mice were treated with the phthalate mixture (1-500μg/ml) and samples were collected across the ovulatory period. The treated cells presented decreased ovulation rates in a dose-dependent manner, in addition to decreased levels of prostaglandin, progesterone, progesterone receptor (PGR) signaling and dysregulation on extracellular matrix. These data suggest that phthalate exposure could inhibit ovulation by altering oocyte biochemical markers [85].

The limited human data available also indicate associations between phthalate exposures and disruption of folliculogenesis. The relationship between maternal blood concentrations of MEHP and earlier menarche was observed in adolescent girls and associated with a reduction in early follicular phase of serum follicle stimulating hormone (FSH) and serum anti-Mullerian hormone

(AMH) [86]. Low levels of these hormones, which are markers of antral follicles and granulosa cell function, may suggest a diminished ovarian reserve. Among women seeking infertility care, urinary concentrations of DEHP metabolites were associated with significant decreases in antral follicle count, a well-established marker of ovarian reserve in sub fertile populations [87]. Also, in a recent study performed in the same type of population, urinary concentrations of the sum of DEHP metabolites were negatively associated with the number of total oocytes, mature oocytes, fertilized oocytes, and top-quality embryos, suggesting that DEHP may impair early *in vitro* fertilization (IVF) outcomes [88]. The effects of phthalates in women seeking infertility care will be better explored in this review, however, it is important to mention that it is still not clear whether such results are generalizable to women without fertility concerns. Although few epidemiological studies have examined the associations between phthalates exposure and folliculogenesis, extensive experimental data suggested that these chemicals can induce a disruption in this process. Ovarian folliculogenesis is vital for reproduction and overall female health, however, further experimental and epidemiological studies are necessary to understand the mechanisms and extent of phthalate effects and whether such effects could directly cause infertility.

3.2-OVARIAN STEROIDOGENESIS

The production of sex steroid hormones is one of the primary functions of the ovary. Steroidogenesis is conducted by the follicles and corpus luteum in a process that requires both the theca cells and granulosa cells, a significant volume of enzymes, and a coordinated secretion of pituitary hormones, FSH and LH. The ovarian hormones produced in this process include dehydroepiandrosterone (DHEA), progesterone, androstenedione, testosterone, and estradiol. The steroid hormones synthesized in the ovary act on numerous target tissues associated or not with reproductive function. Therefore, proper steroidogenesis is required for fertility as well as female general health [50]. Similar as observed in the folliculogenesis process, exposure to phthalates can also affect ovarian steroidogenesis in multiple life stages usually by decreasing key steroidogenic enzyme levels. Prenatal exposure to phthalates has been shown to alter steroidogenesis in female offspring on experimental studies. Exposure to MEHP during the gestational period altered estrous cyclicity in female mice offspring in association with increased levels of serum FSH and estradiol [61]. A similar experiment performed with maternal oral exposure to DiBP reported increased anogenital distance in the female rat offspring, suggesting a masculinizing effect, but also increased mRNA levels of Cyp19a1 (aromatase) in the ovaries of prepubertal female offspring [89]. However, other developmental rat studies with phthalates failed to detect changes in anogenital distance of the exposed female offspring [90, 91]. In mice, a study of maternal exposure to DEHP reported decreased mRNA levels of key steroidogenic enzymes and hormone receptors in the ovaries of adult offspring, including decreased levels of

Cyp19a1, Cyp17a1, progesterone receptor, FSH receptor, and LH receptor [92]. During adulthood, phthalates can also disrupt ovarian steroidogenesis. Experimental studies demonstrated that oral exposure to DEHP can cause an imbalance in hormone levels that leads to anovulation in adult rats. Liu and colleagues [93] showed that oral exposure of adult female Wistar rats to DEHP at 1000 and 3000 mg/kg/day, decreased serum estradiol levels, which prevented the LH surge needed for ovulation, resulting in anovulation. Diisononyl phthalate (DiNP) is often used as a DEHP replacement, and because of the increased use of DiNP, humans are increasingly exposed to this compound over time. Chiang and colleagues [94] tested whether short-term exposure to DEHP and DiNP could alter mice ovarian follicle populations and levels of circulating hormone. Female CD-1 mice aged 39–40 days were orally dosed with DEHP (20 μg/kg/day–200 mg/kg/day) or DiNP (20 μg/kg/day–200 mg/kg/day) for 10 days and the outcomes were assessed at 3-, 6-, and 9-months post-dosing. Both DEHP and DiNP altered the levels of several different hormones, including testosterone, estradiol, FSH at multiple doses and multiple time points, including increased FSH levels up to 6 months post-dosing in the groups exposed to DEHP at 20 and 200 mg/kg/day and decreased testosterone in 100 μg/kg/day DiNP exposed mice up to 6 months. These results indicate that short-term exposure to either DEHP or DiNP has long-term consequences that persist long after cessation of exposure. This pattern of hormonal and cycle disruption following DEHP exposure is observed in several works with different time and dose levels [58, 64, 93, 95]. These studies are corroborated by multiple *in vitro* culture model systems. Similar to the effects seen in animal studies, exposure of isolated rat ovarian cells to MEHP suppressed estradiol production specifically in granulosa cells [96, 97], possibly by a mechanism related to decreased mRNA levels, protein expression and availability of aromatase enzyme [96, 98]. Also, MEHP exposure decreased progesterone production and FSH-induced cAMP accumulation in rat granulosa cells [99]. However, longer exposure to higher doses increased progesterone and protein levels of steroidogenic acute regulatory protein (STAR) in rat granulosa cells [100]. Studies performed in isolated human ovarian cell cultures also show that phthalates have the potential to disrupt steroidogenesis in humans. Human granulosa-lutein cells isolated from women undergoing *in vitro* fertilization show decreased production of estradiol when cultured with MEHP [101], an alteration attributed to a decrease in the mRNA levels and activity of aromatase. Also, in human luteal cells isolated from *corpora lutea,* exposure to DEHP, DnBP and BBP decreased progesterone production associated with the decreased secretion of prostaglandin E2 and prostaglandin F2α and inhibited luteal cell release of vascular endothelial growth factor. These molecules are regulators of *corpora lutea* survival and their deficiency could result in reduced cell viability altering hormonal balance and pregnancy maintenance. Recent studies have suggested that oxidative stress could be a mechanism of action of phthalate-induced hormonal disruption [102], including for effects on steroidogenesis. Tripathi and colleagues [103] investigated whether DEHP exposure could affect steroidogenesis trough oxidative stress

mechanism. Rat ovarian granulosa cells were exposed to various concentrations of DEHP (0.0, 100, 200, 400 and 800 μM) for 72 h *in vitro*. The results showed that DEHP at 400 μM significantly increased oxidative stress biomarkers while significantly decreasing steroid hormones levels (estradiol and progesterone) and lowering the expression of steroidogenic responsive genes (Cyp11a1, Cyp19A1, Star, ERβ1) in treated granulosa cells. Overall, phthalates appear to directly disrupt steroidogenesis by decreasing steroid hormone and steroidogenic enzyme levels in human ovarian cells as well as in animals and *in vitro* studies, but the specific mechanisms remain to be studied.

Limited epidemiologic evidence explores the associations between phthalate exposure and steroidogenic defects. The Western Australian Pregnancy Cohort Study demonstrated that maternal phthalate metabolites concentrations, specifically monoethyl phthalate (MEP), had a negative association with Anti-mullerian hormone (AMH) in the adolescent daughters, and the sum of DEHP metabolites was associated with a trend for earlier age at menarche in the adolescent female offspring, indicating disruptions of steroidogenesis [104]. Anti-mullerian hormone (AMH) has a inhibitory role in the process of follicular development and recruitment, contributing to follicular arrest, with studies already correlating increased AMH levels in plasma samples with endocrine alterations in patients with PCOS [105]. Maternal phthalate metabolites in urine were also associated with altered steroidogenesis in other studies, including associations between MEHP and testosterone levels in the cord serum of female human infants [106] and monoethyl phthalate (MEP) and monobenzyl phthalate (MBzP) exposure and increased testosterone levels in young female offspring [107]. Some studies also evaluated pos-natal outcomes in diverse periods of development. In 8-year-old girls, urinary levels of DEHP metabolites were positively associated with increased serum progesterone and FSH levels [108]. Similarly, the urinary levels of several phthalate metabolites were associated with decreased total serum testosterone levels in females aged 6–20 and 40–60 years from the National Health and Nutrition Examination Survey [109]. A recent human study examined the interaction of phthalates exposure and diet or exercise habits with hormonal secretion in a Chinese population. The authors observed that high urinary mono(2-ethyl-5-oxohexyl) phthalate MEOHP concentrations, a secondary DEHP metabolite, in specific association with exercise frequency, were related to abnormal serum progesterone, FSH, and LH levels, indicating different susceptibility to phthalates exposure following individual lifestyle [110]. Also, in pregnant women, urinary levels of DEHP metabolites and mono-n-butyl phthalate (MnBP, the main DnBP metabolite) were associated with decreased testosterone levels in maternal plasma [111]. Regulation of ovarian steroidogenesis is vital for female health and although experimental studies indicate that phthalates can dysregulate steroidogenesis, the doses used fall far from the range estimated of human exposure levels. On the other hand, some epidemiological data indicate plausible associations between exposure to certain phthalates and

hormonal changes in women. Future work should elucidate the mechanisms by which phthalates disrupt ovarian steroidogenesis and investigate whether such changes can lead to infertility and non-reproductive adverse outcomes.

3.3- POLYCYSTIC OVARIAN SYNDROME (PCOS)

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders among reproductive-age women. With a diverse diagnostic criterion, its prevalence can be estimated between 5% to 21%, depending on the symptoms considered and the population assessed [112]. Despite the high prevalence rates, it is estimated that 70% of women with PCOS remain undiagnosed [113]. There is a great diversity of symptoms that women may experience with PCOS, comprising hormonal and metabolic alterations that may result in reproductive disruption. The clinical presentation of PCOS includes oligomenorrhea, chronic anovulation, hyperandrogenism, presence of polycystic ovarian morphology, metabolic features – obesity, dyslipidemia, diabetes, and finally subfertility and/or infertility [114]. However, of all the symptoms listed the key feature is PCOS-associated hyperandrogenism, which is a key characteristic for clinical phenotypes and fertility dysregulation [115]. The etiology of PCOS is multifactorial, suggesting clinical importance of both genetic and environmental factors, including lifestyle and exposure to EDCs [115]. In this context, substantial evidence points to EDCs as having a potential role in the initiation of PCOS in some cases [116, 117, 118]. Both animal and human studies already described evidence that supraphysiological maternal androgen levels could lead to hormonal alterations in female offspring that are an indicative of PCOS phenotype, leading to the hypothesis of a fetal origin for the development of PCOS [118, 119]. It has been demonstrated that testosterone has an important impact on fetal tissue differentiation and elevated prenatal testosterone levels lead to disruption in reproductive and metabolic systems that mimic PCOS [119, 120]. In relation to EDCs, most of the available studies emphasize possible associations between PCOS and Bisphenol A exposures, but experimental results also suggest that phthalates could play a role in the etiopathogenesis of PCOS, particularly DEHP and MEHP [6]. In the prenatal context, exposure of gestating animals to phthalates could lead to PCOS-like characteristics in the female offspring. In rats gestationally exposed to DnBP and DEHP it was found that all the females among the first-generation (F1) and third generation (F3) offspring had a significantly higher incidence of polycystic ovaries, morphologically characterized by increased ovarian cysts [74]. Also, exposure to DEHP in adult rats induced traits like the ones found in PCOS, such as prolonged estrous cycles, decreased ovulation or anovulatory cycles, changes in hormonal balance, and the development of polycystic ovaries [60]. However, dose levels in these studies are much higher than the average daily human exposure, indicating that the extrapolation of these results to humans should be done with caution.

Few studies investigated the associations between exposure to phthalates and PCOS in humans, and the results in the literature are conflicting. Some studies even suggest a protective effect of phthalate exposure in PCOS, given their antiandrogenic effect that could lower testosterone levels and reduce the hyperandrogenemia, one of the main features of the disorder [121, 122]. Hart and colleagues [86] found that higher maternal DEP exposure, measured through serum levels of monoethyl phthalate (MEP), was associated with a reduced prevalence of PCOS in the exposed daughters. However, this study also found that maternal levels of MEP were associated to reduced antral follicle count and reduced serum AMH in the adolescent offspring, which are considered markers of ovarian reserve and granulosa cell function, respectively. In a case control-study, lower urine concentrations of MnBP and monobenzyl phthalate (mBzP) were detected in women with PCOS, but the small sample size is considered an important limitation [104]. In a study performed in young (12-18 years) girls with PCOS diagnosis, no relationship between DEHP exposure and PCOS was observed [122]. On the other hand, in a study performed in women attending infertility clinics higher levels of DEHP were found in the follicular fluid of women with PCOS when compared with control. Also, the pregnancy rate in patients with higher levels of DEHP was significantly lower [123]. In a recent study, Akin and colleagues [124] did not find any differences in the concentrations of serum DEHP and MEHP between adolescents with or without PCOS, nonetheless, they observed significant associations between DEHP/MEHP levels and insulin resistance and serum triglycerides in PCOS adolescents, independently of their body mass indexes (BMIs). These results show that despite not being specifically linked with PCOS, phthalates could be associated with the metabolic disturbances of the syndrome. Also, taken into consideration the diverse alterations that involve and could lead to PCOS other alterations should be taken into considerations when evaluating the risk of PCOS. It is important to mention that cross-sectional and case-control study designs cannot clearly identify causal relationships due to the difficulty in determining the temporal sequence of exposure and disease, especially in conditions that are multifactorial and possibly involve pre and postnatal environmental factors, such as PCOS. These conflicting results could be explained by differences in study designs, including route, timing and dose levels of exposure. Yet, animal studies show that DEHP exposure could lead to dysfunction of the hypothalamus-pituitary-ovarian axis and altered pulsatile LH secretion [64, 93]. Considering that increased levels of LH and altered periodicity of its secretion are a trademark of PCOS alterations it is possible that those alterations could also lead to a PCOS [118]. Additional studies are necessary to clarify the divergences from previous studies and to understand the mechanisms involved in the alterations caused by phthalate exposure and how those disruptions could establish a PCOS or PCOs-like status in females.

Data obtained from several *in vitro* and experimental studies as well as a few clinical and epidemiological studies suggest that phthalates stand as a potential hazard to the ovary, being able

to disrupt its balance and function. The ovary is a complex tissue responsible for remarkable functions as folliculogenesis, steroidogenesis, oocyte maturation and ovulation. Exposure to phthalates and particularly, as seem in this review, to DEHP or its main metabolite MEHP can impair all these processes. Regarding folliculogenesis, DEHP impairs germ cell cyst breakdown and follicle assembly, and accelerates primordial follicle recruitment, while MEHP negatively affect oocyte maturation. Phthalates also are capable to alter steroidogenesis and most of the deleterious effects are related to exposure to DEHP and MEHP, which act mostly by disrupting hormonal levels through effects on mRNA, protein levels, and activity of steroidogenic enzymes. However, further work must be done to elucidate the mechanisms by which phthalates disrupt folliculogenesis and steroidogenesis. Regarding PCOS, the majority of studies have focused on the effects of prenatal phthalate exposures in the development of PCOS [118]. Yet, some recent studies suggest that phthalates, including DEHP, might have a role in the development and aggravation of PCOS through effects on insulin resistance, obesity and androgenization [116, 124, 125]. Therefore, further investigations are necessary to clarify mechanisms of action and better determined the effects of phthalates on the development of PCOS, but the large number of experimental results demonstrate sufficient evidence to consider phthalate exposures, and in particular DEHP exposure, as potential risks for ovarian health and consequently to fertility.

4- UTERINE/ ENDOMETRIAL DISORDERS

The uterus is a key organ in mammalian development, being an important component for reproduction and embryogenesis processes. Uterine cells respond to estrogen and progesterone, which regulate endometrial cell proliferation and differentiation [126, 127]. Estrogen promotes endometrial cell proliferation and growth and increases vascular permeability. Progesterone reduces estrogen levels and promotes cell differentiation and angiogenesis [128]. The harmonization of steroid hormones with endometrial cell function is essential for successful implantation and reproduction [126]. Some phthalates are capable to disrupt estrogen signaling *in vitro*, increasing estrogen levels and possibly leading to uterine disorders [129]. Higher levels of estrogen could induce cystic endometrial hyperplasia, squamous metaplasia, adenomyosis, and myometrial and general uterine hypoplasia [130]. Mice overexpressing uterine estrogen receptors present increased number of apoptotic cells in the endometrial epithelium and decreased number of implantation sites [131]. It has been already suggested in animal models that exposure to DEHP can alter the number of endometrial glands and disrupt their structure in rats and mice [132, 133]. In this section, we will discuss the possible relation of phthalates exposure with uterine leiomyoma and endometriosis (Figure 2), two uterine disorders with high prevalence in women and significant influence on reproductive capacity.

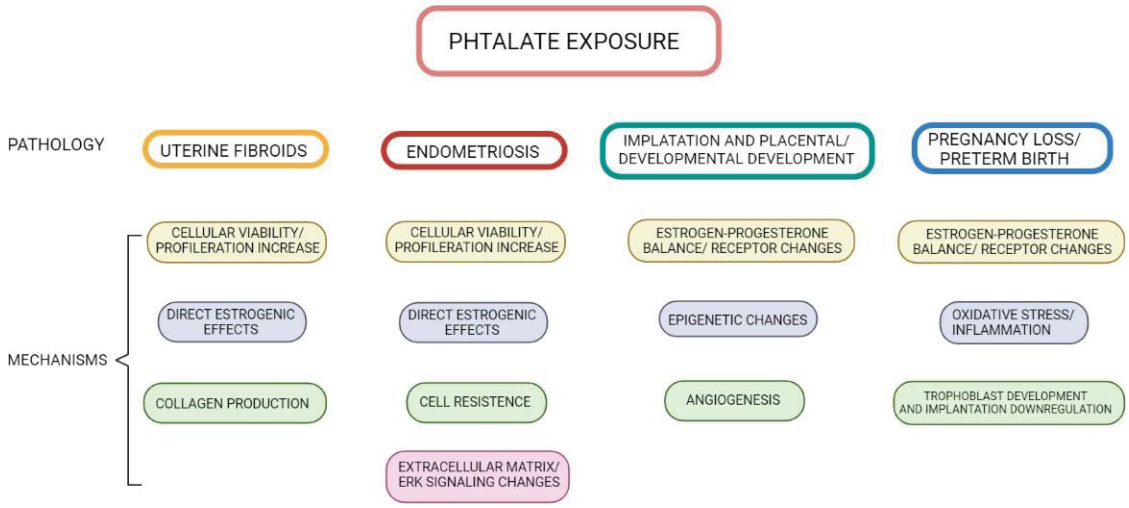

Figure 2. Potential uterine, placental, and gestational adverse outcomes related to phthalate exposures and some proposed mechanisms.

4.1 UTERINE LEIOMYOMA

Uterine leiomyoma or fibroids is a common disorder among reproductive-age women, with a clinically relevant prevalence of 20-50% depending on the population and method of diagnosis [134]. The disorder is defined as a benign tumor composed of smooth muscle cells, growing from the underlying myometrial tissue in the uterus [135]. Most patients show no symptoms; however, some face a devastating disorder and experience symptoms of menorrhagia, pelvic pressure or pain, dysuria, reproductive dysfunction (infertility or subfertility) [136], or even more drastic outcomes, being the leading indication for hysterectomy [137].

The pathophysiology of uterine fibroids is yet unknown, but it is related to impaired action of endogenous sex steroid hormones, estrogen and progesterone [138], as abnormal myometrial or fibroid cells exhibit an exaggerated response to these hormones [139]. However, other factors have been suggested to be responsible for initiating or promoting this disease, including regulatory changes of hormonal receptors, as well as demographic, physiological, and lifestyle risk factors [136]. In the context of environmental risk factors, epidemiological studies have reported that several chemicals are associated with the presence of uterine fibroids, including phthalates [140, 141, 142, 143]. In a case-control study, patients with uterine leiomyoma had significantly higher levels of several phthalates in comparison with controls, and logistic regression analyses demonstrated that leiomyomata were positively associated with individual and the sum of DEHP and DnBP metabolites [143]. In a similar case-control study by Kim and colleagues [142], urinary levels of 16 phthalate metabolites were measured and the sum of DEHP metabolites was associated with increased odds of leiomyoma. Results from *in vitro* and human studies indicate that phthalate exposure could influence the pathogenesis and pathophysiology of uterine leiomyoma. *In vitro*, treatment of human myometrial cells with DEHP increase cellular

viability, proliferation, and antiapoptotic protein expression in normal myometrial cells as well as in leiomyoma cells [144], alterations that have been reported to be a critical component of uterine leiomyoma pathogenesis [145]. The treatment with DEHP also increased type I collagen expression in both cells, an important result since type I collagen levels are increased in human uterine leiomyoma. Also, in the same study, urinary concentrations of mono(2-ethyl-5-carboxypentyl) phthalate (MECPP), a secondary DEHP metabolite, were higher in women with surgically confirmed uterine leiomyoma compared with controls. A more recent study recruited women seeking surgical care for their fibroids and examined the relationship between concentrations of 14 biomarkers of phthalate exposure measured in spot urine samples and the diameter of the largest fibroid and uterine dimensions of those patients [146]. The authors found that concentrations of several phthalate biomarkers, including the secondary DEHP metabolites mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and mono-2-carboxymethyl hexyl phthalate (MECPP), were positively associated with uterine volume but not with the size of the largest fibroid. However, there are divergent studies in the literature. The National Health and Nutrition Examination Survey (NHANES, 1999–2004) reported that MEHP is inversely associated with uterine leiomyoma, while MnBP levels are positively associated with self-reported uterine leiomyoma [147]. Also, results from the SELF study (Study of the Environment Lifestyle and Fibroids) indicated that in black women aged 23–35 years MEHP urinary concentrations show weak-to-moderate positive associations with uterine fibroids, being considered by the authors not appreciably associated with a higher risk of development of the disorder [148]. A meta-analysis addressing this matter, analyzed the results of 5 case-control studies and found no significant association between total urinary phthalate metabolites and risk of uterine leiomyoma. However, total urinary DEHP metabolites were significantly associated with the risk of uterine leiomyoma, which suggested that DEHP exposure might play a more important role than other phthalates in uterine leiomyoma [140]. A recent case-control study agrees with previous studies that suggest associations of phthalates with uterine fibroids. In this study, urinary concentrations of metabolites of DEHP and the non-phthalate plasticizer di-2-ethylhexyl terephthalate (DEHTP) were significantly associated with uterine fibroids in reproductive aged women. The same result was observed for organophosphate esters and alternative plasticizers [149]. In a recent study, data from 1204 women aged 20-54 years old in the National Health and Nutrition Examination Survey (NHANES 2001-2006) were evaluated to better understand the impact of EDCs in uterine leiomyomata and endometriosis [150]. Exposure data of ten commonly EDCs, including urinary phthalates, were examined in relationship to self-reported uterine leiomyomata and endometriosis. The authors found that MEHP was negatively associated with uterine leiomyomata and endometriosis by combining the results of two statistical models, but also observed a positive association between the chemical mixture and endometriosis, with mono-isobutyl phthalate (MiBP) and MBzP having the highest

weight in the model [150] Yet, taking into consideration that phthalates can interact with epigenetic modifications a study was conducted to examine the associations between phthalate exposures and miRNA expression levels in fibroid tumors of pre-menopausal women undergoing surgery for fibroid treatment. It was observed that biomarkers of certain phthalates were associated with miRNA expression in fibroid tumors [151], especially downregulation of the miR-29 family that has been already described as an alteration that contributes to extracellular collagen matrix formation in fibroid cells [152]. A recent review examining the link between EDCs and uterine fibroids described that, based on *in vitro* and *in vivo* data, DEHP can impact biological pathways critical to fibroid pathogenesis and that both human and experimental studies indicate that epigenetic processes may play an important role in fibroid pathogenesis linked to EDCs [153]. It is already clear that DEHP and its metabolites bind to estrogen receptors (ERs) and induce ER expression and therefore could adversely affect uterine histology [129]. However, whether phthalate exposure, or specifically DEHP, can induce utero leiomyoma and in this way contribute to decreased female fertility remains inconclusive.

4.2 ENDOMETRIOSIS

Endometriosis is one of the main causes of infertility in women, responsible for 30%-50% of all observed cases of difficulty or inability to conceive [154]. This gynecologic disease is estrogen-dependent and commonly defined as the presence of endometrial tissue outside the endometrial cavity [155, 156]. The disorder has multifactorial causes, but emerging evidence has suggested that environmental chemicals could be associated with its pathophysiology [157, 158] and that the disease may have a developmental or *in utero* origin [159]. Being an estrogen-dependent disease, some studies have investigated the possible association between phthalate exposure and endometriosis. Several *in vitro* and experimental studies suggest that these chemicals could play a role in endometriosis physiopathology. *In vitro* studies show that treatment of endometrial stromal cells with phthalates, specifically DEHP and MEHP, increased their viability and resistance under stressful conditions [63, 160]. These studies suggested that phthalate exposure could promote and facilitate the establishment of endometriosis, through an increase of refluxed endometrial cells viability under the stress of the immune cell response outside the uterus. Also, treatment of endometrial cells with DEHP led to biological changes, specifically increased matrix metalloproteinases (MMPs), MMP-2 and MMP -9 activities, an increase in Erk phosphorylation, and increased Pak4 expression, alterations that can result in increased cell invasion and may trigger the establishment of endometriosis [161]. The activation of these biochemical markers provides resistance to apoptosis and promotes cell survival [162], being alterations already described in patients with advanced-stage endometriosis [163]. These findings were confirmed in animal studies, where endometrial tissue of DEHP-treated mice presented an increase of the same biological markers, such as MMP-2, MMP-9, and Pak4 [161]. Furthermore, there are also

morphological findings showing that ovariectomized mice implanted with fragments of human endometrial tissue presented endometriotic lesions of higher volume after oral treatment with DEHP in comparison to the control group [161]. These studies indicate that exposure to DEHP promotes cellular proliferation and invasiveness facilitating the environment for endometriosis.

In humans, many case-control studies evaluated the plasma concentrations of phthalate metabolites in relation to the occurrence of endometriosis [164, 165], and in particular associations with DEHP exposures. More recently, studies started to investigate the relation of endometriosis with concentrations of urinary phthalate metabolites, which are more sensitive biomarkers for exposure to phthalate diesters. A study on the association between phthalate metabolites in urine and endometriosis was conducted by Buck Louis and colleagues [158], revealing a two-fold increase in the odds of an endometriosis diagnosis for certain phthalate metabolites, including MEHP, MEHHP, and MEOHP. Also, a prospective cohort study revealed increased urinary levels of MEHHP and MEOHP, secondary DEHP metabolites, in women with advanced endometriosis compared with controls [161]. Recently, Chou and colleagues [166] showed associations between MnBP urinary levels and the presence of endometriosis in female patients. Also, human granulosa cells treated with MnBP exhibited altered expression of genes that play a pivotal role in the pathophysiology of this disorder, such as IL-1β, TNF-α, and inhibin-B. However, an earlier study did not found associations of MnBP or other phthalate metabolites, such as MBzP, MEHP, MEHHP and MEOHP, with endometriosis when comparing women with confirmed diagnostic and controls [167]. Similarly, in a case-control study no significant differences were seen in the urinary levels of several phthalate metabolites between controls and endometriosis patients with confirmed histological diagnosis [168]. In a meta-analysis performed by Cai and colleagues [169], the associations between five different phthalate metabolites (MEHHP, MEHP, MEP, MBzP and MEOHP) and endometriosis were examined. Data from 8 studies were analyzed and no associations were found, except for a positive association with MEHHP levels. However, the authors suggest that well-designed cohort studies, with large sample sizes, should be conducted and analyzed for better assessments of the relationship between phthalate exposures and endometriosis. A similar meta-analysis examined the relationship between endometriosis and exposures to four classes of EDCs (BPA, polychlorinated biphenyls, organochlorine pesticides, and phthalates). All classes, with the exception of BPA, were significantly associated with endometriosis [170]. A recent systematic review and meta-analysis by Conforti [171] has also summarized the current literature regarding the link between phthalate exposures and occurrence of endometriosis. The authors analyzed the data of 14 studies considering more than 20 phthalates and phthalate metabolites and observed associations between endometriosis and increased urinary levels of MnBP, MEOHP, and MEHHP and a link between blood-derived analysis of BBP, DEHP, DnBP, and MEHP with endometriosis. However, the

authors highlight the methodological weaknesses of the available data as major limitations and stand out the necessity of more rigorous studies [171]. It is suggested that the inconsistent results could be caused by differences in study designs, reflecting variation in exposure, sample size and population analyzed. Although several *in vitro*, animal and human studies strongly support the hypothesis that phthalates can play a crucial role in the pathogenesis of endometriosis, more studies are necessary to understand the mechanism of influence of these EDCs in the endometriosis disorder.

Uterine disorders can significantly impact women's reproductive health and consequently their fertility. Although studies showed a possible association between exposure to some phthalates and uterine conditions, as endometrioses and uterine leiomyomata, we still do not have sufficient data to fully understand the impacts of phthalates on those disorders and to comprehend their possible mechanism of action.

5-GESTATION

Pregnancy is a vulnerable period for a woman and her offspring. Pregnancy complications occur in approximately 19% of pregnancies, including metabolic alterations, gestational hypertension, preeclampsia, eclampsia, preterm birth, and placenta disorders [172]. During the gestational period, a change in reproductive hormone levels is required to maintain and develop a healthy pregnancy. Human chorionic gonadotropin or hCG is produced by the differentiated syncytio-trophoblasts of the conceptus and rises rapidly after implantation [173] and a significant increase in steroid hormone receptor expression occurs in the placenta [174]. The vulnerability of the pregnancy state and the high demand for hormones makes gestation a stage of life especially sensitive to EDCs exposure. Abnormal levels of hormones during this period can influence implantation, placental development, and general maintenance of human pregnancy, being well-accepted that abnormal levels of progesterone and estrogen are an important cause of pregnancy loss and preterm birth [175]. In the context of the influence of EDCs during pregnancy, phthalates have the potential to influence the normal activity of hormones during pregnancy. In naturally conceiving pregnant women, the early pregnancy urinary concentration of monoisobutyl phthalate (MiBP), MBzP, and metabolites of DEHP were positively associated with maternal estrogen plasma levels measured during the same period. Also, monocarboxyisononyl phthalate (MCNP), a metabolite of di-isodecyl phthalate (DiDP), and metabolites of DEHP were inversely associated with maternal testosterone levels [176]. Yet, several studies show that EDCs can reach the placenta through maternal blood (178, 27). Placentation process is highly susceptible to endocrine disruption and exposure to phthalates can lead to disturbances in the placental function. Studies have already described that EDCs can act on fetoplacental vasculature, indirectly increasing blood pressure and oxidative stress [178]. Therefore, phthalate exposure could have

the potential to affect not only the length and processes of pregnancy, but also increase the risk of developing pregnancy complications and predisposing the fetus to adverse health risks later in life (Figure 3) (27).

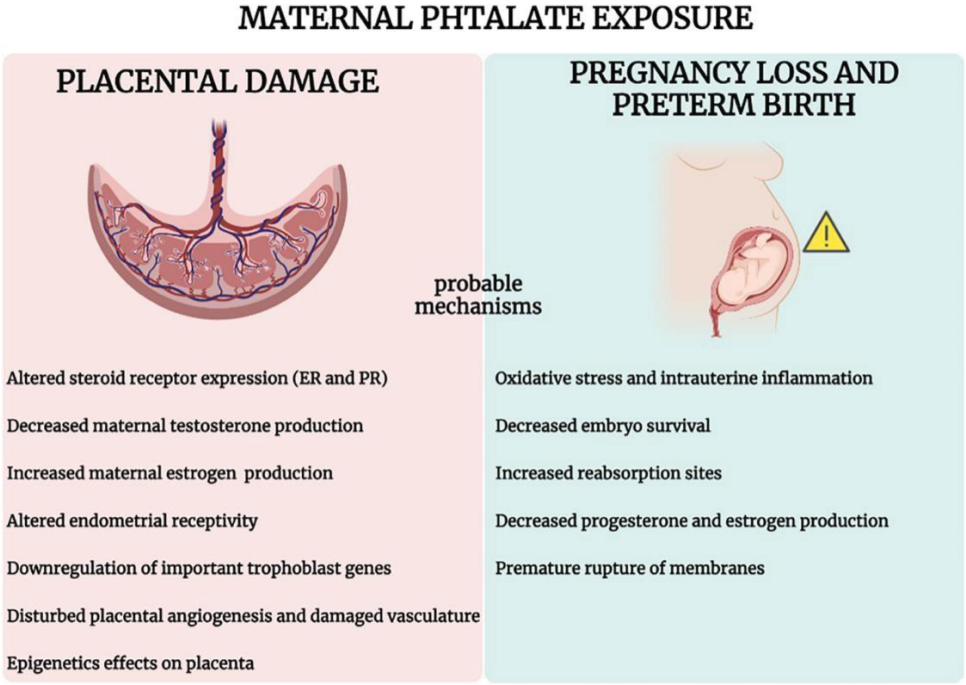

Figure 3. Potential adverse gestational outcomes related to phthalate exposures and some proposed mechanisms.

5.1 -IMPLANTATION AND PLACENTAL DEVELOPMENT

Embryo implantation is a process that involves the accurate early development of an embryo associated with a receptive state of the uterus. This process is an important part of reproduction and any disruption in its development could lead to pregnancy loss [179]. The receptive status of the uterus or as defined, the implantation window, is maintained for a short period and consists of morphological and endocrine changes that capacitate the endometrium to receive the embryo [180]. The endometrial receptivity, like most processes in female reproduction, is regulated by estrogen and progesterone and, therefore, susceptible to the effects of EDCs exposure. In animal studies, phthalates, specifically DEHP, are capable to alter mouse cycling causing endocrine disorders [58, 96]. The process of implantation is still not completely understood, and few studies have examined how chemicals affect this process or explore specific compounds as phthalates. A study assessing the effects of DEHP exposure on endometrial receptivity and embryo implantation in pregnant mice observed that DEHP exposure had significant effects on this process [181]. Daily oral exposure to high doses of DEHP (1000 mg/kg/day), between gestation days 1 and 6, reduced the number of implantation sites in mouse endometrium compared to controls. Also, exposure to DEHP upregulated the expression levels of receptors to estrogen and progesterone impairing endometrial receptivity and embryo implantation. Zhang and colleagues

[182] treated pregnant mice daily with DEHP and examined the uterine and placental tissue to investigate the effect of DEHP exposure on the growth and development of the placenta. They observed that the group with higher DEHP exposure (500 mg/kg/day) showed a significantly reduced number of embryo implantation, suggesting that blastocyst implantation could be impaired. Also, exposure to DEHP reduced in a dose-dependent manner the placental weight and area of spongiotrophoblasts, demonstrating that DEHP exposure significantly affects the growth and development of the placenta. The effects of DEHP exposure were also observed on the transcriptome of trophoblast cells isolated from trophectoderm of blastocyst from Macaca mulatta, one of the closest human placental models. DEHP treatment downregulated the expression of genes related to trophoblast development, implantation, and immunomodulation leading to a significant decrease in cell growth and proliferation, cell invasion, and endothelial development [183]. However, these results have not been yet demonstrated in humans. In a study recently performed with women undergoing assisted reproduction, no association was observed between the implantation process or embryo development and DEHP exposure [185]. A recent systematic review summarizes all experimental studies carried out on mice models that assessed the EDCs effects on the implantation process. A total of 34 studies were included. A primary effect was the reduction of the number of implantation sites and pregnancy rates especially associated with BPA and phthalate exposure. The effects of phthalates on the implantation process was described especially for MBP, DBP and DEHP – but only 3 studies were included in the analyses. Overall, the authors suggest that EDCs could deeply affect blastocyst implantation, at least in mice models [185].

Placental development is a primordial process for future fetal development, and disorders in this process can have serious effects on fetal and maternal health. Alterations in the placentation process could result in preeclampsia, miscarriage, intrauterine fetal growth re- striction, and preterm births, among others [186]. Placental development begins when the blastocyst outer layer, trophectoderm, adheres in the endometrium, a process also dependent of hormonal regulation and therefore susceptible to EDCs interference, including phthalates [187]. Placentation is highly sensitive to EDCs as these substances can alter primary human cytotrophoblast differentiation, impairing the proper invasion of the maternal decidua and, consequently, disrupting the spiral artery remodeling and placental function [27]. In this recent review, Lorigo and Cairrao [27] summarize the literature regarding maternal exposure to EDCs and adverse maternal health and fetoplacental vascular function outcomes. The authors suggest that considering the current literature, BPA and phthalates are the most damaging EDCs to fetoplacental vasculature during pregnancy, which is reinforced by the vast literature associating these compounds to preeclampsia, preterm birth, gestational diabetes mellitus and other gestacional complications [27].

Studies have already demonstrated, in animal and *in vitro* models, that DEHP is capable to alter angiogenesis during placental development. Pregnant mice exposed daily to DEHP by oral gavage presented significantly lowered placental weight and total area of the placenta in dose-dependent manner (125, 250, and 500 mg/kg) when compared with the control group [182]. Also, DEHP treatment downregulates the expression of angiogenic factors and impairs endothelial development [183]. In humans, Ferguson and colleagues [188] observed that pregnant women with higher urinary concentrations of DEHP metabolites present decreased serum levels of placental growth factor, an important angiogenic placental biomarker. Cellular proliferation is an important factor of the placental growth process that can also be affected by phthalate exposure. In *in vitro* experiments, mouse and human trophoblast cells exposed to DEHP and MEHP show decreased cellular proliferation associated with downregulation of the progesterone receptor, but it is yet to be determined whether this alteration could impact placental size in humans [189]. In this context exposure to DEHP was already associated with changes in placental weight in American and European populations [190, 191]. In both studies the sum of DEHP metabolites in urinary samples has been inversely associated with placental weight, suggesting placental insufficiency. Maternal DEHP exposure by gavage at 125, 250, and 500 mg/kg/day during pregnancy, lead to reduced placental size and disruption of placental structure in an animal model study performed in mice [192]. However, the underlying mechanisms are unclear. Zhang and colleagues [193] hypothesized that DEHP disturbs the endocrine function of placenta by inhibit the proliferation of placental cells. Therefore, they explored the effects of oral DEHP treatment at doses of 50 mg/kg and 200 mg/kg on estradiol and progesterone secretion in pregnant mice, but also *in vitro* using human placenta trophoblast JEG-3 cells exposed to MEHP for 7 days at concentrations of 20, 200 and 500 μM. They reported that DEHP and its active metabolite MEHP altered placenta endocrine function through increased progesterone levels in pregnant mice and in JEG-3 cells, respectively, and in a dose and concentration dependent manner. Also, they observed that DEHP and MEHP can restrain the proliferation of placental cells.

Recently, studies started to explore the placental epigenetics effects of phthalates. Epigenetic mechanisms, especially DNA methylation are crucial during the early stages of placental development [194] and recent studies, performed in animal models and humans, have suggested that exposure to DEHP could impair DNA methylation possibly leading to disruption of placental development. In placenta of pregnant mice orally exposed to DEHP for five days, an alteration in the expression of imprinted genes was described. The study reported a low but significant relaxation of imprinted expression of genes involved with late fetal and/or neonatal lethality in mice [195]. In humans, Zhao and colleagues [196], examined associations between prenatal phthalate exposure and global DNA methylation in human placenta samples. The authors observed that higher maternal urinary concentrations of the secondary DEHP metabolite MEHHP

and the sum of DEHP metabolites were negatively associated with placental DNA LINE-1 sequences methylation, a marker for global methylation in studies of environmental exposures. Yet, the sum of DEHP metabolites in urine of women in early pregnancy stages was associated with decrease in placental tissue DNA methylation of the non-coding gene H19 [197]. H19 plays a major role in embryonic and placental growth, however, the alteration did not have a significant impact on birth length or birth weight. A recent review summarizing the effects of BPA and phthalates on placental outcomes in animal and cell models suggests that these chemicals can impact placental hormones, epigenetic endpoints, increase inflammation and oxidative stress, and decrease cell viability and nutrient transfer. However, few animal or cell studies have assessed these outcomes at concentrations relevant to humans. Yet, regarding phthalates, most of the studies evaluated the effects of MEHP with few data on other phthalate metabolites [198]. In another recent review paper by Warner and colleagues [199], the impact of phthalate exposures on placental morphophysiology is discussed in light of available *in vitro*, experimental, and humans studies. The authors suggest that although the biological mechanisms are still poorly understood, exposure to phthalates is associated with morphological alterations in the placenta and changes in gene expression and epigenetic mechanisms, which can potentially impair the placental health and fetal development [199].

Other phthalates have also been associated with epigenetic alterations of placental development, including exposures to BBP, DiBP, and DnBP, which displayed associations with altered DNA methylation and expression levels of epigenetic regulators or long noncoding RNAs [88,200]. Both studies analyzed epigenetic alterations in human placental tissue associated with concentrations of maternal urinary phthalate metabolites. However, the number of samples in both studies is small and more studies would be necessary to understand the effects of specific phthalate exposures on placental epigenetic development. Nevertheless, there is mounting evidence from *in vitro*, *in vivo*, and human epidemiological studies showing that exposure to phthalates, and in particular DEHP, can disrupt the early process of implantation and placentation.

5.2 – PREGNANCY LOSS

Pregnancy loss is the most common adverse pregnancy outcome in humans, accountable for approximately 35% of the adverse results [201]. The adverse ending of gestation is responsible for psychological traumas for pregnant women and even physical and economic damage. A pregnancy loss can occur in several stages being classified as (A) sub-clinical, early, or biochemical pregnancy loss when the loss occurs before the pregnancy is clinically recognized (no gestational sac is observed at 6 weeks ultrasonography control). (B) clinical pregnancy loss is defined as a miscarriage of a clinically recognized pregnancy (gestational weeks >6) that involuntarily ends before 28 complete weeks. Clinical pregnancy loss can also be categorized as

embryonic loss (gestational weeks 6 to 10) and fetal loss (gestational weeks 11 to 27) [202]. A few environmental factors that are capable of increase the risk of pregnancy loss have already been established, including lifestyle factors such as alcohol consumption [203], physical strain [204], and certain environmental contaminants, including EDCs. The adverse effects of chemicals on endocrine functioning could change the circulating levels of hormones responsible for maintaining pregnancy, being already well established in the literature that phthalates can alter steroidal hormone levels. Also, exposure to phthalates has been already associated with oxidative stress and inflammation. Ferguson and colleagues [205] analyzed urine samples of pregnant women to assess exposure to nine phthalates and measured the markers of oxidative stress 8-hydroxydeoxyguanosine and 8-isoprostane. They observed an association of MnBP, MiBP and DEHP metabolites with increased levels of the oxidative stress biomarkers. In a similar study, bilirubin, a potent antioxidant, was inversely associated with several phthalates metabolites including metabolites of DEHP and DnBP, in particular MBzP and MCPP. The same metabolites were also associated with biomarkers of inflammation, including alkaline phosphatase, absolute neutrophil count and ferritin [206]. Those studies indicate an association between phthalate exposure and oxidative stress and inflammation, alterations that are suggested as mediators of pregnancy loss and failure [207]. Therefore, there is biological support for the hypothesis that phthalates are associated with pregnancy loss. In animal studies, exposure to specific phthalates has significantly decreased embryo survival, increased the incidence of resorptions, reduced the number and size of litters, and increased the abortion rate [208,209], being directly associated with preimplantation and post-implantation losses [210]. In humans, numerous data have demonstrated an increased risk of pregnancy loss with exposure to phthalates. A study performed with a cohort of couples planning their first pregnancy observed increased pregnancy loss among women with higher urinary concentrations of MEHP [211]. This prospective cohort study collected urinary samples during pre-conception and conception time of 128 Danish women with naturally conceived pregnancies. Their data show a statistically significant association between elevated pre-conceptional MEHP exposure and pregnancy loss, also observing that early pregnancy loss was more frequent at higher MEHP concentration levels. However, they did not find any significant association between pregnancy loss and metabolites concentration in the conception sample. In contrast, the North Carolina Early Pregnancy Study reported somewhat different results [212]. They examined urinary concentrations of phthalate metabolites in a cohort of women attempting to become pregnant naturally and observed that higher concentrations of MEOHP and the sum of DEHP metabolites were associated with reduced early pregnancy loss. However, the authors suggest that exposure to DEHP could lead to undetected early pregnancy loss, occurring before the detection of hCG in maternal urine, resulting, therefore, in a decrease in the frequency of observed pregnancy loss with higher DEHP exposure.

Regarding analyses of pos-conceptional time of exposure, Mu and colleagues [213] investigated post-conceptional phthalate exposures in women who underwent clinical pregnancy loss in comparison to healthy pregnant women. They observed that urinary concentrations of the metabolites MEP, MiBP, and MEHP were significantly higher in women with pregnancy loss than those in the control group, being these metabolites also significantly associated with clinical pregnancy loss with a clear dose-response relationship. The study also observed increased clinical pregnancy loss at the highest quartiles of several other measured phthalate metabolites, but these associations were not significant. In a similar study, Yi and collegues [214], analyzed the urinary metabolites concentrations of patients having missed miscarriage (fetal death without expulsion before 20 weeks of gestation). They observed that levels of DEHP metabolites (MECPP, MEHHP, MEOHP, and MEHP) and the sum of all four DEHP metabolites were significantly higher in missed miscarriage cases than in normal pregnancies. Also, exposures to DEHP and dimethyl phthalate were associated with an increased risk of missed miscarriage. Interestingly the authors also found that levels of serum progesterone and estradiol were significantly reduced in the missed miscarriage cases, suggesting that the association of phthalate exposure with pregnancy loss could be mediated by hormonal alterations. Peng et al. [215] demonstrated that urinary levels of MEHP and MiBP were higher among women with unexplained recurrent miscarriage, however, the small sample size is an important limitation. Contrary to these results, Liao et al. [216] found that MEP, MiBP, and the sum of DEHP metabolites were not associated with pregnancy loss, although the results were borderline significant. However, they did observe that the sum of DnBP metabolites was significantly associated with the risk of recurrent pregnancy loss. A large sample size study recruited pregnant women at early stages of pregnancy (gestational weeks 5 to 14) and observed that embryonic loss was more sensitive to phthalate exposure [217]. In this study, urinary concentrations of MEP, MnBP, MEOHP, and MEHHP during the early stages of pregnancy were significantly associated with an increased risk of clinical pregnancy loss during the 6-10 weeks of gestation, suggesting that exposure to phthalates in the early stages of pregnancy is an independent risk factor for early clinical pregnancy loss. A recent case-control study performed in a Taiwan population assessed the estimated daily intake of phthalates, based on the levels of urinary metabolites, in relation to recurrent pregnancy loss of unknown etiology. They observed that patients with recurrent pregnancy loss had a significantly higher cumulative exposure to phthalates than controls, being the risk strongly linked to phthalate intake, in particular to the higher quartiles of DEHP exposure [218].

In women undergoing medically assisted reproduction, the Environment and Reproductive Health Study (EARTH) reported an association between cycle-specific urinary concentrations of phthalates metabolites and pregnancy loss [219]. The study showed that concentrations of individual and sum of DEHP metabolites were associated with biochemical pregnancy loss, with

more strong associations for the secondary metabolites MEHHP and MEOHP. Also, the study reported elevated odds of total loss (early and clinical loss combined) with higher exposure to DEHP. The study suggests involvement of phthalate exposure in the early stages of implantation, decidualization, placentation, or embryogenesis. Such associations might be the result of direct actions of phthalates on these processes, but also consequence of altered hormonal balance and therefore, affecting the support to pregnancy. Finally, in a recent meta-analysis, which included most of the studies described in this session, MBP, MEHP, MEHHP, and MEOHP were associated with increased risk of spontaneous pregnancy loss [220]. Overall, the current data suggest that phthalate exposure might be a possible risk factor for spontaneous pregnancy loss, but additional large-scale, well-designed, population-based studies are still needed to clarify the relationship between phthalate exposure and spontaneous pregnancy loss.

5.3– PRETERM BIRTH

Preterm birth is defined as an infant who is born alive before 37 completed weeks of pregnancy [221]. Worldwide, it is estimated that each year 15 million babies are born preterm, with rates increasing yearly in several countries [222]. Prematurity is a leading cause of neonatal mortality and is associated in several epidemiological studies with long-term adverse health outcomes [223, 224, 225]. Most preterm births result from spontaneous preterm labor or preterm premature rupture of the membranes (pPROM) and they are likely the consequences of intrauterine inflammation [226]. Previous studies suggest that phthalate exposure could lead to an inflammatory state [227], suggesting a possible involvement in preterm births. Considering that preterm birth is a condition highly associated with inflammation in the placenta, Alsubaie and colleagues [228] investigated the effects of DBP on the production of biomarkers for placental inflammation. Placental explants from women undergoing elective caesarean were treated with DBP and, to understand how DBP may affect the placental immune response to bacteria, with DBP in the presence of *E.coli*. The concentrations of IL-1β, TNF-α, IL-10, heme-oxygenase-1 (HO-1), and BDNF were quantified. The results showed that DBP enhance the levels of IL-6 in basal cultures and IL-1β and TNF-α in cultures co-stimulated with E. coli., suggesting that DBP could have some influence on placental inflammation. A recent Chinese birth cohort study examined the mRNA levels of several biomarkers of inflammation in 2469 placentas sampled at birth in relation to concentrations of seven phthalate metabolites (MMP, MEP, MBP, MBzP, MEHP, MEHHP e MEOHP) measured in first trimester urine samples [229]. The data showed that maternal phthalate exposure was associated with inflammatory biomarkers in placental tissues. The associations were stronger in placentas of male than of female fetuses. Specifically, Mono-n-butyl phthalate (MBP) was associated with higher IL-1β, IL-6, and CRP expression in placentas of male fetuses and with higher IL-6, CRP, MCP-1, IL-8, IL-10, and CD68 expression in placentas of female fetuses. Mono benzyl phthalate (MBzP) levels were associated with the

increased expression of TNF-α, MCP-1, and CD68 only in placentas of male fetuses. Mono (2-ethyl-5-oxohexyl) phthalate (MEOHP) was negatively associated with CRP, MCP-1, and CD68 in placentas of female fetuses [229].

Taking into consideration that most preterm births are spontaneous [230] and exposure to phthalates is widespread, the population susceptible to the possible effects of phthalates exposure in preterm birth is quite large. Several studies have already investigated this association [220, 231, 232]. Exposure to phthalates was associated with preterm delivery in pregnant Chinese women [141]. Cord blood levels of several phthalates were associated with preterm birth in preterm infants with or without a history of intravenous (IV) therapy, excluding a possible interference of medical equipment use alone. Similarly, in a nested case-control study Ferguson and colleagues [227] demonstrated an association of urinary phthalate metabolite concentrations with increased odds of preterm birth in pregnant women. Maternal urinary levels of MEHP, MECPP and the sum of DEHP metabolites showed a strong and dose-dependent relationship with odds of birth before 37 weeks gestation in the 130 cases of preterm birth. Also, when considering only spontaneous preterm births (spontaneous preterm labor or pPROM) a higher subset of phthalate metabolite levels was associated with significantly elevated odds of prematurity. Later, the same group observed that urinary phthalate metabolites were associated with increases in urinary 8-isoprostane, a biomarker of oxidative stress. Both, phthalate exposure and 8-isoprostane, were associated with increased risk of preterm birth [188,205]. More recently, this group demonstrated that the relationship between phthalate exposure and spontaneous preterm birth is mediated in part by phthalate-induced oxidative stress [233]. Considering that oxidative stress is tightly linked to inflammation, they suggest that it may play a relevant role in spontaneous preterm birth, possibly being the origin of inflammation in the pathway to spontaneous preterm birth. The group also examined the effects of stress in association with phthalate exposure, observing an association between the sum of urinary DEHP metabolites in third trimester and preterm birth that was significant only in women that experience one or more stressful life event during pregnancy [234].

However, there are also conflicting results on the relationship between phthalates and preterm birth. Shoaff and colleagues [235], found that in participants of the Health Outcomes and Measures of the Environment (HOME) study, maternal urinary phthalate metabolite concentrations were not associated with gestational duration. Similarly, in a Canadian population, the Maternal-Infant Research on Environmental Chemicals (MIREC) Study, no clear association between phthalate exposure during early pregnancy and the risk of preterm birth was found [236]. On the other hand, the Generation R Study, a Dutch study that examined data from 1379 women, observed associations between maternal urinary phthalate metabolites concentrations at three-time points in pregnancy and preterm birth and others birth disorders [237]. In another study, Gao

and colleagues [238] observed that the overall risk of preterm birth was elevated with the increase of urinary levels of phthalate metabolites. The observed associations related preterm birth with exposure to specific phthalates during different times windows of pregnancy, suggesting that the time of exposure also influences the association. Searching for a new exposure approach, a case-control study performed in Denmark observed the effects of phthalate exposure through drugs used during gestation [239]. The study includes pregnancies exposed to selected study drugs compared with pregnancies exposed to phthalate containing drugs and pregnancies exposed to phthalate-free generic drugs, and the results observed were that exposure to diethyl phthalate (DEP) in medication, specifically during the third trimester, was associated with an increased risk of preterm birth. Lastly, in couples seeking infertility treatment, results of the Environment and Reproductive Health (EARTH) Study demonstrated that the sum of DEHP urinary metabolite concentrations, measured in mothers before conception, was associated with a higher risk of preterm birth [220]. In a subsequent publication from the EARTH study, maternal DEHP and paternal DEHP exposure before conception were positively associated with preterm birth [240]. Taken together, the results of the EARTH study suggest a relationship between phthalate exposure before conception with preterm birth, also suggesting that preterm birth may be a couple-based pregnancy outcome. A recent systematic review and meta-analysis on the relationship between maternal exposure to EDCs and preterm birth, which included 59 studies from January 1990 to July 2021, reported that both maternal exposure to metals and phthalates were related to an increased risk for preterm birth. Data on phthalates revealed that maternal exposure to MEP, MECPP, MBzP and DEHP were significantly associated with preterm birth, based on nine selected studies [241]. A recent prospective cohort study by Yland et al. [242] examined the associations between maternal urinary concentrations phthalate and phthalate alternative metabolites and the risk of preterm birth. The study was performed with urine samples collected at each trimester from 386 pregnant women that underwent fertility treatment and compared the concentrations of exposure biomarkers in women with term and preterm births. The results demonstrated that maternal urinary concentrations of summed DEHP metabolites were higher among women with preterm than term delivery, especially late in gestation [242]. Despite some divergent results in the literature, the majority of recent studies showed a significant association between phthalate exposure and preterm birth. The mechanisms through which phthalates can increase the risk of preterm birth are still inconclusive, but the induction of inflammation and oxidative stress seems to be a possible pathway. Although we still need more studies to reach definite conclusions on the effects and mechanisms of phthalates on preterm birth and other gestational outcomes, expecting mothers should be advised to reduce the exposure to these chemicals to protect pregnancy and normal fetal development.

Gestation is a delicate window for the action of EDCs and phthalate exposures have been associated with impaired pregnancy maintenance, pregnancy loss, and preterm birth. However, the studies evaluating the relationship between these compounds and adverse pregnancy outcomes still present inconsistent results.

6- CONCLUSIONS

The objective of this review was to analyze the effects of phthalate exposures on the reproductive health of women, comprising ovarian (Figure 1), uterine (Figure 2) and gestational (Figure 3) outcomes. This paper was elaborated trough a review of the most significant works from the last few years of *in vitro*, *in vivo*, and epidemiological studies with focus on female health and fertility. Phthalates represent a serious concern for female reproductive health and an economic burden, due to their high daily prevalence and their capacity to interfere with the endocrine system. The several studies presented in this review indicate that phthalates exposure could possibly be an important environmental etiological factor of uterine and ovarian disorders, such as PCOS and endometriosis. However, further studies are required to better understand the relationship between phthalate exposures and female reproductive health and their impact on fertility, since the results are several times conflicting and study designs not always appropriate to imply causality. The design of the studies is of huge influence on the observed outcomes and, therefore, a more diverse set of studies, exploring how different populations respond to phthalates will be of great value to clarify the reported associations. On the other hand, a large number of animal and cell-based studies lend support to the biological plausibility of several associations between phthalate exposures and adverse health effects in females. However, additional studies are needed to clearly establish the adverse outcome pathways leading to female reproductive disorders in experimental conditions that mimic more closely the human exposure scenarios, including testing of human relevant dose levels, as well as studies with environmentally relevant mixtures of phthalates and other EDCs. Also, there is a rise of interest in understanding the relationship between phthalate exposures, pregnancy-related problems, and epigenetic effects, adding new concerns to the adverse reproductive outcomes linked to exposures to phthalates and other EDCs. In summary, understanding the impacts and the underlying mechanisms of phthalates on reproductive health would possibly improve the development of strategies to reduce the burden of environmental factors on females and their negative consequences on health and fertility.

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The authors declare that they have no known competing interest or personal relationships that could have appeared to influence the work reported in this paper.

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10- REFERENCES

[1] Fritz MA and Speroff S. Clinical gynecologic endocrinology and infertility. Baltimore (MD): Lippincott Wilkins and Williams (2011) (8th ed).

[2] Piazza MJ, Urbanetz AA. Environmental toxins and the impact of other endocrine disrupting chemicals in women's reproductive health. JBRA Assist Reprod. 2019 Apr 30;23(2):154-164. doi: 10.5935/1518-0557.20190016.

[3] Zoeller RT, Brown TR, Doan LL, Gore AC, Skakkebaek NE, Soto AM, Woodruff TJ, Vom Saal FS. Endocrine-disrupting chemicals and public health protection: a statement of principles from The Endocrine Society. Endocrinology. 2012 Sep;153(9):4097-110. doi: 10.1210/en.2012-1422.

[4] Di Renzo GC, Conry JA, Blake J, DeFrancesco MS, DeNicola N, Martin JN Jr, McCue KA, Richmond D, Shah A, Sutton P, Woodruff TJ, van der Poel SZ, Giudice LC. International Federation of Gynecology and Obstetrics opinion on reproductive health impacts of exposure to toxic environmental chemicals. Int J Gynaecol Obstet. 2015 Dec;131(3):219-25. doi: 10.1016/j.ijgo.2015.09.002.

[5] Rogers JA, Metz L, Yong VW. Review: Endocrine disrupting chemicals and immune responses: a focus on bisphenol-A and its potential mechanisms. Mol Immunol. 2013 Apr;53(4):421-30. doi: 10.1016/j.molimm.2012.09.013.

[6] Gore AC, Chappell VA, Fenton SE, Flaws JA, Nadal A, Prins GS, Toppari J, Zoeller RT. EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. Endocr Rev. 2015 Dec;36(6):E1-E150. doi: 10.1210/er.2015-1010.

[7] Huang HB, Chen HY, Su PH, Huang PC, Sun CW, Wang CJ, Chen HY, Hsiung CA, Wang SL. Fetal and Childhood Exposure to Phthalate Diesters and Cognitive Function in Children Up to 12 Years of Age: Taiwanese Maternal and Infant Cohort Study. PLoS One. 2015 Jun 29;10(6):e0131910. doi: 10.1371/journal.pone.0131910. PMID: 26121592; PMCID: PMC4488303.

[8] Benjamin S, Masai E, Kamimura N, Takahashi K, Anderson RC, Faisal PA. Phthalates impact human health: Epidemiological evidences and plausible mechanism of action. J Hazard Mater. 2017 Oct 15;340:360-383. doi: 10.1016/j.jhazmat.2017.06.036. Epub 2017 Jun 19. PMID: 28800814.

[9]Bølling AK, Sripada K, Becher R, Bekö G. Phthalate exposure and allergic diseases: Review of epidemiological and experimental evidence. Environ Int. 2020 Jun;139:105706. doi: 10.1016/j.envint.2020.105706.

[10] Mariana M, Cairrao E. Phthalates Implications in the Cardiovascular System. J Cardiovasc Dev Dis. 2020 Jul 22;7(3):26. doi: 10.3390/jcdd7030026. PMID: 32707888; PMCID: PMC7570088.

[11] Muñoz-Cruz S, Togno-Pierce C, Morales-Montor J. Non-reproductive effects of sex steroids: their immunoregulatory role. Curr Top Med Chem. 2011;11(13):1714-27. doi: 10.2174/156802611796117630. PMID: 21463251.

[12]Palacios-Arreola MI, Morales-Montor J, Cazares-Martinez CJ, Gomez-Arroyo S, Nava-Castro KE. Environmental pollutants: an immunoendocrine perspective on phthalates. Front Biosci (Landmark Ed). 2021 Jan 1;26:401-430. doi: 10.2741/4899. PMID: 33049675.

[13] Hoppin JA, Jaramillo R, London SJ, Bertelsen RJ, Salo PM, Sandler DP, Zeldin DC. Phthalate exposure and allergy in the U.S. population: results from NHANES 2005-2006. Environ Health Perspect.

2013 Oct;121(10):1129-34. doi: 10.1289/ehp.1206211. Epub 2013 Jun 25. PMID: 23799650; PMCID: PMC3801456.

[14] Bekö G, Weschler CJ, Langer S, Callesen M, Toftum J, Clausen G. Children's phthalate intakes and resultant cumulative exposures estimated from urine compared with estimates from dust ingestion, inhalation and dermal absorption in their homes and daycare centers. PLoS One. 2013 Apr 23;8(4):e62442. doi: 10.1371/journal.pone.0062442. PMID: 23626820; PMCID: PMC3633888.

[15] Bertelsen RJ, Carlsen KC, Calafat AM, Hoppin JA, Håland G, Mowinckel P, Carlsen KH, Løvik M. Urinary biomarkers for phthalates associated with asthma in Norwegian children. Environ Health Perspect. 2013 Feb;121(2):251-6. doi: 10.1289/ehp.1205256. Epub 2012 Nov 16. PMID: 23164678; PMCID: PMC3569683.

[16]Preece AS, Knutz M, Lindh CH, Bornehag CG, Shu H. Prenatal phthalate exposure and early childhood wheeze in the SELMA study. J Expo Sci Environ Epidemiol. 2021 Sep 2. doi: 10.1038/s41370-021-00382-w

[17] Imai Y, Kondo A, Iizuka H, Maruyama T, Kurohane K. Effects of phthalate esters on the sensitization phase of contact hypersensitivity induced by fluorescein isothiocyanate. Clin Exp Allergy. 2006 Nov;36(11):1462-8. doi: 10.1111/j.1365-2222.2006.02574.x.

[18] Lourenço AC, Galbiati V, Corti D, Papale A, Martino-Andrade AJ, Corsini E. The plasticizer dibutyl phthalate (DBP) potentiates chemical allergen-induced THP-1 activation. Toxicol In Vitro. 2015 Dec;29(8):2001-8. doi: 10.1016/j.tiv.2015.08.011.

[19] Sekiguchi K, Kurohane K, Tsutsumi M, Mochizuki N, Orii A, Nose M, Imai Y. Enhancement of mouse contact hypersensitivity appears with a short chain triacylglycerol but not with a long chain one. Toxicology. 2019 Jan 15;412:48-54. doi: 10.1016/j.tox.2018.11.014.

[20] McCracken E, Monaghan M, Sreenivasan S. Pathophysiology of the metabolic syndrome. Clin Dermatol. 2018 Jan-Feb;36(1):14-20. doi: 10.1016/j.clindermatol.2017.09.004. Epub 2017 Sep 8. PMID: 29241747.

[21] Milošević N, Milanović M, Sudji J, Bosić Živanović D, Stojanoski S, Vuković B, Milić N, Medić Stojanoska M. Could phthalates exposure contribute to the development of metabolic syndrome and liver disease in humans? Environ Sci Pollut Res Int. 2020 Jan;27(1):772-784. doi: 10.1007/s11356-019-06831-2. Epub 2019 Dec 6. PMID: 31808097.

[22] Zamora AN, Jansen EC, Tamayo-Ortiz M, Goodrich JM, Sánchez BN, Watkins DJ, Tamayo-Orozco JA, Téllez-Rojo MM, Mercado-García A, Baylin A, Meeker JD, Peterson KE. Exposure to Phenols, Phthalates, and Parabens and Development of Metabolic SyndromeAmong Mexican Women in Midlife. Front Public Health. 2021 Feb 26;9:620769. doi: 10.3389/fpubh.2021.620769. PMID: 33718320; PMCID: PMC7952420.

[23] Trasande L, Attina TM, Sathyanarayana S, Spanier AJ, Blustein J. Race/ethnicity-specific associations of urinary phthalates with childhood body mass in a nationally representative sample. Environ Health Perspect. 2013 Apr;121(4):501-6. doi: 10.1289/ehp.1205526. Epub 2013 Feb 4. PMID: 23428635; PMCID: PMC3620751.

[24] Wang Y, Qian H. Phthalates and Their Impacts on Human Health. Healthcare (Basel). 2021 May 18;9(5):603. doi: 10.3390/healthcare9050603. PMID: 34069956; PMCID: PMC8157593.

[25] Thomsen AM, Riis AH, Olsen J, Jönsson BA, Lindh CH, Hjollund NH, Jensen TK, Bonde JP, Toft G. Female exposure to phthalates and time to pregnancy: a first pregnancy planner study. Hum Reprod. 2017 Jan;32(1):232-238. doi: 10.1093/humrep/dew291

[26] Mínguez-Alarcón L, Messerlian C, Bellavia A, Gaskins AJ, Chiu YH, Ford JB, Azevedo AR, Petrozza JC, Calafat AM, Hauser R, Williams PL; Earth Study Team. Urinary concentrations of bisphenol A, parabens and phthalate metabolite mixtures in relation to reproductive success among women undergoing in vitro fertilization. Environ Int. 2019 May;126:355-362. doi: 10.1016/j.envint.2019.02.025.

[27] Lorigo M, Cairrao E. Fetoplacental vasculature as a model to study human cardiovascular endocrine disruption. Mol Aspects Med. 2021 Nov 25:101054. doi: 10.1016/j.mam.2021.101054. Epub ahead of print. PMID: 34839931.

[28] Blount BC, Silva MJ, Caudill SP, Needham LL, Pirkle JL, Sampson EJ, Lucier GW, Jackson RJ, Brock JW. Levels of seven urinary phthalate metabolites in a human reference population. Environ Health Perspect. 2000 Oct;108(10):979-82. doi: 10.1289/ehp.00108979.

[29] Heudorf U, Mersch-Sundermann V, Angerer J. Phthalates: toxicology and exposure. Int J Hyg Environ Health. 2007 Oct;210(5):623-34. doi: 10.1016/j.ijheh.2007.07.011.

[30] Giuliani A, Zuccarini M, Cichelli A, Khan H, Reale M. Critical Review on the Presence of Phthalates in Food and Evidence of Their Biological Impact. Int J Environ Res Public Health. 2020 Aug 5;17(16):5655. doi: 10.3390/ijerph17165655.

[31] Lucattini L, Poma G, Covaci A, de Boer J, Lamoree MH, Leonards PEG. A review of semi-volatile organic compounds (SVOCs) in the indoor environment: occurrence in consumer products, indoor air and dust. Chemosphere. 2018 Jun;201:466-482. doi: 10.1016/j.chemosphere.2018.02.161.

[32] Müller AK, Nielsen ELO. Human Exposure to Selected Phthalates in Denmark by the Danish Veterinary and Food Administration (2003).

[33] Yen TH, Lin-Tan DT, Lin JL. Food safety involving ingestion of foods and beverages prepared with phthalate-plasticizer-containing clouding agents. J Formos Med Assoc. 2011 Nov;110(11):671-84. doi: 10.1016/j.jfma.2011.09.002.

[34] Petrovi˘cová, I, Kolena, B, Pilka, T. The human biomonitoring of occupational exposure to phthalates. Mediterranean Journal Social Science 2014, 5, 101–107.

[35] National Center for Biotechnology Information, 2020. n.d. Bisphenol A. CID¼6623 [WWW Document]. PubChem Database. https://pubchem.ncbi.nlm.nih.gov/compound/Bisphenol-A.

[36] The Endocrine Disruption Exchange, 2019. TEDX list of potential endocrine disruptors. Phthalates [WWW Document]. https://endocrinedisruption.org/ interactive-tools/critical-windows-of-development/about-the-timeline/ phthalates (accessed 2.20.21).

[37] Centers for Disease Control and Prevention, (2017) Phthalates factsheet. (https://www.cdc.gov/biomonitoring/Phthalates_FactSheet.html) (accessed 2.28.21).

[38] Li XN, Li HX, Yang TN, Li XW, Huang YQ, Zhu SY, Li JL. Di-(2-ethylhexyl) phthalate induced developmental abnormalities of the ovary in quail (Coturnix japonica) via disruption of the hypothalamic-pituitary-ovarian axis. Sci Total Environ. 2020 Nov 1;741:140293. doi: 10.1016/j.scitotenv.2020.140293.

[39] Wittassek M, Angerer J. Phthalates: metabolism and exposure. Int J Androl. 2008 Apr;31(2):131-8. doi: 10.1111/j.1365-2605.2007.00837.x.

[40] Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, Zoeller RT, Gore AC. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocr Rev. 2009 Jun;30(4):293-342. doi: 10.1210/er.2009-0002.

[41] Kortenkamp A. Low dose mixture effects of endocrine disrupters and their implications for regulatory thresholds in chemical risk assessment. Curr Opin Pharmacol. 2014 Dec;19:105-11. doi: 10.1016/j.coph.2014.08.006.

[42] Foster PM. Disruption of reproductive development in male rat offspring following in utero exposure to phthalate esters. Int J Androl. 2006 Feb;29(1):140-7; discussion 181-5. doi: 10.1111/j.1365-2605.2005.00563.x

[43] Mesquita I, Lorigo M, Cairrao E. Update about the disrupting-effects of phthalates on the human reproductive system. Mol Reprod Dev. 2021 Oct;88(10):650-672. doi: 10.1002/mrd.23541. Epub 2021 Oct 6. PMID: 34617353.

[44] Gray LE Jr, Barlow NJ, Howdeshell KL, Ostby JS, Furr JR, Gray CL. Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: added value of assessing multiple offspring per litter. Toxicol Sci. 2009 Aug;110(2):411-25. doi: 10.1093/toxsci/kfp109.

[45] Andrade AJ, Grande SW, Talsness CE, Grote K, Golombiewski A, Sterner-Kock A, Chahoud I. A dose-response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): effects on androgenic status, developmental landmarks and testicular histology in male offspring rats. Toxicology. 2006 Aug 1;225(1):64-74. doi: 10.1016/j.tox.2006.05.007.

[46] Andrade AJ, Grande SW, Talsness CE, Grote K, Chahoud I. A dose-response study following in utero and lactational exposure to di-(2-ethylhexyl)-phthalate (DEHP): non-monotonic dose-response and low dose effects on rat brain aromatase activity. Toxicology. 2006 Oct 29;227(3):185-92. doi: 10.1016/j.tox.2006.07.022.

[47] Do RP, Stahlhut RW, Ponzi D, Vom Saal FS, Taylor JA. Non-monotonic dose effects of in utero exposure to di(2-ethylhexyl) phthalate (DEHP) on testicular and serum testosterone and anogenital distance in male mouse fetuses. Reprod Toxicol. 2012 Dec;34(4):614-21. doi: 10.1016/j.reprotox.2012.09.006.

[48] Zhou C, Gao L, Flaws JA. Exposure to an Environmentally Relevant Phthalate Mixture Causes Transgenerational Effects on Female Reproduction in Mice. Endocrinology. 2017 Jun 1;158(6):1739-1754. doi: 10.1210/en.2017-00100.

[49] López-Rodríguez D, Aylwin CF, Delli V, Sevrin E, Campanile M, Martin M, Franssen D, Gérard A, Blacher S, Tirelli E, Noël A, Lomniczi A, Parent AS. Multi- and Transgenerational Outcomes of an Exposure to a Mixture of Endocrine-Disrupting Chemicals (EDCs) on Puberty and Maternal Behavior in the Female Rat. Environ Health Perspect. 2021 Aug;129(8):87003. doi: 10.1289/EHP8795.

[50] Hannon PR, Flaws JA. The effects of phthalates on the ovary. Front Endocrinol (Lausanne). 2015 Feb 2;6:8. doi: 10.3389/fendo.2015.00008.

[51] Hu FB, Grodstein F, Hennekens CH, Colditz GA, Johnson M, Manson JE, Rosner B, Stampfer MJ. Age at natural menopause and risk of cardiovascular disease. Arch Intern Med. 1999 May 24;159(10):1061-6. doi: 10.1001/archinte.159.10.1061.

[52] Woods NF, Mariella A, Mitchell ES. Patterns of depressed mood across the menopausal transition: approaches to studying patterns in longitudinal data. Acta Obstet Gynecol Scand. 2002 Jul;81(7):623-32. doi: 10.1034/j.1600-0412.2002.810708.x.

[53] Bhattacharya P, Keating AF. Impact of environmental exposures on ovarian function and role of xenobiotic metabolism during ovotoxicity. Toxicol Appl Pharmacol. 2012 Jun 15;261(3):227-35. doi: 10.1016/j.taap.2012.04.009.

[54] Craig ZR, Wang W, Flaws JA. Endocrine-disrupting chemicals in ovarian function: effects on steroidogenesis, metabolism and nuclear receptor signaling. Reproduction. 2011 Nov;142(5):633-46. doi: 10.1530/REP-11-0136.

[55] Kay VR, Chambers C, Foster WG. Reproductive and developmental effects of phthalate diesters in females. Crit Rev Toxicol. 2013 Mar;43(3):200-19. doi: 10.3109/10408444.2013.766149.

[56] Vabre P, Gatimel N, Moreau J, Gayrard V, Picard-Hagen N, Parinaud J, Leandri RD. Environmental pollutants, a possible etiology for premature ovarian insufficiency: a narrative review of animal and human data. Environ Health. 2017 Apr 7;16(1):37. doi: 10.1186/s12940-017-0242-4.

[57] Craig ZR, Ziv-Gal A. Pretty Good or Pretty Bad? The Ovary and Chemicals in Personal Care Products. Toxicol Sci. 2018 Apr 1;162(2):349-360. doi: 10.1093/toxsci/kfx285.

[58] Hannon PR, Peretz J, Flaws JA. Daily exposure to Di(2-ethylhexyl) phthalate alters estrous cyclicity and accelerates primordial follicle recruitment potentially via dysregulation of the phosphatidylinositol 3-kinase signaling pathway in adult mice. Biol Reprod. 2014 Jun;90(6):136. doi: 10.1095/biolreprod.114.119032.

[59] Zhang XF, Zhang LJ, Li L, Feng YN, Chen B, Ma JM, Huynh E, Shi QH, De Felici M, Shen W. Diethylhexyl phthalate exposure impairs follicular development and affects oocyte maturation in the mouse. Environ Mol Mutagen. 2013 Jun;54(5):354-61. doi: 10.1002/em.21776.

[60] Xu C, Chen JA, Qiu Z, Zhao Q, Luo J, Yang L, Zeng H, Huang Y, Zhang L, Cao J, Shu W. Ovotoxicity and PPAR-mediated aromatase downregulation in female Sprague-Dawley rats following combined oral exposure to benzo[a]pyrene and di-(2-ethylhexyl) phthalate. Toxicol Lett. 2010 Dec 15;199(3):323-32. doi: 10.1016/j.toxlet.2010.09.015.

[61] Moyer B, Hixon ML. Reproductive effects in F1 adult females exposed in utero to moderate to high doses of mono-2-ethylhexylphthalate (MEHP). Reprod Toxicol. 2012 Aug;34(1):43-50. doi: 10.1016/j.reprotox.2012.02.006.

[62] Craig ZR, Hannon PR, Wang W, Ziv-Gal A, Flaws JA. Di-n-butyl phthalate disrupts the expression of genes involved in cell cycle and apoptotic pathways in mouse ovarian antral follicles. Biol Reprod. 2013 Jan 31;88(1):23. doi: 10.1095/biolreprod.112.105122

[63] Wang W, Craig ZR, Basavarajappa MS, Gupta RK, Flaws JA. Di (2-ethylhexyl) phthalate inhibits growth of mouse ovarian antral follicles through an oxidative stress pathway. Toxicol Appl Pharmacol. 2012 Jan 15;258(2):288-95. doi: 10.1016/j.taap.2011.11.008.

[64] Herreros MA, Gonzalez-Bulnes A, Iñigo-Nuñez S, Contreras-Solis I, Ros JM, Encinas T. Toxicokinetics of di(2-ethylhexyl) phthalate (DEHP) and its effects on luteal function in sheep. Reprod Biol. 2013 Mar;13(1):66-74. doi: 10.1016/j.repbio.2013.01.177.

[65] Mu X, Liao X, Chen X, Li Y, Wang M, Shen C, Zhang X, Wang Y, Liu X, He J. DEHP exposure impairs mouse oocyte cyst breakdown and primordial follicle assembly through estrogen receptor-dependent and independent mechanisms. J Hazard Mater. 2015 Nov 15;298:232-40. doi: 10.1016/j.jhazmat.2015.05.052.

[66] Li L, Zhang T, Qin XS, Ge W, Ma HG, Sun LL, Hou ZM, Chen H, Chen P, Qin GQ, Shen W, Zhang XF. Exposure to diethylhexyl phthalate (DEHP) results in a heritable modification of imprint genes DNA methylation in mouse oocytes. Mol Biol Rep. 2014 Mar;41(3):1227-35. doi: 10.1007/s11033-013-2967-7.

[67] Zhang T, Li L, Qin XS, Zhou Y, Zhang XF, Wang LQ, De Felici M, Chen H, Qin GQ, Shen W. Di-(2-ethylhexyl) phthalate and bisphenol A exposure impairs mouse primordial follicle assembly in vitro. Environ Mol Mutagen. 2014 May;55(4):343-53. doi: 10.1002/em.21847.

[68] Liu JC, Yan ZH, Li B, Yan HC, De Felici M, Shen W. Di (2-ethylhexyl) phthalate impairs primordial follicle assembly by increasing PDE3A expression in oocytes. Environ Pollut. 2021 Feb 1;270:116088. doi: 10.1016/j.envpol.2020.116088. Epub 2020 Nov 17. PMID: 33234378.

[69] Ray B, D'Souza AS, Kumar V, Pugazhandhi B, D'Souza MR, Nayak D, Sushma RK, Shetty P, Singh H, Krishna L, Bhat KM, Rao AC, Chakraborti S, Kumar N, Saxena A. Ovarian development in Wistar rat treated prenatally with single dose diisobutyl phthalate. Bratisl Lek Listy. 2012;113(10):577-82. doi: 10.4149/bll_2012_129.

[70] Sen N, Liu X, Craig ZR. Short term exposure to di-n-butyl phthalate (DBP) disrupts ovarian function in young CD-1 mice. Reprod Toxicol. 2015 Jun;53:15-22. doi: 10.1016/j.reprotox.2015.02.012.

[71] Muczynski V, Lecureuil C, Messiaen S, Guerquin MJ, N'tumba-Byn T, Moison D, Hodroj W, Benjelloun H, Baijer J, Livera G, Frydman R, Benachi A, Habert R, Rouiller-Fabre V. Cellular and molecular effect of MEHP Involving LXRα in human fetal testis and ovary. PLoS One. 2012;7(10):e48266. doi: 10.1371/journal.pone.0048266.

[72] Gupta RK, Singh JM, Leslie TC, Meachum S, Flaws JA, Yao HH. Di-(2-ethylhexyl) phthalate and mono-(2-ethylhexyl) phthalate inhibit growth and reduce estradiol levels of antral follicles in vitro. Toxicol Appl Pharmacol. 2010 Jan 15;242(2):224-30. doi: 10.1016/j.taap.2009.10.011.

[73] Grande SW, Andrade AJ, Talsness CE, Grote K, Golombiewski A, Sterner-Kock A, Chahoud I. A dose-response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): reproductive effects on adult female offspring rats. Toxicology. 2007 Jan 5;229(1-2):114-22. doi: 10.1016/j.tox.2006.10.005.

[74] Manikkam M, Tracey R, Guerrero-Bosagna C, Skinner MK. Plastics derived endocrine disruptors (BPA, DEHP and DBP) induce epigenetic transgenerational inheritance of obesity, reproductive disease and sperm epimutations. PLoS One. 2013;8(1):e55387. doi: 10.1371/journal.pone.0055387.

[75] Rasmussen LM, Sen N, Vera JC, Liu X, Craig ZR. Effects of in vitro exposure to dibutyl phthalate, mono-butyl phthalate, and acetyl tributyl citrate on ovarian antral follicle growth and viability. Biol Reprod. 2017 May 1;96(5):1105-1117. doi: 10.1095/biolreprod.116.144691

[76] Meling DD, Warner GR, Szumski JR, Gao L, Gonsioroski AV, Rattan S, Flaws JA. The effects of a phthalate metabolite mixture on antral follicle growth and sex steroid synthesis in mice. Toxicol Appl Pharmacol. 2020 Feb 1;388:114875. doi: 10.1016/j.taap.2019.114875.

[77] Grossman D, Kalo D, Gendelman M, Roth Z. Effect of di-(2-ethylhexyl) phthalate and mono-(2-ethylhexyl) phthalate on in vitro developmental competence of bovine oocytes. Cell Biol Toxicol. 2012 Dec;28(6):383-96. doi: 10.1007/s10565-012-9230-1.

[78] Ambruosi B, Uranio MF, Sardanelli AM, Pocar P, Martino NA, Paternoster MS, Amati F, Dell'Aquila ME. In vitro acute exposure to DEHP affects oocyte meiotic maturation, energy and oxidative stress parameters in a large animal model. PLoS One. 2011;6(11):e27452. doi: 10.1371/journal.pone.0027452.

[79] Dalman A, Eimani H, Sepehri H, Ashtiani SK, Valojerdi MR, Eftekhari-Yazdi P, Shahverdi A. Effect of mono-(2-ethylhexyl) phthalate (MEHP) on resumption of meiosis, in vitro maturation and embryo development of immature mouse oocytes. Biofactors. 2008;33(2):149-55. doi: 10.1002/biof.5520330207.

[80] Lu Z, Zhang C, Han C, An Q, Cheng Y, Chen Y, Meng R, Zhang Y, Su J. Plasticizer Bis(2-ethylhexyl) Phthalate Causes Meiosis Defects and Decreases Fertilization Ability of Mouse Oocytes in Vivo. J Agric Food Chem. 2019 Mar 27;67(12):3459-3468. doi: 10.1021/acs.jafc.9b00121.

[81] Li FP, Zhou JL, Guo AW, Liu Y, Zhang F, Xu BH, Liu R, Wang YL, Chen MH, Lin YH, He SW, Liao BQ, Fu XP, Wang HL. Di(n-butyl) phthalate exposure impairs meiotic competence and development of mouse oocyte. Environ Pollut. 2019 Mar;246:597-607. doi: 10.1016/j.envpol.2018.12.077.

[82] Liu JC, Lai FN, Li L, Sun XF, Cheng SF, Ge W, Wang YF, Li L, Zhang XF, De Felici M, Dyce PW, Shen W. Di (2-ethylhexyl) phthalate exposure impairs meiotic progression and DNA damage repair in fetal mouse oocytes in vitro. Cell Death Dis. 2017 Aug 3;8(8):e2966. doi: 10.1038/cddis.2017.350. PMID: 28771232; PMCID: PMC5596541.

[83] Mirihagalle S, You T, Suh L, et al. Prenatal exposure to di-(2-ethylhexyl) phthalate and high-fat diet synergistically disrupts mouse fetal oogenesis and affects folliculogenesis†. Biol Reprod. 2019;100(6):1561-1570. doi:10.1093/biolre/ioz051

[84] Kalo D, Vitorino Carvalho A, Archilla C, Duranthon V, Moroldo M, Levin Y, Kupervaser M, Smith Y, Roth Z. Mono(2-ethylhexyl) phthalate (MEHP) induces transcriptomic alterations in oocytes and their derived blastocysts. Toxicology. 2019 Jun 1;421:59-73. doi: 10.1016/j.tox.2019.04.016.

[85] Land KL, Lane ME, Fugate AC, Hannon PR. Ovulation is Inhibited by an Environmentally Relevant Phthalate Mixture in Mouse Antral Follicles In Vitro. Toxicol Sci. 2021 Jan 28;179(2):195-205. doi: 10.1093/toxsci/kfaa170. PMID: 33316053; PMCID: PMC8454202.

[86] Hart R, Doherty DA, Frederiksen H, Keelan JA, Hickey M, Sloboda D, Pennell CE, Newnham JP, Skakkebaek NE, Main KM. The influence of antenatal exposure to phthalates on subsequent female reproductive development in adolescence: a pilot study. Reproduction. 2014 Mar 2;147(4):379-90. doi: 10.1530/REP-13-0331.

[87] Messerlian C, Souter I, Gaskins AJ, Williams PL, Ford JB, Chiu YH, Calafat AM, Hauser R; Earth Study Team. Urinary phthalate metabolites and ovarian reserve among women seeking infertility care. Hum Reprod. 2016 Jan;31(1):75-83. doi: 10.1093/humrep/dev292.

[88] Machtinger R, Zhong J, Mansur A, Adir M, Racowsky C, Hauser R, Brennan K, Karlsson O, Baccarelli AA. Placental lncRNA Expression Is Associated With Prenatal Phthalate Exposure. Toxicol Sci. 2018 May 1;163(1):116-122. doi: 10.1093/toxsci/kfy013.

[89] Boberg J, Metzdorff S, Wortziger R, Axelstad M, Brokken L, Vinggaard AM, Dalgaard M, Nellemann C. Impact of diisobutyl phthalate and other PPAR agonists on steroidogenesis and plasma insulin and leptin levels in fetal rats. Toxicology. 2008 Sep 4;250(2-3):75-81. doi: 10.1016/j.tox.2008.05.020.

[90] Parks LG, Ostby JS, Lambright CR, Abbott BD, Klinefelter GR, Barlow NJ, Gray LE Jr. The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat. Toxicol Sci. 2000 Dec;58(2):339-49. doi: 10.1093/toxsci/58.2.339.

[91] Grande SW, Andrade AJ, Talsness CE, Grote K, Chahoud I. A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicol Sci. 2006 May;91(1):247-54. doi: 10.1093/toxsci/kfj128.

[92] Pocar P, Fiandanese N, Secchi C, Berrini A, Fischer B, Schmidt JS, Schaedlich K, Borromeo V. Exposure to di(2-ethyl-hexyl) phthalate (DEHP) in utero and during lactation causes long-term pituitary-

gonadal axis disruption in male and female mouse offspring. Endocrinology. 2012 Feb;153(2):937-48. doi: 10.1210/en.2011-1450

[93] Liu T, Li N, Zhu J, Yu G, Guo K, Zhou L, Zheng D, Qu X, Huang J, Chen X, Wang S, Ye L. Effects of di-(2-ethylhexyl) phthalate on the hypothalamus-pituitary-ovarian axis in adult female rats. Reprod Toxicol. 2014 Jul;46:141-7. doi: 10.1016/j.reprotox.2014.03.006.

[94] Chiang C, Lewis LR, Borkowski G, Flaws JA. Exposure to di(2-ethylhexyl) phthalate and diisononyl phthalate during adulthood disrupts hormones and ovarian folliculogenesis throughout the prime reproductive life of the mouse. Toxicol Appl Pharmacol. 2020 Apr 15;393:114952. doi: 10.1016/j.taap.2020.114952. Epub 2020 Mar 10. PMID: 32165126; PMCID: PMC7138141.

[95] Li N, Liu T, Zhou L, He J, Ye L. Di-(2-ethylhcxyl) phthalate reduces progesterone levels and induces apoptosis of ovarian granulosa cell in adult female ICR mice. Environ Toxicol Pharmacol. 2012 Nov;34(3):869-75. doi: 10.1016/j.etap.2012.08.013.

[96] Davis BJ, Weaver R, Gaines LJ, Heindel JJ. Mono-(2-ethylhexyl) phthalate suppresses estradiol production independent of FSH-cAMP stimulation in rat granulosa cells. Toxicol Appl Pharmacol. 1994 Oct;128(2):224-8. doi: 10.1006/taap.1994.1201.

[97] Lovekamp-Swan T, Jetten AM, Davis BJ. Dual activation of PPARalpha and PPARgamma by mono-(2-ethylhexyl) phthalate in rat ovarian granulosa cells. Mol Cell Endocrinol. 2003 Mar 28;201(1-2):133-41. doi: 10.1016/s0303-7207(02)00423-9.

[98] Lovekamp TN, Davis BJ. Mono-(2-ethylhexyl) phthalate suppresses aromatase transcript levels and estradiol production in cultured rat granulosa cells. Toxicol Appl Pharmacol. 2001 May 1;172(3):217-24. doi: 10.1006/taap.2001.9156.

[99] Treinen KA, Dodson WC, Heindel JJ. Inhibition of FSH-stimulated cAMP accumulation and progesterone production by mono(2-ethylhexyl) phthalate in rat granulosa cell cultures. Toxicol Appl Pharmacol. 1990 Nov;106(2):334-40. doi: 10.1016/0041-008x(90)90252-p.

[100] Svechnikova K, Svechnikova I, Söder O. Gender-Specific Adverse Effects of Mono-Ethylhexyl Phthalate on Steroidogenesis in Immature Granulosa Cells and Rat Leydig cell Progenitors in vitro. Front Endocrinol (Lausanne). 2011 Apr 25;2:9. doi: 10.3389/fendo.2011.00009.

[101] Reinsberg J, Wegener-Toper P, van der Ven K, van der Ven H, Klingmueller D. Effect of mono-(2-ethylhexyl) phthalate on steroid production of human granulosa cells. Toxicol Appl Pharmacol. 2009 Aug 15;239(1):116-23. doi: 10.1016/j.taap.2009.05.022.

[102] Panagiotou EM, Ojasalo V, Damdimopoulou P. Phthalates, ovarian function and fertility in adulthood. Best Pract Res Clin Endocrinol Metab. 2021 Sep;35(5):101552. doi: 10.1016/j.beem.2021.101552. Epub 2021 Jun 2. PMID: 34238683.

[103] Tripathi A, Pandey V, Sahu AN, Singh A, Dubey PK. Di-(2-ethylhexyl) phthalate (DEHP) inhibits steroidogenesis and induces mitochondria-ROS mediated apoptosis in rat ovarian granulosa cells. Toxicol Res (Camb). 2019 Feb 4;8(3):381-394. doi: 10.1039/c8tx00263k. PMID: 31160972; PMCID: PMC6505384.

[104] Vagi SJ, Azziz-Baumgartner E, Sjödin A, Calafat AM, Dumesic D, Gonzalez L, Kato K, Silva MJ, Ye X, Azziz R. Exploring the potential association between brominated diphenyl ethers, polychlorinated biphenyls, organochlorine pesticides, perfluorinated compounds, phthalates, and bisphenol A in polycystic ovary syndrome: a case-control study. BMC Endocr Disord. 2014 Oct 28;14:86. doi: 10.1186/1472-6823-14-86.

[105] Garg D, Tal R. The role of AMH in the pathophysiology of polycystic ovarian syndrome. Reprod Biomed Online. 2016 Jul;33(1):15-28. doi: 10.1016/j.rbmo.2016.04.007.

[106] Lin LC, Wang SL, Chang YC, Huang PC, Cheng JT, Su PH, Liao PC. Associations between maternal phthalate exposure and cord sex hormones in human infants. Chemosphere. 2011 May;83(8):1192-9. doi: 10.1016/j.chemosphere.2010.12.079.

[107] Watkins DJ, Téllez-Rojo MM, Ferguson KK, Lee JM, Solano-Gonzalez M, Blank-Goldenberg C, Peterson KE, Meeker JD. In utero and peripubertal exposure to phthalates and BPA in relation to female sexual maturation. Environ Res. 2014 Oct;134:233-41. doi: 10.1016/j.envres.2014.08.010

[108] Su PH, Chen JY, Lin CY, Chen HY, Liao PC, Ying TH, Wang SL. Sex steroid hormone levels and reproductive development of eight-year-old children following in utero and environmental exposure to phthalates. PLoS One. 2014 Sep 10;9(9):e102788. doi: 10.1371/journal.pone.0102788.

[109] Meeker JD, Ferguson KK. Urinary phthalate metabolites are associated with decreased serum testosterone in men, women, and children from NHANES 2011-2012. J Clin Endocrinol Metab. 2014 Nov;99(11):4346-52. doi: 10.1210/jc.2014-2555.

[110] Zhang J, Yin W, Li P, Hu C, Wang L, Li T, Gao E, Hou J, Wang G, Wang X, Wang L, Yu Z, Yuan J. Interaction between diet- and exercise-lifestyle and phthalates exposure on sex hormone levels. J Hazard Mater. 2019 May 5;369:290-298. doi: 10.1016/j.jhazmat.2019.02.011.

[111] Sathyanarayana S, Barrett E, Butts S, Wang C, Swan SH. Phthalate exposure and reproductive hormone concentrations in pregnancy. Reproduction. 2014 Mar 2;147(4):401-9. doi: 10.1530/REP-13-0415.

[112] Boyle J, Teede HJ. Polycystic ovary syndrome - an update. Aust Fam Physician. 2012 Oct;41(10):752-6.

[113] March WA, Moore VM, Willson KJ, Phillips DI, Norman RJ, Davies MJ. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Hum Reprod. 2010 Feb;25(2):544-51. doi: 10.1093/humrep/dep399.

[114] Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 2004 Jan;81(1):19-25. doi: 10.1016/j.fertnstert.2003.10.004.

[115] Lizneva D, Gavrilova-Jordan L, Walker W, Azziz R. Androgen excess: Investigations and management. Best Pract Res Clin Obstet Gynaecol. 2016 Nov;37:98-118. doi: 10.1016/j.bpobgyn.2016.05.003.

[116] Rutkowska AZ, Diamanti-Kandarakis E. Polycystic ovary syndrome and environmental toxins. Fertil Steril. 2016 Sep 15;106(4):948-58. doi: 10.1016/j.fertnstert.2016.08.031.

[117] Fowler PA, Bellingham M, Sinclair KD, Evans NP, Pocar P, Fischer B, Schaedlich K, Schmidt JS, Amezaga MR, Bhattacharya S, Rhind SM, O'Shaughnessy PJ. Impact of endocrine-disrupting compounds (EDCs) on female reproductive health. Mol Cell Endocrinol. 2012 May 22;355(2):231-9. doi: 10.1016/j.mce.2011.10.021.

[118] Hewlett M, Chow E, Aschengrau A, Mahalingaiah S. Prenatal Exposure to Endocrine Disruptors: A Developmental Etiology for Polycystic Ovary Syndrome. Reprod Sci. 2017 Jan;24(1):19-27. doi: 10.1177/1933719116654992.

[119] Abbott DH, Barnett DK, Bruns CM, Dumesic DA. Androgen excess fetal programming of female reproduction: a developmental aetiology for polycystic ovary syndrome? Hum Reprod Update. 2005 Jul-Aug;11(4):357-74. doi: 10.1093/humupd/dmi013.

[120] Veiga-Lopez A, Pu Y, Gingrich J, Padmanabhan V. Obesogenic Endocrine Disrupting Chemicals: Identifying Knowledge Gaps. Trends Endocrinol Metab. 2018 Sep;29(9):607-625. doi: 10.1016/j.tem.2018.06.003.

[121] Kandaraki E, Chatzigeorgiou A, Livadas S, Palioura E, Economou F, Koutsilieris M, Palimeri S, Panidis D, Diamanti-Kandarakis E. Endocrine disruptors and polycystic ovary syndrome (PCOS): elevated serum levels of bisphenol A in women with PCOS. J Clin Endocrinol Metab. 2011 Mar;96(3):E480-4. doi: 10.1210/jc.2010-1658.

[122] Akgül S, Sur Ü, Düzçeker Y, Balcı A, Kızılkan MP, Kanbur N, Bozdağ G, Erkekoğlu P, Gümüş E, Kocer-Gumusel B, Derman O. Bisphenol A and phthalate levels in adolescents with polycystic ovary syndrome. Gynecol Endocrinol. 2019 Dec;35(12):1084-1087. doi: 10.1080/09513590.2019.1630608.

[123] Jin Y, Zhang Q, Pan JX, Wang FF, Qu F. The effects of di(2-ethylhexyl) phthalate exposure in women with polycystic ovary syndrome undergoing in vitro fertilization. J Int Med Res. 2019 Dec;47(12):6278-6293. doi: 10.1177/0300060519876467.

[124] Akın L, Kendirci M, Narin F, Kurtoğlu S, Hatipoğlu N, Elmalı F. Endocrine Disruptors and Polycystic Ovary Syndrome: Phthalates. J Clin Res Pediatr Endocrinol. 2020 Nov 25;12(4):393-400. doi: 10.4274/jcrpe.galenos.2020.2020.0037.

[125] Al-Saleh I. The relationship between urinary phthalate metabolites and polycystic ovary syndrome in women undergoing in vitro fertilization: Nested case-control study. Chemosphere. 2022 Jan;286(Pt 1):131495. doi: 10.1016/j.chemosphere.2021.131495. Epub 2021 Jul 14. PMID: 34293567

[126] Cheon YP. Di-(2-ethylhexyl) Phthalate (DEHP) and Uterine Histological Characteristics. Dev Reprod. 2020 Mar;24(1):1-17. doi: 10.12717/DR.2020.24.1.1.

[127] Singh H, Endo Y, Nie G. Decidual HtrA3 negatively regulates trophoblast invasion during human placentation. Hum Reprod. 2011 Apr;26(4):748-57. doi: 10.1093/humrep/der019

[128] Ma W, Tan J, Matsumoto H, Robert B, Abrahamson DR, Das SK, Dey SK. Adult tissue angiogenesis: evidence for negative regulation by estrogen in the uterus. Mol Endocrinol. 2001 Nov;15(11):1983-92. doi: 10.1210/mend.15.11.0734.

[129] Cavanagh JE, Trought K, Mitchell C, Northcott G, Tremblay LA. Assessment of endocrine disruption and oxidative potential of bisphenol-A, triclosan, nonylphenol, diethylhexyl phthalate, galaxolide, and carbamazepine, common contaminants of municipal biosolids. Toxicol In Vitro. 2018 Apr;48:342-349. doi: 10.1016/j.tiv.2018.02.003.

[130] Houston KD, Copland JA, Broaddus RR, Gottardis MM, Fischer SM, Walker CL. Inhibition of proliferation and estrogen receptor signaling by peroxisome proliferator-activated receptor gamma ligands in uterine leiomyoma. Cancer Res. 2003 Mar 15;63(6):1221-7.

[131] Tomic D, Frech MS, Babus JK, Symonds D, Furth PA, Koos RD, Flaws JA. Effects of ERalpha overexpression on female reproduction in mice. Reprod Toxicol. 2007 Apr-May;23(3):317-25. doi: 10.1016/j.reprotox.2006.08.004.

[132] Somasundaram DB, Manokaran K, Selvanesan BC, Bhaskaran RS. Impact of di-(2-ethylhexyl) phthalate on the uterus of adult Wistar rats. Hum Exp Toxicol. 2017 Jun;36(6):565-572. doi: 10.1177/0960327116657601.

[133] Richardson KA, Hannon PR, Johnson-Walker YJ, Myint MS, Flaws JA, Nowak RA. Di (2-ethylhexyl) phthalate (DEHP) alters proliferation and uterine gland numbers in the uteri of adult exposed mice. Reprod Toxicol. 2018 Apr;77:70-79. doi: 10.1016/j.reprotox.2018.01.006.

[134] Stewart EA, Cookson CL, Gandolfo RA, Schulze-Rath R. Epidemiology of uterine fibroids: a systematic review. BJOG. 2017 Sep;124(10):1501-1512. doi: 10.1111/1471-0528.14640.

[135] Schwartz SM, Marshall LM, Baird DD. Epidemiologic contributions to understanding the etiology of uterine leiomyomata. Environ Health Perspect. 2000 Oct;108 Suppl 5:821-7. doi: 10.1289/ehp.00108s5821.

[136] Parker WH. Etiology, symptomatology, and diagnosis of uterine myomas. Fertil Steril. 2007 Apr;87(4):725-36. doi: 10.1016/j.fertnstert.2007.01.093

[137]Falcone T, Walters MD. Hysterectomy for benign disease. Obstet Gynecol. 2008 Mar;111(3):753-67. doi: 10.1097/AOG.0b013e318165f18c.

[138] Flake GP, Andersen J, Dixon D. Etiology and pathogenesis of uterine leiomyomas: a review. Environ Health Perspect. 2003 Jun;111(8):1037-54. doi: 10.1289/ehp.5787.

[139] Bulun SE. Uterine fibroids. N Engl J Med. 2013 Oct 3;369(14):1344-55. doi: 10.1056/NEJMra1209993.

[140] Fu Z, Zhao F, Chen K, Xu J, Li P, Xia D, Wu Y. Association between urinary phthalate metabolites and risk of breast cancer and uterine leiomyoma. Reprod Toxicol. 2017 Dec;74:134-142. doi: 10.1016/j.reprotox.2017.09.009.

[141] Huang Y, Li J, Garcia JM, Lin H, Wang Y, Yan P, Wang L, Tan Y, Luo J, Qiu Z, Chen JA, Shu W. Phthalate levels in cord blood are associated with preterm delivery and fetal growth parameters in Chinese women. PLoS One. 2014 Feb 4;9(2):e87430. doi: 10.1371/journal.pone.0087430.

[142] Kim YA, Kho Y, Chun KC, Koh JW, Park JW, Bunderson-Schelvan M, Cho YH. Increased Urinary Phthalate Levels in Women with Uterine Leiomyoma: A Case-Control Study. Int J Environ Res Public Health. 2016 Dec 15;13(12):1247. doi: 10.3390/ijerph13121247.

[143] Sun J, Zhang MR, Zhang LQ, Zhao D, Li SG, Chen B. Phthalate monoesters in association with uterine leiomyomata in Shanghai. Int J Environ Health Res. 2016;26(3):306-16. doi: 10.1080/09603123.2015.1111310.

[144]Kim JH, Kim SH, Oh YS, Ihm HJ, Chae HD, Kim CH, Kang BM. In vitro effects of phthalate esters in human myometrial and leiomyoma cells and increased urinary level of phthalate metabolite in women with uterine leiomyoma. Fertil Steril. 2017 Apr;107(4):1061-1069.e1. doi: 10.1016/j.fertnstert.2017.01.015.

[145] Maruo T, Ohara N, Wang J, Matsuo H. Sex steroidal regulation of uterine leiomyoma growth and apoptosis. Hum Reprod Update. 2004 May-Jun;10(3):207-20. doi: 10.1093/humupd/dmh019.

[146] Zota AR, Geller RJ, Calafat AM, Marfori CQ, Baccarelli AA, Moawad GN. Phthalates exposure and uterine fibroid burden among women undergoing surgical treatment for fibroids: a preliminary study. Fertil Steril. 2019 Jan;111(1):112-121. doi: 10.1016/j.fertnstert.2018.09.009.

[147] Weuve J, Hauser R, Calafat AM, Missmer SA, Wise LA. Association of exposure to phthalates with endometriosis and uterine leiomyomata: findings from NHANES, 1999-2004. Environ Health Perspect. 2010 Jun;118(6):825-32. doi: 10.1289/ehp.0901543.

[148] Fruh V, Claus Henn B, Weuve J, Wesselink AK, Orta OR, Heeren T, Hauser R, Calafat AM, Williams PL, Baird DD, Wise LA. Incidence of uterine leiomyoma in relation to urinary concentrations of phthalate and phthalate alternative biomarkers: A prospective ultrasound study. Environ Int. 2021 Feb;147:106218. doi: 10.1016/j.envint.2020.106218.

[149] Lee G, Kim S, Bastiaensen M, Malarvannan G, Poma G, Caballero Casero N, Gys C, Covaci A, Lee S, Lim JE, Mok S, Moon HB, Choi G, Choi K. Exposure to organophosphate esters, phthalates, and alternative plasticizers in association with uterine fibroids. Environ Res. 2020 Oct;189:109874. doi: 10.1016/j.envres.2020.109874.

[150] Zhang Y, Lu Y, Ma H, Xu Q, Wu X. Combined Exposure to Multiple Endocrine Disruptors and Uterine Leiomyomata and Endometriosis in US Women. Front Endocrinol (Lausanne). 2021 Aug 20;12:726876. doi: 10.3389/fendo.2021.726876. PMID: 34489871; PMCID: PMC8418539.

[151] Zota AR, Geller RJ, VanNoy BN, Marfori CQ, Tabbara S, Hu LY, Baccarelli AA, Moawad GN. Phthalate Exposures and MicroRNA Expression in Uterine Fibroids: The FORGE Study. Epigenet Insights. 2020 Feb 18;13:2516865720904057. doi: 10.1177/2516865720904057.

[152] Marsh EE, Steinberg ML, Parker JB, Wu J, Chakravarti D, Bulun SE. Decreased expression of microRNA-29 family in leiomyoma contributes to increased major fibrillar collagen production. Fertil Steril. 2016 Sep 1;106(3):766-72. doi: 10.1016/j.fertnstert.2016.05.001. Epub 2016 May 24. PMID: 27233758; PMCID: PMC5011009.

[153] Bariani MV, Rangaswamy R, Siblini H, Yang Q, Al-Hendy A, Zota AR. The role of endocrine-disrupting chemicals in uterine fibroid pathogenesis. Curr Opin Endocrinol Diabetes Obes. 2020 Dec;27(6):380-387. doi: 10.1097/MED.0000000000000578. PMID: 33044243; PMCID: PMC8240765.

[154] Macer ML, Taylor HS. Endometriosis and infertility: a review of the pathogenesis and treatment of endometriosis-associated infertility. Obstet Gynecol Clin North Am. 2012 Dec;39(4):535-49. doi: 10.1016/j.ogc.2012.10.002.

[155] Giudice LC. Clinical practice. Endometriosis. N Engl J Med. 2010 Jun 24;362(25):2389-98. doi: 10.1056/NEJMcp1000274.

[156] Zondervan KT, Cardon LR, Kennedy SH. What makes a good case-control study? Design issues for complex traits such as endometriosis. Hum Reprod. 2002 Jun;17(6):1415-23. doi: 10.1093/humrep/17.6.1415.

[157] Lai GL, Yeh CC, Yeh CY, Chen RY, Fu CL, Chen CH, Tzeng CR. Decreased zinc and increased lead blood levels are associated with endometriosis in Asian Women. Reprod Toxicol. 2017 Dec;74:77-84. doi: 10.1016/j.reprotox.2017.09.001.

[158] Buck Louis GM, Sundaram R, Schisterman EF, Sweeney AM, Lynch CD, Gore-Langton RE, Maisog J, Kim S, Chen Z, Barr DB. Persistent environmental pollutants and couple fecundity: the LIFE study. Environ Health Perspect. 2013 Feb;121(2):231-6. doi: 10.1289/ehp.1205301.

[159] Signorile PG, Baldi F, Bussani R, D'Armiento M, De Falco M, Boccellino M, Quagliuolo L, Baldi A. New evidence of the presence of endometriosis in the human fetus. Reprod Biomed Online. 2010 Jul;21(1):142-7. doi: 10.1016/j.rbmo.2010.04.002.

[160] Kim YH, Kim SH, Lee HW, Chae HD, Kim CH, Kang BM. Increased viability of endometrial cells by in vitro treatment with di-(2-ethylhexyl) phthalate. Fertil Steril. 2010 Nov;94(6):2413-6. doi: 10.1016/j.fertnstert.2010.04.027.

[161] Kim SH, Cho S, Ihm HJ, Oh YS, Heo SH, Chun S, Im H, Chae HD, Kim CH, Kang BM. Possible Role of Phthalate in the Pathogenesis of Endometriosis: In Vitro, Animal, and Human Data. J Clin Endocrinol Metab. 2015 Dec;100(12):E1502-11. doi: 10.1210/jc.2015-2478.

[162] Dart AE, Wells CM. P21-activated kinase 4--not just one of the PAK. Eur J Cell Biol. 2013 Apr-May;92(4-5):129-38. doi: 10.1016/j.ejcb.2013.03.002.

[163] Chen CY, Chou YY, Wu YM, Lin CC, Lin SJ, Lee CC. Phthalates may promote female puberty by increasing kisspeptin activity. Hum Reprod. 2013 Oct;28(10):2765-73. doi: 10.1093/humrep/det325.

[164] Cobellis L, Latini G, De Felice C, Razzi S, Paris I, Ruggieri F, Mazzeo P, Petraglia F. High plasma concentrations of di-(2-ethylhexyl)-phthalate in women with endometriosis. Hum Reprod. 2003 Jul;18(7):1512-5. doi: 10.1093/humrep/deg254

[165] Reddy BS, Rozati R, Reddy S, Kodampur S, Reddy P, Reddy R. High plasma concentrations of polychlorinated biphenyls and phthalate esters in women with endometriosis: a prospective case control study. Fertil Steril. 2006 Mar;85(3):775-9. doi: 10.1016/j.fertnstert.2005.08.037.

[166] Chou YC, Chen YC, Chen MJ, Chang CW, Lai GL, Tzeng CR. Exposure to Mono-n-Butyl Phthalate in Women with Endometriosis and Its Association with the Biological Effects on Human Granulosa Cells. Int J Mol Sci. 2020 Mar 5;21(5):1794. doi: 10.3390/ijms21051794.

[167] Itoh H, Iwasaki M, Hanaoka T, Sasaki H, Tanaka T, Tsugane S. Urinary phthalate monoesters and endometriosis in infertile Japanese women. Sci Total Environ. 2009 Dec 15;408(1):37-42. doi: 10.1016/j.scitotenv.2009.09.012.

[168] Moreira Fernandez MA, Cardeal ZL, Carneiro MM, André LC. Study of possible association between endometriosis and phthalate and bisphenol A by biomarkers analysis. J Pharm Biomed Anal. 2019 Aug 5;172:238-242. doi: 10.1016/j.jpba.2019.04.048. Epub 2019 Apr 28. PMID: 31063881.

[169] Cai W, Yang J, Liu Y, Bi Y, Wang H. Association between Phthalate Metabolites and Risk of Endometriosis: A Meta-Analysis. Int J Environ Res Public Health. 2019 Sep 30;16(19):3678. doi: 10.3390/ijerph16193678. PMID: 31574938; PMCID: PMC6801736.

[170] Wen X, Xiong Y, Qu X, Jin L, Zhou C, Zhang M, Zhang Y. The risk of endometriosis after exposure to endocrine-disrupting chemicals: a meta-analysis of 30 epidemiology studies. Gynecol Endocrinol. 2019 Aug;35(8):645-650. doi: 10.1080/09513590.2019.1590546. Epub 2019 Mar 23. PMID: 30907174.

[171] Conforti A, Carbone L, Simeon V, Chiodini P, Marrone V, Bagnulo F, Cariati F, Strina I, Alviggi C. Unravelling the link between phthalate exposure and endometriosis in humans: a systematic review and meta-analysis of the literature. J Assist Reprod Genet. 2021 Oct;38(10):2543-2557. doi: 10.1007/s10815-021-02265-3. Epub 2021 Jul 6. PMID: 34227050; PMCID: PMC8581146.

[172] Cavazos-Rehg PA, Krauss MJ, Spitznagel EL, Bommarito K, Madden T, Olsen MA, Subramaniam H, Peipert JF, Bierut LJ. Maternal age and risk of labor and delivery complications. Matern Child Health J. 2015 Jun;19(6):1202-11. doi: 10.1007/s10995-014-1624-7.

[173] Choi J, Smitz J. Luteinizing hormone and human chorionic gonadotropin: origins of difference. Mol Cell Endocrinol. 2014 Mar 5;383(1-2):203-13. doi: 10.1016/j.mce.2013.12.009.

[174] Fowden AL, Forhead AJ, Sferruzzi-Perri AN, Burton GJ, Vaughan OR. Review: Endocrine regulation of placental phenotype. Placenta. 2015 Apr;36 Suppl 1:S50-9. doi: 10.1016/j.placenta.2014.11.018.

[175] Karwacka A, Zamkowska D, Radwan M, Jurewicz J. Exposure to modern, widespread environmental endocrine disrupting chemicals and their effect on the reproductive potential of women: an overview of current epidemiological evidence. Hum Fertil (Camb). 2019 Apr;22(1):2-25. doi: 10.1080/14647273.2017.1358828.

[176] Sathyanarayana S, Butts S, Wang C, Barrett E, Nguyen R, Schwartz SM, Haaland W, Swan SH; TIDES Team. Early Prenatal Phthalate Exposure, Sex Steroid Hormones, and Birth Outcomes. J Clin Endocrinol Metab. 2017 Jun 1;102(6):1870-1878. doi: 10.1210/jc.2016-3837.

[177] Rager, J.E., Bangma, J., Carberry, C., Chao, A., Grossman, J., Lu, K., Manuck, T.A., Sobus, J.R., Szilagyi, J., Fry, R.C., 2020. Review of the environmental prenatal exposome and its relationship to maternal and fetal health. Reprod. Toxicol. 98, 1–12. https://doi.org/10.1016/j.reprotox.2020.02.004.

[178] Blake BE, Fenton SE. Early life exposure to per- and polyfluoroalkyl substances (PFAS) and latent health outcomes: A review including the placenta as a target tissue and possible driver of peri- and postnatal effects. Toxicology. 2020 Oct;443:152565. doi: 10.1016/j.tox.2020.152565. Epub 2020 Aug 27. PMID: 32861749; PMCID: PMC7530144

[179] Paria BC, Reese J, Das SK, Dey SK. Deciphering the cross-talk of implantation: advances and challenges. Science. 2002 Jun 21;296(5576):2185-8. doi: 10.1126/science.1071601.

[180] Zorn TM, Soto-Suazo M, Pellegrini CR, Oliveira JG, Stumpf WE. Estradiol receptor binding to the epithelium of uterine lumen and glands: region- and time-related changes during preimplantation and periimplantation periods studied by autoradiography. Histochem Cell Biol. 2003 Jul;120(1):1-12. doi: 10.1007/s00418-003-0534-3.

[181] Li R, Yu C, Gao R, Liu X, Lu J, Zhao L, Chen X, Ding Y, Wang Y, He J. Effects of DEHP on endometrial receptivity and embryo implantation in pregnant mice. J Hazard Mater. 2012 Nov 30;241-242:231-40. doi: 10.1016/j.jhazmat.2012.09.038

[182] Zhang L, Zhang TL, Zong T, Chen YL, Ren M, Yu XC, Kuang HB. [Effect of di-(2-ethylhexyl) phthalate exposure on placental development in pregnant mice]. Nan Fang Yi Ke Da Xue Xue Bao. 2016 Apr;36(4):467-71. Chinese.

[183] Midic U, Goheen B, Vincent KA, VandeVoort CA, Latham KE. Changes in gene expression following long-term in vitro exposure of Macaca mulatta trophoblast stem cells to biologically relevant levels of endocrine disruptors. Reprod Toxicol. 2018 Apr;77:154-165. doi: 10.1016/j.reprotox.2018.02.012

[184] Deng T, Du Y, Wang Y, Teng X, Hua X, Yuan X, Yao Y, Guo N, Li Y. The associations of urinary phthalate metabolites with the intermediate and pregnancy outcomes of women receiving IVF/ICSI treatments: A prospective single-center study. Ecotoxicol Environ Saf. 2020 Jan 30;188:109884. doi: 10.1016/j.ecoenv.2019.109884.

[185] Caserta D, Costanzi F, De Marco MP, Di Benedetto L, Matteucci E, Assorgi C, Pacilli MC, Besharat AR, Bellati F, Ruscito I. Effects of Endocrine-Disrupting Chemicals on Endometrial Receptivity and Embryo Implantation: A Systematic Review of 34 Mouse Model Studies. Int J Environ Res Public Health. 2021 Jun 25;18(13):6840. doi: 10.3390/ijerph18136840. PMID: 34202247; PMCID: PMC8297133.

[186] Martínez-Razo LD, Martínez-Ibarra A, Vázquez-Martínez ER, Cerbón M. The impact of Di-(2-ethylhexyl) Phthalate and Mono(2-ethylhexyl) Phthalate in placental development, function, and pathophysiology. Environ Int. 2021 Jan;146:106228. doi: 10.1016/j.envint.2020.106228.

[187] Gingrich J, Ticiani E, Veiga-Lopez A. Placenta Disrupted: Endocrine Disrupting Chemicals and Pregnancy. Trends Endocrinol Metab. 2020 Jul;31(7):508-524. doi: 10.1016/j.tem.2020.03.003.

[188] Ferguson KK, McElrath TF, Chen YH, Loch-Caruso R, Mukherjee B, Meeker JD. Repeated measures of urinary oxidative stress biomarkers during pregnancy and preterm birth. Am J Obstet Gynecol. 2015 Feb;212(2):208.e1-8. doi: 10.1016/j.ajog.2014.08.007.

[189] Zhang H, Gao F, Ben Y, Su Y. Association between phthalate exposure and risk of spontaneous pregnancy loss: A systematic review and meta-analysis. Environ POLLUT. 2020 Dec;267:115446. doi: 10.1016/j.envpol.2020.115446.

[190] Philippat C, Heude B, Botton J, Alfaidy N, Calafat AM, Slama R; EDEN Mother–Child Cohort Study Group. Prenatal Exposure to Select Phthalates and Phenols and Associations with Fetal and Placental

Weight among Male Births in the EDEN Cohort (France). Environ Health Perspect. 2019 Jan;127(1):17002. doi: 10.1289/EHP3523.

[191] Mustieles V, Mínguez-Alarcón L, Christou G, Ford JB, Dimitriadis I, Hauser R, Souter I, Messerlian C; Environment and Reproductive Health (EARTH) Study Team. Placental weight in relation to maternal and paternal preconception and prenatal urinary phthalate metabolite concentrations among subfertile couples. Environ Res. 2019 Feb;169:272-279. doi: 10.1016/j.envres.2018.11.022.

[192] Zong T, Lai L, Hu J, Guo M, Li M, Zhang L, Zhong C, Yang B, Wu L, Zhang D, Tang M, Kuang H. Maternal exposure to di-(2-ethylhexyl) phthalate disrupts placental growth and development in pregnant mice. J Hazard Mater. 2015 Oct 30;297:25-33. doi: 10.1016/j.jhazmat.2015.04.065. Epub 2015 Apr 24. PMID: 25935407

[193] Zhang S, Sun C, Zhao S, Wang B, Wang H, Zhang J, Wang Y, Cheng H, Zhu L, Shen R, Sun M, Xu T, Zhao L. Exposure to DEHP or its metabolite MEHP promotes progesterone secretion and inhibits proliferation in mouse placenta or JEG-3 cells. Environ Pollut. 2020 Feb;257:113593. doi: 10.1016/j.envpol.2019.113593. Epub 2019 Nov 17. PMID: 31771930.

[194] Lewis A, Mitsuya K, Umlauf D, Smith P, Dean W, Walter J, Higgins M, Feil R, Reik W. Imprinting on distal chromosome 7 in the placenta involves repressive histone methylation independent of DNA methylation. Nat Genet. 2004 Dec;36(12):1291-5. doi: 10.1038/ng1468.

[195] Kang ER, Iqbal K, Tran DA, Rivas GE, Singh P, Pfeifer GP, Szabó PE. Effects of endocrine disruptors on imprinted gene expression in the mouse embryo. Epigenetics. 2011 Jul;6(7):937-50. doi: 10.4161/epi.6.7.16067.

[196] Zhao Y, Shi HJ, Xie CM, Chen J, Laue H, Zhang YH. Prenatal phthalate exposure, infant growth, and global DNA methylation of human placenta. Environ Mol Mutagen. 2015 Apr;56(3):286-92. doi: 10.1002/em.21916.

[197] LaRocca J, Binder AM, McElrath TF, Michels KB. The impact of first trimester phthalate and phenol exposure on IGF2/H19 genomic imprinting and birth outcomes. Environ Res. 2014 Aug;133:396-406. doi: 10.1016/j.envres.2014.04.032.

[198] Strakovsky RS, Schantz SL. Using Experimental Models to Assess Effects of Bisphenol A (BPA) and Phthalates on the Placenta: Challenges and Perspectives. Toxicol Sci. 2018 Dec 1;166(2):250-268. doi: 10.1093/toxsci/kfy224. PMID: 30203063; PMCID: PMC6260157

[199] Warner GR, Dettogni RS, Bagchi IC, Flaws JA, Graceli JB. Placental outcomes of phthalate exposure. Reprod Toxicol. 2021 Aug;103:1-17. doi: 10.1016/j.reprotox.2021.05.001. Epub 2021 May 17. PMID: 34015474; PMCID: PMC8260441

[200] Grindler NM, Vanderlinden L, Karthikraj R, Kannan K, Teal S, Polotsky AJ, Powell TL, Yang IV, Jansson T. Exposure to Phthalate, an Endocrine Disrupting Chemical, Alters the First Trimester Placental Methylome and Transcriptome in Women. Sci Rep. 2018 Apr 17;8(1):6086. doi: 10.1038/s41598-018-24505-w.

[201] Gupta S, Agarwal A, Banerjee J, Alvarez JG. The role of oxidative stress in spontaneous abortion and recurrent pregnancy loss: a systematic review. Obstet Gynecol Surv. 2007 May;62(5):335-47; quiz 353-4. doi: 10.1097/01.ogx.0000261644.89300.df.

[202] Porter TF, Scott JR. Evidence-based care of recurrent miscarriage. Best Pract Res Clin Obstet Gynaecol. 2005 Feb;19(1):85-101. doi: 10.1016/j.bpobgyn.2004.11.005.

[203] Henriksen TB, Hjollund NH, Jensen TK, Bonde JP, Andersson AM, Kolstad H, Ernst E, Giwercman A, Skakkebaek NE, Olsen J. Alcohol consumption at the time of conception and spontaneous abortion. Am J Epidemiol. 2004 Oct 1;160(7):661-7. doi: 10.1093/aje/kwh259.

[204] Hjollund NH, Jensen TK, Bonde JP, Henriksen TB, Andersson AM, Kolstad HA, Ernst E, Giwercman A, Skakkebaek NE, Olsen J. Spontaneous abortion and physical strain around implantation: a follow-up study of first-pregnancy planners. Epidemiology. 2000 Jan;11(1):18-23. doi: 10.1097/00001648-200001000-00006.

[205] Ferguson KK, McElrath TF, Chen YH, Mukherjee B, Meeker JD. Urinary phthalate metabolites and biomarkers of oxidative stress in pregnant women: a repeated measures analysis. Environ Health Perspect. 2015 Mar;123(3):210-6. doi: 10.1289/ehp.1307996.

[206] Ferguson KK, Loch-Caruso R, Meeker JD. Exploration of oxidative stress and inflammatory markers in relation to urinary phthalate metabolites: NHANES 1999-2006. Environ Sci Technol. 2012 Jan 3;46(1):477-85. doi: 10.1021/es202340b.

[207] Kwak-Kim J, Bao S, Lee SK, Kim JW, Gilman-Sachs A. Immunological modes of pregnancy loss: inflammation, immune effectors, and stress. Am J Reprod Immunol. 2014 Aug;72(2):129-40. doi: 10.1111/aji.12234.

[208] Schmidt JS, Schaedlich K, Fiandanese N, Pocar P, Fischer B. Effects of di(2-ethylhexyl) phthalate (DEHP) on female fertility and adipogenesis in C3H/N mice. Environ Health Perspect. 2012 Aug;120(8):1123-9. doi: 10.1289/ehp.1104016

[209] Mankidy R, Wiseman S, Ma H, Giesy JP. Biological impact of phthalates. Toxicol Lett. 2013 Feb 13;217(1):50-8. doi: 10.1016/j.toxlet.2012.11.025.

[210] Ema M, Miyawaki E, Kawashima K. Effects of dibutyl phthalate on reproductive function in pregnant and pseudopregnant rats. Reprod Toxicol. 2000 Jan-Feb;14(1):13-9. doi: 10.1016/s0890-6238(99)00066-0.

[211] Toft G, Jönsson BA, Lindh CH, Jensen TK, Hjollund NH, Vested A, Bonde JP. Association between pregnancy loss and urinary phthalate levels around the time of conception. Environ Health Perspect. 2012 Mar;120(3):458-63. doi: 10.1289/ehp.1103552.

[212] Jukic AM, Calafat AM, McConnaughey DR, Longnecker MP, Hoppin JA, Weinberg CR, Wilcox AJ, Baird DD. Urinary Concentrations of Phthalate Metabolites and Bisphenol A and Associations with Follicular-Phase Length, Luteal-Phase Length, Fecundability, and Early Pregnancy Loss. Environ Health Perspect. 2016 Mar;124(3):321-8. doi: 10.1289/ehp.1408164.

[213] Mu D, Gao F, Fan Z, Shen H, Peng H, Hu J. Levels of Phthalate Metabolites in Urine of Pregnant Women and Risk of Clinical Pregnancy Loss. Environ Sci Technol. 2015 Sep 1;49(17):10651-7. doi: 10.1021/acs.est.5b02617.

[214] Yi H, Gu H, Zhou T, Chen Y, Wang G, Jin Y, Yuan W, Zhao H, Zhang L. A pilot study on association between phthalate exposure and missed miscarriage. Eur Rev Med Pharmacol Sci. 2016 May;20(9):1894-902.

[215] Peng F, Ji W, Zhu F, Peng D, Yang M, Liu R, Pu Y, Yin L. A study on phthalate metabolites, bisphenol A and nonylphenol in the urine of Chinese women with unexplained recurrent spontaneous abortion. Environ Res. 2016 Oct;150:622-628. doi: 10.1016/j.envres.2016.04.003.

[216] Liao KW, Kuo PL, Huang HB, Chang JW, Chiang HC, Huang PC. Increased risk of phthalates exposure for recurrent pregnancy loss in reproductive-aged women. Environ Pollut. 2018 Oct;241:969-977. doi: 10.1016/j.envpol.2018.06.022.

[217] Gao H, Zhang YW, Huang K, Yan SQ, Mao LJ, Ge X, Xu YQ, Xu YY, Sheng J, Jin ZX, Zhu P, Tao XG, Hao JH, Tao FB. Urinary concentrations of phthalate metabolites in early pregnancy associated with clinical pregnancy loss in Chinese women. Sci Rep. 2017 Jul 28;7(1):6800. doi: 10.1038/s41598-017-06450-2.

[218] Chang WH, Chou WC, Waits A, Liao KW, Kuo PL, Huang PC. Cumulative risk assessment of phthalates exposure for recurrent pregnancy loss in reproductive-aged women population using multiple hazard indices approaches. Environ Int. 2021 Sep;154:106657. doi: 10.1016/j.envint.2021.106657. Epub 2021 May 27. PMID: 34052604

[219] Messerlian C, Wylie BJ, Mínguez-Alarcón L, Williams PL, Ford JB, Souter IC, Calafat AM, Hauser R; Earth Study Team. Urinary Concentrations of Phthalate Metabolites and Pregnancy Loss Among Women Conceiving with Medically Assisted Reproduction. Epidemiology. 2016 Nov;27(6):879-88. doi: 10.1097/EDE.0000000000000525. PMID: 27299194; PMCID: PMC5248552.

[220] Zhang Y, Mustieles V, Yland J, Braun JM, Williams PL, Attaman JA, Ford JB, Calafat AM, Hauser R, Messerlian C. Association of Parental Preconception Exposure to Phthalates and Phthalate Substitutes

With Preterm Birth. JAMA Netw Open. 2020 Apr 1;3(4):e202159. doi: 10.1001/jamanetworkopen.2020.2159

[221] WHO (World Health Organization), 2018. http://www.who.int/mediacentre/factsheets/ fs363/en/.

[222] Chang HH, Larson J, Blencowe H, Spong CY, Howson CP, Cairns-Smith S, Lackritz EM, Lee SK, Mason E, Serazin AC, Walani S, Simpson JL, Lawn JE; Born Too Soon preterm prevention analysis group. Preventing preterm births: analysis of trends and potential reductions with interventions in 39 countries with very high human development index. Lancet. 2013 Jan 19;381(9862):223-34. doi: 10.1016/S0140-6736(12)61856-X.

[223] Gyamfi-Bannerman C. The scope of the problem: the epidemiology of late preterm and early-term birth. Semin Perinatol. 2011 Oct;35(5):246-8. doi: 10.1053/j.semperi.2011.05.013

[224] Quigley MA, Poulsen G, Boyle E, Wolke D, Field D, Alfirevic Z, Kurinczuk JJ. Early term and late preterm birth are associated with poorer school performance at age 5 years: a cohort study. Arch Dis Child Fetal Neonatal Ed. 2012 May;97(3):F167-73. doi: 10.1136/archdischild-2011-300888.

[225] Reddy UM, Bettegowda VR, Dias T, Yamada-Kushnir T, Ko CW, Willinger M. Term pregnancy: a period of heterogeneous risk for infant mortality. Obstet Gynecol. 2011 Jun;117(6):1279-1287. doi: 10.1097/AOG.0b013e3182179e28.

[226] McElrath TF, Hecht JL, Dammann O, Boggess K, Onderdonk A, Markenson G, Harper M, Delpapa E, Allred EN, Leviton A; ELGAN Study Investigators. Pregnancy disorders that lead to delivery before the 28th week of gestation: an epidemiologic approach to classification. Am J Epidemiol. 2008 Nov 1;168(9):980-9. doi: 10.1093/aje/kwn202.

[227] Ferguson KK, McElrath TF, Meeker JD. Environmental phthalate exposure and preterm birth. JAMA Pediatr. 2014 Jan;168(1):61-67. doi: 10.1001/jamapediatrics.2013.3699.

[228] Alsubaie AM, Arita Y, Atwater M, Mahfuz A, Peltier MR. Enhancement of placental inflammation by Dibutyl Phthalate. J Reprod Immunol. 2021 Sep;147:103368. doi: 10.1016/j.jri.2021.103368. Epub 2021 Aug 24. PMID: 34461555

[229] Wang JQ, Hu YB, Gao H, Sheng J, Huang K, Zhang YW, Mao LJ, Zhou SS, Cai XX, Zhang LJ, Wang SF, Hao JH, Yang LQ, Tao FB. Sex-specific difference in placental inflammatory transcriptional biomarkers of maternal phthalate exposure: a prospective cohort study. J Expo Sci Environ Epidemiol. 2020 Sep;30(5):835-844. doi: 10.1038/s41370-020-0200-z. Epub 2020 Feb 3. PMID: 32015430.

[230] Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008 Jan 5;371(9606):75-84. doi: 10.1016/S0140-6736(08)60074-4.

[231] Ferguson KK, Rosen EM, Rosario Z, Feric Z, Calafat AM, McElrath TF, Vélez Vega C, Cordero JF, Alshawabkeh A, Meeker JD. Environmental phthalate exposure and preterm birth in the PROTECT birth cohort. Environ Int. 2019 Nov;132:105099. doi: 10.1016/j.envint.2019.105099.

[232] Zhong Q, Liu HL, Fu H, Niu QS, Wu HB, Huang F. Prenatal exposure to phthalates with preterm birth and gestational age: A systematic review and meta-analysis. Chemosphere. 2021 Nov;282:130991. doi: 10.1016/j.chemosphere.2021.130991.

[233] Ferguson KK, Chen YH, VanderWeele TJ, McElrath TF, Meeker JD, Mukherjee B. Mediation of the Relationship between Maternal Phthalate Exposure and Preterm Birth by Oxidative Stress with Repeated Measurements across Pregnancy. Environ Health Perspect. 2017 Mar;125(3):488-494. doi: 10.1289/EHP282.

[234] Ferguson KK, Rosen EM, Barrett ES, Nguyen RHN, Bush N, McElrath TF, Swan SH, Sathyanarayana S. Joint impact of phthalate exposure and stressful life events in pregnancy on preterm birth. Environ Int. 2019 Dec;133(Pt B):105254. doi: 10.1016/j.envint.2019.105254.

[235] Shoaff JR, Romano ME, Yolton K, Lanphear BP, Calafat AM, Braun JM. Prenatal phthalate exposure and infant size at birth and gestational duration. Environ Res. 2016 Oct;150:52-58. doi: 10.1016/j.envres.2016.05.033.

[236] Hu JMY, Arbuckle TE, Janssen P, Lanphear BP, Braun JM, Platt RW, Chen A, Fraser WD, McCandless LC. Associations of prenatal urinary phthalate exposure with preterm birth: the Maternal-Infant Research on Environmental Chemicals (MIREC) Study. Can J Public Health. 2020 Jun;111(3):333-341. doi: 10.17269/s41997-020-00322-5.

[237] Santos S, Sol CM, van Zwol-Janssens C, Philips EM, Asimakopoulos AG, Martinez-Moral MP, Kannan K, Jaddoe VWV, Trasande L. Maternal phthalate urine concentrations, fetal growth and adverse birth outcomes. A population-based prospective cohort study. Environ Int. 2021 Jun;151:106443. doi: 10.1016/j.envint.2021.106443.

[238] Gao H, Wang YF, Huang K, Han Y, Zhu YD, Zhang QF, Xiang HY, Qi J, Feng LL, Zhu P, Hao JH, Tao XG, Tao FB. Prenatal phthalate exposure in relation to gestational age and preterm birth in a prospective cohort study. Environ Res. 2019 Sep;176:108530. doi: 10.1016/j.envres.2019.108530.

[239] Broe A, Pottegård A, Hallas J, Ahern TP, Lamont RF, Damkier P. Phthalate exposure from drugs during pregnancy and possible risk of preterm birth and small for gestational age. Eur J Obstet Gynecol Reprod Biol. 2019 Sep;240:293-299. doi: 10.1016/j.ejogrb.2019.07.023.

[240] Zhang Y, Mustieles V, Williams PL, Wylie BJ, Souter I, Calafat AM, Demokritou M, Lee A, Vagios S, Hauser R, Messerlian C. Parental preconception exposure to phenol and phthalate mixtures and the risk of preterm birth. Environ Int. 2021 Jun;151:106440. doi: 10.1016/j.envint.2021.106440.

[241] Wu Y, Wang J, Wei Y, Chen J, Kang L, Long C, Wu S, Shen L, Wei G. Maternal exposure to endocrine disrupting chemicals (EDCs) and preterm birth: A systematic review, meta-analysis, and meta-regression analysis. Environ Pollut. 2022 Jan 1;292(Pt A):118264. doi: 10.1016/j.envpol.2021.118264. Epub 2021 Oct 1. PMID: 34606968.

[242] Yland JJ, Zhang Y, Williams PL, Mustieles V, Vagios S, Souter I, Calafat AM, Hauser R, Messerlian C; Earth Study Team. Phthalate and DINCH urinary concentrations across pregnancy and risk of preterm birth. Environ Pollut. 2022 Jan 1;292(Pt B):118476. doi: 10.1016/j.envpol.2021.118476. Epub 2021 Nov 8. PMID: 34763012.

5.2 ARTIGO 2: USE OF MILD ANALGESICS AND REPRODUCTIVE OUTCOMES OF WOMEN UNDERGOING ASSISTED REPRODUCTION.

Artigo aborda a influência da exposição a analgésicos de venda livre nos desfechos reprodutivos de pacientes em tratamento de reprodução assistida. Escrito nas normas do periódico Human Reproduction

Use of mild analgesics and reproductive outcomes of women undergoing assisted reproduction.

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ABSTRACT

Study question: Does female mild analgesics consumption previous or during assisted reproduction treatment influence on reproductive outcomes?

Summary answer: Consumption of paracetamol and dipyrone during assisted reproduction treatment was negatively associated with embryo quality and collection rate, respectively.

What is known already: Studies have shown that mild analgesics can disturb the reproductive system and act as potential endocrine-disrupting chemicals. Epidemiological studies also indicate that analgesics consumption could be related to measures of human fecundity, being associated with longer time to pregnancy. However, data on literature still is conflicted and taken the biological plausibility for endocrine and reproductive effects of analgesic medications, further research is needed to investigate their effects.

Study design, size, duration: This is a prospective cohort study that comprises data of 98 reproductive aged women (n=125 cycles of fertility treatment) who were recruited during their infertility treatment, between 2018 and 2021 in the city of Curitiba in South Brazil.

Participants/materials, setting, methods: There were 98 reproductive aged women (20-40 years) included in our study, comprising 125 cycles of infertility treatment. Participants were diagnosed with infertility and had indication to undergo high complexion assisted reproduction treatment. They responded to baseline and follow-up questionnaires regarding their medication use prior or during the fertility treatment, being questioned specifically over their consumption of paracetamol, dipyrone, ibuprofen, acetylsalicylic acid and sodium diclofenac - which are the most commonly used mild analgesics by the general Brazilian population. Reproductive outcomes information includes number of retrieved oocytes, collection rate, maturation rate, fertilization rate, quality embryo rate, blastulation rate, utilization rate and clinical outcomes. General linear models and logistic regression were used to estimate the associations between each analgesic use and the continuous and categorical reproductive outcomes, respectively.

Main results and the role of chance: In our study, the use of mild analgesics in both time periods was associated with some of the reproductive outcomes in women trying to conceive. Previous use of paracetamol and dipyrone was positively associated with the maturation rate and utilization rate, respectively. However, when the model was adjusted to indication of use the associations were no longer significant. Previous use of dipyrone also shown a negative association with collection rate in the model adjusted to indication of use. Regarding the use of mild analgesics during the controlled ovarian hyperstimulation (COH) protocol, we observed that paracetamol use was negatively associated with the embryo quality rate in all models tested. Also, the use of dipyrone was positively associated with the embryo blastulation rate, and ibuprofen use was positively associated with collection rate and negatively associated with fertilization rate, but these associations were not sustained in the model adjusted for indication of use.

Limitations, reasons for caution: Although our study have accounted for confounding by indication, the infertility conditions of the patients in our study may result in the use of mild analgesics and both the medical conditions per se and the resulting use of analgesics have a potential to directly affect the outcomes of our study. Also, the interpretation of our findings in relation to the literature and other populations is challenging due to the lack of data regarding associations between mild analgesics exposure during the preconception period, especially in assisted reproduction settings, and reproductive outcomes

Wider implications of the findings: The high prevalence of analgesic use among women seeking medically assisted reproduction both in the period before infertility treatment and during the IVF procedure, as well as certain associations between such exposures and assisted reproduction outcomes, warrant further research in this particular realm

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Trial registration number: N/A

Key words: Mild analgesics, assisted reproduction, reproductive outcomes, paracetamol, dipyrone, embryo quality, endocrine disruptors, infertility.

Introduction

Pain relievers are currently the most used pharmaceutical drugs worldwide (Kristensen et al., 2016), with estimations of regular use by approximately 12% of the adult population (Zhou et al., 2014). The extensive consumption of these pharmaceuticals could be due to their high prescription rates, but also to their over-the-counter distribution, which leads consumers to not always identify mild analgesics as drugs (Kristensen et al., 2011, Hassoun-Barhamji et al., 2015). Mild analgesics include NSAIDs - as acetylsalicylic acid and ibuprofen - paracetamol (acetaminophen), and dipyrone (metamizole), and despite their high prescription and intake rate, recent studies indicate that several organ systems, including the reproductive and endocrine systems, might be vulnerable to these medications (Brune et al., 2015, Roberts et al., 2016, Zafeiri et al., 2021, Patel et al., 2022). NSAIDs act as analgesics by inhibiting the enzymes that synthesize prostaglandins (Cashman, 1996). Therefore, it is not surprising that they can affect the reproductive capacity, as prostaglandins are essential for reproduction, being involved in several reproductive processes, including ovulation and implantation (Agrawal and Alvin Jose., 2009, Sehring et al., 2022). Also, a number of studies have indicated that NSAIDs and other mild analgesics can disturb male and female reproductive development by acting as endocrine-disrupting chemicals (Ben Maamar et al., 2017; Kristensen et al., 2011, 2012, 2016; van den Driesche et al., 2015).

In contrast to NSAIDs, paracetamol and dipyrone do not exhibit marked anti-inflammatory effects, as the inhibition of cyclooxygenase (COX) enzyme activity by these drugs appears to be limited to non-inflamed tissues (Botting., 2000; Ouellet and

Percival, 2001; Rezende et al., 2008; Graham et al., 2013). However, inhibition of prostaglandin biosynthesis alone does not appear to be the sole mechanism for the analgesic actions of paracetamol and dipyrone (Rezende et al., 2008; Graham et al., 2013). Experimental data indicates that paracetamol has the potential to act as an antiandrogen by inhibiting testosterone production, which may result in adverse reproductive effects (Kristensen et al., 2011, 2012; van den Driesche et al., 2015). Likewise, *in vitro* and *in vivo* data indicate that dipyrone and its main metabolites may have the potential to disrupt steroidogenesis (Passoni et al., 2018; Passoni et al., 2022). Therefore, it is possible that NSAIDs and mild analgesics can alter the endocrine control of reproduction and consequently affect fertility and reproductive capacity through several different mechanisms.

In recent years, there has been increasing concerns on a possible temporal decline in human reproductive health and the role of environmental factors, including exposure to industrial chemicals and pharmaceuticals, in negative trends in human fertility (Skakkebaek et al., 2022; Levine et al., 2017). On the other hand, the use of medically assisted reproduction has substantially increased over the recent years (Skakkebaek et al., 2022). A few epidemiological studies indicate that analgesics consumption could be related to measures of human fecundity. Smarr and colleagues (2016) reported that the highest quartile (>73.47 ng/ml) of urinary concentrations of paracetamol in men were associated with longer time to pregnancy in a partner-specific and couple-based models' prospective study, although the same was not observed for female participants. Nevertheless, the literature is still conflicted regarding the effects of these drugs on the reproductive capacity of men and women. Use of non-aspirin NSAIDs or paracetamol were not associated with fecundability of women trying to conceive in any of the three different menstrual cycle windows – pre-ovulation, peri-ovulation and implantation (Jukic et al., 2020). Also, Akande and colleagues (2006) described that the consumption of diclofenac (100mg) by patients undergoing infertility treatment at the time of egg collection does not appear to affect implantation or pregnancy rates. To date, little is known about the effects of pain-relieving medication use on human fertility and, in particular, on the outcomes of medically assisted reproduction. Yet given the large percentage of reproductive age women who take over-the-counter pain medications, and the biological plausibility for endocrine and reproductive effects of analgesic medications, further research is needed to investigate the use of NSAIDs, paracetamol

and other pain-relieving medications and their associations with reproductive capacity. Therefore, the objective of this work is to evaluate the effect of analgesics consumption on reproductive outcomes of women undergoing infertility treatment.

Material and methods

Study population

Study population comprises 98 reproductive aged women (n=125 cycles of fertility treatment) who were recruited during their infertility treatment. The patients were recruited in a fertility clinic in the city of Curitiba in South Brazil, between 2018 and 2021. Women aged 20–40 years who were diagnosed with infertility and had indication to undergo high complexion assisted reproduction treatment (in vitro fertilization – IVF - or intracytoplasmatic sperm injection - ICSI) were eligible to participate and approximately 55% of those contacted by the research staff signed up to the study. Participants were enrolled previously to their controlled ovarian hyperstimulation (COH) protocol and followed through their treatment. We enrolled only women undergoing treatment for female factor infertility, excluding patients in treatment for severe male infertility. Further exclusion criteria included use of oocyte donor or cryopreserved gametes during treatment. Participants completed a survey with a baseline questionnaire, providing detailed data on demographic, lifestyle, and behavioral factors; anthropometrics; and reproductive and medical history.

Ethical approval

Institutional review board approvals were obtained from all collaborating institutions. Patients gave written informed consent prior to study participation and any data collection.

Ovarian hyperstimulation protocol

To reduce possible confounding by treatment protocol, all enrolled patients were treated with controlled ovarian stimulation using Gonadotrophin-releasing hormone (GnRH) antagonist (Orgalutran; Schering-plough) protocol. For ovarian stimulation, a daily dose of recombinant follicle-stimulating hormone (FSH, Gonal-F, Merck-Serono or Puregon, Merck Sharp & Dohme) followed by purified human menopausal gonadotrophin (hMG, Menopur; Ferring) was used starting on the third day of the menstrual cycle. The dose administered was dependent on the age, body mass index (BMI), and treatment history.

Patients were monitored during gonadotropin stimulation for serum estradiol, follicle size measurements and counts, and endometrial thickness. Human chorionic gonadotropin (hCG) (Ovidrelle, Merck Serono) or GnRH agonist (Gonapeptyl Daily; Ferring) were administered approximately 36 h before the scheduled oocyte retrieval.

Assessment of medication usage

On the baseline and follow-up questionnaires (Fig.1), women were asked whether they had used any medications in the 12 months prior to the start of the fertility treatment (baseline questionnaire) or during the fertility treatment protocol (follow-up questionnaires). In baseline questionnaire, participants were asked over their pharmaceutical consumption overall and specifically over their consumption of paracetamol, dipyrone, ibuprofen, acetylsalicylic acid, and sodium diclofenac - which are the most commonly used mild analgesics by the general Brazilian population (Moreira de Barros et al., 2019). The patients who reported using analgesics were asked to provide indication of use, frequency of consumption, and number of pills taken for each medication. Regarding the follow-up questionnaires, during the CHO protocol, the patients completed a medication diary recording their daily medication intake, comprising type, frequency, dose and indication of use of analgesics and any other medication. Participants were also asked to carefully report any consumption of the five selected analgesics (paracetamol, dipyrone, ibuprofen, acetylsalicylic acid and sodium diclofenac), during the COH protocol.

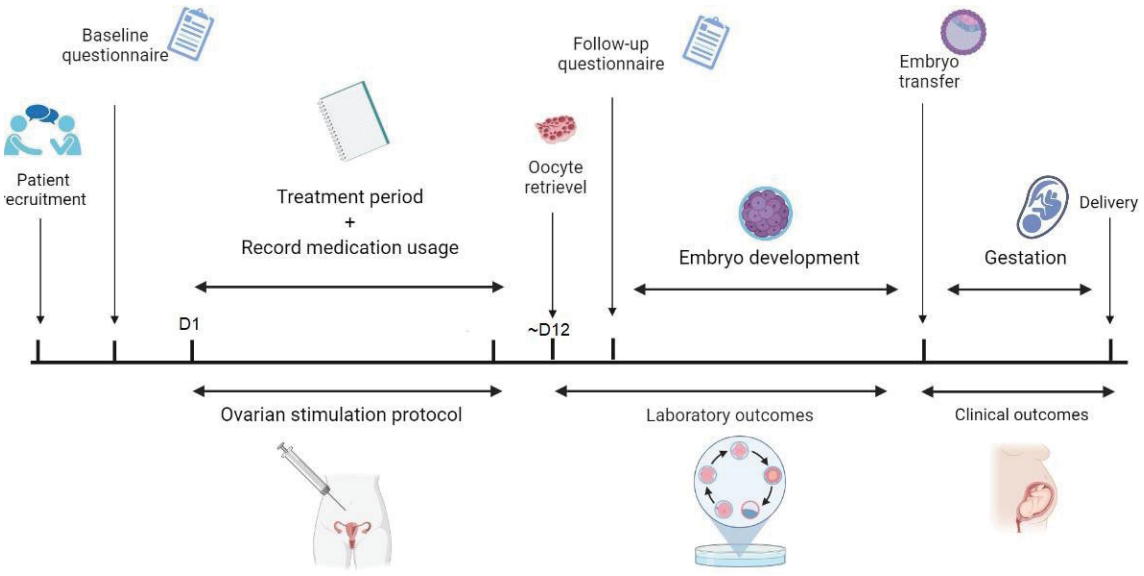

Figure 1. Timeline of data collection.

Assessment of reproductive outcomes

Laboratorial and clinical outcomes information was abstracted from the patient's electronic medical record by the research staff. The reproductive data collection initiated at the time of oocyte retrieval (Fig 1). The number of follicles was registered, and the number of retrieved oocytes was used to calculate the collection rate (number of oocytes/number of follicles). Embryologists classified oocytes as germinal vesicle, metaphase I, metaphase II (MII), or degenerated oocytes, and the number of oocytes in metaphase II was used to determine the maturation rate (number of oocytes in metaphase II/total number of oocytes). Fertilization was determined 16 to 18 hours after ICSI or IVF– and a normal fertilization comprised an injected egg cell with the presence of two pronuclei and two polar bodies. The fertilization rate of each patient was recorded as the number of normal fertilizations/ total number of injected oocytes. Embryos were further assessed on day 3 for cell number, symmetry, and fragmentation. High quality embryos were classified as those with > 8 symmetric cells and < 10% fragmentation, being recorded the quality embryo rate (number of high-quality embryos/total number of embryos) (Alpha Scientists, 2011). Embryo assessment was also performed at day 5 for control of blastulation, being recorded the blastulation rate (number of blastocysts/ total number of embryos). We also recorded the utilization rate, which considered the number of transferred, frozen or biopsied embryos in relation to the total number of embryos (number of utilized embryos/ total number of embryos). For clinical outcomes, positive β-hCG (i.e., successful implantation) was defined as a serum β-hCG level > 25 mIU/ mL, typically measured 12 days after embryo transfer. Clinical pregnancy was defined as the presence of an intrauterine gestational sac and fetal heartbeat confirmed by ultrasound by 7 weeks of gestation, and live birth as the delivery of a live neonate. Miscarriage, biochemical and ectopic pregnancies were recorded.

Statistical analysis

Descriptive statistics for continuous and categorical variables and demographic data were presented as mean and/or frequency and were compared between users and non-users of all the assessed analgesics. Chi-square tests were used to evaluate significance for all categorical variables. Participants were permitted to contribute with more than one cycle of infertility treatment. We categorized each analgesic medication use as a dichotomous variable (use versus non-use). General linear models were used to estimate the associations between each analgesic use and the continuous reproductive outcomes

(laboratorial outcomes). Logistic regression was used to estimate the associations between each analgesic use and the categorical reproductive outcomes (clinical outcomes). The reference group for all analyses was non-use of analgesic medications (being considered only the use of each specific analgesic in the model). Potential confounders were identified using prior data found in literature (with a focus on variables consistently related to ART outcomes and analgesics consumption) and descriptive statistics from our cohort. Each confounder was previously tested in the model to analyze its potential influence: age (continuous), race (white/black/other), BMI (continuous), smoking status (nonsmoker or current or previous smoker), and infertility cause (anatomical; ovarian insufficiency; endocrine (PCOS); endometrioses; unexplained (ESCA); or others). These potential confounders were considered indicators of both analgesic medications use and result of reproductive outcomes, and the adjusted model included age, BMI and infertility cause (model I). Also, the indication of use of each medication was included as an additional covariate to account for confounding by indication errors (model II). Indication of use was categorized for each medication, being selected the categories: headache, pain, cramps, fever, colds, or other reason of use. Considering better adjustment of the model, the 2 most frequent indications of use of each medication were taken into consideration (Table 1). All analyses were carried out using SPSS version 22 (IBM, Armonk, NY: IBM Corp. USA).

Table 1. Indication of use reported by the study participants.

Previous use[a]		**Use during COH[b]**	
	N (%)		N (%)
Paracetamol			
Headache	43 (49%)	Pain	23(67%)
Pain	33 (38%)	Cramp	6(18%)
Other	11(13%)	Other	5(15%)
Dipyrone			
Headache	36(66%)	Pain	8(61.5%)
Pain	9(16%)	Cold	4(31%)
Other	10(18%)	Other	1(7.5%)
Acetylsalicylic acid			
Headache	18(53%)	Infertility[c]	35 (94.5%)
Pain	1(3%)	Pain	2(5.5%)

Infertility	4(11%)		
Other	11(32%)		
Ibuprofen			
Cramps	49(80%)	Cramps	29 (96%)
Pain	11(18%)	Others	1(4%)
Other	1(2%)		
Diclofenac			
Pain	10(67%)		
Other	5(33%)		

[a] Use of analgesics in the 12 months prior to the start of the fertility treatment.

[b] Use of analgesics during the Controlled Ovarian Hyperstimulation (COH) protocol.

*Indication for infertility includes doctor recommendations and miscarriage prevention treatment.

Results

Sociodemographic and lifestyle characteristics of study population

Among the 127 recruited patients, 98 (77%) completed all study stages and 29 women (23%) were lost to follow-up after completing one or more study questionnaires. General data on our study population are shown in Table 2. The cohort comprised mostly Caucasian women (81.6%) with high household incomes (72.4% above 6 minimum wages) and high education (70.4% with college degree). Females in our study tended to be older (48% aged 36 - 40 years), had low body mass index (BMI) and a low prevalence of smoking (69.4% never smoked). The mean age (± standard deviation) at enrollment was 34.8 ± 3.9 years. The mean BMI (± standard deviation) was 23.9 kg/m2 (±4.8) and 11.2 % of women were obese (BMI ≥ 30.0 kg/m2). Concerning the infertility status, almost all patients declared primary infertility (89,8%). Patients present varied causes of infertility, being categorized as ovary insufficiency (21.4%), anatomical (18.4%), endocrine (20.4%), endometrioses (17.4%), unexplained infertility (7.1%), and others (15.3%) – including genetic disorders, low sexual frequency, and immunological disorders.

Table 2. Sociodemographic characteristics of study participants

Variable	Mean ± SD or N (%)[a]
Number of participants	98
Age	34.8±3.99
< 31 years (%)	14 (14.3%)
31-35 years (%)	37 (37.7%)
36-40 years (%)	47 (48%)
BMI	23.9±4.8
<18.5 - 24.9 (%)	62 (63.3%)
25 – 29.9 (%)	25 (25.5%)
> 30 (%)	11 (11.2%)
Race/Ethinicity	
White (%)	80 (81.6%)
Other (%)	18 (18.4%)
Education	
< College Degree (%)	29 (29.6%)
≥ College Degree (%)	69 (70.4%)
Household income	
< 6 minimum wage	27 (27.6%)
6-10 minimum wage	38 (38.8%)
> 10 minimum wage	33 (33.6%)
Smoker	
Never smoker (%)	68 (69.4%)
Current or Previous smoker (%)	9 (9.2%)
Passive smoker (%)	21 (21.4%)
Infertility	
Primary (%)	88 (89.8%)
Secondary (%)	10 (10.2%)
Anatomical (%)	18 (18.4%)
Ovarian insufficiency (%)	21 (21.4%)
Endocrine (PCOS)(%)	20 (20.4%)
ESCA (%)	7 (7.1%)
Endometrioses (%)	17 (17.3%)
Others (%)	15 (15.3%)

[a]Data on continuous variables are presented as mean ± standard deviation and categorical data as counts and percentage.

Analgesic use

The use of at least one analgesic one year previous to the IVF treatment was reported by 84.7% of participants (Table 3). In this study we specifically asked about the use of paracetamol, dipyrone, ibuprofen, acetylsalicylic acid (AAS), and diclofenac. Paracetamol was by far the most used analgesic (69.4%), followed by ibuprofen (50%) and dipyrone (41.8%). AAS and diclofenac were used by only 27.6% and 13.3% of study participants, respectively. From the 98 patients that concluded the study 125 cycles were analyzed. In addition to assessment of analgesic use in 12 months prior to IVF treatment, we also recorded the medication consumption during the COH protocol, from day 1 (D1) until the oocyte collection date (~D12), comprehending the period of beginning to the end of COH protocol. During the COH protocol, 65.5% of the patients reported the consumption of at least one analgesic. Again, the participants were asked to specifically report the use of paracetamol, dipyrone, ibuprofen, acetylsalicylic acid (AAS), and diclofenac. AAS was the most used analgesic (39.6%) during the cycle induction followed by paracetamol (26.4%) and ibuprofen (24%). Dipyrone had a lower consumption (10.4%) and no patient reported the consumption of diclofenac during the cycle treatment. We analyzed the consumption between the different sociodemographic groups and no significant difference was found (Data not shown).

Table 3. Use of mild analgesics of study participants

Variable	**N (%)**
Participants	98
Previous analgesic consumption[a]	
Yes	83(84.7%)
No	15(15.3%)
Paracetamol	68(69.4%)
Dypirone	41(41.8%)
Ibuprofen	49(50%)
Acetilsalicilic acid	27(27.6%)
Diclofenac	13(13.3%)
Cycles included	125

COH analgesic consumption[b]	
Yes	82(65.6%)
No	43(34.4%)
Paracetamol	22(26.4%)
Dypirone	13(10.4%)
Ibuprofen	30(24%)
Acetilsalicilic acid	37(29.6%)
Diclofenac	-

[a] Consumption of any of the five most commonly used analgesics (paracetamol, dipyrone, ibuprofen, acetylsalicylic acid, or diclofenac) in the 12 months prior to the start of the fertility treatment.

[b] Consumption of any of the five most commonly used analgesics (paracetamol, dipyrone, ibuprofen, acetylsalicylic acid, or diclofenac) during the Controlled Ovarian Hyperstimulation (COH) protocol.

Reproductive outcomes

The outcomes of 125 cycles were analyzed. The mean rates observed for the reproductive outcomes followed normal rates expected for assisted reproduction procedures (ESHRE, 2017) and of the 125 cycles 107 resulted in embryo transfer (85.6%). The cycles that did not result in embryo transfer were due to no embryo development or no viable embryo available for transfer. In our study 53 (49.5%) patients presented a positive pregnancy test, 44 (41%) a clinical pregnancy, 34 (31.7%) deliveries and 6 (5.6%) ongoing pregnancies (Table 4).

Table 4. Reproductive outcomes of study participants.

Reproductive outcomes	**Mean ± SD or %(N)[a]**
Cycles included	125
Number of follicles	12.2 ±6.3
Number of oocytes	9.1 ±6.1
Collection rate	73%
Maturation rate	78%
Fertilization rate	82%
Quality rate	65.5%
Blastulation rate	37.4%
Utilization rate	46.6%
Transfer rate	85.6% (107)
BHCG positive	49.5% (53)
Clinical gestation	41% (44)

Bioquemical pregnancy	8.5% (9)
Ectopic pregnancy	0% (0)
Miscarriage	3.7% (4)
Deliverys	31.7% (34)
Ongoing pregnancy	5.6% (6)

[a]Data on continuous variables are presented as mean ± standard deviation and categorical data as counts (when applied) and mean percentage.

Analgesic use as predictor reproductive outcomes

Previous analgesic use

The analgesic use was assessed as a predictor of the reproductive outcomes, separately for each drug. In the unadjusted model we observed significant associations between the use of paracetamol, dipyrone and diclofenac and some reproductive outcomes. The use of paracetamol was positively associated with the maturation rate in the unadjusted model [p=0.018; 11.76 (2.08 to 21.45)], but also after adjustment for confounders (age, BMI, infertility cause) [p= 0.018; 12.17(-2.10 to 22.23)] (Model I). However, when the model was adjusted to indication of use (Model II), we no longer observed a significant association for this outcome [p=0.40; 6.99(-9.53 to 23.52)] (Table 5). The use of dipyrone was positively associated with the utilization rate [p=0.03; 9.89(0.84 to 18.93)], however in the adjusted models this association was no longer observed (Table 6). Also, a negative association was observed between dipyrone consumption and collection rate [p=0.01; -19.02(-33.32 to -4.73)] when the model was adjusted to indication of use (Model II) (Table 6). The use of diclofenac was negatively associated with the maturation rate in the unadjusted model [p=0.01; -18.13(-32.65 to 3.61)]. This association was still significant in the model I [p=0.01; -18.04(-32.67 to 3.42)], but not after adjustment for indication of use (Table 7). No significant associations were observed for AAS or Ibuprofen use (Data not shown). We also analyzed the possible associations between the use of AINEs and analgesics with clinical reproductive outcomes, however no significant results were observed (Data not shown).

Table 5. Previous use of paracetamol and reproductive outcomes of participants undergoing infertility treatment.

Variables	Crude B (95% CI)	p-value	Model I B (95% CI)[a]	p-value	Model II B (95% CI)[b]	p-value
Nº follicules	-1.85(-4.30 - 0.60)	0.138	-2.19(-4.64 - 0.26)	0.080	-0.54(-4.56 - 3.48)	0.790
Nºoocytes	0.23 (-2.09 - 2.65)	0.814	0.25(-2.18 - 2.69)	0.836	0.15(-3.86 - 4.16)	0.941
Collection rate	8.80(-0.94 - 18.53)	0.076	9.86(-0.12 - 19.84)	0.053	-2.15(-18.34 - 14.05)	0.793
Maturation rate	11.76(2.08 - 21.45)	0.018*	12.17(-2.10 - 22.23)	0.018*	6.99(-9.53 - 23.52)	0.404
Fertilization rate	1.79(-7.82 - 11.39)	0.713	2.09(-7.60 - 11.78)	0.670	5.43(-10.50 - 21.37)	0.501
Quality rate	-7.43(-18.09 - 3,24)	0.171	-8.02(-19.04 - 3.01)	0.152	4.77(-13.15 - 22.68)	0.599
Blastulation rate	0.15(-9.69 - 9.99)	0.977	0.19(-9.67 - 10.05)	0.970	8.43(-7.68 - 24.55)	0.302
Utilization rate	3.83(-6.03 - 13.70)	0.443	4.31(-5.65 - 14.27)	0.393	7.03(-9.36 - 23.41)	0.397

Self-reported use of paracetamol (yes/no) 12 months before enrollment in the study. [a]Model I: adjusted for age, BMI and cause of infertility. [b]Model II: adjusted for all covariates of Model I and for self-reported indication of use. *p-value was considered significant when $p \leq 0.05$. CI: confidence interval.

Table 6. Previous use of dipyrone and reproductive outcomes of participants undergoing infertility treatment.

Variables	Crude B (95% CI)	p-value	Model I B (95% CI)[a]	p-value	Model II B (95% CI)[b]	p-value
Nº follicules	0.46(-1,84 - 2.77)	0.69	0.77(-1.56 - 3.11)	0.51	1.40(-2.20 - 5.01)	0.44
Nºoocytes	-0.93(-3.13 - 1.27)	0.40	-0.61(-2.90 - 1.68)	0.60	-2.62(-6.13 - 0.88)	0.14
Collection rate	-6.99(-16.10 - 2.11)	0.13	-4.73(-14.26 - 4.78)	0.32	-19.02(-33.32 - -4.73)	0.01*
Maturation rate	-2.92(-12.15 - 6.30)	0.53	-4.79(-14.48 - 4.88)	0.32	-4.23(-19.18 - 10.71)	0.57
Fertilization rate	7.42(-1.43 - 16.28)	0.09	4.86(-4.24 - 13.96)	0.29	-0.59(-14.58 - 13.40)	0.93
Quality rate	-2.31(-12.32 - 7.70)	0.64	-2.58(-13.06 -7.89)	0.62	10.92(-4.87 - 26.81)	0.17
Blastulation rate	1.79(-7.37 - 10.96)	0.70	-0.33(-9.62 - 8.96)	0.94	7.66(-6.55 - 21.88)	0.28
Utilization rate	9.89(0.84 - 18.93)	0.03*	6.78(-2.56 - 16.12)	0.15	5.24(-9.17 - 19.66)	0.47

Self-reported use of dipyrone (yes/no) 12months before enrollment in the study. [a]Model I: adjusted for age, BMI and cause of infertility. [b]Model II: adjusted for all covariates of Model I and for self-reported indication of use. *p-value was considered significant when $p \leq 0.05$. CI: confidence interval.

Table 7. Previous use of diclofenac and reproductive outcomes of participants undergoing infertility treatment.

Variables	Crude B (95% CI)	p-value	Model I B (95% CI)[a]	p-value	Model II B (95% CI)[b]	p-value
Nº follicules	-1.66(-5.36 - 2.04)	0.37	-1.65(-5.25 - 1.95)	0.36	-3.61(-16.74 - 9.50)	0.58
Nºoocytes	-3.34(-6.85 - 0.16)	0.06	-3.34(-6.83 - 0.14)	0.06	-3.03(-15.75 - 9.68)	0.63
Collection rate	-11.65(-26.30 - 2.99)	0.11	-11.83(-26.42 - 2.76)	0.11	0.06(-53.08 - 53.21)	0.99
Maturation rate	-18.13(-32.65 - 3.61)	0.01*	-18.04(-32.67 - 3.42)	0.01*	-34.30(-87.52 - 18.92)	0.20
Fertilization rate	-3.31(-11.09 - 17.72)	0.65	-3.82(-10.26 - 17.91)	0.59	-33.87(-84.73 - 16.97)	0.19
Quality rate	-1.25(-14.87 - 17.38)	0.97	-1.43(-14.74 - 17.61)	0.86	-17.02(-75.89 - 41.84)	0.56
Blastulation rate	-8.84(-23.53 - 5.83)	0.23	-8.35(-22.61 - 5.89)	0.24	12.74(-39.06 - 64.55)	0.62
Utilization rate	-3.56(-18.39 - 11.26)	0.63	-3.10(-17.63 - 11.41)	0.67	-10.26(-63.18 - 42.66)	0.70

Self-reported use of diclofenac (yes/no) 12 months before enrollment in the study. [a]Model I: adjusted for age, BMI and cause of infertility. [b]Model II: adjusted for all covariates of Model I and for self-reported indication of use. *p-value was considered significant when $p \leq 0.05$. CI: confidence interval.

COH analgesic use

All patients selected for the study underwent a cycle of gonadotropin administration for COH. During the CHO protocol, the patients completed a medication diary for recording the use of analgesics and any other medication. The analgesic consumption during this protocol was assessed as a predictor of the reproductive outcomes for each medication. The report of paracetamol use during COH was negatively associated with the embryo quality rate in the unadjusted model [p=0.01; -16.61(-27.52 to -5.70)] and this association was sustained in both adjusted models [p=0.01; -16.47(-27.46 to -5.47) and p<0.01; -29.02(-48.88 to -9.16)] as show in Table 8. The use of dipyrone during the treatment was positively associated with the embryo blastulation rate, both in the unadjusted model [p=0.04; 15.45(0.95 to 29.95)] and model I [p=0.02; 16.70(2.42 to 30.98)], but no effect was observed in the model II, adjusted for indication of use (Table 9). The analyses of self-reported ibuprofen consumption during the COH protocol revealed a positive association with the collection rate in the unadjusted model [p=0.02; 12.21(1.71 to 22.71)] and in the model I [p=0.04; 10.97(0.33 to 21.62)], but not in model II. Also, we observed a negative relation between ibuprofen use and the fertilization rate in the unadjusted model [p=0.02; -12.29(-22.51 to -2.07)] and model I [p=0.02; -11.69(-21.83 to -1.55)], but again no significant association after adjustment for indication of ibuprofen use (Table 10). No significant associations were observed with the self-reported consumption of AAS (Data not shown). In relation to clinical reproductive outcomes, no significant results were observed for any analgesic.

Table 8. During COH protocol use of paracetamol and reproductive outcomes of participants undergoing infertility treatment.

Variables	Crude B (95% CI)	p-value	Model I B (95% CI)[a]	p-value	Model II B (95% CI)[b]	p-value
Nº follicules	-2.80(-5.36 - -0.24)	0.03	-2.57(-5.08 - -0.07)	0.04	-4.19(-8.74 - 0.36)	0.07
Nºoocytes	-2.25(-4.71 - 0.22)	0.07	-2.07(-4.54 - 0.39)	0.10	-1.51(-6.01 - 2.98)	0.51
Collection rate	-5.92(-16.25 - 4.41)	0.25	-5.30(-15.65 - 5.05)	0.31	5.52(-13.21 - 24.24)	0.56
Maturation rate	-8.80(-19.12 - 1.52)	0.09	-9.15(-19.58 - 1.28)	0.08	-3.99(-22.98 - 15.00)	0.68
Fertilization rate	-3.93(-14.03 - 6.16)	0.44	-4.61(-14.51 - 5.29)	0.36	-22.89(-40.50 - -5.29)	0.10
Quality rate	-16.61(-27.52 - -5.70)	0.01*	-16.47(-27.46 - -5.47)	0.01*	-29.02(-48.88 - -9.16)	< 0.01*
Blastulation rate	-2.57(-12.92 - 7.78)	0.62	-2.75(-12.84 - 7.33)	0.59	0.15(-18.24 - 18.54)	0.99
Utilization rate	-6.44(-16.78 - 3.91)	0.22	-7.54(-17.68 - 2.61)	0.14	-8.07(-26.58 - 10.44)	0.39

Self-reported use of paracetamol (yes/no) during COH protocol. [a]Model I: adjusted for age, BMI and cause of infertility. [b]Model II: adjusted for all covariates of Model I and for self-reported indication of use. *p-value was considered significant when $p \leq 0.05$. CI: confidence interval.

Table 9. During COH use of dipyrone and reproductive outcomes of participants undergoing infertility treatment.

Variables	Crude B (95% CI)	p-value	Model I B (95% CI)[a]	p-value	Model II B (95% CI)[b]	p-value
Nº follicules	0.23(-3.49 - 3.95)	0.90	1.15(-2.53 - 4.82)	0.54	-0.43(-9.18 - 8.32)	0.92
Nºoocytes	1.13(-2.43 - 4.69)	0.53	1.82(-1.77 - 5.41)	0.32	1.88(-6.68 - 10.44)	0.66
Collection rate	7.88(-6.86 - 22.61)	0.29	9.78(-5.14 - 24.70)	0.20	23.70(-11.74 - 59.14)	0.19
Maturation rate	14.09(-0.57 - 28.76)	0.06	14.21(-0.84 - 29.26)	0.06	18.94(-16.90 - 54.79)	0.30
Fertilization rate	-7.03(-21.40 - 7.34)	0.33	-8.22(-22.51 - 6.07)	0.26	-18.50(-52.49 - 15.48)	0.28
Quality rate	4.06(-12.06 - 20.18)	0.62	5.64(-10.81 - 22.09)	0.50	-14.81(-53.78 - 24.16)	0.45
Blastulation rate	15.45(0.95 - 29.95)	0.04*	16.70(2.42 - 30.98)	0.02*	-9.43(-43.03 - 24.18)	0.58
Utilization rate	5.13(-9.68 - 19.95)	0.49	3.10(-11.70 - 17.89)	0.68	-29.81(-64.42 - 4.79)	0.09

Self-reported use of dipyrone (yes/no) during the induction treatment for hyperovulation. [a]Model I: adjusted for age, BMI and cause of infertility. [b]Model II: adjusted for all covariates of Model I and for self-reported indication of use. *p-value was considered significant when $p \leq 0.05$. CI: confidence interval.

Table 10. During COH use of ibuprofen and reproductive outcomes of participants undergoing infertility treatment.

Variables	Crude B (95% CI)	p-value	Model I B (95% CI)[a]	p-value	Model II B (95% CI)[b]	p-value
Nº follicules	-0.40(-3.10 - 2.29)	0.77	-0.86(-3.51 - 1.79)	0.52	-0.40(-3.10 - 2.29)	0.77
Nºoocytes	1.29(-1.28 - 3.86)	0.32	0.93(-1.67 - 3.53)	0.48	1.29(-1.28 - 3.86)	0.32
Collection rate	12.21(1.71 - 22.71)	0.02*	10.97(0.33 - 21.62)	0.04*	12.21(1.71 - 22.71)	0.20
Maturation rate	-2.70(-13.48 - 8.08)	0.62	-2.10(-13.10 - 8.91)	0.71	-2.70(-13.48 - 8.08)	0.62
Fertilization rate	-12.29(-22.51 - -2.07)	0.02*	-11.69(-21.83 - -1.55)	0.02*	-12.29(-22.51 - -2.07)	0.12
Quality rate	-0.16(-11.86 - 11.53)	0.98	-0.90(-12.78 - 10.98)	0.88	-0.16(-11.86 - 11.53)	0.98
Blastulation rate	-5.57(-16.23 - 5.09)	0.30	-5.74(-16.21 - 4.74)	0.28	-5.57(-16.23 - 5.09)	0.30
Utilization rate	-5.31(-16.02 - 5.41)	0.33	-3.67(-14.32 - 6.98)	0.50	-5.31(-16.02 - 5.41)	0.33

Self-reported use of ibuprofen (yes/no) during the induction treatment for hyperovulation. [a]Model I: adjusted for age, BMI and cause of infertility. [b]Model II: adjusted for all covariates of Model I and for self-reported indication of use. *p-value was considered significant when $p \leq 0.05$. CI: confidence interval.

Discussion

In the present study, we evaluated the associations of preconception use of mild analgesics with reproductive outcomes in assisted reproduction patients. We assessed the use of analgesic both previous (12 months before) and during the COH protocol for IVF treatment. In our prospective study, the use of mild analgesics in both time periods was associated with some of the examined reproductive outcomes in women trying to conceive, but the direction and magnitude of the reported associations varied for the different types of analgesics and the covariates added in the models. The prevalence of mild analgesic use in our study was high, being paracetamol the most used drug among patients before the assisted reproduction treatment.

Previous use of paracetamol was positively associated with the maturation rate, an association that was sustained in model I (adjusted for age, BMI, and cause of infertility) but not significant in model II, which additionally controlled for indication of use. However, the use of paracetamol during COH was negatively associated with the embryo quality rate in all models tested. Previous literature already reveals a concern regarding endocrine and reproductive effects of paracetamol. Epidemiological and experimental data suggest that intrauterine exposure to paracetamol could lead to hormonal imbalances (Kristensen et al., 2016), including reduced testosterone production by human fetal testes in a xenograft model (van den Driesche et al., 2015). The experimental data is corroborated by some epidemiological studies indicating associations between prenatal exposure to paracetamol and the development of reproductive adverse outcomes linked to endocrine disruption (Patel et al., 2022, Ernst et al., 2019, Fisher et al., 2016). However, studies of preconception paracetamol exposure are still scarce. Yet, paracetamol use has already been associated in the literature with longer time to pregnancy. In the study by Smarr et al. (2016), high urinary concentrations of paracetamol in men were associated with longer time to conceive.

Several multidirectional mechanisms of action are being investigated to understand the potential role of paracetamol exposure in the disruption of hormonal homeostasis, including inhibition of steroidogenesis and depletion of multiple sulfated metabolites of androstenediol, pregnenolone, and dehydroepiandrosterone (DHEA) (Cohen et al., 2018). Steroid hormones are an integral part of ovarian follicular development, and DHEA plays

an special role as a metabolic intermediate in this process (Mo, Lu, & Simon, 2006; Artini et al., 2012), triggering important signaling cascades that result in oocyte maturation and activation (Chimote and Chimote., 2018). Considering that DHEA-S is the most abundant steroid in follicular fluid (FF) of preovulatory follicles (Dehenin, Jondet, & Scholler, 1987) and that paracetamol exposure could deplete sulfated sex hormones, it is biological plausible that this drug could affect oocyte maturation. However, the use of paracetamol in our study was positively associated with maturation rate and, although the association was not observed in the model controlling for indication of use (model II), it is possible to speculate that paracetamol could lead to alterations in oocyte maturation. In an experimental mice study, *in utero* exposure to therapeutic doses of paracetamol and ibuprofen deregulated the expression of genes involved in later oocyte meiosis. The alteration in gene expression led to increased primordial follicle pool and oocyte activation in the F1 (exposed) and F2 (offspring of exposed F1 parents) animals exposed to these drugs in comparison with controls (Rossitto et al., 2019). Therefore, more studies are necessary to understand the effects and mechanisms of paracetamol exposure on hormone levels changes and oocyte maturation, especially in patients undergoing assisted reproduction treatment.

Additionally, an interesting result of our analyses was that exposure to paracetamol during the COH protocol was negatively associated with embryo quality, an association sustained in all models. Experimental studies have already observed alterations in embryo development in association with paracetamol exposure. Animal models have shown degenerative and necrotic changes in pulmonary, reproductive and nervous systems following exposure to paracetamol during the period of embryogenesis or organogenesis (Van den Anker et al., 2018). Therefore, it is possible that paracetamol could exert a negative influence in embryo development, acting at even earlier embryonic stages. Paracetamol presents inhibition of cyclooxygenases, involvement in the endocannabinoid system, serotonergic pathways, potassium and calcium channels and ultimately L-arginine in the nitric oxide synthesis pathways (Przybyła et al., 2020). All of these mechanisms have key roles in hemostatic function of several regulatory systems that could possibly disrupt embryo development. However more studies are necessary to understand those mechanisms.

Similar to the literature observed for paracetamol, NSAIDs might also be able to act as endocrine disruptors. Several studies have already demonstrated that Ibuprofen and AAS

might inhibit fetal testosterone production (Kilcoyne and Mitchell, 2019; Sharpe, 2020; Stukenborg et al., 2021). Also, selective cyclooxygenase (COX) inhibitors have the potential to substantially inhibit ovulation (Pall et al., 2001), since COX is one of the key enzymes involved in ovulation and implantation (Duffy, 2015). However, limited data is available on the impact of non-selective COX inhibitors, such as ibuprofen, on oocyte quality and embryo development. In our study, the consumption of ibuprofen during the COH protocol was positively associated with the collection rate and negatively associated with the fertilization rate. In contrast, in an observational study comparing ibuprofen exposed and unexposed women undergoing natural cycle IVF, Kohl Schwartz and colleagues (2020) reported no differences in relation to oocyte maturation rate, fertilization rate, embryo development, and embryo quality. However, this study was performed under natural cycle IVF conditions, while our results were observed during a COH protocol. In this context, Matyas and colleagues (2015) described that Ibuprofen exposure during early follicular phase was associated with higher follicle stimulating hormone (FSH) and with lower estradiol concentrations in healthy premenopausal women. Therefore, it is possible that ibuprofen could interfere with oocyte development trough disruption of steroid hormones levels. Yet, it is important to mention that ibuprofen is a highly effective drug in the treatment of symptoms of dysmenorrhea, disorder regularly associated with SOP and endometriosis, conditions that are also associated with altered collection and fertilization rates. Accordingly, the associations between reproductive outcomes and ibuprofen exposure reported here were not significant in the model controlling for indication of use. Regarding others NSAIDs, our study did not show any significant association except for a negative association of previous diclofenac exposure with maturation rate. A previous study examined the possible influence of diclofenac in outcomes of IVF treatment, showing that the use of diclofenac sodium did not significantly compromise the implantation and pregnancy rates when used prior to oocyte retrieval, but the laboratorial outcomes were not evaluated (Kailasam et al., 2008). In line with these results, our study did not find associations between diclofenac consumption and clinical outcomes of patients. Yet, more studies are necessary to understand the potential effects of diclofenac and other NSAIDs during IVF treatment.

Lastly, our study also noted some associations of laboratory reproductive outcomes with dipyrone (metamizole), in particular a negative association was observed between dipyrone consumption previous to treatment and collection rate (model II only). Also, a

positive association between dipyrone use prior to fertility treatment and utilization rate (unadjusted model only), as well as a positive association between dipyrone use during the COH protocol and embryo blastulation rate. However, none of these associations were significant after adjustment for indication of use (model II). In experimental studies conducted by our group, we found evidence for endocrine-disruptive properties of dipyrone and its main metabolites (Passoni et al., 2018, 2022), including decreased testosterone and increased 17-OH progesterone concentrations in the H295R adenocarcinoma cell line following in vitro exposure to dipyrone (Passoni et al., 2018). In vivo, gestational rat exposure to dipyrone impaired parturition and induced changes in biomarkers of endocrine disruption in male and female offspring (Passoni et al., 2022), indicating a possible influence of dipyrone on endocrine mediated processes.

Overall, our data indicate a high prevalence of analgesic use among women seeking for medically assisted reproduction both in the period before infertility treatment and during the IVF procedure as well. We also report some associations between analgesic use and reproductive outcomes, but most of them were not significant after controlling by indication of use. Interpretation of our findings in relation to the literature and other populations is challenging due to the lack of data regarding associations between mild analgesics exposure during the preconception period, especially in assisted reproduction settings, and reproductive outcomes. It is important to highlight that the infertility conditions of study participants may result in the use of mild analgesics and that both the medical conditions per se and the resulting use of analgesics have a potential to directly affect the outcomes of our study. Therefore, our data also demonstrate the importance of accounting for confounding by indication when studying medication usage in association to health outcomes.

Author's roles

CGB conducted the literature review, samples collection, execution of study, statistical analyses, interpretation of the results and drafted the manuscript. IRH conducted the samples collection and execution of the study. FFF conducted the recruitment of patients and critical discussion. RVR conducted statistical analyses, interpretation of the results and critical discussion. AJMA conducted the literature review, orientation of the study, interpretation of the results and critical revision of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Reference list

Agrawal SS, Alvin Jose M. Anti-implantation activity of H2 receptor blockers and meloxicam, a COX-inhibitor, in albino Wistar rats. *Eur J Contracept Reprod Health Care*. 2009 Dec;**14(6)**:444-50.

Akande V, Garas A, Cahill D. The effect of diclofenac and paracetamol on pregnancy and implantation rates in infertile women undergoing IVF treatment. *J Obstet Gynaecol*. 2006 Nov;**26(8)**:785-7.

Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. *Hum Reprod*. 2011 Jun;**26(6):**1270-83

Artini PG, Simi G, Ruggiero M, Pinelli S, Di Berardino OM, Papini F, Papini S, Monteleone P, Cela V. DHEA supplementation improves follicular microenviroment in poor responder patients. *Gynecol Endocrinol*. 2012 Sep;**28(9)**:669-73.

Ben Maamar M, Lesné L, Hennig K, Desdoits-Lethimonier C, Kilcoyne KR, Coiffec I, Rolland AD, Chevrier C, Kristensen DM, Lavoué V, et al. Ibuprofen results in alterations of human fetal testis development. *Sci Rep*. 2017 Mar **10(7)**:44184.

Brune K, Renner B, Tiegs G. Acetaminophen/paracetamol: A history of errors, failures and false decisions. *Eur J Pain.* 2015 Aug;**19(7)**:953-65.

Botting RM. Mechanism of action of acetaminophen: is there a cyclooxygenase 3? *Clin Infect Dis.* 2000 Oct;**31 Suppl 5**:S202-10.

Cashman JN. The mechanisms of action of NSAIDs in analgesia. *Drugs* 1996;**52 Suppl 5**:13–23.

Chimote BN, Chimote NM. Dehydroepiandrosterone (DHEA) and Its Sulfate (DHEA-S) in Mammalian Reproduction: Known Roles and Novel Paradigms. *Vitam Horm*. 2018;**108**:223-250.

Cohen IV, Cirulli ET, Mitchell MW, Jonsson TJ, Yu J, Shah N, Spector TD, Guo L, Venter JC, Telenti A. Acetaminophen (Paracetamol) Use Modifies the Sulfation of Sex Hormones. *EBioMedicine*. 2018 Feb;**28**:316-323.

Dehennin L, Jondet M, Scholler R. Androgen and 19-norsteroid profiles in human preovulatory follicles from stimulated cycles: an isotope dilution-mass spectrometric study. *J Steroid Biochem*. 1987 Mar;**26(3)**:399-405.

Duffy DM. Novel contraceptive targets to inhibit ovulation: the prostaglandin E2 pathway. *Hum Reprod Update*. 2015 Sep-Oct;**21(5)**:652-70.

Ernst A, Brix N, Lauridsen LLB, Olsen J, Parner ET, Liew Z, Olsen LH, Ramlau-Hansen CH. Acetaminophen (Paracetamol) Exposure During Pregnancy and Pubertal Development in Boys and Girls From a Nationwide Puberty Cohort. *Am J Epidemiol*. 2019 Jan 1;**188(1)**:34-46.

Fisher BG, Thankamony A, Hughes IA, Ong KK, Dunger DB, Acerini CL. Prenatal paracetamol exposure is associated with shorter anogenital distance in male infants. *Hum Reprod*. 2016 Nov;**31(11)**:2642-2650.

Graham GG, Davies MJ, Day RO, Mohamudally A, Scott KF. The modern pharmacology of paracetamol: therapeutic actions, mechanism of action, metabolism, toxicity and recent pharmacological findings. *Inflammopharmacology*. 2013 Jun;**21(3)**:201-32.

Hassoun-Barhamji R, Raia Barjat T, Chauleur C. In the Era Of Self-Medication, What Do Pregnant Women Know about Anti-Inflammatory Drugs?. *Therapie*. 2015 Jul-Aug;**70(4)**:369-76.

Jukic AMZ, Padiyara P, Bracken MB, McConnaughey DR, Steiner AZ. Analgesic use at ovulation and implantation and human fertility. *Am J Obstet Gynecol*. 2020 May;**222(5)**:476.e1-476.e11.

Kailasam C, Hunt LP, Ryder I, Bhakri I, Gordon UD. Safety and effectiveness of diclofenac sodium in assisted reproduction treatment: a randomized prospective double-blind study. *Reprod Biomed Online*. 2008 May;**16(5)**:724-9.

Kilcoyne KR, Mitchell RT. Effect of environmental and pharmaceutical exposures on fetal testis development and function: a systematic review of human experimental data. *Hum Reprod Update*. 2019 Jul 1;**25(4)**:397-421.

Kristensen DM, Hass U, Lesné L, Lottrup G, Jacobsen PR, Desdoits-Lethimonier C, Boberg J, Petersen JH, Toppari J, Jensen TK, et al. Intrauterine exposure to mild analgesics is a risk factor for development of male reproductive disorders in human and rat. *Hum Reprod*. 2011 Jan;**26(1)**:235-44.

Kristensen DM, Lesné L, Le Fol V, Desdoits-Lethimonier C, Dejucq-Rainsford N, Leffers H, Jégou B. Paracetamol (acetaminophen), aspirin (acetylsalicylic acid) and indomethacin are anti-androgenic in the rat foetal testis. *Int J Androl*. 2012 Jun;**35(3)**:377-84.

Kristensen DM, Mazaud-Guittot S, Gaudriault P, Lesné L, Serrano T, Main KM, Jégou B. Analgesic use - prevalence, biomonitoring and endocrine and reproductive effects. *Nat Rev Endocrinol*. 2016 Jul;**12(7)**:381-93.

Kohl Schwartz AS, Burkard S, Mitter VR, Leichtle AB, Fink A, Von Wolff M. Short-term application of ibuprofen before ovulation. *Facts Views Vis Obgyn.* 2020 Oct 8;**12(3)**:179-184.

Levine H, Jørgensen N, Martino-Andrade A, Mendiola J, Weksler-Derri D, Mindlis I, Pinotti R, Swan SH. Temporal trends in sperm count: a systematic review and meta-regression analysis. *Hum Reprod Update*. 2017 Nov 1;**23(6)**:646-659.

Matyas RA, Mumford SL, Schliep KC, Ahrens KA, Sjaarda LA, Perkins NJ, Filiberto AC, Mattison D, Zarek SM, Wactawski-Wende J, Schisterman EF. Effects of over-the-counter analgesic use on reproductive hormones and ovulation in healthy, premenopausal women. *Hum Reprod*. 2015 Jul;**30(7)**:1714-23.

Moreira de Barros GA, Calonego MAM, Mendes RF, Castro RAM, Faria JFG, Trivellato SA, Cavalcante RS, Fukushima FB, Dias A. Uso de analgésicos e o risco da automedicação em amostra de população urbana: estudo transversal [The use of analgesics and risk of self-medication in an urban population sample: cross-sectional study]. *Braz J Anesthesiol*. 2019 Nov-Dec;**69(6)**:529-536.

Mo Q, Lu SF, Simon NG. Dehydroepiandrosterone and its metabolites: differential effects on androgen receptor trafficking and transcriptional activity. *J Steroid Biochem Mol Biol*. 2006 Apr;**99(1)**:50-8.

Ouellet M, Percival MD. Mechanism of acetaminophen inhibition of cyclooxygenase isoforms. *Arch Biochem Biophys*. 2001 Mar 15;**387(2)**:273-80.

Patel R, Sushko K, van den Anker J, Samiee-Zafarghandy S. Long-Term Safety of Prenatal and Neonatal Exposure to Paracetamol: A Systematic Review. *Int J Environ Res Public Health*. 2022 Feb 14;**19(4)**:2128.

Passoni MT, Kristensen MN, Morais RN, Woitkowiak C, Boareto AC, da Silva Amaral BA, Grechi N, Dalsenter PR, Munkboel CH, Styrishave B, et al. Assessment of the analgesic dipyrone as a possible (anti)androgenic endocrine disruptor. *Toxicol Lett*. 2018 Mar 15;**285**:139-147.

Passoni MT, Krebs Ribeiro DC, França de Almeida SC, Furtado da Costa B, Grechi N, Lima Tolouei SE, Curi TZ, Degraf Cavallin M, Romano RM, Romano MA, et al. The Analgesic Dipyrone Affects Pregnancy Outcomes and Endocrine-Sensitive Endpoints in Female and Male Offspring Rats. *Toxicol Sci*. 2022 Apr 26;**187(1)**:80-92.

Pall M, Fridén BE, Brännström M. Induction of delayed follicular rupture in the human by the selective COX-2 inhibitor rofecoxib: a randomized double-blind study. *Hum Reprod*. 2001 Jul;**16(7)**:1323-8.

Przybyła GW, Szychowski KA, Gmiński J. Paracetamol - An old drug with new mechanisms of action. *Clin Exp Pharmacol Physiol*. 2020 **Aug 7**.

Rezende RM, França DS, Menezes GB, dos Reis WG, Bakhle YS, Francischi JN. Different mechanisms underlie the analgesic actions of paracetamol and dipyrone in a rat model of inflammatory pain. *Br J Pharmacol*. 2008 Feb;**153(4)**:760-8.

Roberts E, Delgado Nunes V, Buckner S, Latchem S, Constanti M, Miller P, Doherty M, Zhang W, Birrell F, Porcheret M, et al. Paracetamol: not as safe as we thought? A systematic literature review of observational studies. *Ann Rheum Dis*. 2016 Mar;**75(3)**:552-9.

Rossitto M, Ollivier M, Déjardin S, Pruvost A, Brun C, Marchive C, Nguyen AL, Ghettas A, Keime C, de Massy B, et al. In utero exposure to acetaminophen and ibuprofen leads to intergenerational accelerated reproductive aging in female mice. *Commun Biol*. 2019 Aug 13;**2:**310.

Skakkebæk NE, Lindahl-Jacobsen R, Levine H, Andersson AM, Jørgensen N, Main KM, Lidegaard Ø, Priskorn L, Holmboe SA, Bräuner Evet al. Environmental factors in declining human fertility. *Nat Rev Endocrinol*. 2022 Mar;**18(3)**:139-157.

Smarr MM, Grantz KL, Sundaram R, Maisog JM, Honda M, Kannan K, Buck Louis GM. Urinary paracetamol and time-to-pregnancy. *Hum Reprod*. 2016 Sep;**31(9)**:2119-27.

Sehring J, Beltsos A, Jeelani R. Human implantation: The complex interplay between endometrial receptivity, inflammation, and the microbiome. *Placenta*. 2022 Jan;**117:**179-186.

Sharpe RM. Androgens and the masculinization programming window: human-rodent differences. *Biochem Soc Trans. 2020* Aug 28;**48(4)**:1725-1735

Stukenborg JB, Mitchell RT, Söder O. Endocrine disruptors and the male reproductive system. *Best Pract Res Clin Endocrinol Metab.* 2021 Sep;**35(5)**:101567.

van den Driesche S, Macdonald J, Anderson RA, Johnston ZC, Chetty T, Smith LB, Mckinnell C, Dean A, Homer NZ, Jorgensen A, et al.. Prolonged exposure to acetaminophen reduces testosterone production by the human fetal testis in a xenograft model. *Sci Transl Med*. 2015 May 20;**7(288)**:288ra80.

van den Anker JN, Allegaert K. Acetaminophen in the Neonatal Intensive Care Unit: Shotgun Approach or Silver Bullet. *J Pediatr*. 2018 Jul;**198:**10-11.

Zhou Y, Boudreau DM, Freedman AN. Trends in the use of aspirin and nonsteroidal anti-inflammatory drugs in the general U.S. population. *Pharmacoepidemiol Drug Saf*. 2014 Jan;23**(1):**43-50.

Zafeiri A, Mitchell RT, Hay DC, Fowler PA. Over-the-counter analgesics during pregnancy: a comprehensive review of global prevalence and offspring safety. *Hum Reprod Update.* 2021 Jan 4;**27(1)**:67-95.

5.3 ARTIGO 3: ASSOCIATIONS OF URINARY AND FOLLICULAR FLUID CONCENTRATIONS OF PHTHALATE METABOLITES AND REPRODUCTIVE OUTCOMES OF WOMEN UNDERGOING ASSISTED REPRODUCTION.

Artigo aborda as associações entre as concentrações de metabólitos de ftalatos em urina e líquido folicular de pacientes em tratamento de reprodução assistida e seus desfechos reprodutivos. Escrito nas normas do periódico Journal of Assisted Reproduction and Genetics.

Associations of urinary and follicular fluid concentrations of phthalate metabolites and reproductive outcomes of women undergoing assisted reproduction.

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Abstract

Purpose: To assess phthalate exposure in urine and follicular fluid of Brazilian women undergoing medical assisted reproduction and explore the relationship between phthalate exposures and reproductive outcomes of ART.

Methods: Study population comprises 98 reproductive aged women (n=119 cycles of fertility treatment) recruited during their infertility treatment, between 2018 and 2021. Participants provide urine sample and agree with the collection of follicular fluid sample during oocyte collection. We measured the urinary and follicular fluid concentrations of 15 phthalate metabolites representing 10 parent phthalate diesters. Trough multivariate generalized linear mixed models we evaluate the association between urinary and follicular fluid phthalate metabolites and IVF outcomes.

Results: Our adjusted model observed associations of urinary metabolites and oocyte and embryo development parameters. Specifically, mEOHP were associated with number of

follicles and number of blastocysts, while mEHHP was associated with number of follicules, number of mature oocytes, number of fertilized and good quality embryos. Also, sum of DEHP metabolites was associated with number of fertilized embryos. Regarding follicular fluid concentrations no associations were observed for oocyte parameters. However, in the adjusted model, follicular fluid concentrations of mEP and mEOHP were associated with embryo development parameters, specifically, number of fertilized embryos and number of good quality embryos

Conclusions: Our study observed that the associations between urinary DEHP metabolites and early IVF parameters was variable and dependent on the specific metabolite assessed. However, our results agree with previous data that suggest an involvement of phthalates as competing factors in reproductive processes, including early IVF parameters.

Keywords

Phthalates, endocrine disruptors, assisted reproduction, infertility.

Statements and declarations

The authors declare no competing interest.

Introduction

Infertility, described as the inability to achieve pregnancy after 12 months of attempted conception, is a major clinical and public health concern, being estimated to affect about 15% of reproductive-aged couples [1]. Several studies appoint that the environment may play a role in the increasing rates of infertility, suggesting that exposure to endocrine-disrupting chemicals (EDC) that appear in several daily products could contribute to impaired fecundity [2,3]. Among different EDCs identified so far, phthalate esters have emerged as chemicals of major concern to human reproductive health. These compounds are broadly used in plastics and other industrial applications, including the manufacturing of personal care products, and have been classified as EDCs that may interfere with the biosynthesis and/or action of sex-steroid hormones, having important implications for reproduction [4, 5]. The effects of phthalates are better understood for male than female reproductive system, but literature already shows that phthalates can be toxic to the ovaries, affecting essential points of female reproduction as folliculogenesis and steroidogenesis [6].

Exposure to phthalates can impair several processes of female reproduction, displaying potential deleterious effects in general female reproductive health as in their reproductive capacity [7,8]. Phthalates have been demonstrated to have anti-oestrogenic, anti-androgenic, anti-progestogenic and anti-thyrogenic activities [9, 10, 11]. Unbalances with interactions of androgenic, estrogenic and gonadotropin signaling disrupt the endocrine homeostasis necessary to unsure the competence of strictly regulated processes as folliculogenesis and embryo implantation [12]. In this context, epidemiological data has demonstrated that in couples trying to conceive, phthalate exposure has been associated with impaired outcomes in oocyte collection, embryo development and clinical pregnancy rates [13, 14].

The increase in infertility rates also leads to an increase in the use of assisted reproduction techniques (ART). However, despite the increasing use of medical treatment among couples with decreased fecundity, success rates of live birth among couples undergoing ART treatments have remained stable [15]. Therefore, is possible that modifiable factors, such as phthalate exposure, can interfere with human fertility an influence reproductive outcome of couples undergoing ART. Yet, ART procedures provide the opportunity to monitor phthalates metabolites in follicular fluid, a liquid that provides the micro-environment for oocyte development and is crucial for the production and quality of oocytes and may offer a better understanding of the effects of phthalate exposure as a potential influence in reproductive outcomes [16]. Also, ART procedures provide an opportunity to acquire evidence of the impact of environmental pollutants on early stages and critical events of reproduction such as ovulation, fertilization, embryo development and implantation [17, 18]. Despite this fact, data on measurement of phthalate esters in human follicular fluid and influence of these compounds in ART outcomes are scarce. Also, the profile of exposure to phthalate esters may differ across regions or countries [19]. In the present study, we aim to assess phthalate exposure of Brazilian women undergoing medical assisted reproduction by measuring phthalate metabolites in both follicular fluid (FF) and urine samples and explore the relationship between phthalate exposures and reproductive outcomes of ART.

Material and methods

Study population

Study population comprises 98 reproductive aged women (n=119 cycles of fertility treatment) who were recruited during their infertility treatment. The patients were recruited in a fertility clinic in the city of Curitiba in southern Brazil, between 2018 and 2021. Women aged 20–40 years who were diagnosed with infertility and had indication to undergo high complexion assisted reproduction treatment were eligible to participate and approximately 55% of those contacted by the research staff signed up to the study. Participants were enrolled previously to their controlled ovarian hyperstimulation (COH) protocol and followed through their treatment. We enrolled only women undergoing treatment for female factor infertility, excluding patients in treatment for severe male infertility. Further exclusion criteria included use of oocyte donor or cryopreserved gametes during treatment. Participants enrolled in the study independently and were followed up from study entry through their fertility care, pregnancy and delivery. During the progress of the study, participants provided 1 urine sample and agreed with the collection of follicular fluid sample during oocyte collection. They also completed a survey with a baseline questionnaire, providing detailed data on demographic, lifestyle, and behavioral factors, anthropometrics, and reproductive and medical history.

Ethical approval

Institutional review board approvals were obtained from all collaborating institutions. Patients gave written informed consent prior to study participation and any data collection.

Ovarian hyperstimulation protocol

To reduce possible confounding by treatment protocol, all enrolled patients were treated with controlled ovarian stimulation using Gonadotrophin-releasing hormone (GnRH) antagonist (Orgalutran*;* Schering-plough) protocol. For ovarian stimulation, a daily dose of recombinant follicle-stimulating hormone (FSH, Gonal-F, Merck-Serono or Puregon, Merck Sharp & Dohme) followed by purified human menopausal gonadotrophin (hMG, Menopur; Ferring) was used starting on the third day of the menstrual cycle. The dose administered was dependent on the age, body mass index (BMI), and treatment history. Patients were monitored during gonadotropin stimulation for serum estradiol, follicle size measurements and counts, and endometrial thickness. Human chorionic gonadotropin

(hCG) (Ovidrelle, Merck Serono) or GnRH agonist (Gonapeptyl Daily; Ferring) were administered approximately 36 h before the scheduled oocyte retrieval.

Exposure assessment

Participants provided 1 spot urine sample collected on the oocyte collection day, and follicular fluid samples were collected during egg collection procedure for all participants of the study. Urine samples were collected in polypropylene specimen cups, and the specific gravity (SG) of each sample was quantified with a handheld refractometer. The urine samples were then divided into aliquots and frozen for long-term storage at −80 °C. Follicular fluid (FF) samples were a pool of all punction follicles with the presence of an oocyte. After collection FF samples were centrifuged at 450 g for 10 min at 4°C to remove blood and cells, and were then aliquoted and stored at − 80 °C.

We measured the urinary and follicular fluid concentrations of 15 phthalate metabolites representing 10 parent phthalate diesters (Table 2). Concentrations of monomethyl phthalate (mMP), mono-ethyl phthalate (mEP), mono-(3-carboxypropyl) phthalate (mCPP), mono-isopropyl phthalate (mIPRP), mono-propyl phthalate (mPrP), monobutyl phthalate (mBUP/mIBUP), Mono-(2-ethyl-5-oxohexyl) phthalate (mEOHP), mono(2-carboxymethyl)hexyl phthalate (mCMHP), Mono-(2-ethyl-5-carboxypentyl) phthalate (mECPP), Mono-(2-ethyl-5-hydroxyhexyl) phthalate (mEHHP), mono-benzyl phthalate (mBzP), monocyclohexyl phthalate (mCHP), monoisopentyl phthalate (mIPeP), mono-(4-methyl-7-carboxyheptyl) phthalate (mCIOP) and mono(2-ethylhexyl) phthalate (mEHP) were measured using high performance liquid chromatography-tandem mass spectrometry (LC-ESI-MS/MS) analysis combined with an off-line solid-phase extraction procedure, as described previously [20] with minor modifications at the Laboratory of Toxicology of the University of São Paulo at Ribeirão Preto, Brazil.

Sample preparation and instrumental analysis

The concentrations of these compounds were determined after the hydrolysis of the β-glucuronidase conjugates. To analyze the total concentration of analytes, urine samples were thawed and mixed. Subsequently, an aliquot of 1000 µL of urine (or 1000 µL of follicular fluid, after acid treatment) was transferred to a 15 mL polypropylene tube with a conical bottom, fortified with 20 µL of a solution (1000 ng/mL) containing the internal standards followed by the addition of 500 µL of 1 M ammonium acetate buffer (pH 5.5)

containing 20 µL of the enzyme solution (β-glucuronidase, free of arylsulfatase). After gentle mixing, the samples were incubated for 3 h at 37°C. After sample incubation, the enzymatic reaction was stopped by the addition of 1.0 mL of 0.145 M monobasic phosphate buffer (pH 2.0). The sample was then subjected to solid phase extraction using the ABS Elut NEXUS® cartridge (60 mg, 3 mL) under the following conditions: preconditioning with 1.5 mL of acetonitrile, followed by 1.5 mL of 0.145 monobasic phosphate buffer M (pH 2.0), loading the sample, washing the interferents with 1.5 mL of a solution of formic acid (0.1 M) and 1.5 mL of a solution of methanol (5%) in water and elution of the analytes with 1.5 ml of acetonitrile and 1.5 ml of ethyl acetate. The eluate was collected in a 15 mL polypropylene tube and evaporated to dryness. After the drying step, the residue was reconstituted in 200 µL of a water:methanol mixture (8:2, v/v), vortexed and transferred to an insert inside a vial for further analysis by high performance liquid chromatography coupled to mass spectrometry.

The analysis was performed with a Thermo Scientific LC system equipped with a pump (Accela 600 pump) and an autosampler coupled with Thermo Scientific TSQ Quantum Access Max with an electrospray triple quadrupole mass spectrometer. The Xcalibur software version 2.0 (Thermo Fisher Scientific) was used to control the instruments and to process data. The chromatographic separation was carried out on an Ultra AQ C18 column (2.1 mm ×100 mm, 3 µm). A gradient program for the mobile phase was set as the following: mobile phase A was water (0.1% acetic acid), and mobile phase B was methanol (0.1% acetic acid). The following mass spectrometer conditions were used: capillary voltage kept at − 4000 V and capillary temperature of 220 °C. Nitrogen was used as a sheath gas, and auxiliary gas at flow rates of 10, and 5 arbitrary units, respectively, and vaporizer temperature was set at 200 °C. For tandem mass spectrometry, the deprotonated molecule [M−H]- was used as precursor ion for all analytes. Argon was used as a collision-induced-dissociation (CID) gas at 1.0 mTorr.

Quality assurance/quality control

Quality assurance and quality control parameters included procedural blanks, matrix spikes and analysis of calibration curves. Labeled internal standards were spiked into all samples and quantification was by isotope dilution method. Contamination that arises from laboratory materials and solvents was monitored by the analysis of procedural blanks. A 20-point instrumental calibration curve was prepared in MeOH:Water (2:8) at concentrations that ranged from 0.01 to 100 ng/mL. The regression coefficients of the

calibration curves were higher than 0.99. For each batch of 30 samples analyzed, two procedural blanks and two pre-extraction matrix spikes (prepared by spiking known concentrations (20 ng/mL0 of target compounds) were analyzed by passing them through the entire analytical procedure. A calibration checks standard, and methanol were injected after every 30 samples as a check for drift in instrumental sensitivity and carry-over between samples, respectively. The LODs of phthalates metabolites in urine and follicular liquid varied from 0.002 to 0.015 ng/mL (Table 2).

Assessment of reproductive outcomes

Laboratorial and clinical outcomes information was abstracted from the patient's electronic medical record by the research staff. The reproductive data collection initiated at the time of oocyte retrieval. The number of follicles was registered as well as the number of retrieved oocytes. Embryologists classified oocytes as germinal vesicle, metaphase I, metaphase II (MII), or degenerated oocytes, and the number of oocytes in metaphase II was collected. Fertilization was determined 16 to 18 hours after ICSI – and a normal fertilization comprised an injected egg cell with the presence of two pronuclei and two polar bodies. The fertilization rate of each patient was recorded as the number of normal fertilizations/ total number of injected oocytes. Embryos were further assessed on day 3 for cell number, symmetry, and fragmentation. High quality embryos were classified as those with > 8 symmetric cells and < 10% fragmentation, being recorded the quality embryo rate (number of high-quality embryos/total number of embryos) (Alpha Scientists, 2011). Embryo assessment was also performed at day 5 for control of blastulation, being recorded the blastocyst number. For clinical outcomes positive β-hCG (i.e., successful implantation) was defined as a serum β-hCG level > 25 mIU/ mL, typically measured 12 days after embryo transfer. Clinical pregnancy was defined as the presence of an intrauterine gestational sac and fetal heartbeat confirmed by ultrasound by 7 weeks of gestation, and live birth as the delivery of a live neonate. Miscarriage, biochemical and ectopic pregnancies were recorded.

Statistical analyses

Demographic and clinical characteristics of the study participants were reported using mean ± SD or percentages. Cycle-specific urinary concentrations of phthalate metabolites were adjusted to urinary dilution with the formula $Pc = P[(1.018 – 1)/SG – 1]$, where Pc is the specific gravity (SG) corrected phthalate metabolite concentration (micrograms

per liter), P is the measured phthalate metabolite concentration (micrograms per liter), and 1.018 is the mean (and median) SG level in our study population [21] . We used SG-corrected phthalate metabolite concentrations in all analyses of urinary samples. Urinary and follicular fluid phthalate metabolite concentrations below the limit of detection (LOD) were replaced with a value equal to LOD divided by 2 [19]. All samples who were below the LOD were classified into the first quartile, given that the lowest percentage of detection was 47% (mIPrP). Therefore, all samples with percentage of detection lower than 100% present the lowest quartile including samples with concentrations below the LOD and with low concentrations.

To evaluate the association between urinary and follicular fluid phthalate metabolites and IVF outcomes we applied multivariate generalized linear mixed models accounting for different number of cycles contributed per woman. Poisson distribution and log link function were specified for laboratory outcomes (follicle and oocyte counts, fertilization, embryo quality and blastulation), and a binominal distribution and logit link function were specified for clinical outcomes. Confounding was evaluated using prior knowledge of the literature, and included factors known to be associated with phthalate exposure based on previous research or known predictors of the outcomes. The following covariates were considered for inclusion in the final model: maternal age (continuous), BMI (continuous) and primary infertility diagnosis (anatomical; ovarian insufficiency; endocrine (polycystic ovary syndrome - PCOS); endometrioses; unexplained (ESCA); or others). We conducted all statistical analyses using SPSS version 22 (IBM, Armonk, NY: IBM Corp. USA) and considered significance levels < 0.05 as statistically significant.

Results

General data in our study include 93 women who were on average 34.8 years old, 63.4% of whom had body mass index within the normal range ($\geq 18.5 \leq 25$ category), were mostly caucasian (81.7%), nonsmoker (75.5%) and presented primary infertility (90.8%), as shown in Table 1. Women included in our analysis also showed high household incomes (73.2% above 6 minimum wages) and high education (71% with college degree). The primary diagnosis was 22.6% ovarian insufficiency, followed by 19.4% anatomical female factor and 18.3% polycystic ovarian syndrome and endometrioses. Other types of infertility account for 15.1% of patients and include genetic disorders, low sexual frequency, and immunological disorders.

Table1. Baseline characteristics of 93 patients undergoing ART.

Variable	Mean ± SD or N (%)[a]
Age	34.8±4.0
> 31 years (%)	15.1(14)
31-35 years (%)	37.6(35)
36-40 years (%)	47.3(44)
BMI	23.9±4.9
≥18.5 ≤ 25(%)	63.4(59)
25 ≤ 30 (%)	24.7(23)
>30 (%)	11.8(11)
Race/Ethinicity	
White(%)	81.7(76)
Others(%)	18.3(17)
Education	
> College Degree (%)	29(27)
≤ College Degree (%)	71(66)
Household income	
> 6 minimum wage	26.9 (25)
6-10 minimum wage	40.9 (38)
< 10 minimum wage	32.3(30)
Smoker	
Never smoker (%)	65.5(57)
Current or Previous smoker(%)	10.3(9)
Passive smoker(%)	24.1(21)
Infertility	
Primary (%)	90.8(82)
Secondary (%)	9.2(11)
Anatomical (%)	19.4(18)
Ovarian insufficiency (%)	22.6(21)
Endocrine (PCOS)(%)	18.3(17)
ESCA (%)	6.5(6)
Endometrioses (%)	18.3(17)
Others (%)	15.1(14)

[a]Data on continuous variables are presented as mean ± standard deviation and categorical data as counts and percentage.

A total of 73 urine samples were collected from the 93 women and all 119 cycles resulted in a follicular fluid sample, therefore 78% of the participants provided 1 urine sample and 100% provided a follicular fluid sample. The distribution of each urinary and follicular fluid phthalate metabolite in our population is shown in Table 2.

Phthalates	Primary Metabolites	Secundary Metabolites		LOD	%>LOD	GM	Min	Percentil 25	Percentil 50	Percentil 75	Max
Di-methyl phthalate (DMP)	mMP		FF	0.01	95%	0.51	0.005	0.414	0.630	0.839	5.141
			Urine		93%	1.03	0.003	0.911	1.41	2.524	34.844
Di-ethyl phthalate (DEP)	mEP		FF	0.005	93%	0.32	0.003	0.138	0.348	1.080	26.289
			Urine		100%	41.55	0.961	15.513	43.687	107.314	2.041.535
Di-iso-propyl phthalate (DIPrP)	mIPrP		FF	0.01	0%	**	**	**	**	**	**
			Urine		47%	0.02	0.002	0.003	0.011	0.099	2.516.129
Di-npropyl phthalate (DPrP)	mPrP		FF	0.03	0%	**	**	**	**	**	**
			Urine		93%	0.69	0.008	0.253	0.867	2.224	23.120
Butylbenzyl phthalate (BBzP)	mBzP		FF	0.01	0%	**	**	**	**	**	**
			Urine		89%	0.28	0.003	0.158	0.370	0.846	8.093
	mBuP/mIBuP		FF	0.01	100%	0.66	0.031	0.151	0.406	1.823	13.797
			Urine		100%	35.31	0.974	10.121	53.136	118.801	1.495.628
Di-n-butyl phthalate (DnBP)		mCPP	FF	0.05	0%	**	**	**	**	**	**
			Urine		100%	1.21	0.064	0.473	1.120	2.773	24.550
Di-cyclohexyl phthalate (DCHP)	mCHP		FF	0.005	0%	**	**	**	**	**	**
			Urine		81%	0.06	0.001	0.031	0.076	0.244	30.880
Diisopentyl phthalate (DiPeP)	mIPeP		FF	0.01	0%	**	**	**	**	**	**
			Urine		100%	4.35	0.076	2.546	4.833	9.771	81.967
Di-(2-ethyl-hexyl) phthalate (DEHP)	ΣDEHP#		FF			21.13	0.94	9.85	19.74	44.11	299.88
			Urine			313.73	0.13	182.77	333.33	654.89	1070.43
	mEHP		FF	0.05	100%	1.44	0.074	0.752	1.429	2.784	21.512
			Urine		89%	4.62	0.016	3.380	6.717	13.168	343.882
		mEOHP	FF	0.005	100%	0.18	0.005	0.024	0.108	0.566	4.743
			Urine		100%	9.83	0.001	3.168	17.128	42.791	213.585
		mEHHP	FF	0.005	100%	0.93	0.005	0.146	0.756	3.085	26.243
			Urine		100%	12.82	0.132	4.628	19.016	48.028	291.343
		mCMHP	FF	0.005	100%	0.23	0.014	0.108	0.188	0.383	16.139
			Urine		100%	12.12	0.588	7.071	13.211	20.901	2.433.005
		mECPP	FF	0.01	100%	2.39	0.111	1.064	2.147	3.890	51.409

		Urine		100%	29.15	1.40	20.645	31.740	55.863	1.903.446
Di-iso-decylphthalate (DiDP)	mCIOP	FF	0.005	83%	0.06	0.003	0.016	0.069	0.141	1.566
		Urine		100%	2.61	0.009	1.228	3.918	6.783	24.449

Table 2. Distribution of follicular fluid and urinary* phthalate metabolites

The total number of analysed samples was 119 and 73 for follicular fluid and urine, respectively. %>LOD: Phthalate metabolites above the limits of detection. FF: follicular fluid. GM: geometric mean. Min: minimum. Max: maximum. *Urinary phthalate metabolite concentrations were gravitational density adjusted.

** Phthalate metabolites not observed above the limits of detection. #Molar sum of DEHP metabolites: (MEHP*(1/278)) + (MEHHP*(1/294)) + (mEOHP*(1/292)) + (mCMHP*(1/308))+ (mECPP*(1/308)) * 1000 (nmol/L)

An overview of the 119 IVF cycles is shown in Table 3. In brief, 101 cycles of the initial 119 cycles underwent embryo transfer (84.8%), while 18 cycles resulted in inviable embryos and therefore did not have transfer. Of the cycles that underwent embryo transfer, 50.4% resulted in a positive pregnancy, 41.5% lead to a clinical gestation and 37.6% resulted in delivery or ongoing pregnancy.

Table 3. Reproductive outcomes of study participants

Reproductive outcomes	**Mean ± SD or %(N)[a]**
Cycles included	119
Number of follicles	12.4 ±6.4
Number of oocytes	9.2 ±6.2
Collection rate	72.9%
Number of mature oocytes	7.9 ±5.5
Maturation rate	78%
Fertilized embryos	5.9 ±3.7
Fertilization rate	82%
Good quality embryos	4.0 ±2.8
Quality rate	65.5%
Blastocysts	2.4 ±2.0
Blastulation rate	37.1%
Transfer rate	84.8% (101)
BHCG positive	50.4% (51)
Clinical gestation	41.5% (42)
Biochemical pregnancy	8.9% (9)
Ectopic pregnancy	0% (0)
Miscarriage	3.9% (4)
Deliveries/Ongoing	37.6% (38)

[a]Data on continuous variables are presented as mean ± standard deviation and categorical data as counts (when applied) and mean percentage

Urinary metabolites

In multivariable models adjusted for age, BMI and infertility diagnosis specific oocyte parameters were associate with urinary mEOHP, mEHHP, mBzP and mIPrP (Figure 1). Regarding the number of follicles, we observed a positive association with mEOHP in quartile 4 (p-value for Q4 vs. Q1 = 0.03) and a negative association with mEHHP in quartile 2 and 4 (p-value for Q4 vs. Q1 = 0.03, p-value for Q2 vs Q1=0.005) and with mBzP in quartile 4 (p-value for Q4 vs. Q1 = 0.007). Also, a positive association was

observed between the number of collected oocytes and mIPrP in quartil 2 (p-value for Q4 vs. Q1= 0.04). In accordance with the results observed for the number of follicles, the number of mature oocytes was lower in quartiles 2 and 4 for mEHHP (p-value for Q4 vs. Q1 = 0.03, p-value for Q2 vs Q1=0.04) and in quartile 4 for MBzP (p-value for Q4 vs. Q1 = 0.02) compared with quartile 1. Association was also observed in the non-adjusted model for mEHP and mBzP (Data not shown). Specifically, non-adjusted model shows an negative association of mEHP with number of follicles (p-value for Q2 vs. Q1=0.02), number of collected oocytes (p-value for Q2 vs. Q1 = 0.04) and mature oocytes (p-value for Q2 vs. Q1=0.02) in quartil 2. Also, a positive association of mBzP with collected oocytes (p-value for Q2 vs. Q1 = 0.04) in quartile 2.

After controlling for age, BMI and infertility diagnosis, our models showed associations between urinary phthalate levels of mEHHP, mEOHP and ΣDEHP and embryo development outcomes (Figure 1). Specifically, mEHHP in quartile 2 was negatively associated with the numbers of fertilized embryos (p-value for Q2 vs. Q1 = 0.003) and of good quality embryos (p-value for Q2 vs. Q1 = 0.034) and mEHHP in quartile 4 with the number of fertilized embryos (p-value for Q4 vs. Q1 = 0.037). The sum of DEHP metabolites was negatively associated with the number fertilized embryos (p-value for Q4 vs. Q1=0.009) in the highest quartile. Also, we observed a positive association between the number of blastocysts with urinary mEOHP in quartile 3 and 4 concentrations (p-value for Q3 vs. Q1 = 0.03, p-value for Q4 vs. Q1 = 0.04). The unadjusted multivariate model also showed associations of mEHP, mECPP and mCHP with embryo development parameters (data not shown). Concentrations of mEHP in quartile 2 were negatively associated with good quality embryos (p-value for Q2 vs. Q1 = 0.04) and blastocyst (p-value for Q2 vs. Q1 = 0.025). Urinary mCHP concentrations in quartile 2 and meCPP concentrations in quartile 4 were also negatively associated with number of blastocysts (p-value for Q2 vs. Q1 = 0.01, p-value for Q4 vs. Q1 = 0.04, respectively). In relation to clinical outcomes no association was observed between urinary concentrations of phthalate metabolites and our study outcomes, even after controlling for age, BMI and primary infertility diagnosis (data not shown).

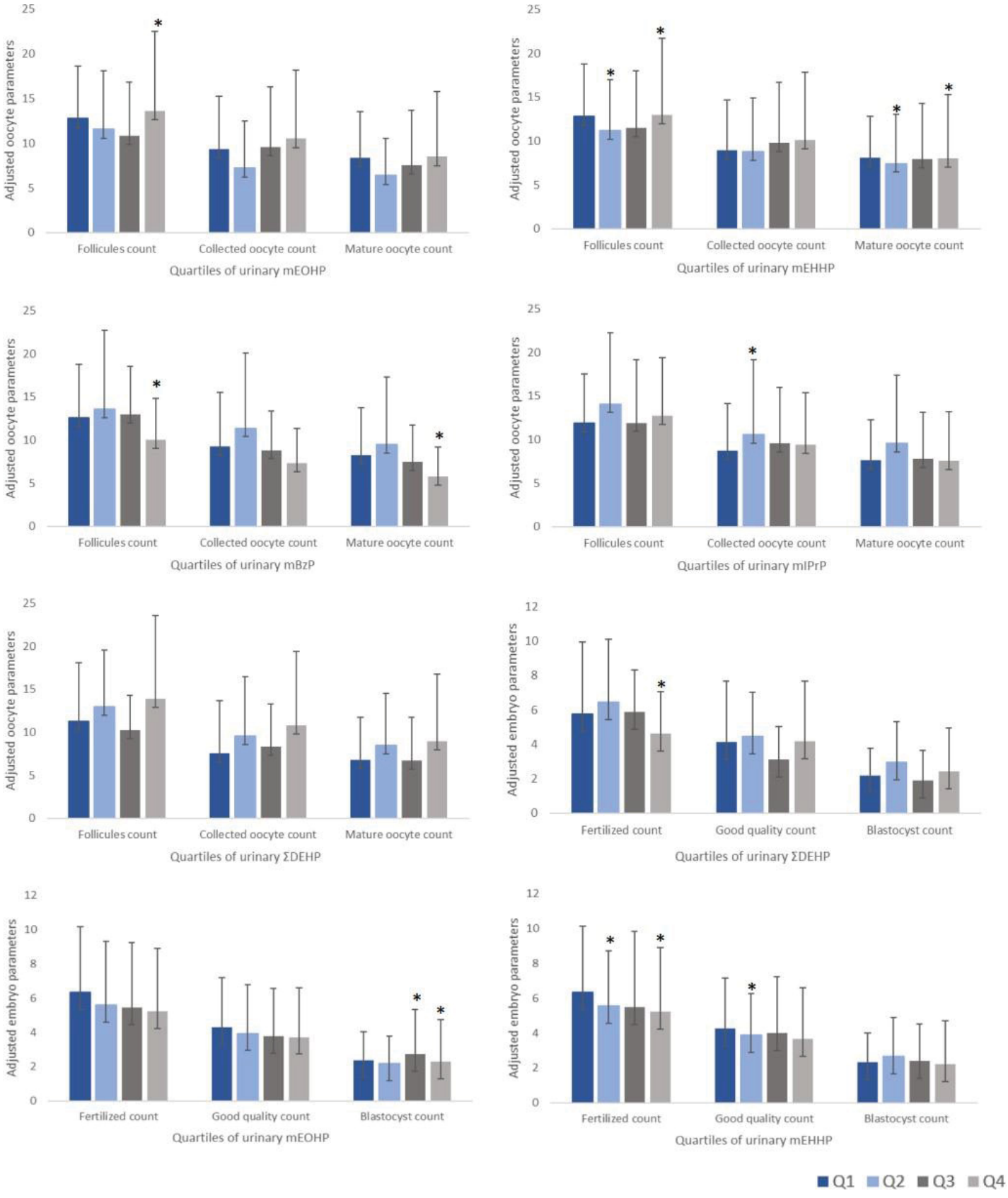

Figure 1. Adjusted mean (95% CI) count of oocyte and embryo parameters by quartile of urinary phthalate metabolites 73 IVF cycles with successful egg retrieval. Adjusted

models control for maternal age (continuous), body mass index (continuous) and primary infertility diagnosis at study entry. *p-value for comparison against Q1 < 0.05

Follicular Fluid metabolites

Regarding follicular fluid phthalate metabolites concentrations, no associations were observed in the adjusted and unadjusted multivariable models for oocyte parameters (data not shown). However, in the adjusted model, follicular fluid concentrations of mEP and mEOHP were associated with embryo development parameters (Figure 2). A positive association was observed between mEP follicular fluid concentrations in quartile 2 and the number of good quality embryos (p-value for Q2 vs. Q1=0.041), as well as, a positive association of quartile 2 and 3 follicular fluid concentrations with the number of fertilized embryos (p-value for Q2 vs. Q1 = 0.019, p-value for Q3 vs. Q1 = 0.021). The unadjusted model also revealed associations of embryo development parameters with mBuP/mIBuP, mEP and mMP follicular fluid concentrations (Data not shown). Follicular fluid concentrations of mEP in quartile 2 (p-value for Q2 vs. Q1 = 0.013) and mBuP/mIBuP in quartile 2 and 4 (p-value for Q2 vs. Q1 = 0.02, p-value for Q4 vs. Q1 = 0.014, respectively) were positively associated with number of fertilized embryos and good quality embryos (mEP p-value for Q2 vs. Q1 = 0.039, mBuP/mIBuP p-value for Q2 vs. Q1 = 0.029 and p-value for Q4 vs. Q1 = 0.013). Follicular fluid concentrations of mMP also show association with good quality number, quartile 2 concentrations were negatively associated with good quality (p-value for Q2 vs. Q1 = 0.043). Lastly, mBuP/mIBuP in quartile 2 and 4 (p-value for Q2 vs. Q1 = 0.01, p-value for Q4 vs. Q1 = 0.039, respectively) were positively associated with number of blastocysts. In relation to clinical outcomes no association was observed between follicular liquid concentrations of phthalate metabolites and our study outcomes, even after controlling for age, BMI and primary infertility diagnosis (data not shown).

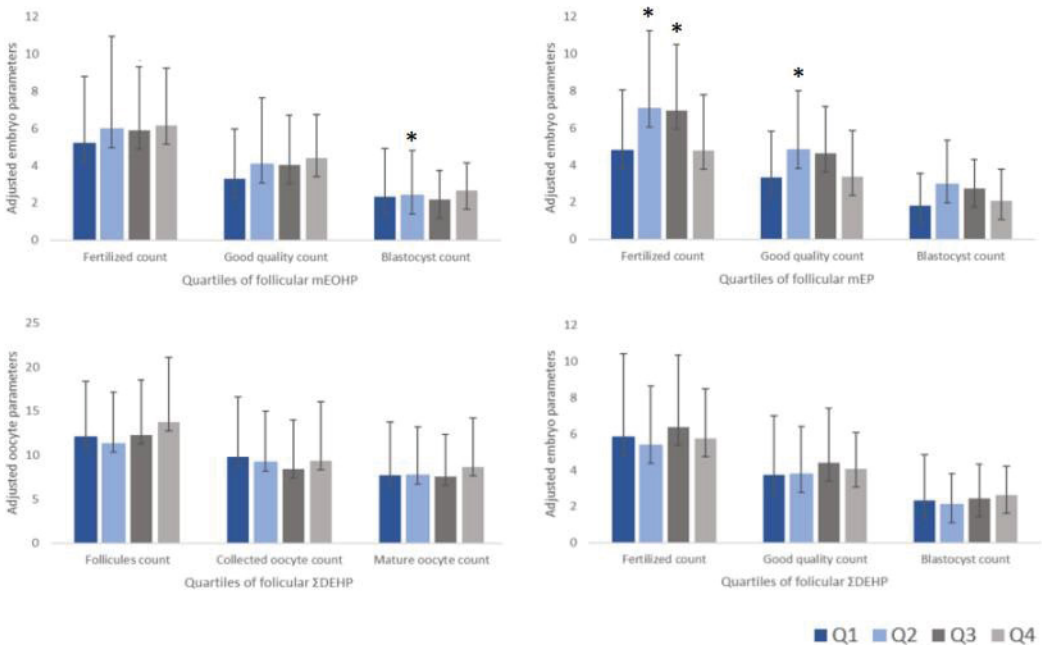

Figure 2. Adjusted mean (95% CI) count of oocyte and embryo parameters by quartile of follicular fluid phthalate metabolites 119 IVF cycles with successful egg retrieval. Adjusted models control for maternal age (continuous), body mass index (continuous) and primary infertility diagnosis at study entry. *p-value for comparison against Q1 < 0.05

Discussion

Although some studies have already investigated the associations of phthalate exposure and assisted reproduction outcomes, the potential influence of these chemicals in infertility treatments has not yet been fully clarified. In our prospective single-center study we investigated the associations between urinary and follicular fluid concentrations of fifteen phthalate metabolites and laboratorial and clinical outcomes of assisted reproduction cycles in Brazilian women. To the best of our knowledge, this is the first study to examine phthalate exposures in women seeking medically assisted reproduction in Brazil.

We observed that higher concentrations of urinary DEHP metabolites, specifically mEHHP and mEOHP, were associated with both oocyte and embryo development parameters. The urinary levels of mEHHP were negatively associated with the numbers of collected follicles and of mature oocytes. In agreement with these data, urinary concentrations of mEHHP were also negatively associated with the numbers of fertilized

embryos and of good quality embryos. Also, urinary sum of DEHP metabolites (ΣDEHP) was negatively associated with number of fertilized embryos in our study. Previous results in the literature also show that metabolites of DEHP may impair early IVF outcomes. An Israeli study reported that the sum of urinary concentrations of DEHP metabolites (ΣDEHP), and the individual metabolites mEOHP and mEHHP, were negatively associated with the number of total oocytes, mature oocytes, fertilized oocytes, and good quality embryos [14]. Similarly, in the EARTH study [13], multivariate models revealed that the highest quartiles of urinary levels of the DEHP metabolites mEHP, mEHHP, mEOHP, and mECPP were associated with lower oocyte yield and lower number of mature (MII) oocytes retrieved. In experimental models, the possible influence of DEHP and its metabolites in oocyte parameters was already described. In mice, exposure to DEHP inhibited follicular growth and reduced levels of estradiol [22]. Also, a similar study reported that DEHP can cause depletion of the primordial follicle pool in the mouse offspring of dams exposed to DEHP [23].

In our study, the direction of the associations between urinary DEHP metabolites and early IVF parameters was variable and dependent on the specific metabolite assessed. Urinary mEOHP concentrations were positively associated with the numbers of follicles and of blastocysts. Also, the number of blastocysts in the 2nd quartile of of mEOHP concentrations in the follicular fluid was significantly higher when compared to the 1st quartile . In a similar prospective cohort study, results disagreed with what was found in our study, where follicular fluid concentrations of women undergoing to infertility treatment show no statistically significant associations with IVF parameters [24].

In addition to the associations seen for DEHP metabolites, we also report associations between some other phthalate metabolites and certain outcomes. The number of good quality embryos was higher in quartile 2 compared with quartile 1 for follicular fluid concentrations of mEP. Also, higher numbers of fertilized embryos was found in quartiles 2 and 3 for follicular fluid mEP concentrations when compared to quartile 1. This data also does not agree with the literature. A recent work assessed urine sample concentrations on the day of oocyte retrieval of women undergoing assisted reproduction treatments and observed a significant correlation of mEP with decreased odds of normal fertilization in medium-concentration group compared to low-concentration group, however the association did not reflect in the odds of good-quality embryos or blastocyst formation [25]. However, the study by Deng et al. [25] used in urine samples whilst our

data report significant associations with mEP in follicular fluid samples. Previous literature already shows that urinary metabolite concentrations are poor predictors of follicular fluid metabolite concentrations [26]. Currently, there are few epidemiologic studies on associations of phthalate concentrations in follicular fluid and early outcomes of *in vitro* fertilization procedures.

In a recent work investigating possible associations of urinary phthalate metabolite concentrations with pre-ovulatory levels of follicular fluid anti-müllerian hormone (AMH) in women undergoing fertility treatment, Sacha et al. [27] observed a negative association of mEOHP, mECPP and mBzP with AMH concentrations [27]. The data observed is consistent with the prior studies in both humans and animals suggesting that ovarian steroid production is susceptible to phthalate exposure in women with infertility [9,22]. In accordance with this data, we observed in our study a negative association of higher concentrations of urinary mBzP with number of follicles and mature oocytes. Both processes are highly dependent of ovarian steroid production. Also, we observed a positive association with number of collected oocytes in quartile 2 concentrations of mIPrP, a chemical already described as having antiestrogenic activity *in vitro* [28].

Our data observed differences associations between urinary and follicular fluid phthalates concentrations. The discrepancies between the two types of samples may be attributed to their biological characteristics, their metabolite filtration and clearance [29]. Also, previous study suggests that, since follicular fluid accumulates in the follicular antrum until ovulation, is possible that the metabolites and toxicity of phthalates in FF might manifest via direct contact with oocyte and granulosa cells [26]. Follicular fluid sampling provides a feasible way to directly estimate the phthalate exposure status of the ovarian and therefore, more studies are needed to understand the phthalate exposure in follicular fluid. In this regard, it is also important to mention that phthalates can affect female reproductive health through multiple mechanisms, including local effects in the ovaries but also by interactions with the hypothalamic-pituitary-ovary axis and other endocrine signaling pathways (e.g., insulin secretion) that can affect ovarian and uterine functions [30; 8]. Such diverse mechanisms might also explain the discrepancies found in studies measuring phthalate levels in urine versus follicular fluid.

The results of our investigation support previous literature that suggest an involvement of phthalates as competing factors in reproductive processes, including early IVF

parameters. Also, to the best of our knowledge, this study is the first to evaluate urinary and follicular fluid phthalate metabolites concentrations in South American women undergoing infertility treatment. However, our study had some limitations, as the inability to extrapolate our findings to the general population since the participants in this study were recruited from a reproductive center. Due to a method limitation we were unable to separate the metabolites of di-n- and di-iso-butyl phthalate, mBuP and mIBuP, respectively. Also, we measured a single-spot urine sample in this study, phthalates are short-lived chemicals and exposures are likely episodic. Yet, exposure to phthalates may be reflective of other unknown lifestyle or fertility factors that might be associated with early reproductive outcomes. Despite the afore mentioned limitations, this study is strengthened by our prospective study design and the application of an in ART setting which provided us an approach to early reproductive outcomes.

Reference List

[1] Inhorn MC, Patrizio P. Infertility around the globe: new thinking on gender, reproductive technologies and global movements in the 21st century. Hum Reprod Update. 2015 Jul-Aug;21(4):411-26. doi: 10.1093/humupd/dmv016. Epub 2015 Mar 22. PMID: 25801630.

[2] Piazza MJ, Urbanetz AA. Environmental toxins and the impact of other endocrine disrupting chemicals in women's reproductive health. JBRA Assist Reprod. 2019 Apr 30;23(2):154-164. doi: 10.5935/1518-0557.20190016. PMID: 30875185; PMCID: PMC6501744.

[3] Zoeller RT, Brown TR, Doan LL, Gore AC, Skakkebaek NE, Soto AM, Woodruff TJ, Vom Saal FS. Endocrine-disrupting chemicals and public health protection: a statement of principles from The Endocrine Society. Endocrinology. 2012 Sep;153(9):4097-110. doi: 10.1210/en.2012-1422. Epub 2012 Jun 25. PMID: 22733974; PMCID: PMC3423612.

[4] Martino-Andrade AJ, Chahoud I. Reproductive toxicity of phthalate esters. Mol Nutr Food Res. 2010 Jan;54(1):148-57. doi: 10.1002/mnfr.200800312.

[5]Kay VR, Bloom MS, Foster WG. Reproductive and developmental effects of phthalate diesters in males. Crit Rev Toxicol. 2014 Jul;44(6):467-98. doi: 10.3109/10408444.2013.875983. Epub 2014 Jun 6. PMID: 24903855.

[6]Hannon PR, Flaws JA. The effects of phthalates on the ovary. Front Endocrinol (Lausanne). 2015 Feb 2;6:8. doi: 10.3389/fendo.2015.00008. PMID: 25699018; PMCID: PMC4313599.

[7]Patel S, Zhou C, Rattan S, Flaws JA. Effects of Endocrine-Disrupting Chemicals on the Ovary. Biol Reprod. 2015 Jul;93(1):20. doi: 10.1095/biolreprod.115.130336. Epub 2015 Jun 10. PMID: 26063868; PMCID: PMC6366440.

[8] Basso CG, de Araújo-Ramos AT, Martino-Andrade AJ. Exposure to phthalates and female reproductive health: A literature review. Reprod Toxicol. 2022 Apr;109:61-79. doi: 10.1016/j.reprotox.2022.02.006. Epub 2022 Mar 3. PMID: 35248714.

[9] Hannon PR, Brannick KE, Wang W, Gupta RK, Flaws JA. Di(2-ethylhexyl) phthalate inhibits antral follicle growth, induces atresia, and inhibits steroid hormone production in cultured mouse antral follicles. Toxicol Appl Pharmacol. 2015 Apr 1;284(1):42-53. doi: 10.1016/j.taap.2015.02.010. Epub 2015 Feb 18. PMID: 25701202; PMCID: PMC4374011.

[10] Morgenstern R, Whyatt RM, Insel BJ, Calafat AM, Liu X, Rauh VA, Herbstman J, Bradwin G, Factor-Litvak P. Phthalates and thyroid function in preschool age children: Sex specific associations. Environ Int. 2017 Sep;106:11-18. doi: 10.1016/j.envint.2017.05.007. Epub 2017 May 27. PMID: 28554096; PMCID: PMC5533628.

[11] Sathyanarayana S, Barrett E, Butts S, Wang C, Swan SH. Phthalate exposure and reproductive hormone concentrations in pregnancy. Reproduction. 2014 Mar 2;147(4):401-9. doi: 10.1530/REP-13-0415. PMID: 24196015; PMCID: PMC3943643.

[12] Dewailly D, Robin G, Peigne M, Decanter C, Pigny P, Catteau-Jonard S. Interactions between androgens, FSH, anti-Müllerian hormone and estradiol during folliculogenesis in the human normal and polycystic ovary. Hum Reprod Update. 2016 Nov;22(6):709-724. doi: 10.1093/humupd/dmw027. Epub 2016 Aug 27. PMID: 27566840.

[13] Hauser R, Gaskins AJ, Souter I, Smith KW, Dodge LE, Ehrlich S, Meeker JD, Calafat AM, Williams PL; EARTH Study Team. Urinary Phthalate Metabolite Concentrations and Reproductive Outcomes among Women Undergoing in Vitro Fertilization: Results from the EARTH Study. Environ Health Perspect. 2016 Jun;124(6):831-9. doi: 10.1289/ehp.1509760. Epub 2015 Nov 6. PMID: 26545148; PMCID: PMC4892919.

[14] Machtinger R, Gaskins AJ, Racowsky C, Mansur A, Adir M, Baccarelli AA, Calafat AM, Hauser R. Urinary concentrations of biomarkers of phthalates and phthalate alternatives and IVF outcomes. Environ Int. 2018 Feb;111:23-31. doi: 10.1016/j.envint.2017.11.011. Epub 2017 Nov 20. PMID: 29161633; PMCID: PMC5800972.

[15] CDC. 2018 Centers for disease control and prevention. Fourth report on human exposure to environmental chemicals, updated tables, (march 2018). Atlanta, ga: U.S. Department of health and human services, centers for disease control and prevention

[16] Tan, J., Zou, Y., Wu, X.W., Tian, L.F., Su, Q., He, J.X., Huang, Z.H., Zhao, Y., Wu, L.P., Wu, Q.F., 2017. Increased SCF in follicular fluid and granulosa cells positively correlates with oocyte maturation, fertilization, and embryo quality in humans. Reprod. Sci. 24 (11), 1544e1550. https://doi.org/10.1177/1933719117697125.

[17] Kadhel P, Monnier P, Boucoiran I, Chaillet N, Fraser WD. Organochlorine pollutants and female fertility: a systematic review focusing on in vitro fertilization studies. Reprod Sci. 2012 Dec;19(12):1246-59. doi: 10.1177/1933719112446077. Epub 2012 May 16. PMID: 22593147.

[18] Machtinger R, Orvieto R. Bisphenol A, oocyte maturation, implantation, and IVF outcome: review of animal and human data. Reprod Biomed Online. 2014 Oct;29(4):404-10. doi: 10.1016/j.rbmo.2014.06.013. Epub 2014 Jul 10. PMID: 25154017.

[19] Bertoncello Souza M, Passoni MT, Pälmke C, Meyer KB, Venturelli AC, Araújo G, de Castilhos BS, Morais RN, Dalsenter PR, Swan SH, Koch HM, Martino-Andrade AJ. Unexpected, ubiquitous exposure of pregnant Brazilian women to diisopentyl

phthalate, one of the most potent antiandrogenic phthalates. Environ Int. 2018 Oct;119:447-454. doi: 10.1016/j.envint.2018.06.042. Epub 2018 Jul 20. PMID: 30031264.

[20]Rocha BA, Asimakopoulos AG, Barbosa F Jr, Kannan K. Urinary concentrations of 25 phthalate metabolites in Brazilian children and their association with oxidative DNA damage. Sci Total Environ. 2017;586:152-162. doi:10.1016/j.scitotenv.2017.01.193

[21]Smith KW, Braun JM, Williams PL, Ehrlich S, Correia KF, Calafat AM, Ye X, Ford J, Keller M, Meeker JD, Hauser R. Predictors and variability of urinary paraben concentrations in men and women, including before and during pregnancy. Environ Health Perspect. 2012 Nov;120(11):1538-43. doi: 10.1289/ehp.1104614. Epub 2012 Jun 21. PMID: 22721761; PMCID: PMC3556607.

[22]Gupta RK, Singh JM, Leslie TC, Meachum S, Flaws JA, Yao HH. Di-(2-ethylhexyl) phthalate and mono-(2-ethylhexyl) phthalate inhibit growth and reduce estradiol levels of antral follicles in vitro. Toxicol Appl Pharmacol. 2010 Jan 15;242(2):224-30. doi: 10.1016/j.taap.2009.10.011. Epub 2009 Oct 27. PMID: 19874833; PMCID: PMC2789888.

[23] Zhang XF, Zhang LJ, Li L, Feng YN, Chen B, Ma JM, Huynh E, Shi QH, De Felici M, Shen W. Diethylhexyl phthalate exposure impairs follicular development and affects oocyte maturation in the mouse. Environ Mol Mutagen. 2013 Jun;54(5):354-61. doi: 10.1002/em.21776. Epub 2013 Apr 26. PMID: 23625783.

[24] Du YY, Fang YL, Wang YX, Zeng Q, Guo N, Zhao H, Li YF. Follicular fluid and urinary concentrations of phthalate metabolites among infertile women and associations with in vitro fertilization parameters. Reprod Toxicol. 2016 Jun;61:142-50. doi: 10.1016/j.reprotox.2016.04.005. Epub 2016 Apr 8. PMID: 27067915

[25]Deng T, Du Y, Wang Y, Teng X, Hua X, Yuan X, Yao Y, Guo N, Li Y. The associations of urinary phthalate metabolites with the intermediate and pregnancy outcomes of women receiving IVF/ICSI treatments: A prospective single-center study. Ecotoxicol Environ Saf. 2020 Jan 30;188:109884. doi: 10.1016/j.ecoenv.2019.109884. Epub 2019 Nov 6. PMID: 31706241.

[26]Yao YC, Du YY, Wang YX, Deng TR, Liu C, Teng XM, Hua X, Yuan XQ, Guo N, Yin L, Zeng Q, Li YF. Predictors of phthalate metabolites in urine and follicular fluid and correlations between urine and follicular fluid phthalate metabolite concentrations among women undergoing in vitro fertilization. Environ Res. 2020 May;184:109295. doi: 10.1016/j.envres.2020.109295. Epub 2020 Feb 26. PMID: 32126376.

[27]Sacha CR, Souter I, Williams PL, Chavarro JE, Ford J, Mahalingaiah S, Donahoe PK, Hauser R, Pépin D, Mínguez-Alarcón L. Urinary phthalate metabolite concentrations are negatively associated with follicular fluid anti-müllerian hormone concentrations in women undergoing fertility treatment. Environ Int. 2021 Dec;157:106809. doi: 10.1016/j.envint.2021.106809. Epub 2021 Aug 7. PMID: 34375942.

[28]Okubo T, Suzuki T, Yokoyama Y, Kano K, Kano I. Estimation of estrogenic and anti-estrogenic activities of some [phthalate diesters and monoesters by MCF-7 cell proliferation assay in vitro. Biol Pharm Bull. 2003 Aug;26(8):1219-24. doi: 10.1248/bpb.26.1219. PMID: 12913283.

[29]Clarke HG, Hope SA, Byers S, Rodgers RJ. Formation of ovarian follicular fluid may be due to the osmotic potential of large glycosaminoglycans and proteoglycans. Reproduction. 2006 Jul;132(1):119-31. doi: 10.1530/rep.1.00960. PMID: 16816338.

[30]Akın L, Kendirci M, Narin F, Kurtoğlu S, Hatipoğlu N, Elmalı F. Endocrine Disruptors and Polycystic Ovary Syndrome: Phthalates. J Clin Res Pediatr Endocrinol. 2020 Nov 25;12(4):393-400. doi: 10.4274/jcrpe.galenos.2020.2020.003

6-DISCUSSÃO

O aumento nos índices de infertilidade gera crescente preocupação com o impacto emocional, financeiro e de saúde pública da redução da capacidade reprodutiva da população. Infertilidade é definida como a falha na obtenção de uma gestação de sucesso após 12 meses de coito frequente sem uso de métodos contraceptivos (Carson et al., 2021). Estudos recentes têm sugerido que o aumento nas taxas de infertilidade pode ocorrer em decorrência de fatores ambientais que, juntamente ao estilo de vida do paciente, podem atuar como causas idiopáticas de infertilidade (Mohamed et al., 2004, No, 2013). Evidências têm sugerido que substâncias que atuam como desreguladores endócrinos apresentam o potencial de ocasionar efeitos deletérios na capacidade reprodutiva e consequentemente nos tratamentos de infertilidade (Foster et al., 2006, Caserta et al., 2008). Dessa forma, a exposição a desreguladores endócrinos, como medicamentos analgésicos, associada a exposição a ftalatos e a outros DEs, tem sido considerada como um importante fator ambiental relacionado a desfechos reprodutivos negativos. Em nosso projeto dois grupos de compostos foram estudados, medicamentos analgésicos e ftalatos. Ambos já foram descritos na literatura como desreguladores endócrinos, entretanto poucos estudos avaliam as associações dessas substâncias com os desfechos obtidos em procedimentos de reprodução assistida. Ainda, são poucos são os dados epidemiológicos que avaliam a população brasileira com infertilidade ou subfertilidade, especialmente considerando dados de exposição a desreguladores endócrinos. Dentro desse contexto nosso estudo avaliou as associações entre exposição a ftalatos e medicamentos analgésicos e desfechos laboratoriais e clínicos de pacientes submetidas a tratamentos para infertilidade através de técnicas complexas de reprodução assistida – sendo observado que desfechos de mulheres em tratamento para infertilidade com técnicas de reprodução assistida apresentam associação entre medicamentos analgésicos e ftalatos específicos.

A primeira etapa do nosso estudo, que avaliou a exposição da população a medicamentos analgésicos, observou alto uso de analgésicos por mulheres buscando tratamento para infertilidade, sendo paracetamol o medicamento mais consumido. Dentro desse contexto, observamos associações entre o uso de medicamentos analgésicos antes e durante o tratamento de reprodução assistida e desfechos laboratoriais. Em relação ao consumo de paracetamol foi observado associação positiva desse composto com a taxa de maturação e associação negativa com a qualidade embrionária. Ainda, observamos associação positiva do consumo de ibuprofeno e taxa de coleta e associação negativa do uso de ibuprofeno com a taxa de fertilização. Em relação à dipirona, foi observada associação negativa entre o consumo de

dipirona e a taxa de coleta e associação positiva com a taxa de utilização e de blastulação. Entretanto os resultados observados variavam de acordo com as covariáveis adicionadas ao modelo, com exceção da associação negativa entre paracetamol e qualidade embrionária que se mostrou significativa em todos os modelos. Dessa forma, é importante considerar que as condições de infertilidade das participantes do estudo podem influenciar nos resultados encontrados, tanto em relação ao consumo de medicamentos quanto ao estilo de vida.

A segunda etapa do nosso projeto consistiu em um estudo prospectivo das associações entre os níveis de metabólitos de ftalatos em urina e líquido folicular de pacientes passando por tratamentos de infertilidade com os desfechos reprodutivos do tratamento. Nesse trabalho específico foi observado que maiores concentrações de metabolitos de DEHP, especificamente mEHHP e mEOHP, foram associados com parâmetros oocitarios e de desenvolvimento embrionário. Maiores níveis urinários de mEHHP foram negativamente associados com número de folículos coletados, oocitos maduros, embriões fertilizados e embriões de qualidade. O metabólito mEOHP por sua vez, mostrou associação positiva com o número de folículos e número de blastocistos em amostras urinárias. Entretanto, as concentrações em líquido folicular do metabólito foram positivamente associadas somente com o número de blastocistos. Observamos, também, correlações positivas dos níveis em líquido folicular do metabólito mEP com número de embriões fertilizados e de boa qualidade. Por fim, nosso estudo também detectou associação negativa entre concentrações urinárias de mBzP e o número de folículos e de oocitos maduros e associação positiva entre concentrações de mIPrP e o número de oocitos coletados.

Dentro do nosso conhecimento, esse é o primeiro estudo a avaliar dois grupos de desreguladores endócrinos, especificamente medicamentos analgésicos e ftalatos, dentro da mesma população de pacientes em tratamento para infertilidade com técnicas de reprodução assistida. A escolha dos grupos de desreguladores endócrinos foi baseada nos dados prévios da literatura que mostram que os dois grupos apresentam potencial de interferir com a capacidade reprodutiva feminina (Brune et al., 2015, Roberts et al., 2016) inclusive em população de pacientes com sub ou infertilidade. Ainda, estudos já demonstraram que ftalatos e medicamentos analgésicos podem exercer seus efeitos deletérios através das mesmas vias. Estudos na literatura já demonstraram que a exposição a ftalatos pode estar associada a desequilíbrios nas vias de prostaglandinas, sendo essa uma das possíveis vias responsáveis pelos efeitos danosos da exposição a ftalatos no sistema reprodutor feminino, uma vez que prostaglandinas estão envolvidas em diversos processos regulatórios no sistema reprodutivo feminino, (Niringiyumukiza et al., 2018, Tran-Guzman e Culty, 2022). Dentro desse contexto,

já é descrito na literatura que ftalatos podem ocasionar desequilíbrios nas vias de prostaglandinas e, consequentemente, alterar processos reprodutivos (Tetz et al., 2015). Por sua vez, medicamentos analgésicos apresentam suas atividades terapêuticas por meio da inibição específica ou não especifica da enzima COX e alteração das cascatas de prostaglandinas (Vane e Botting, 1998). Sendo assim, é possível que fármacos que apresentam mecanismo de ação de inibição de prostaglandinas, como os analgésicos de venda livre também possam atuar como DEs da mesma forma que ftalatos. Por fim, também é importante considerar que estudos já demonstraram que medicamentos podem apresentar o potencial de atuarem como fonte de exposição a ftalatos em humanos, sendo descrito que maiores níveis de consumo de medicamentos estão associados com maiores níveis urinários de metabolitos de ftalatos (Jia et al., 2017; Chung et al., 2019). O estudo de Jia e colegas (2017) reportou a detecção de ftalatos em mais de 90 medicamentos de venda livre, sendo observado a presença mais frequente de DBP e DEHP. Dessa forma, diversos estudos já mostram as possíveis associações entre medicamentos analgésicos e ftalatos atuando como desreguladores endócrinos. Esses trabalhos indicam que exposição a ftalatos e consequentemente seus efeitos deletérios em desfechos reprodutivos podem refletir hábitos de vida, fatores de infertilidade e a exposição a outros compostos com papel de desreguladores endócrinos. Entretanto, mais estudos são necessários para compreender a relação entre o consumo de medicamentos e a possível exposição a ftalatos, assim como os efeitos desses compostos DEs nos desfechos reprodutivos de pacientes em tratamentos de reprodução assistida.

7- CONCLUSÃO

Neste estudo foram avaliadas as associações de desreguladores endócrinos, especificamente ftalatos e medicamentos analgésicos, com desfechos reprodutivos de pacientes em tratamentos de reprodução assistida. Observamos que ambos os desreguladores endócrinos apresentaram associações com parâmetros iniciais do desenvolvimento em ciclos de reprodução assistida, sendo assim sugerido que esses compostos apresentem o potencial de interferir em desfechos de ciclos de reprodução assistida. Nossos resultados em relação a exposição a ftalatos demonstraram-se variáveis e dependentes de metabólitos específicos, mas corroborando com dados da literatura que demonstram as possíveis interferências de exposição a ftalatos em desfechos reprodutivos. As associações observadas com medicamentos analgésicos também demonstraram a importância de considerar variáveis de interferência em estudos que avaliam exposição a desreguladores endócrinos, sendo ainda necessários maiores estudos para entender

as associações entre exposição a desreguladores endócrinos e desfechos reprodutivos em pacientes de reprodução assistida.

REFERÊNCIAS

AGARWAL A, Mulgund A, Hamada A, Chyatte MR. **A unique view on male infertility around the globe**. Reprod Biol Endocrinol. 2015 Apr 26;13:37. doi: 10.1186/s12958-015-0032-1. PMID: 25928197; PMCID: PMC4424520.

AGRAWAL SS, Alvin Jose M. **Anti-implantation activity of H2 receptor blockers and meloxicam, a COX-inhibitor, in albino Wistar rats**. Eur J Contracept Reprod Health Care. 2009 Dec;14(6):444-50. doi: 10.3109/13625180903258695. PMID: 19929648.

AKANDE V, Garas A, Cahill D. **The effect of diclofenac and paracetamol on pregnancy and implantation rates in infertile women undergoing IVF treatment**. J Obstet Gynaecol. 2006 Nov;26(8):785-7. doi: 10.1080/01443610600984578. PMID: 17130031.

AL-SALEH I, Coskun S, Al-Doush I, Abduljabbar M, Al-Rouqi R, Al-Rajudi T, Al-Hassan S. **Couples exposure to phthalates and its influence on in vitro fertilization outcomes.** Chemosphere. 2019 Jul;226:597-606. doi: 10.1016/j.chemosphere.2019.03.146. Epub 2019 Mar 25. PMID: 30954894.

ALUR S, Wang H, Hoeger K, Swan SH, Sathyanarayana S, Redmon BJ, Nguyen R, Barrett ES. **Urinary phthalate metabolite concentrations in relation to history of infertility and use of assisted reproductive technology**. Fertil Steril. 2015 Nov;104(5):1227-35. doi: 10.1016/j.fertnstert.2015.07.1150. Epub 2015 Aug 11. PMID: 26275821; PMCID: PMC4630141.

ANDREA. M, Gore.C, PhD, David Crews, PhD, Loretta L. Doan, PhD, Michele La Merrill, PhD, MPH, Heather Patisaul, PhD, and Ami Zota, ScD, **Introduction to endocrine chemicals (EDCs) a guide for public interest organizations and policy-makers**., Endocr. Soc. (2014).

ANVISAAgência Nacional de Vigilância Sanitária, ANVISA. **12ª Relatório do Sistema Nacional de Produção de Embriões** – SisEmbrio, 2021.

BALA R, Singh V, Rajender S, Singh K. **Environment, Lifestyle, and Female Infertility**. Reprod Sci. 2021 Mar;28(3):617-638. doi: 10.1007/s43032-020-00279-3. Epub 2020 Aug 3. PMID: 32748224.

BASSO CG, de Araújo-Ramos AT, Martino-Andrade AJ. **Exposure to phthalates and female reproductive health: A literature review.** Reprod Toxicol. 2022 Apr;109:61-79. doi: 10.1016/j.reprotox.2022.02.006. Epub 2022 Mar 3. PMID: 35248714.

BEN MAAR M, Lesné L, Hennig K, Desdoits-Lethimonier C, Kilcoyne KR, Coiffec I, Rolland AD, Chevrier C, Kristensen DM, Lavoué V, Antignac JP, Le Bizec B, Dejucq-Rainsford N, Mitchell RT, Mazaud-Guittot S, Jégou B. **Ibuprofen results in alterations of human fetal testis development**. Sci Rep. 2017 Mar 10;7:44184. doi: 10.1038/srep44184. PMID: 28281692; PMCID: PMC5345102.

BENJAMIN S, Masai E, Kamimura N, Takahashi K, Anderson RC, Faisal PA. **Phthalates impact human health: Epidemiological evidences and plausible mechanism of action**. J Hazard Mater. 2017 Oct 15;340:360-383. doi: 10.1016/j.jhazmat.2017.06.036. Epub 2017 Jun 19. PMID: 28800814.

BCERTOCELLO SOUZA M, Passoni MT, Pälmke C, Meyer KB, Venturelli AC, Araújo G, de Castilhos BS, Morais RN, Dalsenter PR, Swan SH, Koch HM, Martino-Andrade AJ. **Unexpected, ubiquitous exposure of pregnant Brazilian women to diisopentyl phthalate, one of the most potent antiandrogenic phthalates**. Environ Int. 2018 Oct;119:447-454. doi: 10.1016/j.envint.2018.06.042. Epub 2018 Jul 20. PMID: 30031264.

BLOUNT BC, Silva MJ, Caudill SP, Needham LL, Pirkle JL, Sampson EJ, Lucier GW, Jackson RJ, Brock JW. **Levels of seven urinary phthalate metabolites in a human reference population.** Environ Health Perspect. 2000 Oct;108(10):979-82. doi: 10.1289/ehp.00108979. PMID: 11049818; PMCID: PMC1240132.

BOTTING RM. **Mechanism of action of acetaminophen: is there a cyclooxygenase 3?** Clin Infect Dis 2000;31 Suppl 5:S202–10. [PubMed: 11113024]

BRUNE, K., Renner, B. & Tiegs, G. **Acetaminophen/ paracetamol: a history of errors, failures and false decisions**. Eur. J. Pain 19, 953–965 (2015).

BURDOF A, Brand T, Jaddoe VW, Hofman A, Mackenbach JP, Steegers EA. **The effects of work-related maternal risk factors on time to pregnancy, preterm birth and birth weight: the Generation R Study**. Occup Environ Med 2011;68:197–204

CAPOROSSI, L., Alteri, A., Campo, G., Paci, E., Tranfo, G., Capanna, S., Papaleo, E., Pigini, D., Viganò, P., & Papaleo, B. **Cross sectional study on exposure to BPA and phthalates and semen parameters in men attending a fertility center**. International Journal of Environmental Research and Public Health, 17(2), 489. 2020 https://doi. org/10.3390/ijerph17020489

CARSON SA, Kallen AN. **Diagnosis and Management of Infertility: A Review**. JAMA. 2021 Jul 6;326(1):65-76. doi: 10.1001/jama.2021.4788. PMID: 34228062; PMCID: PMC9302705.

CASERTA D, Maranghi L, Mantovani A, Marci R, Maranghi F, Moscarini M. **Impact of endocrine disruptor chemicals in gynaecology.** Hum Reprod Update. 2008 Jan-Feb;14(1):59-72. doi: 10.1093/humupd/dmm025. Epub 2007 Dec 10. PMID: 18070835.

CASERTA D, Mantovani A, Marci R, Fazi A, Ciardo F, La Rocca C, Maranghi F, Moscarini M. **Environment and women's reproductive health**. Hum Reprod Update. 2011 May-Jun;17(3):418-33. doi: 10.1093/humupd/dmq061. Epub 2011 Jan 24. PMID: 21266373.

CASHMAN JN. **The mechanisms of action of NSAIDs in analgesia**. Drugs 1996;52 Suppl 5:13–23. [PubMed: 8922554]

CDC Centers for Disease Control and Prevention, (2017) **Phthalates factsheet**. (〈https://www.cdc.gov/biomonitoring/Phthalates_FactSheet.html〉) (accessed 2..38.2022).

CHAVARRO JE, Mínguez-Alarcón L, Chiu YH, Gaskins AJ, Souter I, Williams PL, Calafat AM, Hauser R; EARTH Study Team. **Soy Intake Modifies the Relation Between Urinary Bisphenol A Concentrations and Pregnancy Outcomes Among Women Undergoing Assisted Reproduction**. J Clin Endocrinol Metab. 2016 Mar;101(3):1082-90. doi: 10.1210/jc.2015-3473. Epub 2016 Jan 27. PMID: 26815879; PMCID: PMC4803173.

CHUNG, B.Y., Choi, S.M., Roh, T.H., Lim, D.S., Ahn, M.Y., Kim, Y.J., Kim, H.S., Lee, B.M., 2019. **Risk assessment of phthalates in pharmaceuticals**. J. Toxicol. Environ. Health A

COBELLIS L, Latini G, Felice CD, Razzi S, Paris I, Ruggieri F, et al. 2003. **High plasma concentrations of di-(2-ethylhexyl)- phthalate in women with endometriosis**. Hum Reprod 18:1512–1515

COIPLET E, Courbiere B, Agostini A, Boubli L, Bretelle F, Netter A. **Endometriosis and environmental factors: A critical review**. J Gynecol Obstet Hum Reprod. 2022 Sep;51(7):102418. doi: 10.1016/j.jogoh.2022.102418. Epub 2022 Jun 3. PMID: 35667590.

DU YY, Guo N, Wang YX, Hua X, Deng TR, Teng XM, Yao YC, Li YF. **Urinary phthalate metabolites in relation to serum anti-Müllerian hormone and inhibin B levels among**

women from a fertility center: a retrospective analysis. Reprod Health. 2018 Feb 23;15(1):33. doi: 10.1186/s12978-018-0469-8. PMID: 29471860; PMCID: PMC5824533.

EALES J, Bethel A, Galloway T, Hopkinson P, Morrissey K, Short RE, Garside R. **Human health impacts of exposure to phthalate plasticizers: An overview of reviews**. Environ Int. 2022 Jan;158:106903. doi: 10.1016/j.envint.2021.106903. Epub 2021 Sep 30. PMID: 34601394.

ESTEVES SC, Humaidan P, Roque M, Agarwal A. **Female infertility and assisted reproductive technology**. Panminerva Med. 2019 Mar;61(1):1-2. doi: 10.23736/S0031-0808.18.03553-X. PMID: 30674179.

FOSTER PM. **Disruption of reproductive development in male rat offspring following in utero exposure to phthalate esters**. Int J Androl. 2006 Feb;29(1):140-7; discussion 181-5. doi: 10.1111/j.1365-2605.2005.00563.x. Epub 2005 Aug 11. PMID: 16102138.

FITZGERALD GA. 2003. **COX-2 and beyond: approaches to prostaglandin inhibition in human disease**. Nat Rev Drug Discov 2:879–890.

FORTNER RT, Oh H, Daugherty SE, Xu X, Hankinson SE, Ziegler RG, Eliassen AH. **Analgesic use and patterns of estrogen metabolism in premenopausal women.** Horm Cancer. 2014 Apr;5(2):104-12. doi: 10.1007/s12672-013-0167-5. Epub 2014 Jan 10. PMID: 24407556; PMCID: PMC3976755.

GORE AC, Chappell VA, Fenton SE, Flaws JA, Nadal A, Prins GS, Toppari J, Zoeller RT. **EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals.** Endocr Rev. 2015 Dec;36(6):E1-E150. doi: 10.1210/er.2015-1010. Epub 2015 Nov 6. PMID: 26544531; PMCID: PMC4702494.

GRAY LE Jr, Barlow NJ, Howdeshell KL, Ostby JS, Furr JR, Gray CL. **Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: added value of assessing multiple offspring per litter.** Toxicol Sci. 2009 Aug;110(2):411-25. doi: 10.1093/toxsci/kfp109. Epub 2009 May 29. PMID: 19482887; PMCID: PMC3145405.

GAYTÁN M, Morales C, Bellido C, Sánchez-Criado JE, Gaytán F. **Non-steroidal anti-inflammatory drugs (NSAIDs) and ovulation: lessons from morphology.** Histol Histopathol. 2006 May;21(5):541-56. doi: 10.14670/HH-21.541. PMID: 16493584.

HALPERN, J. A., Fantus, R. J., Chang, C., Keeter, M. K., Helfand, B., Bennett, N. E., et al. (2020). **Effects of Nonsteroidal Anti-inflammatory Drug (NSAID) Use upon Male Gonadal Function: A National, Population-Based Study**. Andrologia 52 (4), e13542. doi:10.1111/and.13542

HANNON PR, Flaws JA. **The effects of phthalates on the ovary**. Front Endocrinol (Lausanne). 2015 Feb 2;6:8. doi: 10.3389/fendo.2015.00008.

HLISNÍKOVÁ H, Petrovičová I, Kolena B, Šidlovská M, Sirotkin A**. Effects and Mechanisms of Phthalates' Action on Reproductive Processes and Reproductive Health: A Literature Review**. Int J Environ Res Public Health. 2020 Sep 18;17(18):6811. doi: 10.3390/ijerph17186811. PMID: 32961939; PMCID: PMC7559247.

HUANG PC, Liao KW, Chang JW, Chan SH, Lee CC. **Characterization of phthalates exposure and risk for cosmetics and perfume sales clerks**. Environ Pollut. 2017 Nov 2;233:577-587.

HART R, Doherty DA, Frederiksen H, Keelan JA, Hickey M, Sloboda D, Pennell CE, Newnham JP, Skakkebaek NE, Main KM. **The influence of antenatal exposure to phthalates on subsequent female reproductive development in adolescence: a pilot study**. Reproduction. 2014 Mar 2;147(4):379-90. doi: 10.1530/REP-13-0331

HAUSER R, Gaskins AJ, Souter I, Smith KW, Dodge LE, Ehrlich S, Meeker JD, Calafat AM, Williams PL; EARTH Study Team. **Urinary Phthalate Metabolite Concentrations and Reproductive Outcomes among Women Undergoing in Vitro Fertilization: Results from the EARTH Study**. Environ Health Perspect. 2016 Jun;124(6):831-9. doi: 10.1289/ehp.1509760. Epub 2015 Nov 6. PMID: 26545148; PMCID: PMC4892919.

HASSOUN-BARHAMJI, R., Raia Barjat, T. & Chauleur, C. **In the era of self-medication, what do pregnant women know about anti-inflammatory drugs?** Therapie 70, 369–376 (2015).

JALA A, Varghese B, Kaur G, Rajendiran K, Dutta R, Adela R, Borkar RM. **Implications of endocrine-disrupting chemicals on polycystic ovarian syndrome: A comprehensive review.** Environ Sci Pollut Res Int. 2022 Aug;29(39):58484-58513. doi: 10.1007/s11356-022-21612-0. Epub 2022 Jul 1. PMID: 35778660.

JIA, L.L., Lou, X.Y., Guo, Y., Leung, K.S.Y., Zeng, E.Y., 2017. **Occurrence of phthalate esters in over-the-counter medicines from China and its implications for human exposure.** Environ. Int. 98, 137–142.

JUKIC AMZ, Padiyara P, Bracken MB, McConnaughey DR, Steiner AZ. **Analgesic use at ovulation and implantation and human fertility**. Am J Obstet Gynecol. 2020 May;222(5):476.e1-476.e11. doi: 10.1016/j.ajog.2019.11.1251. Epub 2019 Nov 15. PMID: 31738897; PMCID: PMC7195999

JURCZEWSKA J, Szostak-Węgierek D. **The Influence of Diet on Ovulation Disorders in Women-A Narrative Review.** Nutrients. 2022 Apr 8;14(8):1556. doi: 10.3390/nu14081556. PMID: 35458118; PMCID: PMC9029579.

KADHEL, P., Monnier, P., Boucoiran, I., Chaillet, N., & Fraser, W. D. (2012). **Organochlorine pollutants and female fertility: A systematic review focusing on in vitro fertilization studies.** Reproductive Sciences, 19(12), 1246–1259.

KAHN LG, Philippat C, Nakayama SF, Slama R, Trasande L. **Endocrine-disrupting chemicals: implications for human health.** Lancet Diabetes Endocrinol. 2020 Aug;8(8):703-718. doi: 10.1016/S2213-8587(20)30129-7. PMID: 32707118; PMCID: PMC7437820.

KARWACKA A, Zamkowska D, Radwan M, Jurewicz J. **Exposure to modern, widespread environmental endocrine disrupting chemicals and their effect on the reproductive potential of women: an overview of current epidemiological evidence.** Hum Fertil (Camb). 2019 Apr;22(1):2-25. doi: 10.1080/14647273.2017.1358828. Epub 2017 Jul 31. PMID: 28758506.

KAVLOCK, R.J., Daston, G.P., DeRosa, C., Fenner-Crisp, P., Gray, L.E., Kaattari, S., et al. ,1996. **Research needs for the assessment of health and environmental effects of endocrine disruptors: A Report of the U.S. EPA-sponsored workshop**. Environ Health Perspect. 104(4):

KELLY-WEEDER S, Cox CL. **The impact of lifestyle risk factors on female infertility**. Women Health 2006;44:1–23.

KRISTENSEN, D. M. et al. **Intrauterine exposure to mild analgesics is a risk factor for development of male reproductive disorders in human and rat**. Hum. Reprod. 26, 235–244 (2011).

KRISTENSEN, D.M., Lesné, L., Le Fol, V., Desdoits-Lethimonier, C., Dejucq-Rainsford, N., Leffers, H., Jégou, B.. **Paracetamol (acetaminophen), aspirin (acetylsalicylic acid) and indomethacin are anti-androgenic in the rat foetal testis**. Int. J. Androl. 35, 377–384 2012. http://dx.doi.org/10.1111/j.1365-2605.2012.01282.x.

KRISTENSEN DM, Mazaud-Guittot S, Gaudriault P, Lesné L, Serrano T, Main KM, Jégou B. **Analgesic use - prevalence, biomonitoring and endocrine and reproductive effects**. Nat Rev Endocrinol. 2016 Jul;12(7):381-93. doi: 10.1038/nrendo.2016.55. Epub 2016 May 6. PMID: 27150289.

LAURETTA R, Sansone A, Sansone M, Romanelli F, Appetecchia M. **Endocrine disrupting chemicals: effects on endocrine glands. Front Endocrinol (Lausanne)**. 2019;10:178. :dito_existshttps:// doi.org/10.3389/fendo.2019.00178

LESSMAN, F., Correia-Sa, L., Calhau, C., Domingues, V. F., Weiss, T., Bruning, T., & Koch, H. M. **Exposure to the plasticizer di(2- ethylhexyl) terephthalate (DEHTP) in Portuguese children—Urinary metabolite levels and estimated daily intakes**. 2017

LEVINE, H., Jørgensen, N., Martino-Andrade, A., Mendiola, J., Weksler-Derri, D., Mindlis, I., et al. (2017). **Temporal Trends in Sperm Count: a Systematic Review and Meta-Regression** Analysis. Hum. Reprod. Update 23 (6), 646–659. doi:10.1093/humupd/dmx022

LI XN, Li HX, Yang TN, Li XW, Huang YQ, Zhu SY, Li JL. **Di-(2-ethylhexyl) phthalate induced developmental abnormalities of the ovary in quail (Coturnix japonica) via disruption of the hypothalamic-pituitary-ovarian axis**. Sci Total Environ. 2020 Nov 1;741:140293. doi: 10.1016/j.scitotenv.2020.140293. Epub 2020 Jun 18. PMID: 32610232.

LU Z, Zhang C, Han C, An Q, Cheng Y, Chen Y, Meng R, Zhang Y, Su J. **Plasticizer Bis(2-ethylhexyl) Phthalate Causes Meiosis Defects and Decreases Fertilization Ability of Mouse Oocytes in Vivo.** J Agric Food Chem. 2019 Mar 27;67(12):3459-3468. doi: 10.1021/acs.jafc.9b00121. Epub 2019 Mar 12. PMID: 30813722.

LUCATTINI L, Poma G, Covaci A, de Boer J, Lamoree MH, Leonards PEG. **A review of semi-volatile organic compounds (SVOCs) in the indoor environment: occurrence in consumer products, indoor air and dust.** Chemosphere. 2018 Jun;201:466-482. doi: 10.1016/j.chemosphere.2018.02.161

MACHTINGER R, Zhong J, Mansur A, Adir M, Racowsky C, Hauser R, Brennan K, Karlsson O, Baccarelli AA. **Placental lncRNA Expression Is Associated With Prenatal Phthalate Exposure.** Toxicol Sci. 2018 May 1;163(1):116-122. doi: 10.1093/toxsci/kfy013.

MARTINO-ANDRADE, A.J., Morais, R.N., Botelho, G.G., Muller, G., Grande, S.W., Carpentieri, G.B., Leao, G.M. and Dalsenter, P.R. **Coadministration of active phthalates results in disruption of fetal testicular function in rats**. International journal of andrology. 2009. 32, 704-712.

MARTINO-ANDRADE AJ, Chahoud I. **Reproductive toxicity of phthalate esters**. Mol Nutr Food Res. 2010 Jan;54(1):148-57. doi: 10.1002/mnfr.200800312. PMID: 19760678.

Mascarenhas, M.N.; Flaxman, S.R.; Boerma, T.; Vanderpoel, S.; Stevens, G.A. **National, Regional, and Global Trends in Infertility Prevalence Since 1990: A Systematic Analysis of 277 Health Surveys**. PLoS Med. 2012, 9, e1001356.

MATYAS RA, Mumford SL, Schliep KC, Ahrens KA, Sjaarda LA, Perkins NJ, Filiberto AC, Mattison D, Zarek SM, Wactawski-Wende J, Schisterman EF**. Effects of over-the-counter analgesic use on reproductive hormones and ovulation in healthy, premenopausal women**. Hum Reprod. 2015 Jul;30(7):1714-23. doi: 10.1093/humrep/dev099. Epub 2015 May 6. PMID: 25954035; PMCID: PMC4472321.

MCINERNEY KA, Hatch EE, Wesselink AK, Rothman KJ, Mikkelsen EM, Wise LA. **Preconception use of pain-relievers and time-to-pregnancy: a prospective cohort study**. Hum Reprod. 2017 Jan;32(1):103-111. doi: 10.1093/humrep/dew272. Epub 2016 Nov 5. PMID: 27816920; PMCID: PMC5165076.

MESQUITA I, Lorigo M, Cairrao E. **Update about the disrupting-effects of phthalates on the human reproductive system.** Mol Reprod Dev. 2021 Oct;88(10):650-672. doi: 10.1002/mrd.23541. Epub 2021 Oct 6. PMID: 34617353.

MESSERLIAN C, Souter I, Gaskins AJ, Williams PL, Ford JB, Chiu YH, Calafat AM, Hauser R; Earth Study Team. **Urinary phthalate metabolites and ovarian reserve among women seeking infertility care.** Hum Reprod. 2016 Jan;31(1):75-83. doi:10.1093/humrep/dev292. Epub 2015 Nov 15. PMID: 26573529; PMCID: PMC4677966.

MÍNGUEZ-ALARCÓN, L., Williams, P. L., Chiu, Y. H., Gaskins, A. J., Nassan, F. L., Dadd, R., Petrozza, J., Hauser, R., Chavarro, J. E., & Earth Study, T. (2018). **Secular trends in semen parameters among men attending a fertility center between 2000 and 2017: Identifying**

potential predictors. Environment International, 121(Pt 2), 1297–1303. https://doi.org/10.1016/j.envint.2018.10.052

MODICK, H., Weiss, T., Dierkes, G., Brüning, T. & Koch, H. M. **Ubiquitous presence of paracetamol in human urine: sources and implications**. Reproduction 147, R105–R117

MOHAMED A.M Hassan, Stephen R Killick, **Negative lifestyle is associated with a significant reduction in fecundity,** Fertility and Sterility, Volume 81, Issue 2, 2004, Pages 384-392

Morgenstern, R., et al., 2017. **Phthalates and thyroid function in preschool age children: sex specific associations**. Environ. Int. 106, 11–18.

NAZIR, S., Usman, Z., Imran, M., Lone, K. P., & Ahmad, G. (2018). **Women diagnosed with endometriosis show high serum levels of diethyl hexyl phthalate.** Journal of Human Reproductive Sciences, 11(2), 131–136. https://doi.org/10.4103/jhrs.JHRS_137_17

NIRINGIYUMUKIZA JD, Cai H, Xiang W. **Prostaglandin E2 involvement in mammalian female fertility: ovulation, fertilization, embryo development and early implantation**. Reprod Biol Endocrinol. 2018 May 1;16(1):43. doi: 10.1186/s12958-018-0359-5. PMID: 29716588; PMCID: PMC5928575.

NO, ACO. **Exposure to toxic environmental agents.** Fertility and Sterility, 100(4), 931–934. (2013).. https://doi.org/10.1016/j.fertnstert. 2013.08.043

PEDNEKAR PP, Gajbhiye RK, Patil AD, Surve SV, Datar AG, Balsarkar GD, Chuahan AR, Vanage GR. **Estimation of plasma levels of bisphenol-A & phthalates in fertile & infertile women by gas chromatography-mass spectrometry**. Indian J Med Res. 2018 Dec;148(6):734-742. doi: 10.4103/ijmr.IJMR_2077_16. PMID: 30778008; PMCID: PMC6396564.

PETRO EM, Leroy JL, Covaci A, Fransen E, De Neubourg D, Dirtu AC, De Pauw I, Bols PE. **Endocrine-disrupting chemicals in human follicular fluid impair in vitro oocyte developmental competence**. Hum Reprod. 2012 Apr;27(4):1025-33. doi: 10.1093/humrep/der448. Epub 2012 Jan 20. PMID: 22267834.

PETROVI˘COVÁ, I, Kolena, B, Pilka, T. **The human biomonitoring of occupational exposure to phthalates.** Mediterranean Journal Social Science 2014, 5, 101–107.

POLANSKA, K.; Ligocka, D.; Sobala, W.; Hanke, W. **Phthalate exposure and child development: The Polish Mother and Child Cohort Study.** Early Hum. Dev. 2014, 90, 477–485. [CrossRef]

PRZYBYLIŃSKA, Paulina A. and Wyszkowski, Mirosław. **Environmental contamination with phthalates and its impact on living organisms** Ecological Chemistry and Engineering S, vol.23, no.2, 2016, pp.347-356.

REPOUSKOU, A.; Panagiotidou, E.; Panagopoulou, L.; Bisting, P.L.; Tuck, A.R.; Sjödin, M.O.D.; Lindberg, J.; Bozas, E.; Rüegg, J.; Gennings, C.; et al. **Gestational exposure to an epidemiologically defined mixture of phthalates leads to gonadal dysfunction in mouse offspring of both sexes**. Sci. Rep. 2019, 9, 6424

ROSSITTO M, Ollivier M, Déjardin S, Pruvost A, Brun C, Marchive C, Nguyen AL, Ghettas A, Keime C, de Massy B, Poulat F, Philibert P, Boizet-Bonhoure B. **In utero exposure to acetaminophen and ibuprofen leads to intergenerational accelerated reproductive aging in female mice.** Commun Biol. 2019 Aug 13;2:310. doi: 10.1038/s42003-019-0552-x. PMID: 31428698; PMCID: PMC6692356.

ROBERTS E, Delgado Nunes V, Buckner S, Latchem S, Constanti M, Miller P, Doherty M, Zhang W, Birrell F, Porcheret M, Dziedzic K, Bernstein I, Wise E, Conaghan PG. **Paracetamol: not as safe as we thought? A systematic literature review of observational studies.** Ann Rheum Dis. 2016 Mar;75(3):552-9. doi: 10.1136/annrheumdis-2014-206914. Epub 2015 Mar 2. PMID: 25732175; PMCID: PMC4789700. Serafin et al., 2022).

SAKHI AK, Lillegaard IT, Voorspoels S, Carlsen MH, Løken EB, Brantsæter AL, Haugen M, Meltzer HM, Thomsen C. **Concentrations of phthalates and bisphenol A in Norwegian foods and beverages and estimated dietary exposure in adults**. Environ Int. 2014 Dec;73:259-69. doi: 10.1016/j.envint.2014.08.005. Epub 2014 Aug 28. PMID: 25173060.

SATHYANARAYANA S, Barrett E, Butts S, Wang C, Swan SH. **Phthalate exposure and reproductive hormone concentrations in pregnancy**. Reproduction. 2014 Mar 2;147(4):401-9. doi: 10.1530/REP-13-0415.

SEO, M.-J., and Oh, D.-K. (2017). **Prostaglandin Synthases: Molecular Characterization and Involvement in Prostaglandin Biosynthesis**. Prog. lipid Res. 66, 50–68. doi:10.1016/j.plipres.2017.04.003

SKAKKEBAEK, N. E., Rajpert-De Meyts, E., & Main, K. M. (2001). **Testicular dysgenesis syndrome: An increasingly common developmental disorder with environmental aspects.** Human Reproduction, 16(5), 972–978. https://doi.org/10.1093/humrep/16.5.972 M.M.

SMARR, K.L. Grantz, R. Sundaram, J.M. Maisog, M. Honda, K. Kannan, G.M. Buck Louis, **Urinary paracetamol and time-to-pregnancy**, Hum. Reprod. 31 (2016) 2119–2127. https://doi.org/10.1093/humrep/dew172.

SOUTER I, Smith KW, Dimitriadis I, Ehrlich S, Williams PL, Calafat AM, Hauser R. **The association of bisphenol-A urinary concentrations with antral follicle counts and other measures of ovarian reserve in women undergoing infertility treatments.** Reprod Toxicol. 2013 Dec;42:224-31. doi: 10.1016/j.reprotox.2013.09.008. Epub 2013 Oct 4. PMID: 24100206; PMCID: PMC4383527.

SRILANCHAKON, K., Thadsri, T., Jantarat, C., Thengyai, S., Nosoognoen, W., & Supornsilchai, V. (2017). **Higher phthalate concentrations are associated with precocious puberty in normal weight Thai girls.** Journal of Pediatric Endocrinology and Metabolism, 30(12), 1293–1298. https://doi.org/10.1515/jpem-2017-0281

TAKEUCHI T, Tsutsumi O, Ikezuki Y, Takai Y, Taketani Y. **Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction**. Endocr J 2004; 51: 165–69. [PubMed: 15118266]

TANG C, Zhang J, Liu P, Zhou Y, Hu Q, Zhong Y, Wang X, Chen L**. Chronic exposure to low dose of bisphenol A causes follicular atresia by inhibiting kisspeptin neurons in anteroventral periventricular nucleus in female mice.** Neurotoxicology. 2020 Jul;79:164-176. doi: 10.1016/j.neuro.2020.04.011. Epub 2020 May 12. PMID: 32407858.

TAVARES IA, Vine ND. **Phthalic acid esters inhibit arachidonate metabolism by rat peritoneal leucocytes.** J Pharm Pharmacol. 1985 Jan;37(1):67-8. doi: 10.1111/j.2042-7158.1985.tb04936.x. PMID: 2858534.

TEDX The Endocrine Disruption Exchange, 2019. **TEDX list of potential endocrine disruptors. Phthalates** [WWW Document]. https://endocrinedisruption.org/ interactive-tools/critical-windows-of-development/about-the-timeline/ phthalates (accessed 2.20.21).

TESARIK J, Mendoza-Tesarik R. **Molecular Clues to Understanding Causes of Human-Assisted Reproduction Treatment Failures and Possible Treatment Options**. Int J Mol Sci.

2022 Sep 8;23(18):10357. doi: 10.3390/ijms231810357. PMID: 36142268; PMCID: PMC9499616.

TETZ LM, Aronoff DM, Loch-Caruso R. **Mono-ethylhexyl phthalate stimulates prostaglandin secretion in human placental macrophages and THP-1 cells.** Reprod Biol Endocrinol. 2015 Jun 3;13:56. doi: 10.1186/s12958-015-0046-8. PMID: 26036283; PMCID: PMC4462084

THIELE, K., Kessler, T., Arck, P., Erhardt, A. & Tiegs, G. **Acetaminophen and pregnancy: short- and long-term consequences for mother and child**. J. Reprod. Immunol. 97, 128–139 (2013)

TRAN-GUZMAN A, Culty M. **Eicosanoid Biosynthesis in Male Reproductive Development: Effects of Perinatal Exposure to NSAIDs and Analgesic Drugs**. Front Toxicol. 2022 Mar 3;4:842565. doi: 10.3389/ftox.2022.842565. PMID: 35295224; PMCID: PMC8915844.

TURUNEN JHO, Mäntyselkä PT, Kumpusalo EA, Ahonen RS. **Frequent analgesic use at population level: prevalence and patterns of use.** Pain. 2005 Jun;115(3):374-381. doi: 10.1016/j.pain.2005.03.013. PMID: 15911164.

VAN DEN DRIESCHE, S., Macdonald, J., Anderson, R.A., Johnston, Z.C., Chetty, T., Smith, L.B., McKinnell, C., Dean, A., Homer, N.Z., Jorgensen, A., Camacho-Moll, M.E., Sharpe, R.M., Mitchell, R.T., 2015. **Prolonged exposure to acetaminophen reduces testosterone production by the human fetal testis in a xenograft model**. Sci. Transl. Med. 7, 288ra80. http://dx.doi.org/10.1126/scitranslmed.aaa4097.

VANE JR, Botting RM. **Anti-inflammatory drugs and their mechanism of action**. Inflamm Res. 1998 Oct;47 Suppl 2:S78-87. doi: 10.1007/s000110050284. PMID: 9831328.

YILMAZ B, Terekeci H, Sandal S, Kelestimur F. **Endocrine disrupting chemicals: exposure, effects on human health, mechanism of action, models for testing and strategies for prevention**. Rev Endocr Metab Disord. 2020 Mar;21(1):127-147. doi: 10.1007/s11154-019-09521-z. PMID: 31792807.

YEN TH, Lin-Tan DT, Lin JL. **Food safety involving ingestion of foods and beverages prepared with phthalate-plasticizer-containing clouding agents**. J Formos Med Assoc. 2011 Nov;110(11):671-84. doi: 10.1016/j.jfma.2011.09.002.,

WHO Word Health Organization. **Infertility**. Available online: https://www.who.int/health-topics/infertility#tab=tab_1 (Acessado em 20/12/21)

WARNER GR, Dettogni RS, Bagchi IC, Flaws JA, Graceli JB. **Placental outcomes of phthalate exposure. Reprod Toxicol**. 2021 Aug;103:1-17. doi: 10.1016/j.reprotox.2021.05.001. Epub 2021 May 17. PMID: 34015474; PMCID: PMC8260441.

WITTASSEK M, Angerer J. **Phthalates: metabolism and exposure**. Int J Androl. 2008 Apr;31(2):131-8. doi: 10.1111/j.1365-2605.2007.00837.x.

ZHANG Y, Mustieles V, Williams PL, Wylie BJ, Souter I, Calafat AM, Demokritou M, Lee A, Vagios S, Hauser R, Messerlian C. **Parental preconception exposure to phenol and phthalate mixtures and the risk of preterm birth**. Environ Int. 2021 Jun;151:106440. doi: 10.1016/j.envint.2021.106440