UNIVERSIDADE ESTADUAL PAULISTA - "JÚLIO DE MESQUITA FILHO"
INSTITUTO DE BIOCIÊNCIAS DE BOTUCATU – IBB
PROGRAMA DE PÓS-GRADUAÇÃO EM FARMACOLOGIA E BIOTECNOLOGIA

ANA CAROLINA CASALI REIS

"INFLUÊNCIA DA EXPOSIÇÃO *IN UTERO* A ONDANSETRONA: POSSÍVEIS EFEITOS TERATOGÊNICOS E REPERCUSSÃO TARDIA EM PARÂMETROS REPRODUTIVOS E COMPORTAMENTAIS EM RATOS MACHOS"

Botucatu - SP
2021

UNIVERSIDADE ESTADUAL PAULISTA - “JÚLIO DE MESQUITA FILHO”
INSTITUTO DE BIOCIÊNCIAS DE BOTUCATU – IBB
PROGRAMA DE PÓS-GRADUAÇÃO EM FARMACOLOGIA E BIOTECNOLOGIA

ANA CAROLINA CASALI REIS

Influência da exposição *in utero* a ondansetrona: Possíveis efeitos teratogênicos e repercussão tardia em parâmetros reprodutivos e comportamentais em ratos machos

Dissertação apresentada ao Programa de Pós-Graduação em Farmacologia e Biotecnologia da Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP), para obtenção do título de Mestre em Farmacologia e Biotecnologia.

Área de concentração: Farmacologia

Orientador: Prof[a]. Dr[a]. Arielle Cristina Arena

Botucatu - SP
2021

R375i

Reis, Ana Carolina Casali

Influência da exposição in utero a ondansetrona : Possíveis efeitos teratogênicos e repercussão tardia em parâmetros reprodutivos e comportametais em ratos machos / Ana Carolina Casali Reis. -- Botucatu, 2022

111 f. : il., tabs., fotos

Dissertação (mestrado) - Universidade Estadual Paulista (Unesp), Instituto de Biociências, Botucatu

Orientadora: Arielle Cristina Arena

1. Medicamentos na gestação. 2. Hiperêmese gravídica. 3. Teratógenos. 4. Diferenciação sexual. 5. Toxicologia. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca do Instituto de Biociências, Botucatu. Dados fornecidos pelo autor(a).

Essa ficha não pode ser modificada.

Dedicatória

Dedico esse trabalho a todos que de alguma forma o tornaram possível.

Agradecimentos

A minha família, que mesmo diante de toda a saudade me apoiou a ir longe pelos meus sonhos, obrigada Evanice Casali, Waldir Edson Reis, Eduardo Casali Reis e Elena Sanches Casali.

A minha nova família, que se cada dia se torna mais importante para mim e deu força para que eu não deixasse de sorrir, Patrick Kwan e Jéssica Casali.

A minha orientadora Arielle Cristina Arena, por abrir as portas do mundo científico para mim, me permitiu criar, testar e evoluir, em qualquer lugar que eu for sempre vou lembrar de onde tudo começou.

Aos meus amigos, que mesmo com a distância continuam sendo meu abrigo Carolina Bernardi e Gustavo Rosário.

As amizades que essa jornada me proporcionou dentro e fora das paredes do laboratório, Bárbara, Júlia, Beatriz e Suyane. Obrigada por entrarem nessa jornada comigo, por além de executar esse trabalho, sentirem por

ele o amor que eu senti, sem vocês tudo isso não teria acontecido, sempre serei grata.

Aos demais discente que ajudaram a executar com maestria uma serie de intermináveis tarefas Ariana Musa, Letícia Cardoso e Carolina Barizan.

Ao técnico de laboratório José Eduardo, por toda a ajuda com diversas técnicas, as conversas e inúmeras brincadeiras.

Aos membros da banca avaliadoras da minha qualificação e defesa, pela disponibilidade e contribuições com o trabalho.

Ao financiamento pelo processo no 2020/08745-9, concedido pela Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

A todos que ajudaram direta ou indiretamente a tornar isso possível.

Muito obrigada.

Epígrafe

"Nada na vida deve ser temido, somente compreendido.
Agora é hora de compreender mais para temer menos."
(Marie Curie)

Resumo

A ondansetrona é um antagonista altamente seletivo do receptor de serotonina 5HT3, utilizado na prevenção e tratamento de náuseas e vômitos. É considerada uma droga de categoria B, contraindicada para mulheres grávidas sem orientação médica ou do cirurgião dentista, no entanto é amplamente utilizada para aliviar náuseas e vômitos na gestação. Poucos dados avaliando o uso deste medicamento durante a período gestacional estão disponíveis na literatura. Dessa forma o objetivo é avaliar os possíveis efeitos teratogênicos e impactos tardios da ondansetrona em parâmetros reprodutivos e comportamentais na prole masculina após exposição intrauterina. Para isso, ratas Wistar com diagnóstico de prenhez foram alocadas em três grupos (n=20/grupo), sendo um grupo controle (água destilada) e dois grupos tratados com diferentes doses de ondansetrona (1,7 ou 2,5 mg/kg/dia, via oral). Parte dos animais n=10/grupo foi exposta ao medicamento do 6° ao 15° dia de prenhez (período correspondente a organogênese), e a outra parte foi exposta durante todo o período de prenhez, abrangendo o primeiro pico de testosterona, essencial para o processo de diferenciação sexual hipotalâmica. Os resultados gerados indicam que ondansetrona não foi capaz de alterar significativamente o processo de diferenciação sexual hipotalâmica, não havendo alterações em marcadores de masculinizacão e puberdade respectivamente: distância anogenital e separação prepucial. Demais parâmetros espermáticos e férteis avaliados permaneceram inalterados, limitando seus impactos a vida peripubere alterando o padrão de comportamento social. Avaliações do caráter teratogênico não indicou que o uso de ondansetrona durante a organogênese aumenta o risco de malformações externas, viscerais ou esqueléticas. No entanto mudanças em parâmetros bioquímicos e histológicos de ratas prenhas tratadas do dia de prenhez 6 ao 15 apontaram para lesão renal.

Palavras-chaves: náuseas e vômitos na gestação, hiperêmese gravídica, teratógenos, diferenciação sexual hipotalâmica, lesão renal.

Abstract

Ondansetron is a highly selective serotonin 5HT3 receptor antagonist used in the prevention and treatment of nausea and vomiting. It is considered a category B drug, contraindicated for pregnant women without medical or dental surgeon guidance, however, it is widely used to relieve nausea and vomiting during pregnancy. Few data evaluating the use of this drug during pregnancy are available in the literature. Thus, the objective is to evaluate the possible teratogenic effects and late impacts of ondansetron on reproductive and behavioral parameters in male offspring after intrauterine exposure. For this, Wistar rats diagnosed with pregnancy were allocated into three groups (n=20/group), being a control group (distilled water) and two groups treated with different doses of ondansetron (1.7 or 2.5 mg/kg /day, orally). Part of the animals n=10/group was exposed to the drug from the 6th to the 15th day of pregnancy (period corresponding to organogenesis), and the other part was exposed throughout the entire pregnancy period, covering the first peak of testosterone, essential for the process of hypothalamic sexual differentiation. The developed results indicate that ondansetron was not able to significantly alter the hypothalamic sexual differentiation process, with no changes in masculinization and puberty markers, anogenital distance, and preputial separation respectively. Other sperm and fertile parameters evaluated remained unchanged, limiting their impacts on peripubertal life, altering the pattern of social behavior. Assessments of teratogenicity did not indicate that the use of ondansetron during organogenesis increases the risk of external, visceral, or skeletal malformations. However, changes in biochemical and histological parameters of pregnant rats treated from pregnancy day 6 to 15 pointed to kidney injury.

key words: nausea and vomiting during pregnancy, hyperemesis gravidarum, teratogens, hypothalamic sex differentiation, kidney injury.

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS E SÍMBOLOS

5HT	5- hidroxitriptamina
AVPV	Núcleo Periventricular Anteroventral
DG	Dia Gestacional
FDA	Food and Drug Administration
GABA	Ácido Gama-aminobutírico
hCG	Gonadotrofina Coriônica Humana
HG	Hiperêmese gravídica
NVG	Náuseas e Vômitos na Gestação
SDN-POA	Núcleo Sexualmente Dimórfico da Area Pré-óptica
SNC	Sistema Nervoso Central

SUMÁRIO

Introdução

Os sintomas de náuseas e vômitos na gestação (NVG) são uma condição clinicamente significativa, capaz de afetar mais de 80% das mulheres grávidas (Matthews et al., 2010). Também conhecido como “mal-estar matinal”, esses sintomas tem início durante as primeiras semanas de gestação, e normalmente perduram até o fim do primeiro trimestre, porém, algumas mulheres sofrem complicações até o final da gestação (Summers, 2012), interferindo com a qualidade de vida (Mazzotta et al., 2009). Tem sido reportado na literatura uma correlação entre a hiperêmese gravídica (HG) e um aumento das taxas de depressão e transtorno de estresse pós-traumático, o que resulta em mau prognóstico para os neonatos, como baixo peso ao nascer, parto prematuro e morte fetal (Christodoulou-Smith et al., 2011).

A patogênese do quadro de NVG pode estar relacionada a múltiplos fatores genéticos, endócrinos, infecciosos, entre outros (Bustos et al., 2017). Um possível mecanismo, relacionado a essa patologia é a produção do hormônio gonadotrofina coriônica humana (hCG), que inicia sua produção, atinge um platô e tem seu declínio de forma semelhante ao período de aparecimento e resolução dos sintomas (Sanu; Lamont, 2011).

A ondansetrona (Zofran, Vonau, Nausedron) é um antagonista altamente seletivo do receptor de serotonina 5HT-3, utilizado na prevenção e tratamento de náuseas e vômitos, induzidos por quimioterapia, radioterapia e pós-operatórios (Farmacêutica, 1999). É considerado pela Food and Drug Administration (FDA USA), como uma droga de categoria B, que não deve ser utilizada por mulheres grávidas sem orientação médica ou do cirurgião-dentista. No entanto é amplamente utilizado nos Estados Unidos da América, sendo o medicamento mais frequentemente prescrito para alívio de NVG (Huybrechts et al., 2018; Siminerio et al., 2016). Na literatura, o uso da ondansetrona durante a gestação apresenta dados conflitantes, gerando uma instabilidade sobre o risco-benefício do uso deste medicamento.

A serotonina é uma amina biogênica amplamente estudada acerca do seu papel em distúrbios psicológicos associados a disponibilidade da mesma no SNC (Wilson et al., 1986). Os efeitos da serotonina são mediados por receptores 5-hidroxitriptamina (5HT) (Hoyer; Hannon; Matin, 2002). A presença de receptores 5HT3 em áreas pré-sinápticas e pós-sinápticas sugerem efeitos autorregulatórios na liberação de serotonina e sinalização excitatória serotoninérgica, dessa forma, a ação de antagonistas de 5HT-3 no organismo, como a ondansetrona, podem interferir nas vias de liberação de serotonina mediada por receptores 5HT-3 (Bang-Andersen et al., 2011)[13]. Além disso, possuem a capacidade de promover a liberação de noradrenalina (Gupta; Prabhakar; Radhakrishnan, 2016) e modular o sistema dopaminérgico (Huang et al., 2015).

Serotonina, Noradrenalina e Dopamina possuem um importante papel durante o desenvolvimento prenatal, e alterações no padrão de liberação desses neurotransmissores podem interferir no desenvolvimento do cérebro, acarretando consequências neurocomportamentais tardias (Bogi et al., 2018), que podem se manifestar somente na vida adulta. Dessa forma, mudanças nos níveis de neurotransmissores ocasionadas pelo uso de antagonistas de 5HT-3 na gestação seriam capazes de promover estímulos adversos em períodos críticos de desenvolvimento do feto, podendo afetar características funcionais e estruturais em processos, como o da diferenciação sexual hipotalâmica (Döhler et al., 1991).

Em mamíferos, para que aconteça o comportamento sexual tipicamente masculino, o hipotálamo precisa ser masculinizado, uma vez que, durante a vida pré-natal, esta região está organizada intrinsecamente do tipo feminino (Maclusky; Naftolin, 1981). Dessa forma, são necessários dois processos distintos: defeminização e masculinização, que irão conferir ao macho a capacidade de apresentar comportamento sexual típico masculino (McEwen et al., 1977). Esses processos são dependentes de testosterona, que por ação da enzima citocromo

P450 aromatase, é metabolizada originando estrógeno no cérebro (Erskine; Tobet; Baum, 1988; Rhoda; Corbier; Roffi, 1984). Em ratos machos, ocorrem dois picos de testosterona de origem testicular, sendo o primeiro no 18º e 19º dia de gestação (Weisz; Ward, 1980), e o segundo, durante as primeiras horas após o nascimento (Baum et al., 1988; Lalau, 1990). Dessa forma, qualquer substância capaz de alterar os picos de testosterona perinatal podem alterar os processos de masculinização e/ou defeminização do hipotálamo (Gore, 2010).

A potente ação antiemética da ondansetrona, tornou sua prescrição cada vez mais frequente para mulheres que apresentam o quadro de NVG e HG, buscando aliviar os sintomas e melhorar a qualidade de vida. Esse estudo busca elucidar questões a respeito do possível caráter teratogênico e, além disso, avaliar os impactos tardios da ondansetrona em parâmetros reprodutivos e comportamentais na prole masculina após exposição intrauterina.

1. Problemática do uso de medicamentos na gestação

Durante o período gestacional existe uma alta taxa de uso de medicamentos, por prescrição ou por automedicação, cerca de 40 a 90% das gestantes utilizam pelo menos uma substância ao longo da gestação, excluindo-se os polivitamínicos (Rang, 2007). No entanto, apesar de comum, esta é uma prática que requer atenção especial, devido aos possíveis desfechos adversos na gestação.

Um conhecido episódio foi a tragédia da talidomida, em que cerca de 10 mil crianças nasceram com malformações congênitas, servindo como alerta internacional quanto à segurança do uso de medicamentos durante essa fase crítica (Lefebvre et al., 2021). Anteriormente, acreditava-se que a placenta funciona como uma “barreira” que protegia o feto de qualquer agente químico, porém, esse conhecimento foi invalidado e atualmente sabe-se que diversas moléculas cruzam a membrana livremente e alcançam a circulação fetal (Janine Schirmer et al., 2000).

Dessa forma, novas substâncias farmacologicamente ativas devem passar por uma série de ensaios clínicos projetados para estabelecer sua segurança e eficácia antes de serem aprovadas para comercialização (Ministério da Saúde, 1997). No entanto, não é eticamente aceitável realizar estudos clínicos em gestantes, sendo assim, grande parte das informações sobre a segurança dos medicamentos nessa população são provenientes de relatos de caso, estudos de teratogenicidade e toxicidade reprodutiva realizados em modelos animais (Ministério da Saúde, 1996). A importância desses estudos se reflete nas diversas substâncias que tem seu potencial teratogênico conhecido, e a partir disso FDA classifica as drogas em categorias (A, B, C, D e X), de acordo com o seu risco na gestação (FDA, 1977).

2. Diferenciação sexual hipotalâmica

Durante o desenvolvimento embrionário alguns órgãos são tidos como órgãos bipotenciais, ou seja, através da ação hormonal são diferenciados em femininos e masculinos (Schwarz; McCarthy, 2008). Sendo assim no início do desenvolvimento, é necessário que mamíferos passem por um processo denominado determinação sexual, nesse processo o sexo genético (definido pelos cromossomos), definirá o sexo gonadal e assim a consequente produção de hormônios, que atuará na diferenciação sexual do cérebro (Koopman et al., 1990).

Em machos (XY), o desenvolvimento dos testículos está condicionado à presença do gene SRY, localizado no braço curto do cromossomo Y, responsável pela síntese do fator determinante de testículos (Koopman et al., 1990). Os testículos em desenvolvimento começam a produzir quantidade significativas de testosterona (Weisz; Ward, 1980), que induzirá a formação do epidídimo, ducto deferente, glândulas sexuais acessórias e pênis (Jost, 1948).

Além dessa diferenciação gonadal a testosterona influenciará mudanças em diversas regiões do hipotálamo, conferindo ao cérebro características correspondentes ao sexo gonadal (Figura 1) (Schwarz; McCarthy, 2008).

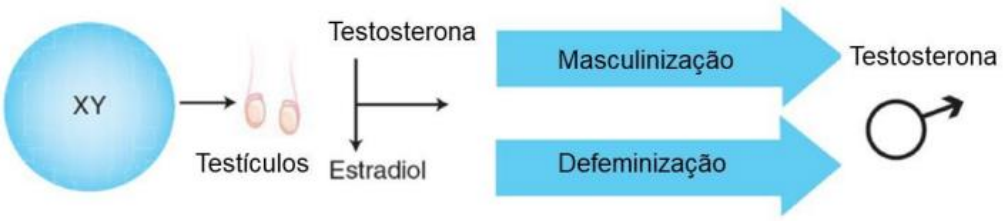

Figura 1. Esquema de determinação sexual, em que o sexo genético define o sexo gonadal e consequente produção de testosterona por parte dos testículos, convertida em estradiol que atua nos processos necessários para diferenciação sexual do cérebro (Adaptado de McCarthy & Arnold, 2011).

O hipotálamo é um centro integrador de informações, funcionando como uma comunicação entre o sistema nervoso central (SNC) e o resto do corpo (Gore, 2010). Antes do período crítico de diferenciação sexual hipotalâmica, o hipotálamo encontra-se organizado intrinsecamente do tipo feminino, determinando dessa forma o comportamento sexual típico de fêmea e um padrão de secreção cíclico de gonadotrofinas (Maclusky; Naftolin, 1981). Nos machos para o correto padrão de secreção de gonadotrofinas e comportamento sexual típico masculino são necessários dois processos distintos: defeminização e masculinização. Sendo a defeminização a perda da capacidade de um adulto em responder aos efeitos do estradiol e progesterona, ou seja, ausência de comportamento feminino (lordose), e a masculinização, a capacidade de apresentar comportamento de monta (Figura 2) (McEwen et al., 1977).

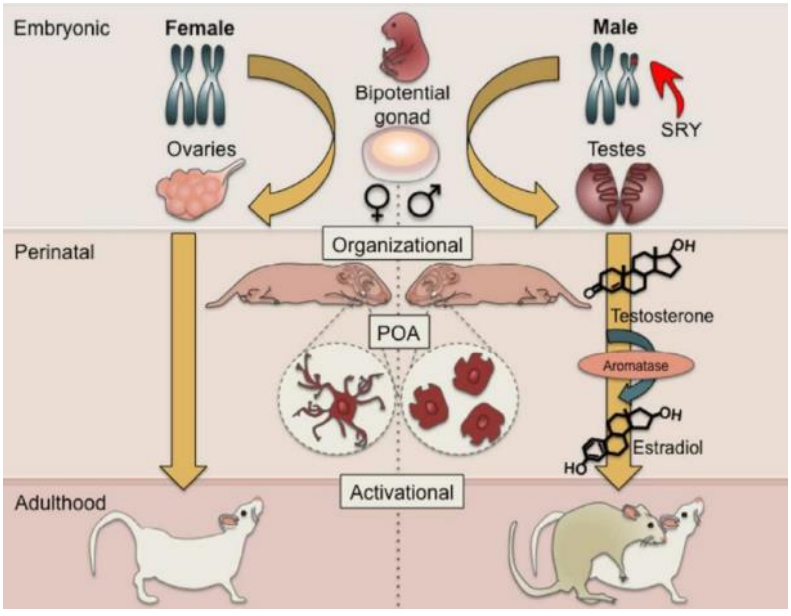

Figura 2. Esquema representando a distinção dos processos de diferenciação sexual, órgãos bipotenciais estimulados pelo gene SRY e consequente produção de testosterona, desencadeia na vida adulta o com portamento sexual típico de macho (Adaptado de Vanryzin; Pickett; McCarthy, 2018).

A testosterona participa ativamente da organização cerebral masculina, através de sua metabolização via enzima citocromo P450 aromatase originando estrógeno no SNC (Erskine; Tobet; Baum, 1988; Rhoda; Corbier; Roffi, 1984), que por sua vez liga-se a dois subtipos de receptores de estrógeno (α e β), sendo o α envolvido principalmente no processo de masculinização e o β com a defeminização (Kudwa et al., 2006).

A aromatização da testosterona é um evento necessário para ocorrer o remodelamento de estruturas cerebrais do SNC dos roedores, estabelecendo o dimorfismo sexual (Hrabovszky; Hutson, 2002). Desse modo, o estradiol atua através de mecanismos distintos em inúmeras regiões do cérebro, induzindo mudanças permanentes relacionadas à masculinização do hipotálamo (Figura 3). Tais mecanismos acarretam alterações no tamanho e volume dos núcleos hipotalâmicos, sendo o núcleo sexualmente dimórfico da área pré-óptica (SDN-POA) maior no sexo masculino e o núcleo periventricular anteroventral (AVPV) maior no sexo feminino; altera a morfologia celular dos neurônios (aumentando as espinhas dendríticas); aumenta a complexidade dos astrócitos no núcleo arqueado de machos, aumentando a síntese e liberação de ácido gama-aminobutírico (GABA) (Schwarz; McCarthy, 2008).

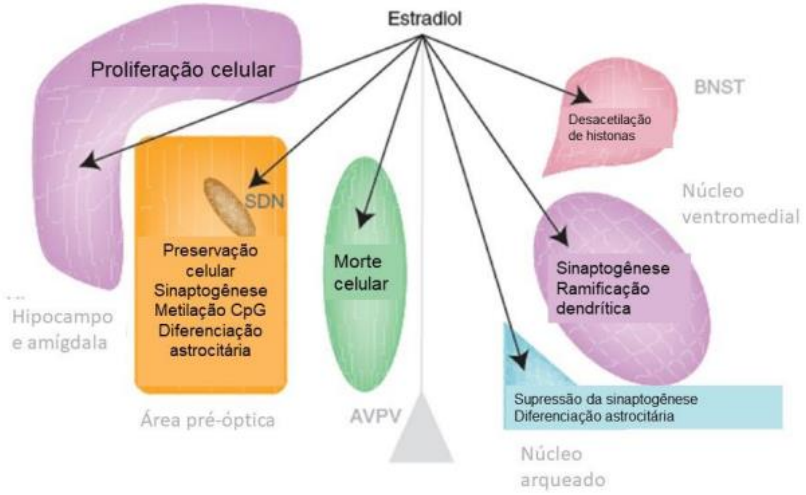

Figura 3. Remodelamento de estruturas cerebrais induzidas pela ação do estradiol (Adaptado de McCarthy & Arnold, 2011).

Desta forma, substâncias capazes de suprimir, atrasar ou retardar o pico de testosterona perinatal ou ainda alterar a expressão de receptores e enzimas envolvidas na diferenciação sexual hipotalâmica, podem alterar o processo de masculinização e defeminização do hipotálamo (Gore, 2010). A exposição a poluentes e o uso de medicamentos no decorrer dos períodos críticos de desenvolvimento podem comprometer processos, alterando o comportamento sexual e a fisiologia reprodutiva, os quais muitas vezes só serão observados na idade adulta reprodutiva (Negri-Cesi et al., 2008).

3. Náuseas e Vômitos na gestação

Náuseas e vômitos são sintomas frequentes durante a gravidez, ocorrendo em 60- 80% das gestações. O termo "enjoo matinal" ou "mal-estar matinal" é amplamente conhecido e utilizado para designar essa sintomatologia (Cunningham; Leveno; Bloom, 2018). Representam um dos grandes incómodos para a grávida, ocasionando uma diminuição do bem-estar e qualidade de vida da mãe, feto e restante família (Heitmann et al., 2017). A náusea é uma sensação subjetiva que precede o vômito, é acompanhada de suores frios, palidez, salivação, perda de tônus gástrico, contrações duodenais e refluxo do conteúdo intestinal para o estômago (Balaban; Yates, 2017).

Diversas pesquisas tentam estabelecer o fator etiológico para NVG, no entanto nenhuma hipótese proposta até o momento responde a todas as perguntas. Portanto, devido às tentativas frustradas para a busca de um único fator etiológico, aceita-se que ela tenha uma base etiológica multifatorial (Febrasgo, 2018). No entanto existe uma teoria mais aceita que envolve o aparecimento do hormônio gonadotrofina coriônica (Furneaux; Langley-Evans; Langley-Evans, 2001). Essa hipótese tem como base a relação temporal entre a concentração máxima de

produção da hCG e o pico de ocorrência das NVG, os quais ocorrem em média entre a 7ª a 10ª semana (Niebyl, 2010). Além disso, as NVG são mais frequentes e mais graves entre gestantes que apresentam aumento das concentrações de hCG, como o caso de gestação múltipla, doença trofoblástica gestacional, gestantes com fetos do sexo feminino e de gestantes com fetos portadores da síndrome de Down (Niebyl, 2010).

Existem diversas formas de apresentação clínica de NVG, que podem solucionar-se sem a necessidade de maiores intervenções ou pode evoluir para quadros clínicos de maior gravidade. No entanto uma pequena, porém relevante porcentagem (0,5-2%) pode manifestar uma sintomatologia mais severa, conhecida por hiperemese gravídica, caracterizada por vómitos persistentes, desidratação, distúrbios hidroeletrolíticos e perda de peso (McCarthy; Lutomski; Greene, 2014). Estudos apontam essa ocorrência associada a complicações na gravidez, como nascimento de bebês de baixo peso, prematuros e pequenos para a idade gestacional (Grooten; Roseboom; Painter, 2015). As possibilidades de tratamento para mulheres portadoras de NVG e HG podem ser divididas em farmacológicas e não farmacológicas (Murphy et al., 2016).

Entre as abordagens que utilizam métodos farmacológicos temos a terapia com antieméticos, os principais antieméticos utilizados são: Ondansetrona, que apresenta alívio dos sintomas em casos leves e graves, com baixa incidência de efeitos colaterais (Oliveira et al., 2014); Metoclopramida, um procinético que leva ao bloqueio dos receptores de dopamina e de serotonina (5HT2), atua em casos de média intensidade, no entanto tem seu uso abandonado devido aos efeitos colaterais como manifestações de ações extra-piramidais; Anti-histaminico, um grupo farmacológico que atua realizando o bloqueio do receptor H1 da histamina, promovendo efeito antiemético nas formas moderadas de NVG (McParlin et al., 2016).

GRUPO FARMACOLÓGICO	MEDICAMENTO	TERATOGÊNESE (FDA)
Antagonista da serotonina (5-HT3)	Ondansetrona	Classe B
Antagonista da dopamina e seratonina (5-HT2)	Metoclopramida	Classe B
Anti-histamínicos	Meclizina, dimenidrato	Classe B

Figura 4. Quadro de tratamentos farmacológicos para NVG e HG (Adaptado de Febrasgo, 2018).

4. Ondansetrona

Ondansetrona é um potente e seletivo antagonista do receptor 5-HT3, utilizado na clínica para tratar náuseas e vômitos induzidos por quimioterapia, radioterapia e cirurgia. Foi desenvolvida pelo Laboratório farmacêutico GlaxoSmithKline, em 1983, sendo aprovada pelo FDA como antiemético em 1991, com o nome comercial de Zofran® (FARMACÊUTICA, 1999).

Encontra-se disponível para comercialização no Brasil em duas apresentações: solução injetável e comprimido. A solução injetável é comercializada em ampolas de 2 ml (4 mg de cloridrato de ondansetrona) e 4 ml (8 mg de cloridrato de ondansetrona). Os comprimidos disponíveis para comercialização são de 4 e 8mg de cloridrato de ondansetrona dihidratado (Simpson; Hicks, 1996).

Tem como base um pó branco, praticamente insolúvel em água, já em sua fórmula comercial, o cloridrato de ondansetrona dihidratado, é um sal solúvel em água ou solução salina à temperatura ambiente (Armando, 2008).

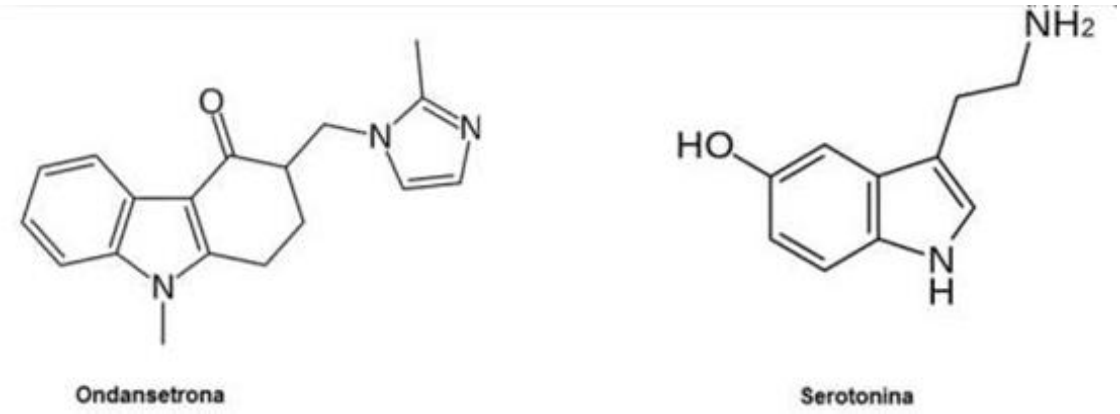

Figura 5. Semelhanças entre a estrutura química da Ondansetrona e Serotonina.

Pertence a uma classe de medicamento que atua especificamente em um receptor, o 5HT-3, que é distribuído em vários locais do organismo, como área postrema (região quimiorreceptora), núcleo do trato solitário (centro do vômito), substância gelatinosa, núcleo trigeminal e complexo vagal dorsal na medula espinhal. Além disto, este receptor serotoninérgico é encontrado em menor concentração no córtex cerebral, hipocampo, amígdala e glândula pineal (Goodin; Cunningham, 2002).

A ondansetrona é absorvida no trato gastrointestinal e ao passar pelo fígado sofre metabolismo de primeira passagem, com eliminação de 30 a 40% do fármaco, atingindo seu pico de concentração plasmática em 1,5 horas após ser administrada. A ligação dela às proteínas plasmáticas é de 70 a 76% e sua concentração no líquido cefalorraquidiano é apenas 15% do nível sérico. Tem alteração em sua biodisponibilidade com a presença de alimento no trato digestivo e não se altera com uso de antiácidos (Simpson; Hicks, 1996).

O metabolismo deste fármaco ocorre em sua maioria pelo fígado, através das enzimas do citocromo P450, particularmente CYP2D6, CYP1A2 e CYP3A4 (Roila; Del Favero, 1995). Após sua passagem no fígado sofre biotransformação em metabólito ativo (7- e 8-hidroxiondansetrona), que em sequência passa por conjugação com o ácido glucurônico e sulfato, produzindo seu metabólito inativo (Simpson; Hicks, 1996). Sua eliminação ocorre

pelos rins: 95% como metabólito inativo e 5% em sua forma inalterada. A meia vida de eliminação é de aproximadamente 4 horas, podendo ser menor em crianças, ou prolongada em portadores de insuficiência hepática (Bozigian et al., 1994).

Seu uso possui baixa taxa de abandono devido ausência de efeitos colaterais severos, raramente possuindo importância clínica, e normalmente quando relevantes, são controlados através de medicamentos (Goodin; Cunningham, 2002). Os efeitos adversos mais comuns são cefaleia (11-24%), sintomas gastrointestinais (constipação 9% e diarreia 5%), fadiga (13%), febre (5%), elevação de enzimas hepáticas, rubor facial e reação de hipersensibilidade (Bozigian et al., 1994; Roila; Del Favero, 1995).

Justificativa

Devido a capacidade da ondansetrona em prevenir e aliviar os sintomas de náuseas e vômitos, sua prescrição tornou-se cada vez mais frequente para mulheres que apresentam o quadro de NVG e HG, buscando assim melhorar a qualidade de vida. No entanto substâncias exógenas podem interferir no desenvolvimento intrauterino e excitabilidade de receptores 5HT3. Como sua segurança para o embrião e feto não está totalmente elucidada, torna-se necessário investigar seus possíveis efeitos teratogênicos e tardios em parâmetros reprodutivos e comportamentais, visto que fortes evidências confirmam o envolvimento do sistema serotoninérgico no processo de diferenciação sexual hipotalâmica.

Objetivo

Objetivo geral

Avaliar os efeitos resultantes da exposição *in utero* a ondansetrona sobre o desenvolvimento neonatal e possíveis repercussões tardias sobre parâmetros reprodutivos e comportamentais em ratos machos.

Objetivos específicos

Analisar os possíveis efeitos tóxicos da exposição a ondansetrona durante o período de prenhez em ratas, bem como analisar o comprometimento do comportamento materno durante esse período.

Avaliar possíveis efeitos teratogênicos do uso do antiemético ondansetrona durante o período da organogênese em ratos.

Examinar os impactos da exposição a ondansetrona na qualidade e quantidade espermática dos descendentes machos.

Avaliar o comprometimento dos comportamentos tipicamente masculinos na vida adulta de ratos machos expostos ao antiemético ondansetrona no período de prenhez.

Verificar a expressão de receptores de andrógenos no hipotálamo da prole masculina exposta ao antiemético ondansetrona no período de prenhez na vida adulta, por meio de western blot.

Referências bibliográficas

BALABAN, C. D.; YATES, B. J. What Is Nausea? A Historical Analysis of Changing Views. *Autonomic neuroscience : basic & clinical*, v. 202, p. 5, 1 jan. 2017. Disponível em: </pmc/articles/PMC5203950/>

BANG-ANDERSEN, B. *et al.* Discovery of 1-[2-(2,4-Dimethylphenylsulfanyl)phenyl]piperazine (Lu AA21004): A Novel Multimodal Compound for the Treatment of Major Depressive Disorder. *Journal of Medicinal Chemistry*, v. 54, n. 9, p. 3206–3221, 12 maio 2011. Disponível em: <https://pubs.acs.org/doi/abs/10.1021/jm101459g>.

BAUM, M. J. *et al.* Immediate Postnatal Rise in Whole Body Androgen Content in Male Rats: Correlation with Increased Testicular Content and Reduced Body Clearance of Testosterone1. *Biology of Reproduction*, v. 38, n. 5, p. 980–986, 1988.

BOGI, E. *et al.* Perinatal exposure to venlafaxine leads to lower anxiety and depression-like behavior in the adult rat offspring. *Behavioural Pharmacology*, v. 29, n. 5, p. 445–452, 1 ago. 2018.

BOZIGIAN, H. P. *et al.* Ondansetron absorption in adults: effect of dosage form, food, and antacids. *Journal of pharmaceutical sciences*, v. 83, n. 7, p. 1011–1013, 1994. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/7965657/>.

BUSTOS, M. *et al.* Nausea and Vomiting of Pregnancy-What's New? HHS Public Access Author manuscript. [S.d.].

CHRISTODOULOU-SMITH, J. *et al.* Posttraumatic stress symptoms following pregnancy complicated by hyperemesis gravidarum. *J Matern Fetal Neonatal Med*, v. 24, n. 11, p. 1307–1311, 2011. Disponível em: <www.HelpHer.org.>.

CUNNINGHAM, F. G.; LEVENO, K. J.; BLOOM, S. L. Chapter 18. Abortion. *Williams OBSTRETRICS 25th Edition*, p. 346–364, 2018.

DÖHLER, K. D. *et al.* Influence of neurotransmitters on sexual differentiation of brain structure and function. *Experimental and clinical endocrinology*, v. 98, n. 2, p. 99–109, 1991. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/1685710/>.

ÊMESE DA GRAVIDEZ SÉRIE ORIENTAÇÕES E RECOMENDAÇÕES FEBRASGO N O 2 • 2018.

ERSKINE, M. S.; TOBET, S. A.; BAUM, M. J. Effect of birth on plasma testosterone, brain aromatase activity, and hypothalamic estradiol in male and female ferrets*. *Endocrinology*, v. 122, n. 2, p. 524–530, 1988.

FARMACÊUTICA, B. CLORIDRATO DE ONDANSETRONA DI-HIDRATADO Comprimidos revestidos 4 mg e 8 mg.

FARMACÊUTICAS, F. D. E. C.; ARMANDO, Y. P. Avaliação da bioequivalência entre comprimido convencional e comprimido de desintegração oral contendo 8 mg de ondansetrona. 2008.

FURNEAUX, E. C.; LANGLEY-EVANS, A. J.; LANGLEY-EVANS, S. C. Nausea and vomiting of pregnancy: endocrine basis and contribution to pregnancy outcome. *Obstetrical & gynecological survey*, v. 56, n. 12, p. 775–782, 2001. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/11753180/>.

General Considerations for the Clinical Evaluation of Drugs | FDA. Disponível em: <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/general-considerations-clinical-evaluation-drugs>.

GOODIN, S.; CUNNINGHAM, R. 5-HT3-Receptor Antagonists for the Treatment of Nausea and Vomiting: A Reappraisal of Their Side-Effect Profile. *The Oncologist*, v. 7, n. 5, p. 424–436, 1 out. 2002. Disponível em: <https://onlinelibrary.wiley.com/doi/full/10.1634/theoncologist.7-5-424>. Acesso em: 25 jan. 2022.

GORE, A. C. Neuroendocrine targets of endocrine disruptors. *Hormones*, v. 9, n. 1, p. 16–27, 2010.

GROOTEN, I. J.; ROSEBOOM, T. J.; PAINTER, R. C. Barriers and Challenges in Hyperemesis Gravidarum Research. *Nutrition and Metabolic Insights*, v. 8, n. Suppl 1, p. 33, jan. 2015. Disponível em: </pmc/articles/PMC4755698/>.

GUPTA, D.; PRABHAKAR, V.; RADHAKRISHNAN, M. 5HT3 receptors: Target for new antidepressant drugs. *Neuroscience and biobehavioral reviews*, v. 64, p. 311–325, 1 maio 2016. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/26976353/>.

HEITMANN, K. *et al.* The burden of nausea and vomiting during pregnancy: severe impacts on quality of life, daily life functioning and willingness to become pregnant again - results from a cross-sectional study. *BMC pregnancy and childbirth*, v. 17, n. 1, p. 75, 28 fev. 2017. Disponível em: <https://bmcpregnancychildbirth.biomedcentral.com/articles/10.1186/s12884-017-1249-0>.

HOYER, D.; HANNON, J. P.; MARTIN, G. R. Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacology, biochemistry, and behavior*, v. 71, n. 4, p. 533–554, 2002. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/11888546/>.

HRABOVSZKY, Z.; HUTSON, J. M. *Androgen imprinting of the brain in animal models and humans with intersex disorders: Review and recommendations*. *Journal of Urology*. [S.l.]: Lippincott Williams and Wilkins. , 1 nov. 2002

HUANG, L. *et al.* Toxicogenomic analysis in the combined effect of tributyltin and benzo[a]pyrene on the development of zebrafish embryos. *Aquatic toxicology (Amsterdam, Netherlands)*, v. 158, p. 157–164, 1 jan. 2015. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/25438121/>.

HUYBRECHTS, K. F. *et al.* Association of maternal first trimester ondansetron use with cardiac malformations and oral clefts in offspring HHS Public Access. *JAMA*, v. 320, n. 23, p. 2429–2437, 2018.

JANINE SCHIRMER ET AL. *Assistência pré-natal*. 2000. v. 16.

JOST, P. A. LE CONTROLE HORMONAL DE LA DIFFÉRENCIATION DU SEXE. *Biological Reviews*, v. 23, n. 2, p. 201–236, 1 abr. 1948. Disponível em: <https://onlinelibrary.wiley.com/doi/full/10.1111/j.1469-185X.1948.tb00462.x>.

KOOPMAN, P. *et al.* Expression of a candidate sex-determining gene during mouse testis differentiation. *Nature*, v. 348, n. 6300, p. 450–452, 1990.

KUDWA, A. E. *et al.* Roles of estrogen receptors α and β in differentiation of mouse sexual

behavior. *Neuroscience*, v. 138, n. 3, p. 921–928, 2006.

LALAU, J. D. ET AL. Reduction in testicular function in rats. *Neuroendocrinology*, 1990.

LEFEBVRE, L. G. *et al.* Ginecologia E Obstetrícia – Caso 1. *Medicina em Casos Clínicos: Uma Coletânea baseada em Casos Reais*, p. 83–87, 2021.

MACLUSKY, N. J.; NAFTOLIN, F. *Sexual differentiation of the central nervous system*. *Science*: Science, 1981. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/6163211/>.

MATTHEWS, A. *et al.* Interventions for nausea and vomiting in early pregnancy. 2010.

MAZZOTTA, P. *et al.* Psychosocial morbidity among women with nausea and vomiting of pregnancy: Prevalence and association with anti-emetic therapy. *http://dx.doi.org/10.3109/01674820009075620*, v. 21, n. 3, p. 129–136, 2009. Disponível em: <https://www.tandfonline.com/doi/abs/10.3109/01674820009075620>.

MCCARTHY, F. P.; LUTOMSKI, J. E.; GREENE, R. A. Hyperemesis gravidarum: current perspectives. *International Journal of Women's Health*, v. 6, n. 1, p. 719, 5 ago. 2014. Disponível em: </pmc/articles/PMC4130712/>. Acesso em: 25 jan. 2022.

MCEWEN, B. S. *et al.* Aromatization: Important for sexual differentiation of the neonatal rat brain. *Hormones and Behavior*, v. 9, n. 3, p. 249–263, 1977.

MCPARLIN, C. *et al.* Treatments for Hyperemesis Gravidarum and Nausea and Vomiting in Pregnancy: A Systematic Review. *JAMA*, v. 316, n. 13, p. 1392–1401, 4 out. 2016. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/27701665/>.

Ministério da saúde. Disponível em: <https://bvsms.saude.gov.br/bvs/saudelegis/cns/1997/res0251_07_08_1997.html>.

Ministério da saúde. Disponível em: <https://bvsms.saude.gov.br/bvs/saudelegis/cns/1996/res0196_10_10_1996.html>.

MURPHY, A. *et al.* Day care versus inpatient management of nausea and vomiting of pregnancy: cost utility analysis of a randomised controlled trial. *European journal of obstetrics, gynecology, and reproductive biology*, v. 197, p. 78–82, 1 fev. 2016. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/26708475/>.

NEGRI-CESI, P. *et al.* Sexual differentiation of the rodent hypothalamus: Hormonal and environmental influences. *Journal of Steroid Biochemistry and Molecular Biology*, v. 109, n. 3–5, p. 294–299, 2008.

NIEBYL, J. R. Clinical practice. Nausea and vomiting in pregnancy. *The New England journal of medicine*, v. 363, n. 16, p. 1544–50, 14 out. 2010. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/20942670>.

OLIVEIRA, L. G. *et al.* Ondansetron compared with doxylamine and pyridoxine for treatment of nausea in pregnancy: a randomized controlled trial. *Obstetrics and gynecology*, v. 124, n. 4, p. 735–742, 10 out. 2014. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/25198265/>.

RANG H.P., D. M. M. Rang & Dale Farmacologia. 2007.

RHODA, J.; CORBIER, P.; ROFFI, J. Gonadal steroid concentrations in serum and hypothalamus of the rat at birth: Aromatization of testosterone to 17β-estradiol. *Endocrinology*,

v. 114, n. 5, p. 1754–1760, 1984.

ROILA, F.; DEL FAVERO, A. Ondansetron Clinical Pharmacokinetics. *Clinical Pharmacokinetics*, v. 29, n. 2, p. 95–109, 1995.

SANU, O.; LAMONT, R. F. Hyperemesis gravidarum: pathogenesis and the use of antiemetic agents. *http://dx.doi.org/10.1517/14656566.2010.537655*, v. 12, n. 5, p. 737–748, abr. 2011. Disponível em: <https://www.tandfonline.com/doi/abs/10.1517/14656566.2010.537655>.

SCHWARZ, J. M.; MCCARTHY, M. M. *Steroid-induced sexual differentiation of the developing brain: Multiple pathways, one goal*. *Journal of Neurochemistry*. [S.l.]: NIH Public Access. Disponível em: </pmc/articles/PMC2565863/>.

SIMINERIO, L. L. *et al.* Ondansetron Use in Pregnancy HHS Public Access Author manuscript. *Obstet Gynecol*, v. 127, n. 5, p. 873–877, 2016.

SIMPSON, K. H.; HICKS, F. M. Clinical pharmacokinetics of ondansetron. A review. *The Journal of pharmacy and pharmacology*, v. 48, n. 8, p. 774–781, 1996. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/8887724/>.

SUMMERS, A. Emergency management of hyperemesis gravidarum. *Emergency nurse : the journal of the RCN Accident and Emergency Nursing Association*, v. 20, n. 4, p. 24–28, 9 jul. 2012. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/22876404/>.

VANRYZIN, J. W.; PICKETT, L. A.; MCCARTHY, M. M. Microglia: Driving critical periods and sexual differentiation of the brain. *Developmental neurobiology*, v. 78, n. 6, p. 580, 1 jun. 2018. Disponível em: </pmc/articles/PMC5980665/>.

WALKER, C. L.; HO, S. M. Developmental reprogramming of cancer susceptibility. *Nature reviews. Cancer*, v. 12, n. 7, p. 479–486, jul. 2012. Disponível em: </pmc/articles/PMC3820510/>.

WEISZ, J.; WARD, I. L. Plasma testosterone and progesterone titers of pregnant rats, their male and female fetuses, and neonatal offspring. *Endocrinology*, v. 106, n. 1, p. 306–316, 1980.

WILSON, C. A. *et al.* The effect of neonatal manipulation of hypothalamic serotonin levels on sexual activity in the adult rat. *Pharmacology, biochemistry, and behavior*, v. 24, n. 5, p. 1175–1183, 1986. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/2425376/>.

Capítulo 1

O manuscrito intitulado **“The long-term effects of ondansetron exposure during the process of hypothalamic sexual differentiation in male rats”** será submetido a revista **Pharmacology Biochemistry and Behavior** (Fator de impacto: 3.533).

The long-term effects of ondansetron exposure during the process of hypothalamic sexual differentiation in male rats

Ana Carolina Casali Reis[1], Bárbara Campos Jorge[1], Júlia Stein[1], Suyane da Silva Moreira[1], Beatriz de Matos Manoel[2], Ariana Musa Aquino[1], Leticia Valente[2], Wellerson Rodrigo Scarano[1], Arielle Cristina Arena[1,3].

[1]Department of Structural and Functional Biology, Institute of Biosciences of Botucatu, Univ. Estadual Paulista – Botucatu (UNESP), São Paulo, Brazil.

[2]College of Health Science, Federal University of Grande Dourados, Dourados, Mato Grosso do Sul, Brazil.

[3]Center of Toxicological Assistance (CEATOX), Institute of Biosciences of Botucatu, Univ. Estadual Paulista – Botucatu (UNESP), São Paulo State, Brazil.

*Corresponding author:

Arielle Cristina Arena

Department of Structural and Functional Biology - Institute of Biosciences of Botucatu

São Paulo State University (UNESP)

Distrito de Rubião Junior, s/n

Caixa Postal – 510; CEP: 18618970; Botucatu - SP

Tel: + 55 14 38800495

E-mail: arielle.arena@unesp.br

Abbreviations

5HT Hydroxytryptamine (serotonin)

AGD Anogenital distance

ALT Alanine Aminotransferase

AR Androgen receptor

AST Aspartate Aminotransferase

AVPV Anteroventral Periventricular Nucleus

BSA Bovine Serum Albumin

CNS Central Nervous System

DSP Daily Sperm Production

GD Gestational day

HG Hyperemesis gravidarum

HHA Hypothalamic-Pituitary-Adrenal axis

HHG Hypothalamic-Pituitary-Gonadal axis

LD Lactational day

MCH Mean Corpuscular Hemoglobin

MCHC Mean Corpuscular Hemoglobin Concentration

MCV Mean Corpuscular Volume

MPOA Medial Preoptic Area

PND Postnatal day

Symbols

µg Micrograms

µL Microliter

g gram

i.p intraperitoneal

kg Kilogram

mg Milligram

mL Milliliter

mm Millimeter

rpm Revolutions per minute

s seconds

s.c subcutaneous

Highlights

- Ondansetron does not impact the hypothalamic differentiation process.
- Levels of testosterone are not impacted by ondansetron intrauterine exposure.
- Sperm quality did not change by intrauterine exposure to ondansetron.

Abstract

Ondansetron is a 5HT3 receptor antagonist considered a category B drug, widely used to relieve the symptoms of hyperemesis gravidarum. 5HT-3 receptors, the target of this drug, can interfere in brain development through changes in neurotransmitter levels, this study evaluated whether the *in utero* treatment with this drug could compromise reproductive and behavioral parameters in male offspring. Wistar rats were exposed to two doses of ondansetron (1.7; 2.5; mg/kg), by gavage, from gestational day 1 to21. Ondansetron exposure did not alter the anogenital distance or age of preputial separation in the male offspring. The group treated with the highest dose (2.5 mg/kg) showed an increase in play behavior, indicating a possible change in the pattern of neutransmitter release. In adulthood, there were no changes in any of the spermatic parameters evaluated, as well as in the levels of testosterone. Exposure during the gestational period did not impact sexual behavior of male offspring remaining with fertility unchanged. In conclusion, the present study demonstrated that maternal exposure to ondansetron proved to be safe for the process of sexual differentiation.

Keywords: 5HT3; sexual behavior; spermatogenesis, play behavior.

1. Introduction

Hyperemesis gravidarum (HG) is a severe form of nausea and vomiting during pregnancy, that persists throughout the gestational period and is accompanied by weight loss, dehydration, and electrolyte imbalances (Koot et al., 2019). Occurs in 0.3% to 2% of pregnant women, although populations with significantly higher rates have been reported (Goodwin, 2008). There is no international agreement on diagnostic criteria for HG, considered a multifactorial disease, that the diagnosis is only made on the basis of the clinical picture with a significant impact on quality of life and is related to negative birth outcomes (Koot et al., 2019).

Ondansetron is a 5HT-3 receptor antagonist approved for use in the treatment of nausea and vomiting associated with surgery and cancer chemotherapy, and become one of the most widely used antiemetics (Goodwin, 2008). It is considered by the Food and Drug Administration (FDA USA) as a category B drug, which should not be used by pregnant women without medical or dental advice.

5HT3Rs are the only serotonergic receptors belonging to the ligand-gated ion channel superfamily (Hoyer, Hannon & Martin 2002). In rats 5HT-3Rs are widely distributed in different regions of the brain, binding studies have shown the presence of 5HT-3Rs in the hippocampus, amygdala, nucleus accumbens, frontal, entorhinal cortex (Kilpatrick et al., 1987, Miller et al., 1992) and high concentrations in the forebrain, including neocortex, anterior olfactory nucleus, and brain stem (Morales et al., 1998).

The presence of 5HT3Rs on presynaptic and postsynaptic loci suggests that they may have autoregulatory effects on the synaptic release of 5HT as well as on fast excitatory signaling of 5HTergic neurotransmission. Two different circuit pathways define the 5HT release or inhibition mediated by 5HT3R: The first is a somatodendritic (DRN) short loop feedback control, whereby activation of 5HT3Rs could increase extracellular 5HT levels, and the second,

a cortical long loop feedback regulation, whereby stimulation of 5HT3Rs in interneuronal (GABA) projections triggers inhibitory control on DRN circuit release (Grupta, Prabhakar & Radhakrishnan, 2016).

5HT plays an important role in brain development, through the activation of 5HT receptors, serotonin can modulate processes such as cell division, differentiation, migration, and synaptogenesis (Azmitia, 2001). Prenatal exposure to drugs that increase serotonin concentrations is known to alter the hypothalamic-pituitary-adrenal (HPA) and gonadal (HPG) axes (Oberlander et al., 2009; Pawluski, 2012; Pawluski et al., 2012a). Alterations in the HPG axis during pregnancy can affect hormone-dependent processes, such as the hypothalamic sexual differentiation process, and bring negative consequences in reproductive and behavioral parameters for the offspring.

During prenatal life, the mammalian hypothalamus is intrinsically organized in the female type. In males, for typical male sexual behavior to occur and for the tonic pattern of gonadotropin secretion to appear, the hypothalamus needs to be masculinized (Maclusky &Naftolin, 1981). In rats the masculinization process of the hypothalamus is dependent on two testosterone peaks on the 18th and 19th day of gestation (Weisz & Ward, 1980), and the second during the first hours after birth (Baum et al., 1988; Corbier et al., 1978; Lalau et al., 1990), which through the action of the cytochrome P450 aromatase enzyme, is metabolized, originating estrogen in the CNS (Erskine et al., 1988; Rhoda et al., 1984).

Thus, substances capable of suppressing or delaying the peak of perinatal testosterone can alter the masculinization and/or defeminization process of the hypothalamus (Gore, 2010). Studies show that stress, exposure to environmental contaminants, as well as drug administration during the critical period of hypothalamic sexual differentiation can compromise this process, leading to changes in reproductive physiology and sexual behavior (Arena &

Pereira, 2002; Negri-Cesi, 2015; Pereira & Piffer, 2005), which are often only detected later, in reproductive adult life. A study conducted by Ladosky & Gaziri (1970) shows that a decreased concentration of serotonin in males may be a consequence of the process of sexual differentiation, indicating that the masculinization process of the rat brain, dependent on testosterone, may be influenced by the concentration of serotonin.

Due to the need for medicines that relieve the symptoms of HG, it is necessary to evaluate the effects of ondansetron exposure during these critical stages of development, since changes in this process, are usually only perceived in puberty or reproductive adulthood (Pereira, 2003; Pereira & Piffer, 2005). Elucidating this issue becomes an important step to assess fetal-maternal safety.

2. Materials and Methods

2.1 Animals

Adult Wistar rats, male (12 weeks, 350g, n = 10) and female (12 weeks, 250g, n = 40) from the Central Biotherium of São Paulo State University (UNESP) were used. The animals were maintained under standard conditions (temperature of 23ºC, photoperiod of 12h light/ 12h dark) and with water and food *ad libitum.* The experimental procedures were following the Ethical Principles in Animal Experimentation adopted by the Brazilian College of Animal Experimentation and were approved by the Ethics Committee on Animal Experimentation at the Institute of Biosciences of Botucatu/UNESP (protocol number: 6353250620).

2.2 Mating and pregnancy diagnostic

To obtain the experimental groups, two nulliparous female rats in estrus (n = 40) were mated with one male (n = 10) in the late afternoon. In the morning of the following day, vaginal lavage was performed, and gestational day (GD) 0 was considered when the presence of sperm was detected in the vaginal lavage. Pregnant rats were weighed and distributed in the experimental groups and maintained in individual cages. The method of determining sample size was based on the "tradition" or "common sense", confirmed by the resource equation (Festing, 2018).

2.3 Experimental groups and treatment

To evaluate the effects of ondansetron, pregnant rats were divided into three experimental groups, control that will receive only distilled water (vehicle) and two groups treated with doses of 1.7 or 2.5 mg/kg, orally (gavage). Pregnant rats were exposed to ondansetron from gestational day (GD) 1 to 21. The doses were based on therapeutic doses and calculated from the body surface area of the animals (Reagan-Shaw et al., 2007). During treatment, pregnant rats, maintained in individual cages, were weighed on alternate days to

calculate the volume of ondansetron to be administered, and clinical signs of toxicity (body weight, diarrhea, piloerection, bleeding, abnormal breathing, tremors, convulsions, and gear changes, posture, and reaction to manipulation) were investigated, as well as the average daily intake of water and food. After birth, the number of pups per litter was reduced to eight pups, four males and four females, to allow everyone to receive similar amounts of milk and to prevent litter size effects on the development of the pups. Male offspring were maintained until adulthood to assess sexual development and reproductive parameters.

2.4 Maternal parameters

2.4.1 Maternal behavior (n= 9 animals/group)

This parameter was evaluated according to Montagnini et al. (2016). On lactational day (LD) 5, all the pups were removed from the cage and the nest destroyed. After 30 min, the puppies were returned to the cage and a mother-pup interaction was observed for 30 min. Latency for collecting behavior and the total time of grouping, cleaning of the pups, puppies without maternal interaction (defined as the time the rat spent without any kind of interaction with the pups), and the construction of the nest were evaluated.

2.4.2 Hematological parameters and maternal biochemical analysis (n= 8-10/group)

After weaning, dams were anesthetized with sodium thiopental 50 mg/kg (i.p) and cardiac puncture was performed for blood collection. The serum obtained after centrifugation (1200 rpm for 20 minutes) was used for dosages of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Gamma-glutamyl transferase (Gamma-GT), Alkaline phosphatase (FA), Urea, Creatinine, Calcium, Cholesterol, Total Protein, and Albumin. These parameters were determined using the BioPlus 2000 semiautomatic equipment, using Bioclin kits.

For hematological analysis, the total count of leukocytes, erythrocytes, and platelets was performed, in addition to hemoglobin, hematocrit, and erythrocyte distribution widths. Except

for hemoglobin levels, which were determined using BioPlus 2000, the remaining hematological parameters were analyzed manually. The organs (thyroid, heart, lung, liver, kidney, adrenal, spleen, uterus, and ovary) were also collected, weighed, and analyzed for external morphology.

2.5 Male offspring parameters

2.5.1 Evaluation of sexual development of male offspring (n= 10/litters/group)

Male pups were weighed on the post-natal day (PND) 1, 13, and PND 22 on an analytical balance. At these same ages, the anogenital distance (AGD), measured between the genital tubercle and anus, was determined with the aid of a digital pachymeter, and thereby the relative anogenital distance was determined, calculated by the ratio between the AGD and the weight cube root. To confirm the puberty installation, from the PND 30 the preputial separation was manually checked to verify complete detachment of the prepuce.

2.5.2 Play behavior (n=10/litters/group)

This test was performed according to Zaccaroni et al. (2018) to evaluate the social behavior of playing in juvenile rats, which is an important marker of neurodevelopment. The animals were evaluated between PND 40 and 45, the age at which the animals exhibit the social behavior of "playing". Four males per litter were observed during the dark cycle, under red light.

2.6 Evaluation of reproductive parameters in adulthood

2.6.1 Organ Weights (n= 8-10/group)

At PND 60 and 90, male rats from different experimental groups (1/litter) were anesthetized with sodium thiopental 50 mg/kg (i.p.). Through cardiac puncture, blood was collected for hormonal dosage and organs such as testis, epididymis, seminal vesicle, vas

deferens, prostate, thyroid, liver, and kidney were removed and weighed on the analytical balance. The right testis and epididymis were stored for sperm analysis.

2.6.2 Sperm count in the testis and epididymis (n=9-10/group)

As described previously by Robb et al. (1978) and with adaptations adopted by Fernandes et al. (2007), homogenization-resistant sperm and sperm in the caput/corpus and cauda of the epididymis were counted. The testis was decapsulated and weighed after collection, and homogenized in 5 mL of 0.9% NaCl containing 0.5% Triton X 100 0.5%. The samples were diluted 10 times and transferred to Neubauer chambers, counting mature spermatids. To calculate the daily sperm production (DSP), the number of spermatids in stage 19 was divided by 6.1 (number of days in the seminiferous cycle in which these spermatids are present in the seminiferous epithelium). Portions of the caput/corpus and cauda epididymis portions were cut into fragments and homogenized, and the sperm count proceeded as described for the testis. The transit time of sperm through the epididymis was determined by dividing the number of sperm in each portion of the organ for DSP.

2.6.3 Sperm morphology (n=7-8/group)

To evaluate the sperm morphology, the interior of the right vas deferens of rats was washed with the aid of a needle and syringe, with 1.5 mL of saline solution. Smears were performed on histological slides, and 200 sperm per animal were evaluated under light microscopy (400 X magnification). The morphological abnormalities found in the sperm were classified into abnormalities of the head and tail (Filler, 1993).

2.6.4 Sperm motility (n=5/group)

Analysis of motility from cauda epididymis sperm was performed as described by Perobelli et al. (2010). A 10 mL aliquot of sperm suspension was immediately transferred to a Makler chamber maintained at 34 ºC. Using a phase-contrast microscope (400X magnification),

100 sperm were counted and classified as Type A (motile with progressive movement), Type B (motile without progressive movement), or Type C (static).

2.6.5 Fertility test (n=10/group)

At 80 days old, one male rat from each litter was paired with untreated females in their cages (1 female/male), late in the afternoon. On the following morning, vaginal smears were collected, and the day on which the presence of sperm was found in the smear was considered the day 0 of pregnancy. On the 20th day of pregnancy, the females were killed for the collection of the uterus and ovaries to record the number of corpora lutea, implants, reabsorptions, live and dead fetuses. From these results, these parameters were calculated: the fertility potential (efficiency of implantation): implantation sites/corpora lutea x100; gestation rate: number of pregnant females/number of inseminated females x100; pre-implantation loss rate (number of corpora lutea - number of implantations/number of corpora lutea X100); post-implantation loss rate (number of implantations - number of live fetuses/number of implantations x100), and sex ratio (number of male fetuses/number of female fetuses).

2.6.6 Sexual behavior (n=10/group)

Two weeks after the fertility test, adult rats, now sexually experienced, were anesthetized with Xylazine (10 mg/kg) and Ketamine (25 mg/kg) and bilaterally castrated. The experiments started after 15 days for adaptation of the animals to the inverted light/dark cycle and recovery from surgery. To assess sexual behavior, the animals were placed in observation cages, during the dark period of the light-dark cycle, under light with a red filter. The orchiectomized animals received testosterone propionate 1 mg/day (s.c.), three times a week, for 15 days, the first injection was administered the day after the surgery and the last one was given the day before the male sexual behavior.

2.6.6.1 Male copulatory behaviour (n = 9/group)

Male rats were placed individually in cages of polycarbonate crystal, staying for at least 5 min for adaptation. Then, a female rat in natural estrus was introduced into the same cage. The animals were observed for 30 min, however, if at the end of 10 min they did not show sexual behavior, the evaluation would be completed and it would be considered that this rat did not show such behavior. Each animal was tested only once and the following parameters were observed for 30 min: latency for the first mount, intromission, and ejaculation; latency to the first mount and intromission after first ejaculation; number of mounts and intromissions until the first ejaculation, total number of mounts, intromissions and, ejaculations during the test.

2.6.6.2 Expression of female-typical behaviour in adult male (n = 9/group)

Fifteen days after the evaluation of male sexual behavior, the same rats from the experimental groups were pre-treated with estradiol 17-benzoate 20 µg/kg (i.p.), 24 hours before the test. Untreated and sexually experienced males were placed individually in acrylic cages, where they remained for 5 min for adaptation. Then, the male from an experimental group was placed in the cage. For 10 min the presentation of lordosis and acceptance of the mount by the males of the experimental groups was evaluated in the presence of an untreated and sexually experienced male.

2.6.6.3 Sexual incentive motivation (n=9/group)

Aims to identify the sexual incentive motivation of rats for another male or female. This test was performed in a rectangular arena (50 x 50 x 100) with two small arenas (25 × 15 cm) positioned on opposite sides, where the stimulus animals were positioned. One arena contained one sexually experienced adult male and the other an estrus female rat. The tested rats did not have direct contact with the stimulus animals and the incentive was the odor that the stimulus animals exhale. The arena floor in front of each stimulus animal was demarcated into male and female incentive zones (30 × 20 cm). The experimental animal was placed in the center of the

arena and the following parameters were measured for 20 minutes: number of visits to each of the sexual incentive zones, total time spent in each of the zones, duration of each visit to each incentive zone. After the end of the test, a sexual preference pattern score was calculated for each animal (time spent in female zone/total time spent in both incentive zones) (Ågmo, 2003).

2.7. Serum testosterone levels (n = 8/group)

The testosterone was quantified by chemiluminescence immunoassay (MILLIPLEX® MAP - Multi-Species Hormone Magnetic Bead Panel - MERCK® MSHMAG-21K). The immunoassay was performed according to the manufacturer's technical guidelines, using internal and sample controls, and read in duplicate. Briefly, 200 µL of assay buffer was added to wash the plate; subsequently, 25 µL of assay buffer, 25 µL of standards, internal controls, and samples were added to the previously designated wells, 25 µL of HRP conjugate and 25 µL of a solution containing anti-testosterone beads. The plate has been sealed and incubated on a plate shaker for 16 - 20 hours at 4 ºC and, after this procedure, the contents of the plate will be gently removed, and the plate was washed 4 times. 25 µL of detection antibody was added to each well and shaken for 1 hour; subsequently, 25 µL of Streptavidin was added and shaken again, and the plate was washed 2 times. Finally, 100 µL of drive fluid was added and the reading will follow in the MAGPIX® equipment with the xPONENT software.

2.8 Histopathological evaluation (n = 5/group)

Left testis and epididymis were fixed in Alfac fixative solution (alcohol, acetic acid and formaldehyde) for 24 h and processed as described by Balin (Balin et al., 2020).

Leydig cell nuclei were counted in 10 random fields in each histological section of the testis. The mean core diameter of the Leydig cells were measured for the calculation of their volume. For this, 50 random nucleus (circular or elliptical) were measured per animal (Mantovani & Funic, 2014). The larger (D) and smaller (d) diameter of the cell nuclei were

obtained using a Nikon E-200 (X40) Microscope coupled to a digital camera and computer with NisElements software (version 4.20 for Windows). The mean diameter (M) was calculated using the formula M = (D x d)/2, the nuclear area (A) and the volume (V) were obtained using the following formulas: A = π x ¼ x M2 and V = π x ⅙ x M3, respectively.

2.9 Androgen receptor (AR) expression in hypothalamic neurons (n = 9/group)

2.9.1 Extraction and quantification of protein

Hypothalamus frozen samples were mechanically homogenized with RIPA buffer, plus proteases inhibitor (Sigma-Aldrich®, USA), in a Tureaux type homogenizer (Ultra Stirrer-Ultra80) in 3 cycles of 10 s around 4 °C. The homogenate was centrifuged at 14,000 rpm for 20 min at 4 °C, and the supernatant was collected. Protein concentration was determined by the Bradford method [30] on 96-well polystyrene plates and reading of absorbance was performed on Biochrom microplate reader (Holliston, Massachusetts, USA).

2.9.2 Western blotting

Aliquots containing 5 µg of proteins samples were separated on SDS-PAGE. Following the electrophoresis, the proteins were transferred to nitrocellulose membranes. The nonspecific binding of proteins was blocked by incubating the membrane in 5% non-fat milk in TBST buffer for 90 min at room temperature. The membranes were incubated with the respective primary antibody in 1% non-fat milk in TBST (1: 350-1,000) overnight at 4 °C: AR (N20) (sc-816- Santa Cruz® Biotechnology, Inc., USA) and ß-Actin (sc-47778-Santa Cruz® Biotechnology, Inc., USA).

The membranes were then incubated with a specific secondary antibody conjugated with peroxidase, which was diluted in TBST for 60 min. The immunoreactive components were revealed by GE Amersham ECL chemiluminescent substrate (GE Healthcare). The optic

density of band was used as the unit of measure with software Image J (version 1.33u—National Institutes of Health, USA), and normalized by β-actin values.

2.10 Statistical Analysis

Values are expressed as mean ± SEM or median (Q1 – Q3). Statistical analysis of variance tests - ANOVA were used to compare the results between the experimental groups, with a post hoc Tukey-Kramer test, or the Kruskal-Wallis, followed by a post hoc Dunn test. The differences were considered significant with $p < 0.05$, performed on the GraphPad InStat (version 5.02).

3. Results

3.1. Maternal

Females treated with ondansetron during pregnancy showed no signs of toxicity. Maternal parameters, such as water and food consumption, weight gain, maternal behavior, organ weights, biochemical and hematological parameters are shown in Supplementary material.

3.2. Offspring

Exposure to ondansetron in utero did not show alterations in the AGD (Fig. 2), as well as the weights of pups measured in these same intervals (Fig.3). The age of preputial separation, an important marker for puberty, was not altered (Fig. 4A), neither body weight measurement on the day of complete preputial separation (Fig. 4B). Play behavior analyzed in PND 40 showed significant results, when the highest dose (2,5 mg/kg) group was compared with the control, revealing changes in the behavioral patterns of these animals (Fig. 5). No differences in body weights and relative weights of the organs were observed in the PND 60 (Table 1) or PND 90 (Table 2).

Important parameters to verify the safety of this medicine for the animal's fertility remained unchanged. The sperm counting on PND 90, morphology (Table 3), and motility (Fig.6) revealed that ondansetron used during pregnancy was not able to harm the sperm production of male offspring, as well as it did not impact the fertility potential, revealed by the analysis of reproductive performance where all criteria evaluated were normalized (Table 4). Testosterone levels corroborate the other results with no statistical differences between groups (Fig. 7). Histopathological analysis of the testis shows that ondansetron exposure did not interfere with Leydig cell nucleus count, (Fig. 8), expression of androgen receptors in the hypothalamus (Fig. 9), as well as the epididymis morphology (Fig. 10). Other parameters evaluated such as

1 copulatory sexual behavior (Table 5), and sexual preference (Table 6), did not present
2 differences among groups.

4. Discussion

More than 59,000 pregnant women are hospitalized annually with hyperemesis gravidarum (Fejzo, 2008). The guiding clinical principle for the treatment of nausea and vomiting of pregnancy and hyperemesis gravidarum is avoidance of unnecessary medications (Siminerio et al., 2016). However, when nonpharmacologic therapies fail it is necessary to start a new approach, avoiding an increase of maternal stress, hyponatremia, malnutrition as well as other vitamin deficiencies.

Derangement during critical periods of development can permanently 'program' physiology and increase the risk of disorders in adulthood (Seckl & Holmes, 2007; Barker & Thornburg, 2013). Our results carry an important contribution being the first study to evaluate exposure to ondansetron during critical development windows that could affect sexual maturation and reproductive function.

Although the focus on the impacts of ondansetron on the process of sexual differentiation in the offspring, maternal toxic effects were evaluated during the exposure period, corresponding to the entire pregnancy. Clinical signs of toxicity are parameters that indicate adverse effects, such as loss of body mass during treatment, diarrhea, piloerection, and changes in behavior, other effects are not apparent, such as changes in hematological, biochemical, or weight of vital organs (Gonzáles & Silva, 2006). In our study, the treatment with different doses of ondansetron during pregnancy did not result in maternal toxicity, since no clinical signs or symptoms of toxicity were observed. However, the absence of signs of maternal toxicity does not necessarily assure the protection of the fetus (Danielsson, 2013) and needs to be further investigated.

Several studies suggest a significant interaction between sex hormones and neurotransmitters (Damoiseaux et al., 2014; Pawluski et al., 2020; Sramek & Cutler, 2011). The

use of drugs during pregnancy can be involved in negative impacts observed on sexual maturation in the reproductive function in rats (Balin et al., 2019). Gonadotropin release in rodents is profoundly influenced by neonatal estrogens. These estrogens are aromatized from testicular androgens and are required to organize the male rodent hypothalamic-pituitary-gonadal axis (HPG) as well as other aspects of reproductive physiology and behavior (Corbier et al., 1978, 1992; Weisz & Ward, 1980; MacLusky & Naftolin, 1981; DonCarlos et al., 1995; Simerly, 2002).

In this present study, exposure to ondansetron during testosterone peaks was not able to alter the AGD, nor the bodyweight at any of the ages evaluated. Shorter AGD can indicate an inadequate action or release of testosterone and can be an early indication of impaired sexual activity in adulthood (Gerardin et al., 2008). Other important parameters for assessing the development of offspring remained without significant results among groups, emphasizing preputial separation, which is a physical marker for puberty onset and is highly dependent on androgens (Thankamony et al., 1999) indicating that intrauterine exposure to this medication was not able to impact hormonal levels inducing a delay or advance puberty.

The development of juvenile social play behavior is influenced by steroids during perinatal life (Meaney, 1983). Since ondansetron treatment contemplated a sensitive window of brain sexual differentiation, exposure to chemical factors can alter neurodevelopment (Meaney & Stewart, 1981). In this study, the social play behavior presented an increase, showing changes in neurotransmitter release patterns, inducing social, cognitive, and motor alterations in development. Behavior is sexually differentiated, as males exhibit a higher frequency in this parameter than females (Argue & Mccarthy, 2015), the frequency found in this study may indicate the complete masculinization of the brain. Corroborating with the

expression of androgen receptors in the hypothalamus was not modified, suggesting the ondansetron doses failed to interfere in the masculinization process.

Decreased sperm count, motility, or morphological alterations are a marker for toxic effects on male reproduction and are related to the diminution of male fertility. In some rodent species, the production of normal sperm can be reduced by up to 90% or more without compromising fertility; however, small changes in sperm parameters in men can have serious consequences for fertility (Aafjes et al., 1980). Our findings indicate no impacts of ondansetron in sperm quality, and the same can be stated regarding the development of the testis and epididymis.

The absence of alterations in the testosterone levels corroborates with the presented results. Testosterone is one of the major androgens biosynthesized during steroidogenesis from cholesterol (Hu et al., 2010). Furthermore, testosterone is a facilitator of sexual behavior, acting on the central nervous system increasing nitric oxide production, modifying neuronal excitability and neurotransmitters releasing, such as dopamine in several brain areas, leading as a response to the male sexual interest (Gerardin et al., 1999), thus decreases in the levels of this hormone have an impact on the animal's sexual behavior and consequently on fertility.

In conclusion, the present study demonstrated that maternal exposure to ondansetron did not affect behavioral and reproductive parameters of male offspring in adult life. Considering these results, the use of ondansetron during pregnancy did not impact the sexual differentiation process. However, more studies are needed to consider this drug's safety during pregnancy.

5. Conflict of interest

The authors report no conflicts of interest.

6. Acknowledgements

The authors are grateful to José Eduardo Bozano, from the Department of Structural and Functional Biology, Institute of Biosciences, UNESP, Botucatu/SP–Brazil, for his technical assistance.

7. Funding information

This study was financed by FAPESP (2020/08745-9) - The State of São Paulo Research Foundation.

8. References

Arena AC, Pereira OC. Neonatal inhalatory anesthetic exposure: reproductive changes in male rats. Comp Biochem Physiol C Toxicol Pharmacol, 2002 133:633-40.

Argue KJ, McCarthy MM. Characterization of juvenile play in rats: importance of sex of self and sex of partner. Biol Sex Differ. 2015 Sep;10; 6:16.

Azmitia EC. Modern views on an ancient chemical: serotonin effects on cell proliferation, maturation, and apoptosis. Brain Res Bull. 2001 Nov 15;56(5):413-24

Balin PDS, Jorge BC, Leite ARR, Borges CS, Oba E, Silva EJR, de Barros AL, Horta-Júnior JAC, Arena AC. Maternal exposure to ibuprofen can affect the programming of the hypothalamus of the male offspring. Regul Toxicol Pharmacol. 2020 Mar; 111:104576.

Baum MJ, Brand T, Ooms M, Vreeburg JT, Slob AK. Immediate postnatal rise in whole body androgen content in male rats: correlation with increased testicular content and reduced body clearance of testosterone. Biol Reprod. 1988 Jun;38(5):980-6.

Carr GV, Lucki I. The role of serotonin receptor subtypes in treating depression: a review of animal studies. Psychopharmacology (Berl). 2011 Feb;213(2-3):265-87.

Corbier P, Edwards DA, Roffi J. The neonatal testosterone surge: a comparative study. Arch Int Physiol Biochim Biophys. 1992 Mar-Apr;100(2):127-31.

Corbier P, Kerdelhue B, Picon R, Roffi J. Changes in testicular weight and serum gonadotropin and testosterone levels before, during, and after birth in the perinatal rat. Endocrinology. 1978 Dec;103(6):1985-91.

Damoiseaux VA, Proost JH, Jiawan VC, Melgert BN. Sex differences in the pharmacokinetics of antidepressants: influence of female sex hormones and oral contraceptives. Clin Pharmacokinet. 2014 Jun;53(6):509-19.

DonCarlos LL, McAbee M, Ramer-Quinn DS, Stancik DM. Estrogen receptor mRNA levels in the preoptic area of neonatal rats are responsive to hormone manipulation. Brain Res Dev Brain Res. 1995 Feb 16;84(2):253-60.

Eisensamer B, Rammes G, Gimpl G, Shapa M, Ferrari U, Hapfelmeier G, Bondy B, Parsons C, Gilling K, Zieglgänsberger W, Holsboer F, Rupprecht R. Antidepressants are functional antagonists at the serotonin type 3 (5-HT3) receptor. Mol Psychiatry. 2003 Nov;8(12):994-1007.

Erskine MS, Tobet SA, Baum MJ. Effect of birth on plasma testosterone, brain aromatase activity, and hypothalamic estradiol in male and female ferrets. Endocrinology. 1988 Feb;122(2):524-30.

Fernandes GS, Arena AC, Fernandez CD, Mercadante A, Barbisan LF, Kempinas WG. Reproductive effects in male rats exposed to diuron. Reprod Toxicol. 2007 Jan;23(1):106-12.

Filler R. Methods for evaluation of rat epididymal sperm morphology. In: Chapin, R.E., Heindel, J.J. Methods in Toxicology: Male Reproductive Toxicology. 1993. Academic Press, San Diego.

Food and Drug Administration - FDA. General Considerations for the Clinical Evaluation of Drugs. Rockville, Md.: U.S. Dept. of Health, Education, and Welfare, Public Health Service, Food and Drug Administration.

Gaspar P, Cases O, Maroteaux L. The developmental role of serotonin: news from mouse molecular genetics. Nat Rev Neurosci. 2003 Dec;4(12):1002-12.

Gerardin DC, Pereira OC, Kempinas WG, Florio JC, Moreira EG, Bernardi MM. Sexual behavior, neuroendocrine, and neurochemical aspects in male rats exposed prenatally to stress. Physiology & Behavior. 2005;84(1):97-104.

Goodwin TM. Hyperemesis gravidarum. Obstet Gynecol Clin North Am. 2008 Sep;35(3):401-17, viii. doi: 10.1016/j.ogc.2008.04.002. PMID: 18760227.

Gore AC. Neuroendocrine targets of endocrine disruptors. Hormones (Athens). 2010 Jan-Mar;9(1):16-27.

Gupta D, Prabhakar V, Radhakrishnan M. 5HT3 receptors: Target for new antidepressant drugs. Neurosci Biobehav Rev. 2016 May; 64:311-25.

Harmer CJ, Reid CB, Ray MK, Goodwin GM, Cowen PJ. 5HT(3) antagonism abolishes the emotion potentiated startle effect in humans. Psychopharmacology (Berl). 2006 May;186(1):18-24.

Homberg JR, Schiepers OJ, Schoffelmeer AN, Cuppen E, Vanderschuren LJ. Acute and constitutive increases in central serotonin levels reduce social play behaviour in peri-adolescent rats. Psychopharmacology (Berl). 2007 Dec;195(2):175-82.

Hoyer D, Hannon JP, Martin GR. Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacol Biochem Behav. 2002 Apr;71(4):533-54.

Hu J, Zhang Z, Shen WJ, Azhar S. Cellular cholesterol delivery, intracellular processing and utilization for biosynthesis of steroid hormones. Nutr Metab (Lond). 2010 Jun 1; 7:47.

Kilpatrick GJ, Jones BJ, Tyers MB. Identification and distribution of 5-HT3 receptors in rat brain using radioligand binding. Nature. 1987 Dec 24-31;330(6150):746-8. doi: 10.1038/330746a0. PMID: 3696238.

Koot MH, Grooten IJ, Gauw NE, Roseboom TJ, Painter RC. Hyperemesis gravidarum [Hyperemesis gravidarum]. Ned Tijdschr Geneeskd. 2019 May 3;163:D3591.

Ladosky W, Gaziri LC. Brain serotonin and sexual differentiation of the nervous system. Neuroendocrinology. 1970;6(3):168-74.

Lalau JD, Aubert ML, Carmignac DF, Grégoire I, Dupouy JP. Reduction in testicular function in rats. II. Reduction by dexamethasone in fetal and neonatal rats. Neuroendocrinology. 1990 Mar;51(3):289-93.

MacLusky NJ, Naftolin F. Sexual differentiation of the central nervous system. Science. 1981 Mar 20;211(4488):1294-302.

Miller K, Weisberg E, Fletcher PW, Teitler M. Membrane-bound and solubilized brain 5HT3 receptors: improved radioligand binding assays using bovine area postrema or rat cortex and the radioligands 3H-GR65630, 3H-BRL43694, and 3H-LY278584. Synapse. 1992 May;11(1):58-66.

Montagnini BG, Silveira KM, Pierone BC, de Azevedo Camim N, Anselmo-Franci JA, de Fátima Paccola Mesquita S, Kiss ACI, Gerardin DCC. Reproductive parameters of female

Wistar rats treated with methylphenidate during development. Physiol Behav. 2016 Dec 1; 167:118-124.

Morales M, Battenberg E, Bloom FE. Distribution of neurons expressing immunoreactivity for the 5HT3 receptor subtype in the rat brain and spinal cord. J Comp Neurol. 1998 Dec 21;402(3):385-401.

Negri-Cesi P. Bisphenol A Interaction With Brain Development and Functions. Dose Response. 2015 Jun 17;13(2):1559325815590394.

Niesler B, Walstab J, Combrink S, Möller D, Kapeller J. Rietdorf J. Characterization of the novel human serotonin receptor subunits 5-HT3C, 5-HT3D, and 5-HT3. Mol. Pharmacol. 2007.

Oberlander TF, Gingrich JA, Ansorge MS. Sustained neurobehavioral effects of exposure to SSRI antidepressants during development: molecular to clinical evidence. Clin Pharmacol Ther. 2009 Dec;86(6):672-7. doi: 10.1038/clpt.2009.201.

Pawluski JL, Brain UM, Underhill CM, Hammond GL, Oberlander TF. Prenatal SSRI exposure alters neonatal corticosteroid binding globulin, infant cortisol levels, and emerging HPA function. Psychoneuroendocrinology. 2012 Jul;37(7):1019-28.

Pawluski JL, Kokras N, Charlier TD, Dalla C. Sex matters in neuroscience and neuropsychopharmacology. Eur J Neurosci. 2020 Jul;52(1):2423-2428.

Pawluski JL, Rayen I, Niessen NA, Kristensen S, van Donkelaar EL, Balthazart J, Steinbusch HW, Charlier TD. Developmental fluoxetine exposure differentially alters central and peripheral measures of the HPA system in adolescent male and female offspring. Neuroscience. 2012 Sep 18; 220:131-41.

Pereira OC, Piffer RC. Puberty installation and adrenergic response of seminal vesicle from rats exposed prenatally to hydrocortisone. Life Sci. 2005 Aug 5;77(12):1381-90.

Pereira OC. Endocrine disruptors and hypothalamic sexual differentiation. Annu Rev Biomed Sci. 2003 5: 87-4.

Perobelli JE, Martinez MF, da Silva Franchi CA, Fernandez CD, de Camargo JL, Kempinas Wde G. Decreased sperm motility in rats orally exposed to single or mixed pesticides. J Toxicol Environ Health A. 2010;73(13-14):991-1002.

Rajkumar R, Mahesh R. The auspicious role of the 5-HT3 receptor in depression: a probable neuronal target? J Psychopharmacol. 2010 Apr;24(4):455-69.

Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. FASEB J. 2008 Mar;22(3):659-61.

Rhoda J, Corbier P, Roffi J. Gonadal steroid concentrations in serum and hypothalamus of the rat at birth: aromatization of testosterone to 17 beta-estradiol. Endocrinology. 1984 May;114(5):1754-60.

Seckl JR, Holmes MC. Mechanisms of disease: glucocorticoids, their placental metabolism and fetal 'programming' of adult pathophysiology. Nat Clin Pract Endocrinol Metab. 2007 Jun;3(6):479-88.

Simerly RB. Wired for reproduction: organization and development of sexually dimorphic circuits in the mammalian forebrain. Annu Rev Neurosci. 2002; 25:507-36.

Sramek JJ, Cutler NR. The impact of gender on antidepressants. Curr Top Behav Neurosci. 2011; 8:231-49.

Thankamony A, Pasterski V, Ong KK, Acerini CL, Hughes IA. Anogenital distance as a marker of androgen exposure in humans. Andrology. 2016 Jul;4(4):616-25.

Vanderschuren LJ, Achterberg EJ, Trezza V. The neurobiology of social play and its rewarding value in rats. Neurosci Biobehav Rev. 2016 Nov; 70:86-105.

Weisz J, Ward IL. Plasma testosterone and progesterone titers of pregnant rats, their male and female fetuses, and neonatal offspring. Endocrinology. 1980 Jan;106(1):306-16.

Zaccaroni M, Massolo A, Beani L, Seta DD, Farabollini F, Giannelli G, Fusani L, Dessì-Fulgheri F. Developmental exposure to low levels of ethinylestradiol affects social play in juvenile male rats. Toxicol Res. 2020 Feb 7;36(4):301-310.

Tables and figures

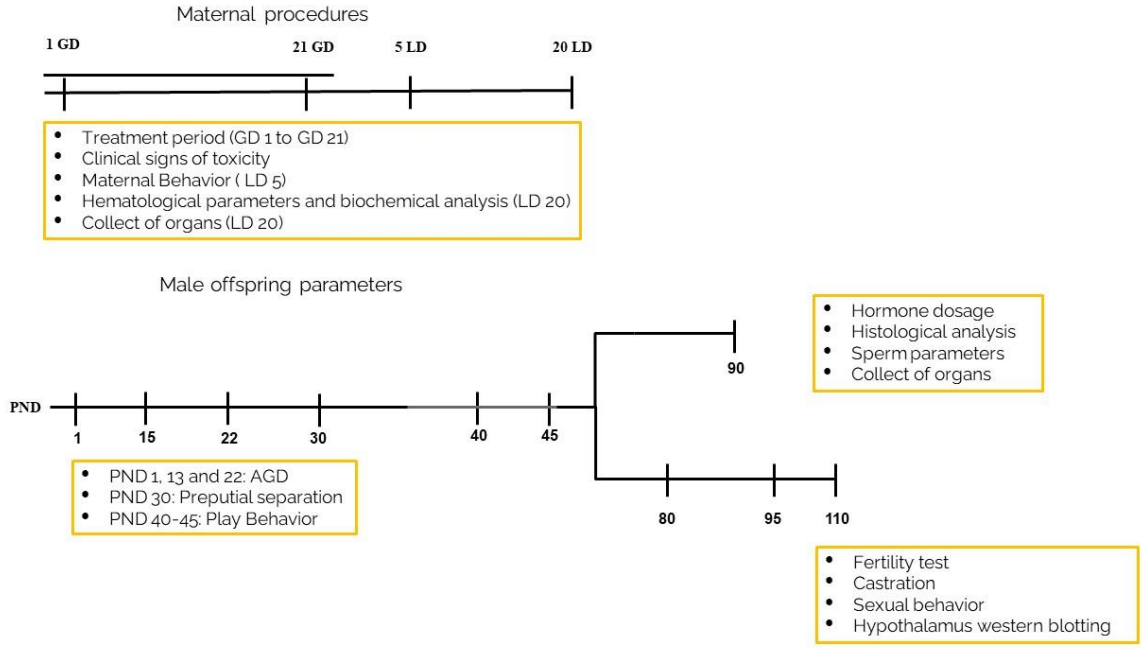

Fig 1. Diagram of the experimental design

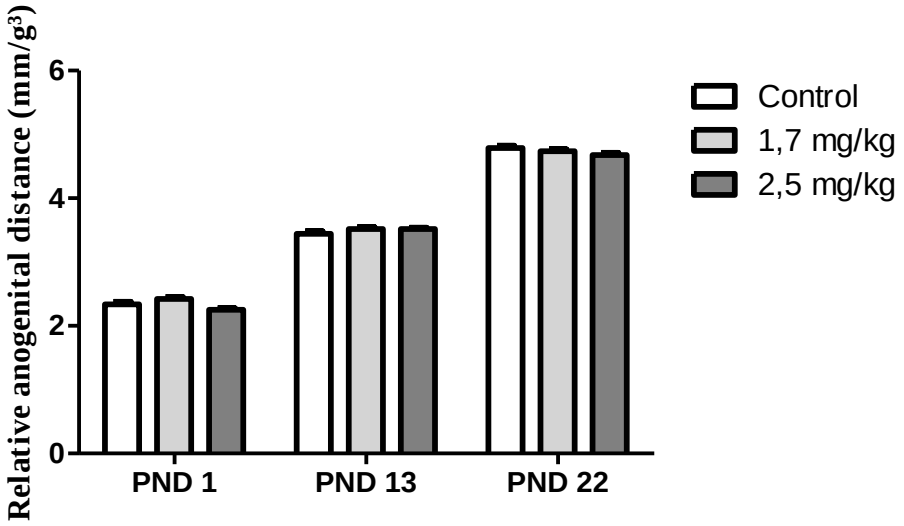

Fig 2. Anogenital distance (mm/g1/3) (A) and body weight (g) (B) in PND 1, 13 and 22 of male offspring exposed to ondansetron. (A) Values expressed as mean ± standard error of the mean (SEM). ANOVA with posterior test Dunnet. $p > 0.05$.

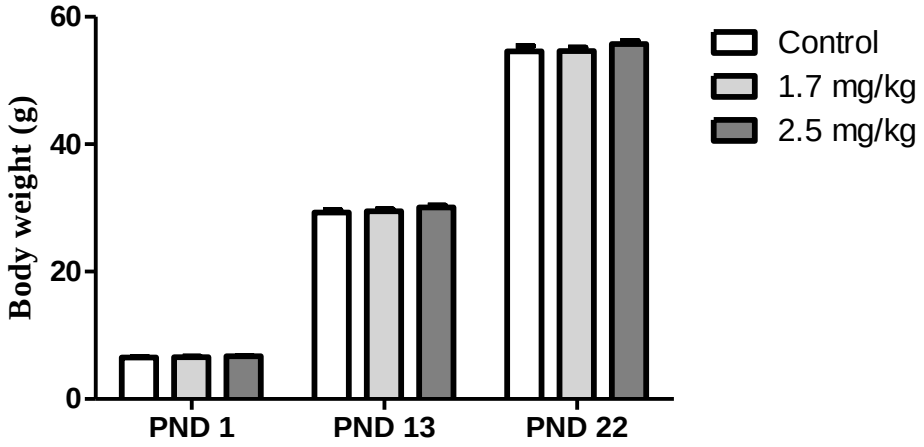

Fig 3. Body weight (g) of male offspring exposed to ondansetron via intrauterine at PND 1, 13 e 22. Values expressed as mean ± standard error of the mean (SEM). ANOVA with posterior test Dunnet. $p > 0.05$.

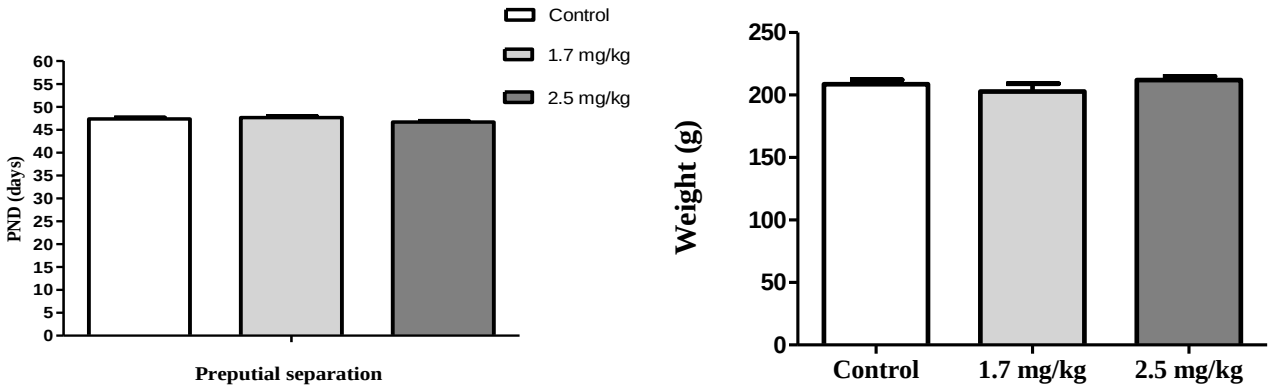

Fig 4. Ages at the time of preputial separation (A) in days and body weight (g) at the time of preputial separation (B) of male offspring exposed to ondansetron. Values expressed as mean ± standard error of the mean (SEM). ANOVA with posterior test Dunnet. $p > 0.05$.

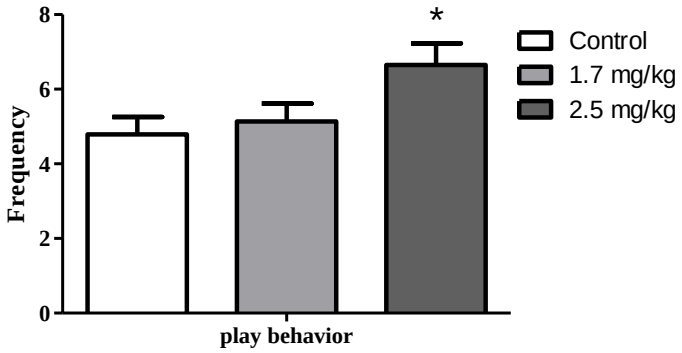

Fig 5. Play Behavior of male offspring exposed to ondansetron. Values expressed as mean ± SEM. n=10 litters/group. ANOVA with posterior Dunnet test. *p<0,05

Table 1. Relative weight of male offspring exposed to ondansetron via intrauterine on PND 60.

Parameters	**Experimental Groups**		
	Control	**1.7 mg/kg**	**2.5 mg/kg**
Body weight (g)	310.63 ± 13.53	335.15 ± 2.52	318.36 ± 7.11
Liver (g)	4.87 ± 0.09	4.12 ± 0.15	4.37 ± 0.28
Kidney (g)	0.45 ± 0.02	0.43 ± 0.02	0.44 ± 0.02
Adrenal Gland (mg)	10.35 ± 1.30	9.68 ± 0.76	9.97 ± 0.51
Spleen (g)	0.24 ± 0.02	0.21 ± 0.01	0.23 ± 0.01
Heart (g)	0.37 ± 0.03	0.32 ± 0.01	0.34 ± 0.02
Lungs (g)	0.69 ± 0.14	0.58 ± 0.07	0.60 ± 0.14
Thyroid (mg)	5.50 ± 0.43	4.44 ± 0.56	4.69 ± 0.30
Testis (g)	0.47 ± 0.02	0.41 ± 0.02	0.45 ± 0.02
Epididymis (mg)	95.28 ± 5.10	87.00 ± 3.10	88.82 ± 4.40
0Vas deferens (mg)	19.54 ± 0.83	18.33 ± 1.02	18.72 ± 0.76
Seminal gland (g)	0.21 ± 0.02	0.16 ± 0.01	0.19 ± 0.01
Prostate (mg)	61.00 ± 6.67	61.71 ± 6.32	63.18 ± 3.67

Values expressed as mean ± SEM. n = 8/group. ANOVA with posterior Dunnet test. $p > 0.05$.

Table 2. Relative weight of male offspring exposed to ondansetron via intrauterine on PND 90.

Parameters	Experimental Groups		
	Control	**1.7 mg/kg**	**2.5 mg/kg**
Body weight (g)	397.23 ± 7.04	397.2 ± 7.01	414.77 ± 7.43
Liver (g)	3.98 ± 0.09	3.82 ± 0.11	4.01 ± 0.05
Kidney (g)	0.43 ± 0.01	0.41 ± 0.01	0.43 ± 0.01
Adrenal Gland (mg)	8.61 ± 0.48	8.23 ± 0.41	9.93 ± 0.31
Spleen (g)	0.20 ± 0.01	0.19 ± 0.01	0.21 ± 0.01
Heart (g)	0.31 ± 0.01	0.32 ± 0.01	0.33 ± 0.02
Lungs (g)	0.41 ± 0.01	0.40 ± 0.01	0.45 ± 0.01
Thyroid (mg)	3.94 ± 0.28	4.44 ± 0.15	4.13 ± 0.25
Testis (g)	0.42 ± 0.01	0.44 ± 0.01	0.46 ± 0.01
Epididymis (mg)	139.87 ± 5.15	144.20 ± 2.07	142.82 ± 2.62
Vas deferens (mg)	22.75 ± 0.66	22.75 ± 0.55	22.60 ± 0.73
Seminal gland (g)	0.33 ± 0.01	0.33 ± 0.02	0.27 ± 0.01
Prostate (mg)	85.67 ± 4.84	87.89 ± 6.26	82.30 ± 3.63

Values expressed as mean ± SEM. n = 10/group. ANOVA with posterior Dunnet test. $p > 0.05$.

Table 3. Number of normal sperm obtained and sperm counts of adult male rats (PND 90) exposed to ondasentron via intrauterine.

Parameters	Experimental groups		
	Control	**1.7 mg/kg**	**2.5 mg/kg**
Sperm morphology (%)			
Normal Sperm	97.50 ± 0.38	99.00 ± 13.78	97.50 ± 1.11
Testis			
Mature spermatids number ($x10^6$/testis)	228.89 ± 12.44	216.58 ± 17.19	196.02 ± 11.90
Relative mature spermatids number ($x10^6$g/testis)	147.12 ± 6.73	148.16 ± 12.55	120.54 ± 5.75
Daily sperm production ($x10^6$ testis/day)	37.53 ± 2.04	35.50 ± 2.81	32.13 ± 1.95
Relative daily sperm production ($x10^6$/testis/g/day)	24.12 ± 1.10	24.29 ± 2.06	19.76 ± 0.94
Epididymis (caput/corpus)			
Sperm number ($x10^6$organ)	111.51 ± 9.62	95.15 ± 7.31	117.88 ± 7.32
Relative sperm number ($x10^6$g/organ)	488.21 ± 24.18	462.81 ± 14.38	468.41 ± 13.58
Sperm transit time (days)	2.96 ± 0.22	28.02 ± 0.31	3.59 ± 0.30
Epididymis (cauda)			
Sperm number ($x10^6$organ)	235.39 ± 27.24	223.93 ± 13.94	213.67 ± 18.61
Relative sperm number ($x10^6$/g/organ)	1322.50 ± 17.07	1360.62 ± 87.44	1409.10 ± 69.13
Sperm transit time (days)	5.95 ± 0.83	6.45 ± 0.42	6.60 ± 0.77

Values expressed as mean ± SEM of 8-9/group. ANOVA with posterior Dunnet test. $p > 0.05$.

Table 4. Fertility test of male offspring exposed to ondansetron via intrauterine.

Parameters	Experimental groups		
	Control	**1.7 mg/kg**	**2.5 mg/kg**
#Fertility potential (%)	96.87 (83.33 – 100.00)	96.42 (80.00 – 100.00)	100.00 (91.67 – 100.00)
Weight gain (g)	131.04 ± 5.65	144.63 ± 3.20	137.31 ± 1.65
Uterine + fetal weight (g)	77.30 ± 2.83	74.20 ± 2.52	72.26 ± 3.310
Placenta weight (g)	0.67 ± 0.01	0.67 ± 0,02	0.69 ± 0.02
Placental efficiency	6.22 ± 0.26	6.22 ± 0.16	6.28 ± 0.22
Fetus weight (g)	4.30 ± 0.05	4.24 ± 0.13	4.25 ± 0.03
Number of live fetuses	12.25 ± 0.67	12.43 ± 0.37	11.00 ± 0.52
Number of implantations	12.75 ± 0.65	12.57 ± 0.30	11.81 ± 0.50
Number of corpora lutea	13.50 ± 0.73	13.00 ± 0.46	12.60 ± 0.45
Number of resorptions	0.50 ± 0.19	0.12 ± 0.12	0.72 ± 0.27
#Preimplantation loss (%)	3.12 (0.00 – 16.67)	3.57 (0.00 – 20.00)	0,00 (0.00 – 8.33)
#Postimplantation loss (%)	3.57 (0.00 – 10.00)	0.00 (0.00 – 8.33)	0.00 (0.00 – 20.00)
Sex ratio (M:F)	0.90 ± 0.14	1.52 ± 0.34	0.82 ± 0.11

Values expressed as mean ± SEM of 8/group. ANOVA with posterior Dunnet test. $p > 0.05$.

Table 5. Male sexual behavior of adult male offspring exposed to ondansetron via intrauterine.

Parameters	Experimental group		
	Control	**1.7 mg/kg**	**2.5 mg/kg**
Latency to the firts mount (s)	29.4 ± 9.31	45.16 ± 11.07	68.00 ± 25.69
Number of mounts	1.80 ± 0.74	3.83 ± 0.79	2.42 ± 0.81
Latency of the first intromission (s)	38.00 ± 9.12	65.66 ± 15.14	72.50 ± 25.45
Number of intromissions	13.00 ± 1.47	20.17± 0.87	21.28 ± 3.01
Latency to the first ejaculation (s)	314.25 ± 18,43	527.50 ± 125.60	683.16 ± 144.90
Latency of the first post-ejaculatory mount (s)	648.50 ± 40.72	787.67 ± 123.11	934.33 ± 145.70
Number of post-ejaculatory mounts	0.25 ± 0.25	1.40 ± 0.74	0.60 ± 0.20
Latency of the first post-ejaculatory intromission (s)	648.75 ± 40.71	803.33 ± 113.93	934.67 ± 145.64
Number of post-ejaculatory intromissions	7.40 ± 0.93	7.80 ± 1.32	7.71 ± 1.85
Number of total ejaculations	2.80 ± 0.37	2.48 ± 0.31	2.48 ± 0.37

Values expressed as mean ± SEM of 8/group. ANOVA with posterior Dunnet test. $p > 0.05$.

Table 6. Sexual incentive motivation of male offspring exposed to ondansetron via intrauterine.

Parameters	Experimental groups		
	Control	**1.7 mg/kg**	**2.5 mg/kg**
Time spent in male zone (s)	403.87 ± 81.52	367.60 ± 46.49	404.72 ± 58.46
Time spent in female zone (s)	456.00 ± 52.78	443.10 ± 72.63	425.27 ± 74.12
Number of visits in male zone	15.75 ± 3.09	17.00 ± 0.55	20.36 ± 1.76
Number of visits in female zone	16.50 ± 2.76	17.20 ± 1.70	17.54 ± 2.20
Preference score	0.57 ± 0.08	0.52 ± 0.07	0.50 ± 0.07

Values expressed as mean ± SEM. n = 8/group. ANOVA with posterior Dunnet test. $p > 0.05$.

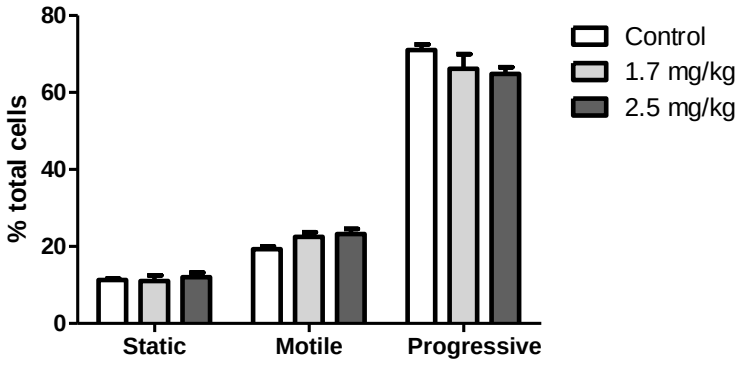

Fig 6. Sperm motility of cauda epididymis sperm of male offspring exposed to ondansetron via intrauterine. Values expressed as mean ± SEM. n= 5 animals/group. ANOVA with posterior Dunnet test. $p > 0.05$.

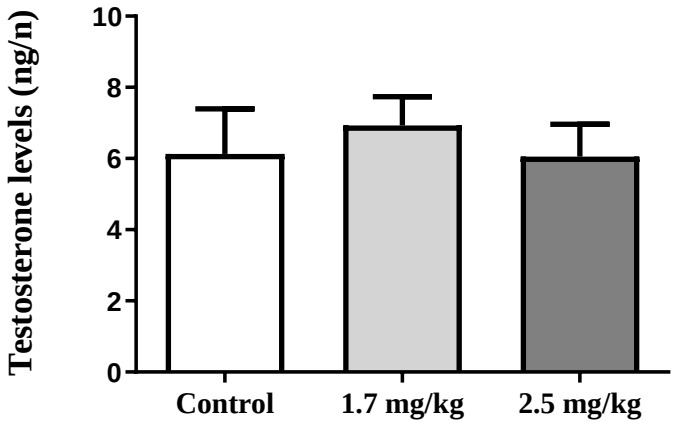

Fig 7. Testosterone serum levels (n = 9-10 animals/group) of male rats exposed to ondansetron. Values expressed as mean ± SEM. n = 5 animals/group. ANOVA with Dunett's posterior test. $p > 0.05$.

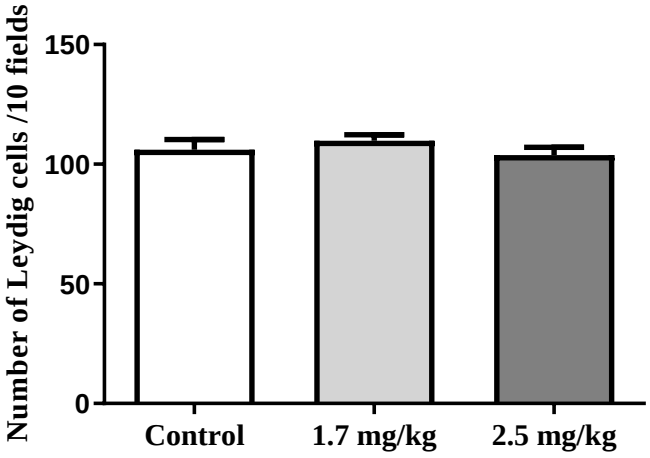

Fig 8. Leydig cell nuclei count (n = 5 animals/group) of male rats exposed to ondansetron. Values expressed as mean ± SEM. n = 5 animals/group. ANOVA with Dunett's posterior test. $p > 0.05$.

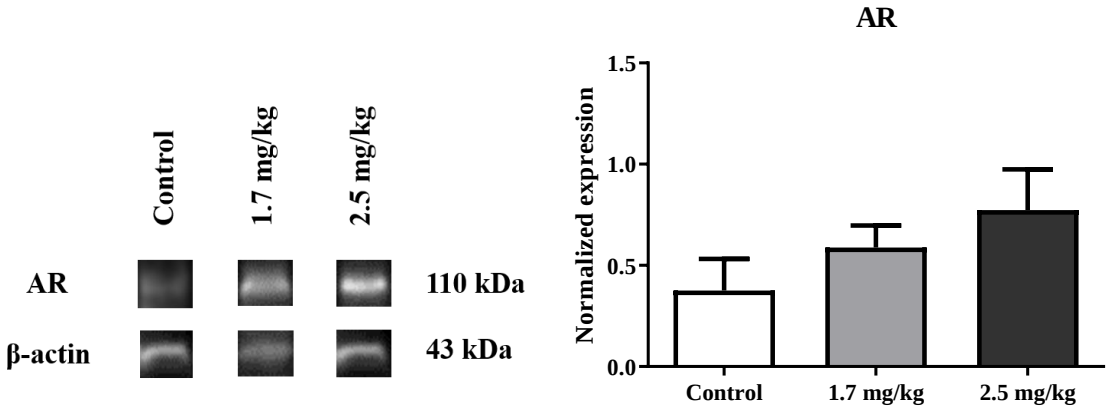

Fig 9. Androgen receptor (AR) expression in hypothalamic neurons of rats exposed to ondansetron in utero (image (A) and graph (B)). Values expressed as mean ± SEM. n = 9/group. ANOVA with posterior Dunnet test. $p > 0.05$.

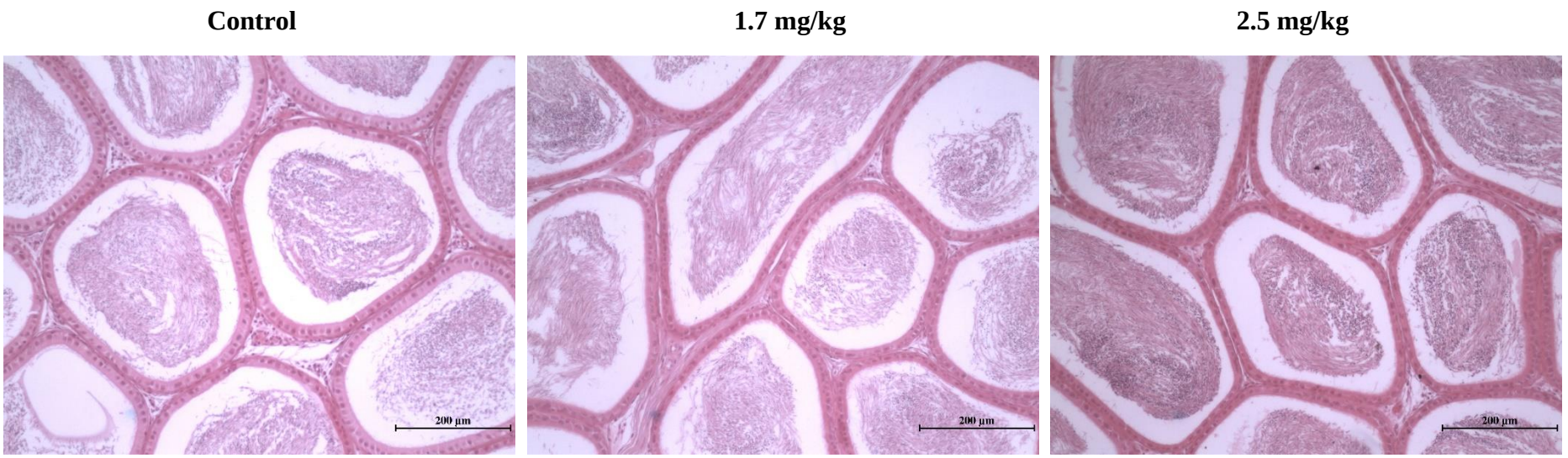

Fig. 9. Photomicrographs of adults epididymis with normal aspect of cauda. Hematoxylin and eosin-stained (magnification: 200×)

Supplementary material

Table S1. Weight gain, water and food consumptions of the dams treated with ondansetron during the during the gestational period.

Parameters	Experimental groups		
	Control	**1.7 mg/kg**	**2.5 mg/kg**
Weight gain (g)	106.28 ± 4.50	102.87 ± 5.89	104.26 ± 2.17
Water consumption (mL/day)	45.49 ± 0.98	41.22 ± 1.46	41.84 ± 1.44
Food intake (g/day)	25.35 ± 0.59	23.98 ± 0.54	24.83 ± 0.77

Values expressed as mean ± SEM. n = 10-11/group. ANOVA with posterior Dunnet test. $p > 0.05$.

Table S2. Maternal behavior of lactating dams treated with ondansetron during the gestational period.

Parameters	Experimental groups		
	Control	**1.7 mg/kg**	**2.5 mg/kg**
Grouping (s)	1089.25 ± 216.02	828.60 ± 196.10	781.36 ± 192.38
Pup grooming (s)	108.25 ± 32.93	196.40 ± 44.08	155.09 ± 32.40
Off pups (s)	1320.37 ± 92.78	1263.10 ± 115.18	1352.90 ± 70.72
Nest	1.75 ± 0.31	2.00 ± 0.36	1.82 ± 0.35

Values expressed as mean ± SEM. n = 9-10/group. ANOVA with posterior Dunnet test. $p > 0.05$.

Table S3. Final body weight and relative organ weight (g/100g body weight) of dams treated with ondansetron during gestational period.

Parameters	Experimental groups		
	Control	**1.7 mg/kg**	**2.5 mg/kg**
Body weight (g)	313.34 ± 6.13	311.63 ± 12.51	310.54 ± 5.28
Liver (g)	5.32 ± 0.12	5.51 ± 0.15	5.60 ± 0.14
Kidney (g)	0.43 ± 0.01	0.43 ± 0.01	0.43 ± 0.01
Adrenal gland (mg)	17.09 ± 0.74	15.38 ± 0.46	15.61 ± 0.79
Spleen (g)	0.23 ± 0.01	0.27 ± 0.01	0.25 ± 0.01
Heart (g)	0.41 ± 0.01	0.42 ± 0.01	0.38 ± 0.01
Lung (g)	0.51 ± 0.03	0.49 ± 0.02	0.49 ± 0.03
Thyroid (mg)	4.42 ± 0.52	4.34 ± 0.56	3.55 ± 0.38
Ovaries (mg)	28.55 ± 1.04	32.44 ± 2.46	27.20 ± 1.06
Uterus (g)	0.17 ± 0.02	0.16 ± 0.01	0.16 ± 0.01

Values expressed as mean ± SEM. n = 9-10/group. ANOVA with posterior Dunnet test. $p > 0.05$.

Table S4. Hematological and biochemical parameters of dams treated with ondansetron during gestational period.

Parameters	Experimental groups		
	Control	**1.7 mg/kg**	**2.5 mg/kg**
Hematological			
Platelets (10^3/µL)	14.75 ± 0.70	19.60 ± 2.87	17.80 ± 1.88
Erythrocytes (10^6/µL)	6.77 ± 0.17	6.71 ± 0.12	6.87 ± 0.14
Hematocrit (%)	47.88 ± 1.58	47.67 ± 0.47	45.64 ± 1.11
Leukocytes (10^3/µL)	8.80 ± 0.92	10.35 ± 0.85	8.34 ± 0.64
Hemoglobin	21.40 ± 0.83	20.66 ± 0.73	19.76 ± 0.69
MCV (fL)	71.16 ± 3.23	72.01 ± 1.01	65.06 ± 1.41
MCH (pg)	31.57 ± 0.82	29.26 ± 1.23	28.58 ± 1.12
MCHC (%)	43.00 ± 2.13	41.70 ± 1.26	45.46 ± 2.59
Biochemical			
AST (U/L)	159.86 ± 11.84	172.14 ± 18.33	145.83 ± 9.03
ALT (U/dL)	148.33 ± 15.38	159.28 ± 15.55	149.28 ± 17.53
Gamma glutamyltransferase (U/L)	0.87 ± 0.35	0.86 ± 0.40	0.25 ± 0.25
Total protein (g/dL)	7.13 ± 0.19	7.22 ± 0.08	7.47 ± 0.14
Albumin (g/dL)	3.30 ± 0.19	2.99 ± 0.07	3.19 ± 0.13
Urea (mg/dL)	84.37 ± 3.50	87.87 ± 3.19	80.87 ± 2.07
Cholesterol (mg/dL)	99.12 ± 7.62	84.50 ± 3.47	85.25 ± 3.68
Alkaline Fosfatase (U/L)	520.00 ± 118.49	635.42 ± 44.30	518.28 ± 87.46

MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; AST- aspartate aminotransferase; ALT- alanine *transaminase*.

Values expressed as mean ± SEM. n = 8/group. ANOVA with posterior Dunnet test. $p > 0.05$.

Capítulo 2

Artigo 2: Evaluations of the teratogenic effects of ondansetron during organogenesis in Wistar rats será submetido a revista **Environmental Toxicology and Pharmacology** (Fator de impacto: 4.860).

Evaluations of the teratogenic effects of ondansetron during organogenesis in Wistar rats

Ana Carolina Casali Reis[1], Bárbara Campos Jorge[1], Suyane da Silva Moreira[1], Júlia Stein[1] Carolina Barizan Perdão[1], Beatriz de Matos Manoel[2], Arielle Cristina Arena[1,3].

[1] Department of Structural and Functional Biology, Institute of Biosciences of Botucatu, Universidade Estadual Paulista – UNESP, Botucatu, SP, Brazil.

[2]College of Health Science, Federal University of Grande Dourados, Dourados, Mato Grosso do Sul, Brazil.

[3]Center of Toxicological Assistance (CEATOX), Institute of Biosciences of Botucatu, Univ. Estadual Paulista – Botucatu (UNESP), São Paulo State, Brazil.

*Corresponding author:

Arielle Cristina Arena

Department of Structural and Functional Biology - Institute of Biosciences of Botucatu

São Paulo State University (UNESP)

Distrito de Rubião Júnior, s/n

Caixa Postal – 510; CEP: 18618970; Botucatu - SP

Tel: + 55 14 38800495

E-mail: arielle.arena@unesp.br

Highlights

- Therapeutic doses of ondansetron did not increase the numbers of visceral or skeletal abnormalities.
- Changes in biochemical parameters in dams exposed to ondansetron may indicate kidney injury.
- Morphologic analysis indicates renal damage in exposed dams.

Abstract

Nausea and vomiting in pregnancy (NVP) are common experiences during the first trimester. Ondansetron is an antiemetic, used for nausea and vomiting after chemotherapy or surgery, nonetheless an extended off-label use occurred for NVP in pregnancy. To evaluate the effects of ondansetron in organogenesis, pregnant rats were exposed to two doses ondansetron (1.7 or 2.5 mg/kg), from gestational day (GD) 6 to 15. Maternal results presented morphologic changes in the kidney and alterations in kidney relative weight. In fetus evaluation there were no statistically significant differences that could indicate a teratogenic potential for ondansetron. Although teratogenic potential of ondansetron has not been evidenced, is important reinforce an integration of results with animal experimentation and epidemiological studies to certify the safety of ondansetron.

Keywords: organogenesis, nausea and vomiting in pregnancy, kidney, safety, teratogenicity.

1. Introduction

Nausea and vomiting in pregnancy (NVP) are common experiences during the first trimester [1]. Metabolic and neuromuscular factors have been implicated in the pathogenesis of NVP, however, their exact cause is unknown [2]. Due to the unspecific cause, finding targets for treatment becomes complicated and, in most cases, the impacts on the fetus are not elucidated. Over the years, several non-pharmacological and pharmacological agents are effective in treating NVP. The most used classes of drugs are antihistamines, which operate as antagonists of H1 receptors, acting on the vestibular system, decreasing the stimulation of the vomiting center [3]; antagonists of dopamine receptors, which work by correcting gastric transit [4] and serotonin receptor antagonists, considered highly effective [5] and target of this study.

Ondansetron (Zofran®) is a serotonin 5HT3 receptor antagonist which is an antiemetic, used for nausea and vomiting after chemotherapy, irradiation, or surgery [6], that works both centrally and peripherally by blocking serotonin receptors in the small bowel and medullary vomiting center [3]. Nonetheless, an extended off-label use has occurred for NVP, even with this use, the fetal safety of this drug is still being evaluated, not knowing the teratogenic risks when used in early pregnancy.

Important regulatory agencies such as the Spanish Agency for Medicines and Health Products (AEMPS) [7] and the National Health Surveillance Agency (ANVISA) from Brazil [8] have warned about the instability of the beneficial use of this drug, with an increased risk of orofacial closure defects, especially cases of cleft palate, which have been identified in children of women exposed during the first trimester of pregnancy to treatment with ondansetron. Recommending that prescribing professionals be cautious when indicating this drug during this period sensitive to exposure to teratogens. However, the purchase of this drug is free, having no obstacles to acquiring it without any guidance.

This insecurity occurs due to prior studies on the fetal safety of ondansetron that produced varied results. Two large human epidemiological studies have shown an association between ondansetron and fetal cardiovascular defects [9], another one suggests a possible association with orofacial clefts [10]. On the other hand, some studies exempt ondansetron from causing or increasing the risks of any anomalies [11].

In these situations, where there is a conflict of information, integration of all relevant data, including results in animal teratology studies with ondansetron can provide valuable information in the risk evaluation process [12]. Being necessary to ascertain the safety of this medicine during the organogenesis, a critical phase of development corresponds to the period with a lot of symptoms of nausea and vomiting and consequently the use of medication to relieve the symptomatology.

2. Materials and Methods

2.1 Animals

Wistar female (n = 30, 12 weeks, 250 g ± 3.12) and male (n = 10, 12 weeks, 400 g ± 2.89) rats were obtained from the Central Biotherium of São Paulo State University (UNESP) were used in this study. Animals were maintained under standard conditions (temperature of 22°C, photoperiod of 12h light/12h dark, relative air humidity of 50%) with commercial food (chow phytoestrogen-free; Nuvilab CR1- Nuvital-PR, Brazil) and water *ad libitum*. All the experimental procedures were following the Ethical Principles in Animal Research adopted by the Brazilian College of Animal Experimentation and approved by the Ethics Committee for Animal Experimentation at the Institute of Biosciences of Botucatu/UNESP (Protocol number: 6353250620)

2.2 Mating and pregnancy diagnostic

After a period of 2 weeks for acclimatization in the experimental room, we performed vaginal lavage to evaluate the estrous cycle of the females, the content was transferred to a slide and analyzed under light microscopy. When the estrous phase was detected, the female was placed with a male sexually active overnight, at the proportion of 1:1. The next morning, the vaginal material was collected again. The presence of sperm was considered the gestational day 0 (GD 0).

2.3 Groups and experimental design

To evaluate the effects of ondansetron, pregnant rats were divided into three experimental groups, control that received only distilled water (vehicle) and two groups treated with doses of 1.7 or 2.5 mg/kg, orally (gavage). The doses were based on therapeutic doses and calculated from the body surface area of the animals [13]. During treatment, pregnant rats, maintained in individual cages, were weighed on alternate days

to calculate the volume of ondansetron to be administered, and clinical signs of toxicity (body weight, diarrhea, piloerection, bleeding, abnormal breathing, tremors, convulsions, and gear changes, posture, and reaction to manipulation) were investigated, as well as the average daily intake of water and food. The treatment was conducted from GD 6 to 15, which comprises the organogenesis period of the rat. The organogenesis is a critical period because the tissues are rapidly differentiating, becoming more susceptible to teratogens [14][15]. The experimental design of this study followed guideline 414 developed by the Organization for Economic Co-operation and Development [16].

2.4 Maternal evaluations

On GD 20, the pregnant females were anesthetized (isoflurane) and killed. The blood was collected by cardiac puncture for hematological and biochemical analyses. Cesarean section was performed with a hysterectomy and bilateral oophorectomy. Ovaries were weighed and carefully analyzed for the number of corpora lutea. The gravid uterus was removed whole and weighted. All fetuses were individually removed, and their placentas were analyzed. The number of fetuses was counted, and several parameters were observed, such as fetal and placental weights, numbers of implantations, resorptions, and live and dead fetuses. Data were used to calculate reproductive rates: rate of implant efficiency, pre-implantation and post-implantation losses, and placental index. The other organs of females (liver, kidney, adrenal gland, spleen, heart, lung, ovary, and thyroid) were removed, weighed. The kidney and liver were fixed in Bouin's solution, and then, embedded in paraffin. The sections of 5 µm were stained with haematoxylin and eosin (H&E) and examined under a light microscopy. Any alterations compared to the normal histology were registered.

2.5 Biochemical and hematological parameters

The serum obtained after centrifugation (1000xg for 20 minutes at 4ºC) from maternal blood were used for dosages of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Gamma-glutamyltransferase (Gamma-GT), alkaline phosphatase (AF), Urea, Creatinine, Calcium, Cholesterol, Total Protein, and Albumin. These parameters were determined using the BioPlus 200 semiautomatic equipment, using Bioclin kits. For hematological analysis, total counts of leukocytes, erythrocytes, and platelets were performed, in addition to the levels of hemoglobin, hematocrit, and erythrocyte distribution width. Except for hemoglobin levels, which were determined using BioPlus 200, the other hematological parameters were analyzed manually.

2.6 Evaluation of fetuses development

External morphological analysis of fetuses: All fetuses were evaluated concerning their external morphology with the aid of a stereoscope, evaluating possible anomalies in the ocular structure, cranial structure, internal bleeding, bruises, limb size, neural tube closure, etc.;

External measurements: With the aid of a digital caliper, the craniocaudal distance (body length), the anogenital distance (AGD - from the genital tubercle to the anus), and the biparietal distance (measured by the distance between the openings of the auditory canal) were measured.

Placental efficiency: Fetuses and their respective placentas were weighed to determine placental efficiency, obtained by the ratio between the weight of the fetus and its placenta [18], to quantify maternal-fetal nutrition.

2.6.1 Visceral parameters

Half of each litter was processed for visceral analysis. The fetuses were placed on an ice plate to maintain their structure unaltered for the following analyzes. The fetuses

were fixed in Bouin solution after evaluation of the external morphology. It was incorporated the cutting plan (transverse and longitudinal sections) of Wilson [19] on the head (five cuts) and the plans proposed by Barrow and Taylor [20] on the thorax (three cuts), to evaluate visceral anomalies in the following tissues: cerebral ventricles, cornea, retina, internal ear, nasal cavity, palate, salivary gland, thyroid, esophagus, trachea, heart, thymus, liver, kidneys, bladder, ureters, gonads, and so forth. The parameters of evaluation of miss formations were based on a study of Manson and Kang [21] and modifications proposed by Oliveira et al [22].

2.6.2 Skeletal parameters

The fetuses selected for this analysis were placed in an acetone solution for 24 h and, after that time, the acetone was replaced by a 0.8% KOH solution, and a saturated Alizarin Red solution was added, resulting in four exchanges in an interval of at least 24 h, following the methods proposed by Staples and Schnell [23]. The evaluated parameters were based on Aliverti et al., [24] responsible for the identification of ossification points that allow determining the degree of fetal development. The analyzes were also based on Taylor [25] who provided a guide to identify anomalies and malformations of the cranial bones, sternum, clavicle, vertebrae, pelvis, bones of the anterior, and posterior limbs. The

2.7. Statistical Analysis

Values are expressed as mean ± SEM or median (Q1 – Q3). To compare the results between the experimental groups, statistical tests of analysis of variance were used. ANOVA, followed by the Dunnet posterior test, or by non-parametric analysis of variance (Kruskal-Wallis), followed by the Dunn a posterior test, when necessary. The differences were considered significant with $p < 0.05$, performed on the GraphPad InStat 18 (version 5.02).

3. Results

3.1 Maternal evaluations

During the treatment with Ondansetron, the pregnant females did not show any clinical signs of toxicity that could be attributed to medication exposure, since the weight evolution, water and food consumption (Table 1) and macroscopic aspects of maternal organs were similar in all experimental groups. However, after biochemical analysis, females exposed in both treated groups presented decreased in serum levels of creatinine (Table 3). In addition, kidney relative weight showed a decrease among exposed groups when compared to the control (Table 2). Alterations in normal aspect of kidney were observed in both treated groups (Fig 2). The others hemato-biochemical parameters were unaltered by the treatment (Table 3). No significant differences were observed in the reproductive parameters (Table 4).

3.2 Fetuses evaluations

There were no statistically significant differences among groups in the analysis of fetal weight, craniocaudal, and encephalic measures (Tables 4 and 5) (Figure 3 and 4). Macroscopic fetal analysis with the stereoscope to evaluate ears implantation, eye structure, internal bleeding, limb length and reflection, neural tube closure, gastroschisis, cauda, hydrops, and anal perforation were similar among groups not showing any alterations, corroborating with skeletal and visceral evaluations that remained without significant changes (Figure 5 and 6).

4. Discussion

Nausea and vomiting in pregnancy are frequent complaints during the first trimester [26] that correspond to organogenesis. Organogenesis is the most critical phase for the embryo to suffer influence from stressors. During this period any disturbance can be critical or fatal to normal development [27], there are conflicting results in human epidemiological studies in relation to risk associated with off-label use of ondansetron against NV in early pregnancy [12] and its ability to act as a teratogen.

Teratogen substances can adversely affect the development of an embryo or a fetus if administered under specific conditions of dose, route of administration, gestational age, and genotype [28]. A wide range of substances has been recognized as teratogens, including some medications [29] [30], demonstrating the need to investigate therapeutic doses of ondansetron. In this study, we reported that ondansetron proved to be safe for development in Wistar rats' offspring, in the conditions analyzed in this research, despite impacting maternal causing systemic toxicity.

In the literature, there are inconsistent and conflicting results regarding the risk of congenital malformations with ondansetron [31]. Our datas did not find evidence of an association between ondansetron and overall risk of birth defects during the organogenesis period, considered the ideal time to evaluate the teratogenicity of medication [27]. Though, it should be emphasized that some analyzes are known to be species-specific, with different magnitudes according to the species or lineage [32]. Studies with Wistar rats administered ondansetron intravenously by the tail vein (0.5, 1.5, and 4 mg/kg/day) during organogenesis demonstrated no effects on maternal body weight gain on gestation, pre-or post-implantation loss, fetal death or sex ratio of the fetuses [33], the same doses in rabbits causes an increase in early intrauterine deaths, post-implantation loss and developmental retardation [33].

Cross information reflects an impossibility of declaring this drug safe for humans only based on studies with Wistar rats. Indeed, Reproductive toxicology guidelines declare that studies of this nature using animal exposure is not only useful but considered essential for the interpretation of study results to assess human safety [34]. Being necessary an integration of results with animal experimentation and epidemiological studies.

In a systematic review conducted by Lavecchia et al., [35] ondansetron use in pregnancy and the correlation with congenital malformations did not yield significant results, but reaffirm that is necessary to keep in mind the limitations of the researchers included on review and their potential biases, which mean that the possibility of teratogenicity still cannot be ruled out.

Despite data exempting this medication from deleterious effects or malformations in the offspring, there were significant maternal alterations, that can indicate impacts in renal function. In front of this, organs were carefully examined for signs of toxicity. Kidneys are excretory organs that receive large amounts of blood, with high oxygen consumption, which makes them more vulnerable to exposure, via the circulation, and consequently injury related to concentrations of chemicals [35]. Relative weight of the kidney was altered in both exposed groups corroborating with our finds in morphological analyses, that indicated a change in the glomeruli of treated animals.

In conclusion, treatment with ondansetron during organogenesis in Wistar rats at these therapeutic doses compromised maternal parameters, being toxic for the organism and possibly leading to kidney injury, other histological analyses must be conducted to determine the commitment of renal function. Although the teratogenic potential of ondansetron has not been evidenced, it is important to reinforce the risks of this substance

causing maternal changes that can compromise maternal-fetal safety. Further studies should be conducted to explore the mechanisms that induced kidney injure. Until the full understanding of ondansetron and their effects in organisms, becomes safer recommend to relieve NVP treatment already established as safe during organogenesis. However, when other options have been exhausted, ondansetron provides a useful and effective treatment for use in NVP.

5. Acknowledgments

The authors are grateful to José Eduardo Bozano, from the Department of Structural and Functional Biology, Institute of Biosciences, UNESP, Botucatu/SP–Brazil, for his technical assistance.

6. Conflict of interest

The authors declare no potential conflicts of interest.

7. Funding information

This study was financed by FAPESP (2020/08745-9) - The State of São Paulo Research Foundation.

8. References

[1] Zambelli-Weiner A, Via C, Yuen M, Weiner DJ, Kirby RS. First trimester ondansetron exposure and risk of structural birth defects. Reprod Toxicol. 2019 Jan; 83:14-20.

[2] Bustos M, Venkataramanan R, Caritis S. Nausea and vomiting of pregnancy - What's new? Auton Neurosci. 2017 Jan; 202:62-72.

[3] Badell ML, Ramin SM, Smith JA. Treatment options for nausea and vomiting during pregnancy. Pharmacotherapy. 2006 Sep;26(9):1273-87.

[4] Lee NM, Saha S. Nausea and vomiting of pregnancy. Gastroenterol Clin North Am. 2011 Jun;40(2):309-34.

[5] Goodwin TM. Hyperemesis gravidarum. Obstet Gynecol Clin North Am. 2008 Sep;35(3):401-17, viii.

[6] Danielsson B, Wikner BN, Källén B. Use of ondansetron during pregnancy and congenital malformations in the infant. Reprod Toxicol. 2014 Dec; 50:134-7.

[7] Agencia Española de Medicamentos y Productos Sanitarios (AEMPS): Ondansetrón: riesgo de defectos de cierre orofacialies (lábio leporino, paladar hendido) tras uso durante el primer trimestre del embarazo. Ministerio de Sanida, Consumo y Bienestar Social. Nota informativa, 2019.

[8] Agência Nacional de Vigilância Sanitária (Anvisa) Acesso: http://portal.anvisa.gov.br/noticias/-/asset_publisher/FXrpx9qY7FbU/content/uso-de-ondansetrona-e-investigado/219201?p_p_auth=4yadZ2rN&inheritRedirect=false.

[9] Andersen J, Jimenez-Solem A, Andersen N, Poulsen H.Ondansetron Use in Early Pregnancy and the Risk of Congenital Malformations - a Registry Based Nationwide Cohort Study. International Society of Pharmaco-epidemiology Montreal, Canada 2013. Abstract 25, Pregnancy session 1.

[10] Anderka M, Mitchell AA, Louik C, Werler MM, Hernández-Diaz S, Rasmussen SA; National Birth Defects Prevention Study. Medications used to treat nausea and vomiting of pregnancy and the risk of selected birth defects. Birth Defects Res A Clin Mol Teratol. 2012 Jan;94(1):22-30.

[11] Fejzo MS, MacGibbon KW, Mullin PM. Ondansetron in pregnancy and risk of adverse fetal outcomes in the United States. Reprod Toxicol. 2016 Jul; 62:87-91.

[12] Danielsson B, Webster WS, Ritchie HE. Ondansetron and teratogenicity in rats: Evidence for a mechanism mediated via embryonic hERG blockade. Reprod Toxicol. 2018 Oct; 81:237-245.

[13] Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. FASEB J. 2008 Mar;22(3):659-61.

[14] Bishop JB, Witt KL, Sloane RA. Genetic toxicities of human teratogens. Mutat Res. 1997 Dec 12;396(1-2):9-43.

[15] Cassina M, Salviati L, Di Gianantonio E, Clementi M. Genetic susceptibility to teratogens: state of the art. Reprod Toxicol. 2012 Sep;34(2):186-91.

[16] OECD – Organisation for Economic Co-operation and Development. Guidelines for Testing of Chemical, Guideline 414, in: OECD (Ed.), Prenatal Developmental Toxicity Study, Paris, 2001.

[17] Soulimane-Mokhtari NA, Guermouche B, Yessoufou A, Saker M, Moutairou K, Hichami A, Merzouk H, Khan NA. Modulation of lipid metabolism by n-3 polyunsaturated fatty acids in gestational diabetic rats and their macrosomic offspring. Clin Sci (Lond). 2005 Sep;109(3):287-95.

[18] Fowden AL, Sferruzzi-Perri AN, Coan PM, Constancia M, Burton GJ. Placental efficiency and adaptation: endocrine regulation. J Physiol. 2009 Jul 15;587(Pt 14):3459-72.

[19] Wilson JG. Methods for administering agents and detecting malformations in experimental animals. In: Wilson JG, Warkany J, eds. Teratology: Principals and Techniques. Chicago: University of Chicago Press; 1965:262-277.

[20] Barrow MV, Taylor WJ. A rapid method for detecting malformations in rat fetuses. J Morphol. 1969 Mar;127(3):291-305.

[21] Manson JN, Kang YJ. Test methods for assessing female reproductive and developmental toxicology. In: Hayes AW, ed. Principles and Methods of Toxicology. New York: Raven Press; 1994:989-1037.

[22] Oliveira RJ, Salles MJ, da Silva AF, Kanno TY, Lourenço AC, Freiria GA, Matiazi HJ, Ribeiro LR, Mantovani MS. Effects of the polysaccharide beta-glucan on clastogenicity and teratogenicity caused by acute exposure to cyclophosphamide in mice. Regul Toxicol Pharmacol. 2009 Apr;53(3):164-73.

[23] Staples RE, Schnell VL. Refinements in rapid clearing technic in the KOH-alizarin red S method for fetal bone. Stain Technol. 1964;39: 61-63.

[24] Aliverti V, Bonanomi L, Giavini E, Leone VG, Mariani L. The extent of fetal ossification as an index of delayed development in teratogenic studies on the rat. Teratology. 1979;20(2):237-242.

[25] Taylor P. Skeletal Examination. Pratical Teratology. Vol 10. Gra-Bretanha: Academic Press; 1986:77-100.

[26] Einarson TR, Piwko C, Koren G. Quantifying the global rates of nausea and vomiting of pregnancy: a meta-analysis. J Popul Ther Clin Pharmacol. 2013;20(2):e171-83.

[27] Alwan S, Chambers CD. Identifying Human Teratogens: An Update. J Pediatr Genet. 2015 Jun;4(2):39-41.

[28] Bánhidy F, Lowry RB, Czeizel AE. Risk and benefit of drug use during pregnancy. Int J Med Sci. 2005;2(3):100-6.

[29] Holmes. Twining's Textbook of Fetal Abnormalities, A. M. Coady & S. Bower, 2014).

[30] Banerjee AK, Zomerdijk IM, Wooder S, Ingate S, Mayall SJ. Post-approval evaluation of effectiveness of risk minimisation: methods, challenges and interpretation. Drug Saf. 2014 Jan;37(1):33-42.

[31] Lavecchia M, Chari R, Campbell S, Ross S. Ondansetron in Pregnancy and the Risk of Congenital Malformations: A Systematic Review. J Obstet Gynaecol Can. 2018 Jul;40(7):910-918.

[32] Kimmel CA, Wilson JG. Skeletal Deviations in Rats: Malformations or Variations? Teratology.1973; 8:309-316.

[33] FDA.NDA Zofran (ondansetron Hydrchloride) Injection. Pharmacologists Review. 2007, p. p49.

[34] ICH Steering Committee Draft ICH Harmonised Guideline S5(R3): Detection of Toxicity to Reproduction for Human Pharmaceuticals (2017).

[35] Radi ZA. Kidney Pathophysiology, Toxicology, and Drug-Induced Injury in Drug Development. Int J Toxicol. 2019 May/Jun;38(3):215-227.

Tables and Figures

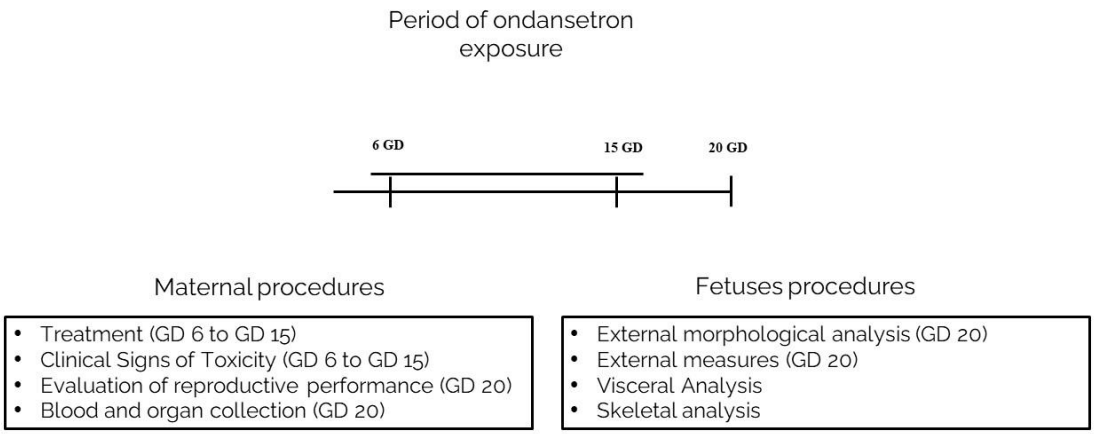

Fig 1. Diagram of the experimental design.

Table 1- Weight gain, water consumption and food intake of the dams treated with ondansetron.

Parameters	**Experimental groups**		
	Control	**1.7 mg/kg**	**2.5 mg/kg**
Weight gain (g)	37.4 ± 4.18	34.37 ± 2.87	29.84 ± 1.45
Water consumption (mL/day)	41.11 ± 2.11	40.90 ± 1.25	40.11 ± 1.49
Food intake (g/day)	25.53 ± 0.93	25.44 ± 1.00	24.49 ± 0.94

Values expressed as mean ± SEM. n = 10-11/group. ANOVA with posterior Dunnet test. P> 0.05.

Table 2 – Final body weight and relative organ weight (g/100g body weight) of dams treated with ondansetron.

Parameters	Experimental groups		
	Control	**1.7 mg/kg**	**2.5 mg/kg**
Body weight (g)	360.52 ± 6.75	375.22 ± 10.12	364.49 ± 9.32
Liver (g)	4.05 ± 0.08	3.95 ± 0.07	4.01 ± 0.06
Kidney (g)	0.31 ± 0.01	0.28 ± 0.00*	0.28 ± 0.01*
Adrenal gland (mg)	16.16 ± 1.22	16.83 ± 0.22	12.57 ± 1.58
Spleen (g)	0.21 ± 0.02	0.22 ± 0.01	0.19 ± 0.01
Lung (g)	0.34 ± 0.01	0.34 ± 0.01	0.34 ± 0.01
Heart (g)	0.24 ± 0.01	0.24 ± 0.00	0.24 ± 0.01
Thyroid (mg)	3.40 ± 0.29	3.47 ± 0.31	3.55 ± 0.20
Ovaries (mg)	27.00 ± 1.62	26.62 ± 0.90	27.22 ± 1.10

Values expressed as mean ± SEM. n = 9-10/group. ANOVA with posteriorDunnet test.
*$p < 0.05$

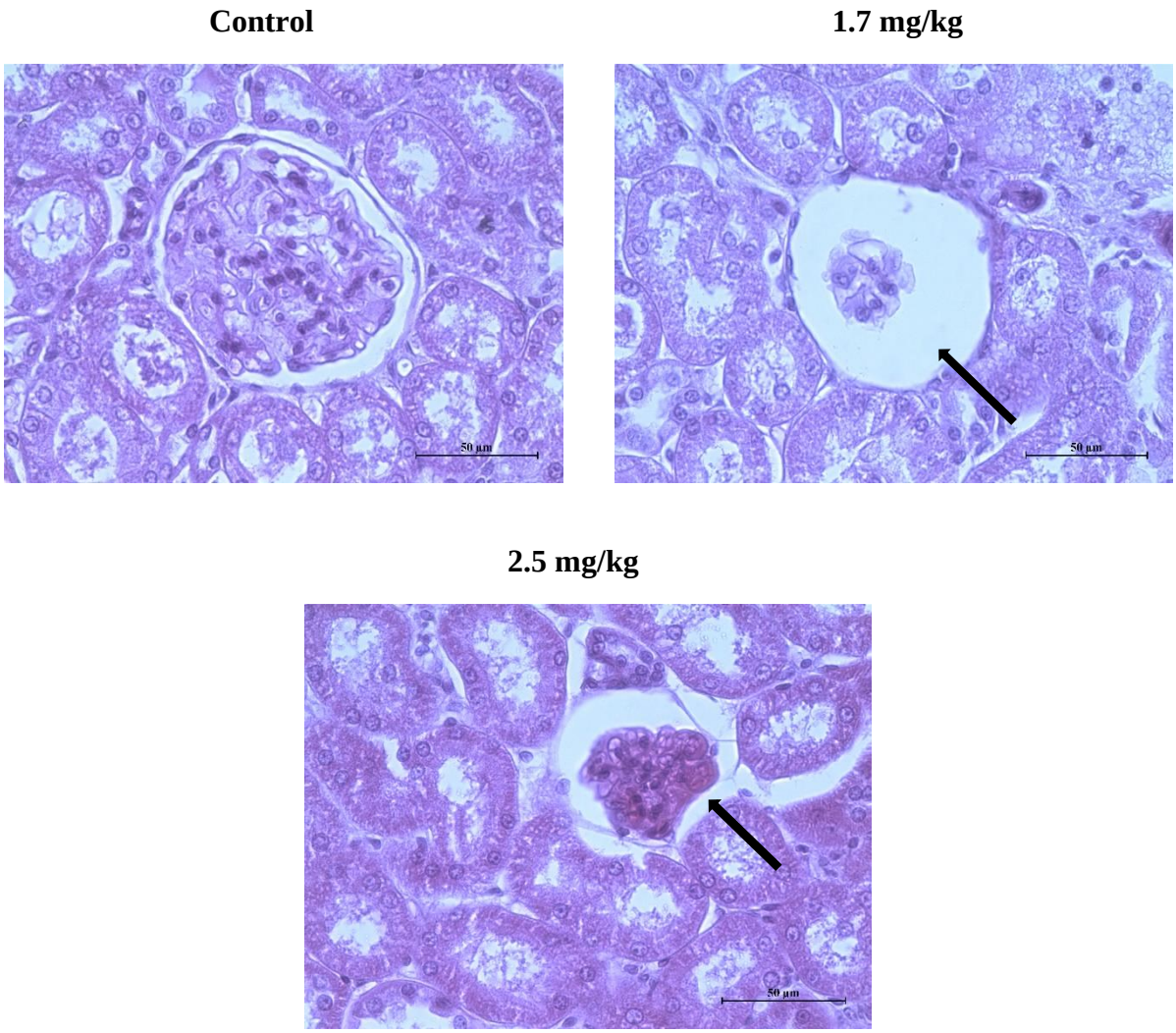

Fig. 2. Histopathological analysis of kidney (H&E / 200X) of the animals treated with Ondansetron. The arrows indicate the changes found in the glomeruli of treated animals.

Table 3 - Hematological and biochemical parameters of dams treated with ondansetron.

Parameters	Experimental groups		
	Control	**1.7 mg/kg**	**2.5 mg/kg**
Hematological			
Platelets (10^3/µL)	12.00 ± 1.27	13.40 ± 1.16	12.00 ± 0.64
Erythrocytes (10^6/µL)	5.91 ± 0.30	5.53 ± 0.13	5.50 ± 0.15
Hematocrit (%)	34.75 ± 0.56	32.30 ± 0.70	31.90 ± 0.90
Leukocytes (10^3/µL)	9.86 ± 1.00	8.64 ± 0.97	7.73 ± 0.60
Hemoglobin	13.85 ± 0.50	13.75 ± 0.30	13.65 ± 10.00
MCV (fL)	58.44 ± 2.27	58.5 ± 1.26	58.30 ± 2.15
MCH (pg)	23.61 ± 0.70	24.97 ± 0.63	24.90 ± 0.87
MCHC (%)	40.57 ± 0.82	42.05 ± 0.68	42.85 ± 0.93
Biochemical			
AST (U/L)	131.86 ± 20.79	111.57 ± 6.18	102.14 ± 3.18
ALT (U/dL)	82.87 ± 7.56	63.25 ± 3.61	73.29 ± 7.10
Creatinine (mg/dL)	4.24 ± 0.22	3.83 ± 0.28	3.50 ± 0.14
Total protein (g/dL)	6.01 ± 0.54	5.27 ± 0.21	5.02 ± 0.13
Calcium (mg/dL)	29.12 ± 5.40	30.89 ± 4.79	30.20 ± 7.10
Albumin (g/dL)	2.19 ± 0.22	1.96 ± 0.08	1.96 ± 0.09
Urea (mg/dL)	72.25 ± 4.97	61.29 ± 4.35	70.37 ± 1.77
Cholesterol (mg/dL)	74.42 ± 4.23	84.71 ± 3.72	79.29 ± 1.37
Alkaline Fosfatase (U/L)	165.43 ± 13.17	165.62 ± 28.40	191.25 ± 14.68

MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; AST- aspartate aminotransferase; ALT- alanine *transaminase.*

Values expressed as mean ± SEM. n = 8/group. ANOVA with posteriorDunnet test. P> 0.05.

Table 4. Fertility test of dams exposed to ondansetron.

Parameters	Experimental groups		
	Control	**1.7 mg/kg**	**2.5 mg/kg**
#Fertility potential (%)	90.90 (83.33 – 100.00)	92.30 (75.00 – 100.00)	94.12 (86.67 – 100.00)
Weight gain (g)	118.99 ± 3.06	123.45 ± 7.73	122.88 ± 7.38
Uterine + fetal weight (g)	62.68 ± 6.56	71.10 ± 3.98	72.89 ± 4.25
Placenta weight (g)	0.66 ± 0.04	0.59 ± 0.02	0.60 ± 0.01
Placental efficiency	6.29 ± 0.45	6.67 ± 0.27	6.67 ± 0.20
Fetus weight (g)	4.14 ± 0.12	3.82 ± 0.10	3.94 ± 0.04
Number of live fetuses	10.87 ± 0.85	12.20 ± 0.72	12.22 ± 0.55
Number of implantations	11.25 ± 0.77	12.90 ± 0.60	12.88 ± 0.48
Number of resorptions	0.33 ± 0.16	0.60 ± 0.27	0.30 ± 0.21
#Preimplantation loss (%)	9.09 (0.00 – 16.67)	7.69 (0.00 – 25.00)	6.78 (0.00 – 76.00)
#Postimplantation loss (%)	0.00 (0.00 – 11.00)	0.00 (0.00 – 18.18)	0.00 (3.85 – 14.28)
Sex ratio (M:F)	0.93 ± 0.14	0.87 ± 0.13	1.42 ± 0.29

Values expressed as mean ± SEM. n = 10/group. ANOVA with posterior Dunnet test. $P > 0.05$.

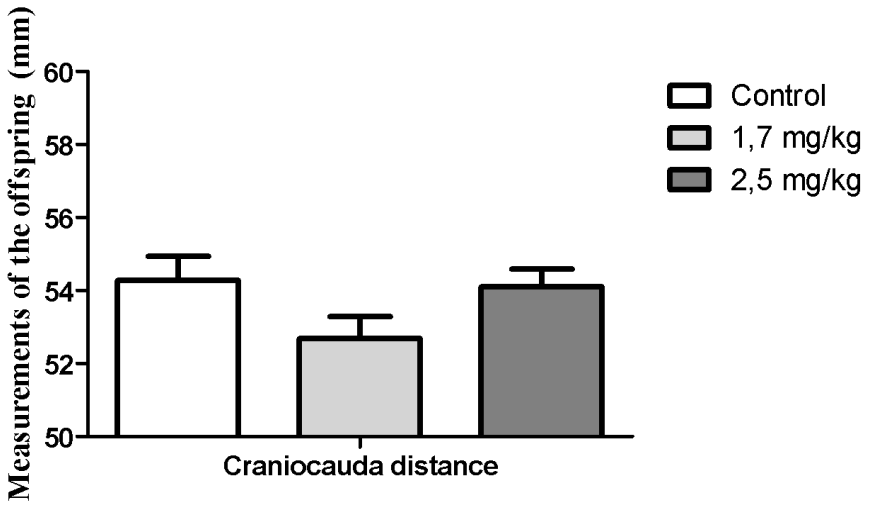

Fig. 3. Craniocaudal distance of offspring exposed to ondansetron via intrauterine. Values expressed as mean ± SEM. n = 10 litters/group. ANOVA/Dunnett test. P> 0.05.

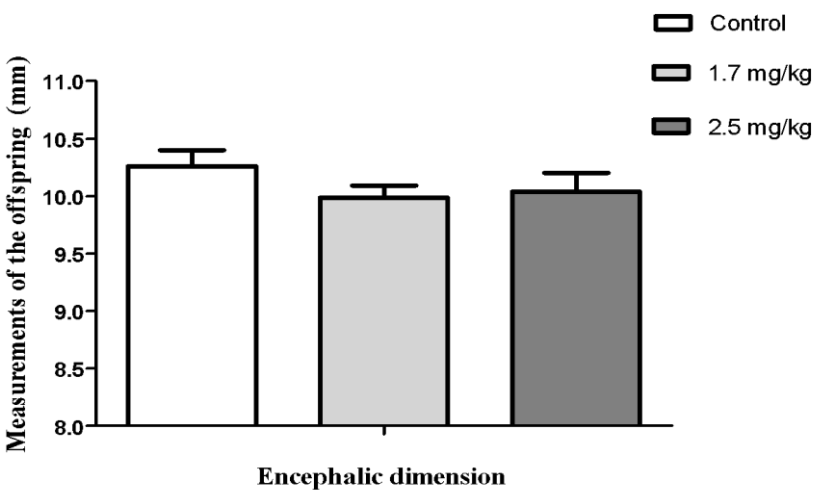

Figure 3. Encephalic dimension of offspring exposed to ondansetron via intrauterine. Values expressed as mean ± SEM. n = 10 litters/group. ANOVA/Dunnett test. P> 0.05.

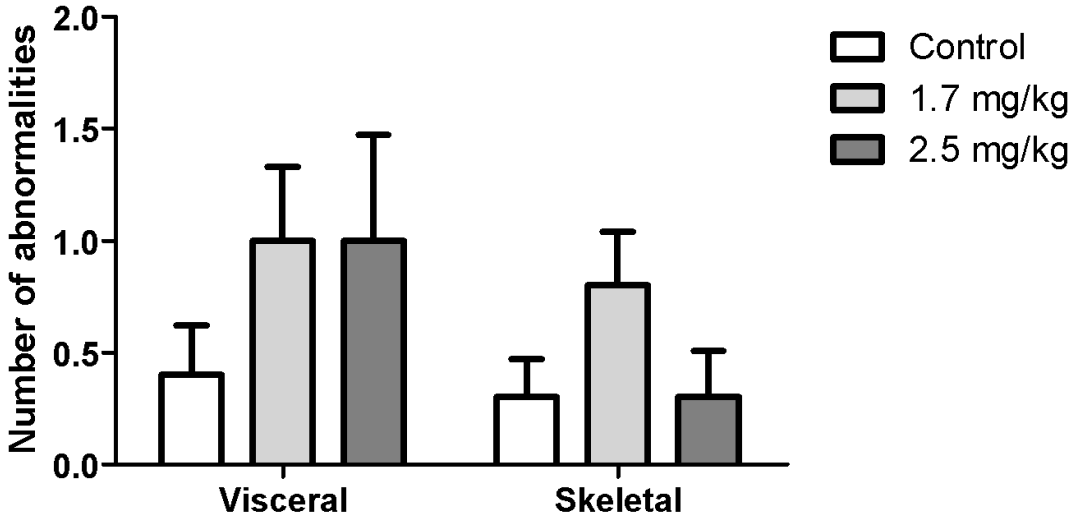

Figure 5. Number of abnormalities in offspring exposed to ondansetron via intrauterine. Values expressed as mean ± SEM. n = 10 litters/group. ANOVA/Dunnett test. P> 0.05.

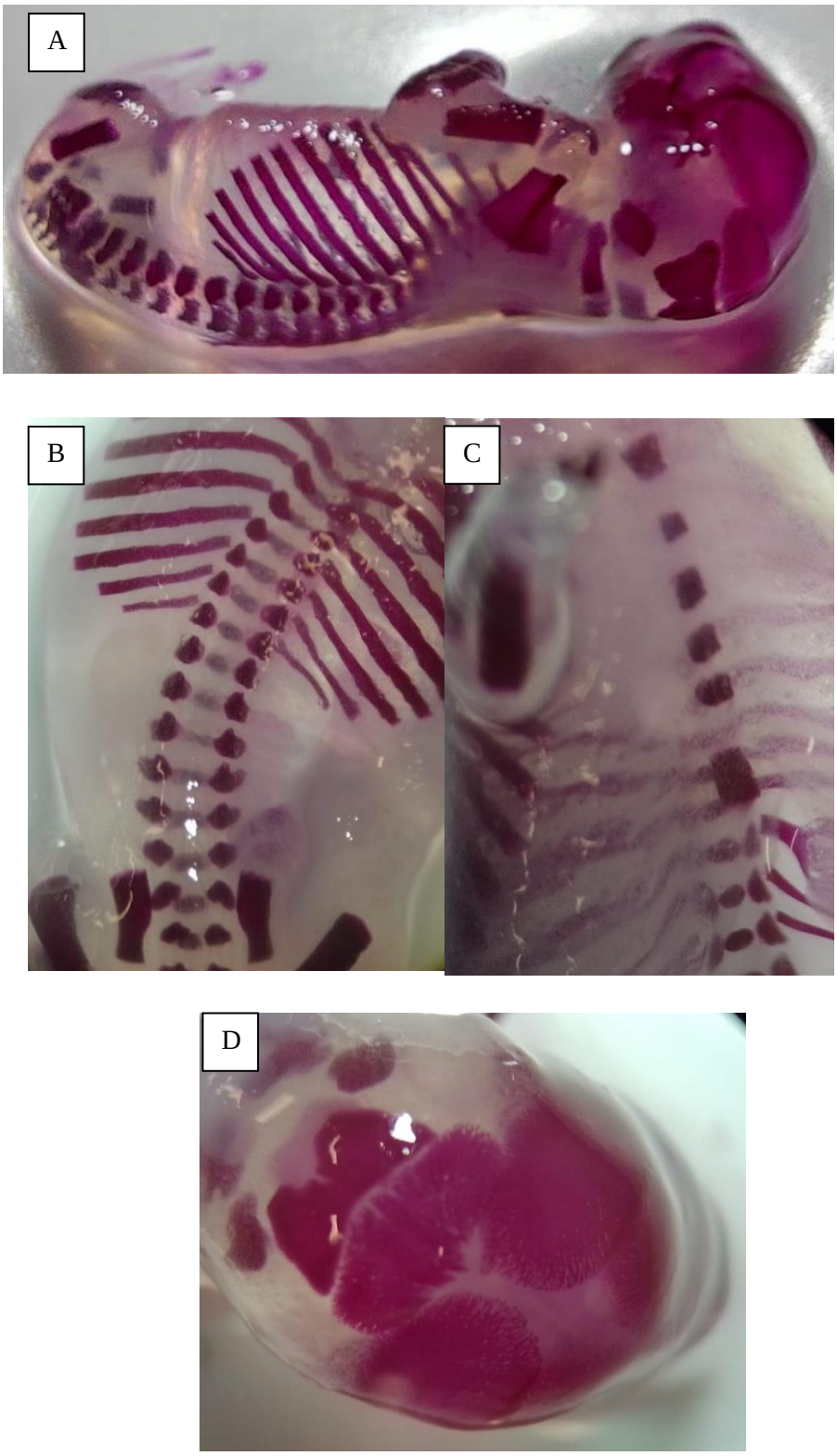

Figure 6. Normal parameters in offspring exposed to ondansetron via intrauterine (A) Fetal overview of skeletal analysis (B) Normal number of vertebrae cervical. (C) Normal ossification of Sternum bones (D) Normal cranial ossification.

7. CONCLUSÃO

Nossos resultados demonstraram que, neste modelo experimental, a ondansetrona não foi capaz de causar impactos no processo de maturação sexual, não ocasionando mudanças comportamentais, assim como alterações significativas em parâmetros espermáticos ou hormonais na vida adulta. Além disso, avaliações do caráter teratogênico dessa substância não demonstraram elevações dos riscos de malformações diante da administração dessa droga em ratas Wistar durante o periodo de organogênese.

8.ANEXO

CERTIFICADO

Certificamos que a proposta intitulada "Influência da exposição in utero a ondansetrona: efeitos teratogênicos e repercussão tardia em parâmetros reprodutivos e comportamentais em ratos machos.", protocolada sob o CEUA nº 6353250620 (ID 000122), sob a responsabilidade de **Arielle Cristina Arena** *e equipe; Ana Carolina Casali Reis* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais do Universidade Estadual Paulista (IBB/UNESP) na reunião de 10/08/2020.

We certify that the proposal "Influence of in utero exposure to ondansetron: teratogenic effects and late repercussions on reproductive and behavioral parameters in male rats.", utilizing 285 Heterogenics rats (males and females), protocol number CEUA 6353250620 (ID 000122), under the responsibility of **Arielle Cristina Arena** *and team; Ana Carolina Casali Reis* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the São Paulo State University (IBB/UNESP) in the meeting of 08/10/2020.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 10/2020 a 05/2022 Área: Ciências Biomédicas

Origem:	Biotério Central da UNESP						
Espécie:	Ratos heterogênicos	sexo:	Machos e Fêmeas	idade:	0 a 5 meses	N:	285
Linhagem:	Wistar			Peso:	4 a 500 g		

Local do experimento: Lugares onde os experimentos e atividades propostas serão realizados: Biotério de mamíferos do departamento de Biologia Estrutural e Funcional Laboratório de toxicologia de produtos naturais e sintéticos

Botucatu, 15 de novembro de 2021

Profa. Dra. Ana Carolina Inhasz Kiss
Coordenadora da Comissão de Ética no Uso de Animais
Universidade Estadual Paulista

Prof. Assoc. Luis Fernando Barbisan
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade Estadual Paulista