UNIVERSIDADE ESTADUAL PAULISTA “JÚLIO DE MESQUITA FILHO”

INSTITUTO DE BIOCIÊNCIAS DE BOTUCATU

PROGRAMA DE PÓS-GRADUAÇÃO EM FARMACOLOGIA E BIOTECNOLOGIA

SUYANE DA SILVA MOREIRA

INFLUÊNCIA DA EXPOSIÇÃO LACTACIONAL AO ANTIDEPRESSIVO VENLAFAXINA: REPERCUSSÃO TARDIA EM PARÂMETROS REPRODUTIVOS E COMPORTAMENTAIS, EM RATOS MACHOS

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Dissertação apresentada ao Instituto de Biociências, Campus de Botucatu, Universidade Estadual Paulista “Júlio de Mesquita Filho” - UNESP, como requisito para a Defesa de Mestrado junto ao Programa de Pós-Graduação em Farmacologia e Biotecnologia.

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Palavras-chave: Comportamento sexual; Depressão pós-parto; Diferenciação sexual hipotalâmica; Venlafaxina.

Dedicatória

Dedico esse trabalho a minha família, Sueleide, Geovana e Hebert. É graças a vocês que essa jornada foi possível.

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Epígrafe

"Compreender as coisas que nos rodeiam é a melhor preparação para compreender o que há mais além."

(Hipátia)

Resumo

A prevalência global de depressão pós-parto é estimada em 100-150 casos a cada 1000 nascimentos, e um dos medicamentos mais indicados para o tratamento é a venlafaxina, um Inibidor da Recaptação da Serotonina e Noradrenalina (IRSN). Como a serotonina possui papel chave no desenvolvimento do cérebro, estudos demonstram que a exposição pré e pós-natal aos IRSNs pode alterar os eixos hipotálamo-hipófise-adrenal (HHA) e hipotálamo-hipófise-gonada (HHG) e afetar processos dependentes de hormônio, como o processo de diferenciação sexual hipotalâmica. Em virtude da importância da testosterona no processo de diferenciação sexual hipotalâmica, torna-se preocupante o uso de antidepressivos durante a gestação e lactação. Desta forma, objetiva-se, neste estudo, avaliar os possíveis efeitos resultantes da exposição lactacional a venlafaxina e suas repercussões tardias sobre parâmetros reprodutivos, em ratos machos. Para tanto, ratas lactantes foram expostas a três doses de venlafaxina (3,85; 7,7 ou 15,4 mg/kg), via gavage, do 1º dia até o 20º dia pós-parto. No 5º dia de lactação, as mães foram submetidas a avaliação de comportamento materno, e ao final do período de exposição, as mesmas foram eutanasiadas e coletou-se sangue e órgãos para investigações posteriores. Após o nascimento, os filhotes machos foram avaliados através dos seguintes parâmetros: determinação do peso corporal, distância anogenital, idades de separação prepucial, descida testicular e *play behavior*. Na vida adulta, estes mesmos animais foram investigados em relação ao: pesos de órgãos reprodutores, contagem espermática, morfologia e motilidade espermática, teste de fertilidade, comportamento sexual masculino, presença de comportamento sexual feminino e preferência sexual, dosagem hormonal e foram coletados hipotálamos para análise da expressão de receptores de andrógenos por meio de western blotting. Em relação aos parâmetros maternos avaliados, houve aumento do peso do baço e uma elevação nos níveis de ureia e ácido úrico séricos, além disso não houve alteração no comportamento materno. A análise do desenvolvimento inicial da prole masculina mostrou um aumento do peso corporal do grupo de menor dose no 13º dia pós-natal, porém essa diferença foi normalizada no 22º dia. Também foi observado uma diminuição em parâmetros do *play behavior* no grupo de dose intermediária. Na idade adulta, os filhotes machos expostos a dose intermediária apresentaram um aumento no peso da vesícula seminal. As análises espermáticas indicaram uma redução do número de espermatozoides progressivos em todos os grupos experimentais, além de um aumento do número de espermatozoides moveis no grupo de maior dose. Foi observado também uma diminuição do índice de masculinização após o teste de fertilidade, e em relação aos testes de comportamento e preferência sexual não houve alterações estatísticas entre os grupos, no entanto, foi detectado uma redução da expressão de receptores de andrógenos no hipotálamo no grupo de maior dose. Conclui-se que a exposição a Venlafaxina foi levemente tóxica para as ratas lactantes e prejudicial para o desenvolvimento cognitivo da prole masculina, além de provocar alterações espermáticas relevantes, apesar de não comprometer parâmetros comportamentais sexuais na vida adulta. São necessários mais estudos

para melhor compreender o papel da Venlafaxina no processo de espermatogênese, bem como no processo de diferenciação sexual hipotalâmica.

Palavras chave: Venlafaxina, diferenciação sexual hipotalâmica, depressão pós-parto, *play behavior*, parâmetros espermáticos.

Abstract

The global prevalence of postpartum depression is estimated at 100-150 cases per 1000 births, and one of the most suitable drugs for treatment is venlafaxine, a serotonin and noradrenaline reuptake inhibitor (IRSN). As serotonin plays a key role in brain development, studies show that prenatal and postnatal exposure to IRSNs can alter the hypothalamic-pituitary-adrenal (HHA) and hypothalamic-pituitary-gonadal (HHG) axes and hormone-dependent processes, such as the hypothalamic sexual differentiation. Due to the importance of testosterone in the hypothalamic sexual differentiation process, the use of antidepressants during pregnancy and lactation is of concern. Thus, the objective of this study is to evaluate the possible effects of lactational exposure to venlafaxine and its late repercussions on reproductive parameters in male rats. Therefore, lactating rats were exposed to three doses of venlafaxine (3.85, 7.7 or 15.4 mg / kg), via gavage, from the 1st day to the 20th day after delivery. On the 5th day of lactation, the females were subjected to maternal behavior assessment, and at the end of the exposure period, the rats were euthanized and blood and organs were collected for further evaluation. After birth, male puppies were assessed using the following parameters: determination of body weight, anogenital distance, ages of preputial separation, testicular descent and play behavior. In adult life, these same animals were investigated in relation to reproductive organ weights, sperm count, sperm morphology and motility, fertility test, male sexual behavior, the presence of female sexual behavior and sexual preference, blood was collected for hormonal dosage and the hypothalamus was collected for analysis of androgen receptor expression by western blotting. In relation to the females evaluated, there was an increase in spleen weight and an increase in serum urea and uric acid levels, moreover there was no change in maternal behavior. The analysis of the initial development showed an increase in the body weight of the offspring of the lowest dose group on the 13th postnatal day, but this difference was normalized on the 22nd. Still in relation to offspring, a decrease in play behavior parameters was observed in the intermediate dose group and an increase in seminal vesicle weight in the same group. Sperm analyzes indicated a reduction in the number of progressive sperm in all experimental groups, in addition to an increase in the number of mobile sperm in the group with the highest dose. There was also a decrease in the masculinization index in the infertility test, and in relation to the behavior and sexual preference tests there were no statistical changes between the groups, however, a reduction in the expression of androgen receptors on the hypothalamus was detected in the higher dose group. It is concluded that exposure to Venlafaxine was slightly toxic to lactating rats and detrimental to the cognitive development of male offspring, in addition to causing relevant sperm changes, despite not compromising sexual behavioral parameters in adulthood. Further studies are needed to better understand the role of Venlafaxine in the development of sperm and in the hypothalamic sexual differentiation process.

Key words: Venlafaxine, hypothalamic sexual differentiation, post-partum depression, play behavior, sperm parameters.

LISTA DE ABREVIATURAS E SIGLAS

5-HT	5-hidroxitriptamina (serotonina)
AVPV	Núcleo Anteroventral Periventricular
HHA	Hipotálamo-Hipófise-Adrenal
HHG	Hipotálamo-Hipófise-Gonadal
IRSN	Inibidor da Recaptação de Serotonina e Noradrenalina
ISRS	Inibidores Seletivos da Recaptação Serotoninérgica
MPOA	Área Pré-óptica Medial
NLET	Núcleo do Leito da Estria Terminal
ODV	O-desmetilvenlafaxina
PGE2	Prostaglandina E2
SDN-POA	Núcleo Sexualmente Dimórfico da Área Pré-óptica
SNC	Sistema Nervoso Central
TDF	Fator determinante do testículo
VEN	Venlafaxina

LISTA DE SÍMBOLOS

Kg	Kilograma
Mg	Miligrama

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Introdução

1. Diferenciação Sexual Hipotalâmica

A diferenciação sexual é um processo que determina o sexo do embrião em desenvolvimento, masculino ou feminino, baseado no sexo cromossômico estabelecido na fecundação. O cérebro, por sua vez, também se diferencia de acordo com o sexo embrionário, momento em que os fenótipos fisiológicos e comportamentais vão ser modificados em concordância com o fenótipo gonadal (McCarthy et al., 2017; McCarthy & Arnold, 2011). O embrião do sexo masculino (XY) possuí o gene SRY, localizado no cromossomo Y, que vai codificar a proteína nomeada de fator determinante do testículo (TDF). Essa proteína é responsável por iniciar as diferenciações celulares que vão dar origem ao testículo e, portanto, iniciar a produção de andrógenos necessários para a organização das características masculinas no indivíduo. Já o embrião do sexo feminino (XX), por não ter presente o gene SRY, não expressará a proteína TDF, o que resultará na formação de ovários (Figura 1) (Turano et al., 2019; VanRyzin et al., 2018).

Estudos indicam que a alta concentração de alfa-fetoproteína presente no embrião exerce um papel protetor contra a ação do estradiol no cérebro em desenvolvimento, que em altas concentrações, leva ao processo de defeminização e masculinização do cérebro. Portanto, o processo de feminização acontece na vida pós-natal, quando há uma queda na produção de alfa-fetoproteína e o estradiol inicia sua atuação no cérebro em menor concentração (Bakker & Baum, 2008). Além disso, sabe-se também do papel de genes específicos no processo de diferenciação feminino do cérebro (McCarthy & Arnold, 2011). Esses dados indicam que o processo de diferenciação do SNC de fêmeas necessita de mecanismos específicos e intrínsecos que garantam a feminização do cérebro e o comportamento correspondente na vida adulta.

Durante o período pré-natal, o cérebro dos machos precisa ser masculinizado para que o comportamento sexual tipicamente masculino e o padrão tônico de secreção de gonadotrofinas sejam estabelecidos (MacLusky & Naftolin, 1981), já que no início do desenvolvimento o hipotálamo de mamíferos está intrinsicamente organizado no modo feminino, o que determina na vida adulta um comportamento sexual típico de fêmea e um padrão de secreção cíclico de gonadotrofinas. Sendo assim, a diferenciação do sistema nervoso central (SNC) em machos é determinada por dois processos distintos: a defeminização e a masculinização. Em modelos experimentais, o processo de defeminização compreende a indução de esterilidade anovulatória e a redução da capacidade de apresentar lordose. Já a masculinização, é definida como a capacidade de apresentar comportamento de monta (McEwen et al., 1977).

O processo de masculinização do hipotálamo é dependente de testosterona, que por ação da enzima citocromo P450 aromatase, é metabolizada originando estrógeno no SNC (Erskine et al., 1988; Rhoda et al., 1984). A exposição ao estrógeno no núcleo sexualmente dimórfico da área pré-óptica (SDN – POA) acarreta um processo de diferenciação das células da microglia para assumirem uma morfologia amebóide e iniciar a produção de prostaglandina (PGE2), que por sua vez atua nos neurônios dessa região para organizar o padrão de sinapses necessário para o comportamento sexual masculino na vida adulta. O SDN - POA masculinizado é ativado pela circulação de esteróides provenientes dos testículos para permitir o comportamento sexual de monta, caraterístico dos machos (Figura 1) (VanRyzin et al., 2018).

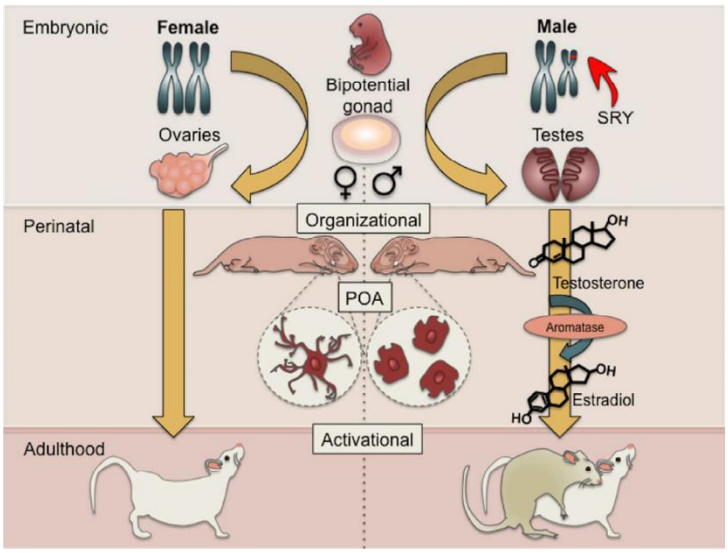


Figura 1 – Processo de diferenciação sexual, organização do cérebro perinatal e ativação do comportamento sexual na vida adulta (VanRyzin et al., 2018).

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 (Figura 2) (Baum et al., 1988; Corbier et al., 1978; Lalau et al., 1990). Corbier et al., (1992), em estudo comparativo, demonstraram que ocorre também uma elevação nos níveis de testosterona, após as primeiras horas do nascimento, numa variedade de espécies de mamíferos machos, corroborando a importância dos andrógenos na diferenciação sexual do SNC desta classe de vertebrados. Evidências sugerem que a conversão de testosterona a estradiol pela aromatase neural, é o mecanismo responsável por regular diversos processos fisiológicos e comportamentais,

como a ativação do comportamento sexual masculino, a diferenciação sexual hipotalâmica e efeitos na retroalimentação negativa sobre a secreção de gonadotrofinas (Balthazart & Ball, 1998).

Dessa forma, substâncias capazes de suprimir ou retardar o pico de testosterona neonatal podem alterar o processo de masculinização e/ou defeminização do hipotálamo (Gore, 2010). Neste sentido, estudos demonstram que o estresse, a exposição a contaminantes ambientais, bem como a administração de fármacos durante o período crítico de diferenciação sexual hipotalâmica pode comprometer esse processo, acarretando em alterações na fisiologia reprodutiva e no comportamento sexual (Arena & Pereira, 2002; Pereira & Piffer, 2005; Negri-Cesi, 2015) as quais muitas vezes só são detectadas mais tarde, na vida adulta reprodutiva.

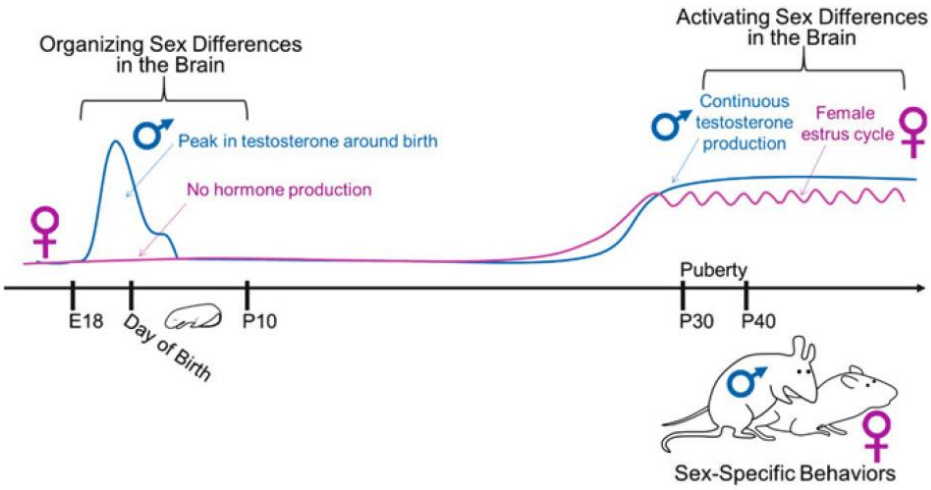


Figura 2 – Picos de testosterona na fase organizacional do cérebro e a produção de hormônios no início da puberdade responsável por ativar as regiões sexualmente dimórficas, resultando nos comportamentos sexuais específicos (Turano et al., 2019).

Assim, para garantir o dimorfismo sexual cerebral, a aromatização da testosterona é um evento necessário, e o estrógeno resultante é o responsável por atuar nos núcleos hipotalâmicos para garantir a masculinização do SNC de roedores (Naftolin et al., 1975). O volume do SDN-POA, por exemplo, é maior em machos do que em fêmeas, já o do núcleo anteroventral periventricular (AVPV) é maior em fêmeas (Gorski et al., 1978; Simerly et al., 1985a; Sumida et al., 1993). Inicialmente, os dois núcleos apresentam o mesmo tamanho e se diferenciam sexualmente somente após ocorrer morte celular por apoptose induzida por hormônios (Bayer & Altman, 1987; Jacobson et al., 1980; Simerly et al., 1985b; Sumida et al., 1993). Dessa forma, hormônios devem atuar nesses núcleos, induzindo ou protegendo as células da apoptose (Schwarz & McCarthy, 2008). As

concentrações de serotonina no hipotálamo diminuem em ratos machos durante a segunda semana pós-natal, influenciada pela concentração de testosterona circulante. Dessa forma, a testosterona pode influenciar a atividade da serotonina, assim como a administração de serotonina pode regular os efeitos da testosterona em núcleos do SNC (Dakin et al., 2008). Um estudo conduzido por Ladosky & Gaziri (1970) mostra que uma concentração diminuída de serotonina em machos pode ser uma consequência do processo de diferenciação sexual, indicando que o processo de masculinização do cérebro de ratos, dependente de testosterona, pode ser influenciado pela concentração de serotonina. A importância do sistema serotoninérgico no cérebro para a diferenciação sexual hipotalâmica em ratos também é demonstrada em estudo de Madden et al. (2016), no qual camundongos machos tratados com agonistas do receptor de serotonina no início da vida pós-natal tiveram o volume do SDN-POA reduzido, o que indica uma feminização desses machos. A serotonina, sendo um neurotransmissor presente no desenvolvimento inicial do cérebro, pode mediar o processo de apoptose induzido por hormônios. Fêmeas tratadas com testosterona ao nascer e posteriormente expostas a um agonista de serotonina na segunda semana de vida, apresentam o núcleo SDN-POA feminizado, comparado as fêmeas que receberam apenas o tratamento com testosterona (Dakin et al., 2008; Murray et al., 2004; Wilson et al., 2009) indicando o efeito anulatório da serotonina sobre a testosterona durante o processo de diferenciação sexual.

Os efeitos dos Inibidores da Recaptação de Serotonina e Noradrenalina (IRSN) no neurodesenvolvimento não são surpreendentes, já que a serotonina (5-HT) possui papel chave no desenvolvimento do cérebro. Através da ativação dos receptores 5-HT, a serotonina modula processos tais como divisão celular, diferenciação, migração e sinaptogênese (Azmitia, 2001). Além disso, uma super ativação de receptores 5-HT leva a mudanças irreversíveis na maturação de alguns circuitos cerebrais (Gaspar et al., 2003). Dessa forma, alterações nos níveis de serotonina, via exposição aos IRSNs, pode afetar processos cruciais do desenvolvimento fetal e neonatal.

A serotonina é também um neurotransmissor envolvido no controle do comportamento sexual durante a vida adulta. Fibras originárias das células sintetizadoras serotoninérgicas na rafe dorsal inervam abundantemente estruturas sexualmente dimórficas, como o núcleo SDN-POA, a área pré-óptica medial (MPOA), o núcleo do leito da estria terminal (NLET) e o núcleo AVPV (Figura 3) (Wilson et al., 1986; Rayen et al, 2013; Simerly et al.1985a). Além disso, sabe-se que a diminuição da neurotransmissão de 5-HT está envolvida com a facilitação do comportamento sexual masculino, enquanto

o aumento da neurotransmissão de 5-HT está relacionado com a inibição deste comportamento (Olivier et al., 2011). Em contrapartida, um estudo usando o antidepressivo fluoxetina, pertencente a classe dos Inibidores Seletivos da Recaptação de Serotonina (ISRS), mostra que a exposição a essa droga facilita o comportamento sexual em ratas adultas (Rayen et al., 2014), evidenciando as diferenças do controle exercido pela serotonina em ambos os sexos.

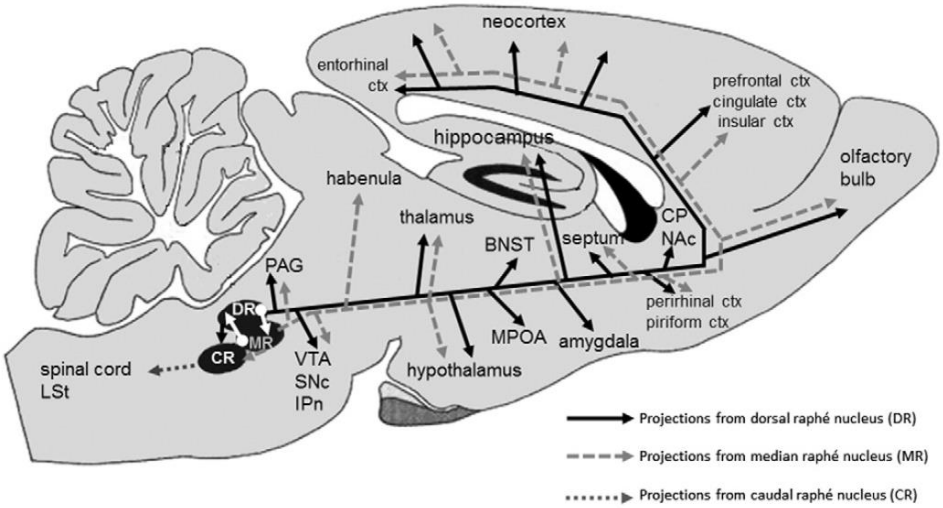


Figura 3 – Projeções de serotonina do núcleo da rafe para outras áreas do cérebro de ratos (Snoeren et al., 2014).

Sabe-se que a exposição pré-natal aos ISRSs altera os eixos hipotálamo-hipófise-adrenal (HHA) e gonadal (HHG) (Oberlander et al., 2009; Pawluski et al., 2012a; Pawluski et al., 2012; Rayen et al., 2011), visto que a serotonina possui importante papel no desenvolvimento desses eixos (Dohler et al., 1991; Jarzab & Dohler, 1984). Uma alteração no eixo HHG durante a gestação e lactação pode afetar processos dependentes de hormônio, como o processo de diferenciação sexual hipotalâmica, e trazer consequências negativas em parâmetros reprodutivos e comportamentais para os descendentes. Neste sentido, estudos que investiguem a relação do aumento da serotonina no cérebro em desenvolvimento são extremamente necessários.

2. Depressão pós-parto e tratamento farmacológico

Desordens depressivas durante a gestação e pós-parto são um importante problema de saúde pública, sendo considerada a complicação obstétrica subdiagnosticada mais comum (Bogi et al., 2018). A prevalência global de depressão pós-parto é estimada em 100-150 casos a cada 1000 nascimentos, podendo ter início durante a gestação e perdurar após o

nascimento (Sriraman et al., 2017; Upadhyay et al., 2017). No entanto, é possível que a estimativa global seja ainda maior, devido as diferenças socioeconômicas e culturais, além de possíveis estigmas em relação a saúde mental (Payne & Maguire, 2019). Como outros quadros depressivos, os sintomas devem estar presentes por 2 semanas para que seja realizado o diagnóstico, os quais incluem perda de interesse e de energia, mudanças nos padrões de sono e alimentação e pensamentos suicidas recorrentes (Kim et al., 2013).

A depressão pós-parto pode interferir e impactar negativamente no relacionamento entre a mãe e a criança, alterando o padrão de interação observado em mães não depressivas, levando a atrasos no desenvolvimento (Field, 2010). Mães depressivas tendem a reproduzir comportamentos negativos em relação a criança, se comunicam menos, interagem menos, tocam menos e de maneira pouco afetiva, o que pode afetar o desenvolvimento neurobiológico, cognitivo, social e emocional dos filhos (O'Hara & McCabe, 2013; Talge et al., 2007; Brummelte & Galea, 2016; Upadhyay et al., 2017).

O desenvolvimento físico também pode ser afetado por esse quadro clínico. Farias (Farías-Antúnez et al., 2018) demonstrou que filhos de mães depressivas possuem maior chance de apresentar baixo peso e baixa estatura no primeiro ano de vida, podendo comprometer o crescimento linear da criança após o primeiro ano. Ademais, estudos mostram que a depressão materna severa e persistente aumenta o risco de problemas de comportamento aos 3,5 anos de vida da criança, bem como gera impacto no desempenho escolar aos 16 anos e aumenta a prevalência de depressão aos 18 anos (Netsi et al., 2018).

Mulheres com depressão moderada a severa precisam fazer uso de medicamentos antidepressivos mesmo durante a gestação e, em alguns casos, dar continuidade ao tratamento farmacológico durante a amamentação (Magalhães & Pinheiro, 2006). O tratamento para depressão materna tem como alvo o sistema serotoninérgico, sendo os Inibidores Seletivos da Recaptação Serotoninérgica (ISRS), a classe de antidepressivos mais comumente prescritas para o tratamento da depressão durante a gestação e pós-parto (Fleschler & Peskin, 2008). No entanto, nos casos em que o uso dos ISRS não surge efeito, ou não é o suficiente para amenizar os sintomas, a classe de escolha para o tratamento é a de Inibidores da Recaptação de Serotonina e Noradrenalina (IRSN), e o principal representante é a Venlafaxina (Ornoy & Koren, 2017; Stewart & Vigod, 2019).

Uma das preocupações do uso de drogas no tratamento da depressão materna é a saúde reprodutiva da mulher, assim como a possibilidade de se aumentar o risco de malformações no feto, complicações na gravidez ou complicações neonatais. Os IRSN são considerados mais seguros que outras classes de antidepressivos para administração

durante o período gestacional e lactacional. Estudos epidemiológicos mostram que não houve associação da administração de venlafaxina com ocorrência de malformações no feto, complicações durante a gestação ou defeitos no desenvolvimento neurológico e neonatal observados no recém-nascido (Ornoy & Koren, 2017; Lusskin et al., 2018). No entanto, existe uma escassez de estudos relacionados a administração dessa droga na vida dos filhos expostos via útero ou via leite, principalmente em relação aos efeitos tardios a essa exposição.

3. Venlafaxina

A venlafaxina (VEN), com fórmula C17H27NO2•HCl (Figura 4), é um sólido cristalino branco derivado sinteticamente de feniletilamina bicíclica. Classificada como Inibidor da Recaptação da Serotonina-Noradrenalina (IRSN), tem como principal mecanismo de ação potencializar os efeitos desses neurotransmissores no Sistema Nervoso Central (SNC). Sua potência de inibição da recaptação de serotonina é 5 vezes maior do que a de noradrenalina, além de possuir baixa afinidade na recaptação de dopamina (Holliday & Benfield, 1995; Bernardi, 2013). Devido a essa interação múltipla, seu potencial terapêutico é superior aos Inibidores Seletivos da Recaptação de Serotonina (ISRS) (Feighner, 1999).

Sua absorção é intestinal, sofrendo intenso metabolismo de primeira passagem no fígado, que resulta em apenas 12,6% da concentração inicial disponível para circulação no sangue. É biotransformada pela ação da isoforma CYP2D6 do citocromo P450 em seu principal metabólito ativo, O-desmetilvenlafaxina (ODV) e mais dois metabólitos de menor relevância. A excreção da VEN e de seus metabólitos é majoritariamente renal, sendo que 87% da droga é eliminada após 48 horas de administração, e cerca de 5 % é identificada na urina em sua forma inalterada, com uma meia-vida de eliminação de 5 horas para a VEN e 10 horas para seu metabólito ativo (Bernardi, 2013; Holliday & Benfield, 1995; Howell et al., 1993; Troy et al., 1997).

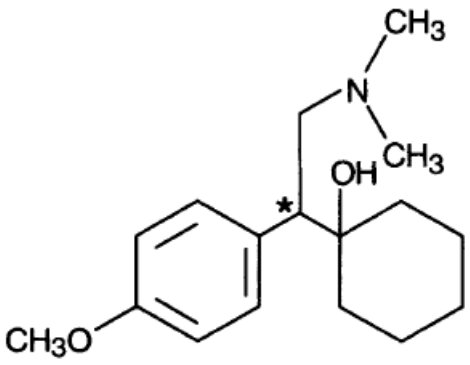


Figura 4 – Estrutura química da venlafaxina.

A VEN é classificada pela Food and Drug Administration (EUA) como uma droga de categoria C, o que significa que os riscos ao embrião/feto não são descartados. Além disso, torna-se preocupante o fato da VEN atravessar a membrana placentária e hemato-encefálica, podendo ser transferida para o recém-nascido através do leite materno em quantidades variadas e imprevisíveis (Dalmizrak et al., 2012; Kristensen et al., 1999). Assim, o uso desse fármaco em fases críticas do desenvolvimento pode aumentar os níveis de monoaminas no feto e recém-nascido (Bellantuono et al., 2015; Tripathi & Majumder, 2010).

A taxa de excreção de VEN e ODV no leite humano é relativamente maior do que de outros antidepressivos devido à alta excreção do ODV, logo, a concentração plasmática da droga em indivíduos recém-nascidos é elevada, e pode acarretar efeitos farmacológicos no organismo. Portanto, o uso na lactação deve ser assistido, principalmente nos casos em que a dose materna for alta. O material disponível na literatura a respeito da VEN no aleitamento materno é escasso, e em sua maioria não relatam efeitos adversos imediatos, embora há relatos de casos de alterações de peso relacionadas com essa exposição (Newport et al., 2009; Orsolini & Bellantuono, 2015; Nielsen & Damkier, 2012). Essa falta de informações ressalvam a necessidade de se investigar os efeitos tardios da exposição indireta a VEN via lactação.

Como a serotonina, noradrenalina e dopamina possuem um importante papel durante o desenvolvimento pré-natal. Drogas com o mecanismo de ação semelhante a VEN, se administradas durante a gestação e lactação, podem interferir com processos que ocorrem durante o desenvolvimento do cérebro, podendo acarretar em consequências neurocomportamentais tardias (Bogi et al., 2018). Desse modo, os possíveis efeitos da

administração da VEN no desenvolvimento neurológico, e suas consequência para o desenvolvimento sexual, devem ser melhor elucidados.

Justificativa

Visto que o uso de IRSN, tais como o antidepressivo venlafaxina, é comum entre mulheres que sofrem com depressão durante a gravidez e no pós-parto, e que este medicamento possui capacidade de passar para o leite materno, seu uso durante fases críticas da amamentação torna-se preocupante. Como sua segurança para o feto e/ou recém-nascido não está totalmente elucidada, torna-se necessário investigar seus possíveis efeitos 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 lactacional ao antidepressivo venlafaxina sobre o desenvolvimento sexual e as possíveis repercussões tardias sobre parâmetros reprodutivos e comportamentais em ratos machos.

Objetivos específicos

Analisar os efeitos tóxicos da exposição a venlafaxina durante o período de lactação em ratas fêmeas, bem como analisar o comprometimento do comportamento materno durante esse período.

Examinar o potencial da exposição lactacional ao antidepressivo venlafaxina na qualidade e quantidade espermática do descentes machos.

Avaliar o comprometimento dos comportamentos tipicamente masculinos na vida adulta de ratos machos expostos ao antidepressivo venlafaxina no período lactacional.

Verificar a expressão de receptores de andrógenos no hipotálamo da prole masculina exposta ao antidepressivo venlafaxina no período lactacional na vida adulta, por meio de western blot.

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Capítulo 1

O manuscrito intitulado **“Influence of lactational exposure to the antidepressant venlafaxine: late repercussion on reproductive and behavioral parameters in male rats”** será submetido para publicação no periódico Reproductive Toxicology, Fator de impacto: 3,143.

INFLUENCE OF LACTATIONAL EXPOSURE TO THE ANTIDEPRESSANT VENLAFAXINE: LATE REPERCUSSION ON REPRODUCTIVE AND BEHAVIORAL PARAMETERS IN MALE RATS

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Abbreviations

5-HT	Hydroxytryptamine (serotonin)
AGD	Anogenital distance
ALT	Alanine Aminotransferase
AR	Androgen receptor
AST	Aspartate Aminotransferase
AVPV	Anteroventral Periventricular Nucleus
BNST	Bed Nucleus of the Stria Terminalis
BSA	Bovine Serum Albumin
CNS	Central Nervous System
DNA	Deoxyribonucleic Acid
DSP	Daily Sperm Production
FSH	Follicle Stimulating Hormone
GD	Gestational day
GGT	Gamma-glutamyl transferase
HHA	Hypothalamic-Pituitary-Adrenal axis
HHG	Hypothalamic-Pituitary-Gonadal axis
LD	Lactational day
LH	Luteinizing Hormone
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MPOA	Medial Preoptic Area
OECD	Organization for Economic Co-operation and Development
PND	Postnatal day
PVDF	Polyvinylidene Difluoride
RIPA	Radioimmunoprecipitation Assay Buffer
SDN-POA	Sexually Dimorphic Nucleus of the Preoptic Area
SDS-PAGE	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
SNRI	Serotonin and Norepinephrine Reuptake Inhibitors
SSRI	Selective Serotonin Reuptake Inhibitors
TBST	Tris Buffered Saline with Tween 20
VEN	Venlafaxine

Symbols

µg	Micrograms
µL	Microliter
fL	Phentoliter
kg	Kilogram
mg	Milligram
mL	Milliliter
mm	Millimeter
pg	Picogram
rpm	Revolutions per minute

Highlights

- Venlafaxine exposure caused mild signs of maternal toxicity.
- Play behavior of male offspring was altered.
- Sperm parameters were negatively affected.
- There was a decrease in AR expression on the hypothalamus of the male offspring

ABSTRACT

Postpartum depression is a public health problem, and one of the most recommended drugs is venlafaxine, an inhibitor of serotonin and norepinephrine reuptake (SNRI). Studies have shown that the exposure to selective serotonin reuptake inhibitors (SSRI) can affect hormone-dependent processes, such as the hypothalamic sexual differentiation. Therefore, the use of antidepressants during lactation is a cause for concern. This study evaluated the possible effects of venlafaxine on lactational exposure and its late repercussions on reproductive parameters in male rats. Lactating rats were exposed to venlafaxine (3.85, 7.7, or 15.4 mg/kg/body weight), via gavage, from lactational day (LD) 1 to 20. The dams were evaluated for maternal behavior, with blood and organs collected for further analysis. Male offspring were assessed using parameters of initial development, puberty installation, play behavior, sperm parameters, fertility test, sexual behavior, hormonal dosage, and evaluation of AR expression in the hypothalamus. Although the maternal behavior was normal, the dams presented an increase in the spleen weight and in the urea and uric acid levels. The male offspring showed a decrease in play behavior parameters in the intermediate dose group. Sperm analysis indicated a reduction in the number of progressive sperm in all experimental groups. The evaluation of the AR expression on the hypothalamus showed a significant reduction on the highest dose group, although the sexual behavior was not affected. The exposure to Venlafaxine caused mild toxicity to lactating rats and it was detrimental to sexual parameters of male offspring.

Keywords: Postpartum depression, serotonin, sexual behavior, hypothalamic sexual differentiation.

1. Introduction

Depressive disorders during pregnancy and postpartum are a major public health problem due to their global prevalence, estimated at 100-150 cases per 1000 births [1–3]. If untreated, this disorder can affect the mother-baby relationship and cause long-term negative consequences on the child's physical and mental development [3,4]. Therefore, women with moderate to severe depression need to take antidepressant medications during breastfeeding [5].

Venlafaxine (VEN), a Serotonin-Norepinephrine Reuptake Inhibitor (SNRI), is an important treatment for maternal depression. It is classified by the Food and Drug Administration (USA) as a category C drug, as it may be of risk to the embryo/fetus, as well as possibly being transferred to the newborn through breast milk in various and unpredictable amounts [6,7].

Fibers originating from serotoninergic synthesizing cells in the dorsal raphe abundantly innervate sexually dimorphic structures, such as the sexually dimorphic nucleus of the preoptic area (SDN-POA), the medial preoptic area (MPOA), the bed nucleus of the stria terminalis (BNST), and the anteroventral periventricular nucleus (AVPV) [8–10] In this way, the administration of drugs with similar action mechanism to VEN during gestation and lactation may interfere with brain developmental processes, with late neurobehavioral consequences [1].

Increased 5-HT neurotransmission is related to inhibition of male sexual behavior in adulthood [11], since serotonin plays an important role in the development of the hypothalamic-pituitary-adrenal (HHA) and gonadal (HHG) axes [12,13]. Alterations in the HHG axis during development can affect hormone-dependent processes, such as the hypothalamic sexual differentiation, and bring negative consequences in reproductive and behavioral parameters for offspring.

For the neonatal brain to be typically male, the hypothalamus needs to be defeminized and masculinized [14]. The process of masculinization depends on testosterone, which, by action of the cytochrome P450 aromatase enzyme, is metabolized to estrogen in the central nervous system (CNS) [15,16]. In male rats, two testosterone peaks of testicular origin occur, the first being at gestational day (GD) 18-19 [17] and the second during the first hours after birth [18–20].

Testosterone can influence serotonin activity, just as serotonin administration can regulate the effects of testosterone on CNS nuclei [21]. Ladosky & Gaziri [22] demonstrated that a decreased serotonin concentration in males may compromised the brain sexual differentiation process. Furthermore, male mice treated with serotonin receptor agonists early in postnatal life had reduced SDN-POA volume, indicating a feminization of these males [23]. The data indicate that the brain sexual differentiation process can be influenced by serotonin concentration.

Therefore, it is necessary to evaluate the effects of exposure to this class of drug during these critical phases of development, since changes in this process are usually only noticed in puberty or in adult reproductive life [24,25]. The experimental model proposed in this study seeks to demonstrate possible interference of the administration of VEN during the lactational period in male offspring.

2. Materials and methods

2.1 Animals

Wistar male (12 weeks, 350g, n = 10) and female (12 weeks, 250g, n = 40) adult rats 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 libitium*. The experimental procedures were in accordance with 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 1152/2019).

2.2 Mating and pregnancy diagnostic

In order to obtain the experimental groups, two adult female rats (n = 40), nulliparous and normally cycling and one adult male (n = 10) were placed to mate, in cages, in the late afternoon. In the morning of the following day, vaginal lavage was performed, and the pregnancy of the female was considered positive when she was in estrus and sperm were detected in the vaginal lavage. The pregnant females were weighed and distributed in the experimental groups and accommodated in individual cages.

2.3 Experimental design

The pregnant rats were allocated into four experimental groups. For the evaluation of the effects of venlafaxine 1-[2-(dimethylamino)-1-(4 methoxyphenyl) ethyl] cyclohexanol, Cruz Vermelha Pharmacy (98% purity), three groups were treated with doses of 3.85, 7.7 or 15.4 mg/kg of body weight, from the 1st lactation day (LD) until the 20th day, orally (gavage). The control group received only the vehicle (distilled water). The offspring were exposed to venlafaxine through milk. The treatment extended until

the end of the rat's lactation phase, as during this period there is rapid growth of the brain, with neuronal migration and differentiation, synaptogenesis, glial multiplication and myelination, thus being considered a critical phase of neuronal maturation [26]. Doses were based on therapeutic doses, with a dose of 3.85 mg/kg of venlafaxine in rats equivalent to a human dose of 37.5 mg/day, 7.7 mg/kg equivalent to a human dose of 75 mg/day and 15.4 mg/kg equivalent to a human dose of 150 mg/day. Equivalent doses for rats were calculated based on the body surface area [27]. During treatment, lactating rats, maintained in individual cages, were weighed on alternate days to calculate the volume of venlafaxine 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, while female offspring were donated to another research group to carry out different analyses.

2.4 Maternal evaluation

2.4.1 Maternal behavior (n= 9 animals/group)

Maternal behavior was assessed according to methodology previously described [28]. The analysis was carried out on the LD5, because in the beginning of the postnatal life the energy cost of lactation is lower and the pups have limited motor capacity. On the day of the test, all the pups were removed from the cage and the nest was 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 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, regardless of their position in the cage) were evaluated, and the construction of the nest.

2.4.2 Analysis of maternal biochemical and hematological parameters (n=9 animals/group)

After weaning, mothers were anesthetized with sodium thiopental (50 mg/kg) and then 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 (GGT), Alkaline phosphatase, Urea, Creatinine, Cholesterol, Total protein, Albumin and Uric acid. These parameters were determined using the BioPlus 2000 semiautomatic equipment, with Bioclin kits.

For hematological analysis, total and differential leukocyte, erythrocyte and platelet counts were performed, in addition to hemoglobin, hematocrit and erythrocyte distribution width. With the exception of hemoglobin levels, which were determined using BioPlus 2000, the remaining hematological parameters were analyzed manually. From these data, the following parameters were determined: mean corpuscular volume (MCV): hematocrit/erythrocyte count, expressed in fL; mean corpuscular hemoglobin (MCH): hemoglobin / erythrocyte count, expressed in pg; mean corpuscular hemoglobin concentration (MCHC): hemoglobin / hematocrit X 100, expressed as a percentage.

The organs (thyroid, heart, lung, liver, kidney, adrenal, spleen, uterus, and ovary) were also collected, weighed and analyzed for the external morphology of these organs.

2.5 Analysis of reproductive parameters of male offspring

After a postnatal day (PND 1) of the pups, the litter was reduced to four males and four females, and all males were assessed for early sexual development. Subsequently, the study was divided into 2 experiments. In experiment 1, male rats from different experimental groups (2/litter) were evaluated for organ collection and sperm parameters. In experiment 2 (2/litter), the following parameters were evaluated: fertility test and male and female sexual behavior.

2.5.1 Evaluation of sexual development of male offspring (n= 4 animals/litter)

Male pups were weighed on PND 1 and PND 22 on an analytical balance. At these same ages, the anogenital distance (AGD), measured between the genital tubercle and the 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 [30].

In order to confirm the beginning of the puberty period, the bilateral descent of the testis was inspected through visualization and palpation of the scrotum, from the PND 15. From the PND 30, the male offspring were examined daily until the complete detachment of the prepuce (preputial separation).

2.5.2 Play behavior (n= 4 animals/litter)

The social behavior of playing in juvenile rats is an important marker of neurodevelopment, since changes in this behavior are associated with disturbances in neurological development. This test was performed according to Zaccaroni et al. [31]. The animals were evaluated between the PND 40 and 45, the age at which the animals exhibit the social behavior of "playing". The four males of the same litter were observed during the dark cycle, under red light, and the behavior was recorded on video for 15 minutes. Social behaviors (touching/smelling the other's body, approaching, jumping and

running, exposing the belly to another, running away, side display, biting, jumping on top of the other, chasing) and non-social behaviors (chewing substrate, lowering, dig, explore the environment, get up, touch yourself).

2.6 Evaluation of reproductive parameters in adulthood

2.6.1 Organs collection (n= 9 animals/ group)

At 90 days of age (sexual maturity), male rats from different experimental groups (1/litter) were anesthetized with sodium thiopental 50 mg/kg. Through cardiac puncture, blood was collected for hormonal measurements and organs such as testis, epididymis, seminal vesicle, vas deferens, prostate, thyroid, liver and kidney were removed and weighed on a precision analytical balance. The right testis and epididymis were stored for sperm analysis.

2.6.2 Sperm parameters

Sperm count in the testis and epididymis (n= 9/group): spermatids resistant to homogenization and sperm in the head/body and cauda of the epididymis were counted, as previously described by Robb et al. [32], with adaptations adopted by Fernandes et al. [33]. Each right testis was decapsulated and weighed after collection, and homogenized in 5 mL of 0.9% NaCl containing 0.5% TritonX100. The sample was diluted 10 times and transferred to Neubauer chambers, counting mature sperm cells. For the calculation of 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). Likewise, portions of the head/body and epididymal cauda were cut into fragments and homogenized, and the sperm count was 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 by the DSP.

Sperm morphology (n= 9/group): To perform the morphology, the animals' right vas deferens was sectioned at the ends, and 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 (400X magnification). The morphological abnormalities found in the sperm were classified as abnormalities of the head and tail [34].

Sperm motility (n = 5 /group): It was evaluated on the tail of the right epididymis, described by Perobelli et al. [35]. For this, a 10 mL aliquot of sperm suspensions 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) and Type C (static).

2.6.3 Fertility test (n= 9 animals/group)

At 80 days old, 1 male rat from each litter was subjected to the fertility test after natural mating. Adult rats were paired, up to a maximum of 15 days, with untreated females, placed in their cages (1 female/male), in the late afternoon. In the following mornings, the vaginal rinses of these rats were collected, and the day on which the presence of sperm was determined and the female was in estrus was considered day 0 of pregnancy. On the 20th day of pregnancy, the sperm-positive females were killed for the collection of the uterus and ovaries to record the number of corpus luteum, implantation sites, resorption, live and dead fetuses, for later determination of the potential for fertility (efficiency implantation): implantation sites/ corpus luteum x 100; gestation rate: number of pregnant females/ number of inseminated females x 100; pre-implantation loss rate: number of corpus luteum - number of implantations/ number of corpus luteum X 100;

post-implantation loss rate: number of implantations - number of live fetuses/ number of implantations x 100, among other parameters.

2.6.4 Sexual behavior (n= 9 animals/group)

After the fertility test, adult rats, now sexually experienced, were submitted to abdominal surgery, bilaterally orchiectomized and collect in a biotherium with an inverted light/ dark cycle. For bilateral orchiectomy, the animals were anesthetized with Xylazine (10 mg/kg) and Ketamine (25 mg/kg). The experiments started after a period of 15 days for adaptation of the animals to the inverted light/dark cycle and recovery from surgery. To assess sexual behavior, the animals were in observation cages, during the dark period of the light-dark cycle, under light with a red filter.

Male sexual behavior: The orchiectomized animals received testosterone propionate 1mg/day (subcutaneous), three times a week, for 15 days, the first administration being carried out the day after the orchiectomy and the last administration the day before the assessment of male sexual behavior [36]. Thus, the androgen levels of the animals evaluated were similar, discarding the hypothesis of a hormonal deficiency, in the absence of male sexual behavior. On the day of the experiment, each male was placed in the observation cage, where they remained for at least 5 min for adaptation. Then, a proven receptive female was introduced into the same box, with natural estrus or previously induced by the administration of estradiol benzoate, 20 µg/kg (intraperitoneal), 24h before the test [37–40]. 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. The following parameters were evaluated: latencies for first mount, intrusion, ejaculation; latencies for 1st mount and intrusion after 1st ejaculation; number of mounts (mounts without penile insertion and intrusions) and intrusions until

the 1st ejaculation, total number of mounts (mounts without penile insertion and intrusions), intrusions and ejaculations during the test. The occurrence of penile intrusion was considered when the following set of signs was observed: mounts lasting more than 2 seconds, lordosis (characteristic curve of the female's back) and cleaning of the male's genital region after mounting. Ejaculation was evidenced by the observation of intromission followed by the raising of the male's trunk and stopped for a few seconds [41,42].

Female sexual behavior: 15 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.), 24h before the evaluation of female sexual behavior [37]. First, untreated and sexually experienced males were placed individually in observation cages, where they remained for 5 min for adaptation. Then, the male from an experimental group was placed in the cage. The observation took place for 10 min and 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.

Sexual Preference: This test aims to identify the sexual preference of rats for another male or female. It was carried out in a semicircular arena (100×50cm) with two cages (25×15cm) positioned on opposite sides outside the arena, where the bait animals were positioned, one cage contained the sexually experienced adult male and the other a female rat (baits) in natural estrus or induced by hormonal manipulation (20 µg/kg of estradiol benzoate, subcutaneous, 24 h before the start of the test). The tested rats had no direct contact with the bait animals and the incentive was the odor that the bait animals gave off. The arena floor in front of each bait animal was demarcated in male and female incentive zones (30 × 20 cm) and the test lasted 20 min. The animals were transferred to the room with an inverted light cycle 2 weeks before the test. Each test rat was placed individually in the center of the arena, for 5 min, 3 times/week, for 1 week, without the

bait animals, so that he could get used to it. On the day of the test, 1 male rat-bait was placed in the incentive box and, in the opposite direction, another box with 1 female rat-bait, 5 min before the test. After the habituation period, 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 incentive zone, total time spent in each zone, duration of each visit to each incentive zone. After the end of the test, a sexual preference pattern score was calculated for each animal, according to Agmo [43]: time spent in female incentive zone/time spent in both incentive zones; this score is an indicative of preference for the female bait.

2.7 Extraction and quantification of protein (n= 8 animals/group)

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 [44] on 96-well polystyrene plates and reading of absorbance was performed on Biochrom microplate reader (Holliston, Massachusetts, USA).

2.7.1. Western blotting

Aliquots containing 5 μg of proteins samples (eight sample) were separated on SDS-PAGE. Following the electrophoresis, the proteins were transferred to nitrocellulose membranes or Polyvinylidene Difluoride (PVDF) (only Caspase-3-cleaved). 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 or 3% BSA (only Caspase-3-

cleaved) 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 (1:10,000–20,000) in TBST for 1 h (IgG goat-antirabbit, ab97051 and IgG goat-anti-mouse, ab97023, Abcam® Inc., USA). The immunoreactive components were revealed by GE Amersham ECL chemiluminescent substrate (GE Healthcare). Analyses were done in eight different biological samples per group. To calculate the mean and SEM, 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. The results were expressed as means ± SEM.

2.8 Statistical analysis

Values were 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 Tukey-Kramer a posteriori test, or by non-parametric analysis of variance (Kruskal-Wallis), followed by the Dunn a posteriori test, when necessary. The differences were considered significant with $p < 0.05$, performed on the GraphPad InStat (version 3.02).

3. Results

During the period of VEN exposure, the lactating rats showed no clinical signs of toxicity. Furthermore, exposure to the drug did not alter water and food consumptions and no changes were observed in final weight and weight gain (Table 1). Analysis of maternal behavior also indicated no statistical differences among groups regarding the parameters evaluated (Table 2). The organs weighed after the euthanasia of these animals, for the most part, were not statistically different from each other, except for the spleen, which had its weight increased in the lower and higher dose groups when compared to the control group (Table 3). The hematological parameters evaluated were not altered, but the biochemical analysis showed an increase in urea and uric acid levels (Table 4).

Regarding the initial development of the male offspring, there was no significant difference among groups in the evaluation of the relative anogenital distance (Figure 2). However, there was a significant increase in the body weight of the offspring of the lower dose group (3.85 mg/kg) compared to the control group at PND 13, but the value was normalized at PND 22 (Figure 3). The ages of testicular descent and preputial separation were not altered (Figure 4A), as well as there was no significant difference in the body weight measurement of these animals (Figure 4B). The parameters analyzed for the evaluation of play behavior in general were not altered (Figure 5A). However, specifically analyzing the frequencies of the parameters Jumping and Exploring the environment, a reduction in the intermediate dose treatment group was observed (Figure 5B), indicating that these animals were less active.

On PND 90, a significant increase in the relative weight of the kidney was detected in the higher dose group, and also of the vas deferens in the intermediate dose group in relation to the control group (Table 5). The weight of the other organs analyzed was not altered by the treatment. The analysis of the sperm parameters showed that there were no

changes regarding count and morphology (Tables 6 and 7); however, the percentage of Type A (motile with progressive movement) spermatozoa was reduced in all experimental groups compared to the control group. Moreover, there was an increase in Type B (motile without progressive movement) spermatozoa in the higher dose group (Figure 6).

The fertility test performed with the males in the experiment did not indicate changes in most of the parameters evaluated, except for the masculinization rate, which was significantly reduced in the lower dose group (Table 8). Venlafaxine exposure during lactation was not able to alter the sexual behavior pattern of rats in adulthood, since there was no change in the parameters evaluated in both the male (Table 9) and female sexual behavior tests. The parameters evaluated in the sexual preference test also showed no statistical differences, although there was a slight decrease in the preference score in the higher dose group (Table 10). However, VEN exposure significantly reduced the expression of androgen receptors in the hypothalamus from animals in the higher dose group (Figure 7).

4. Discussion

With a few exceptions, breast milk contains all the substances that circulate in the maternal bloodstream. Since many drugs can be excreted in milk [45], this study aimed to evaluate the effects of lactation exposure to the antidepressant VEN on reproductive parameters in the male offspring. Our results indicated that this exposure was able to compromise important late reproductive parameters of these animals, reinforcing the need for further investigations on the impact of antidepressant administration during the breastfeeding period.

Mortality is a clear sign of toxicity; however, other variables may be indicative of more subtle adverse effects, such as loss of body mass during treatment, diarrhea, piloerection, and changes in behavior, and a particular substance can cause signs of toxicity that are not apparent, such as changes in hematological, biochemical, and/or weight of vital organs parameters [46]. Treatment with different doses of VEN during the lactation period did not cause any apparent maternal toxicity, since no clinical signs or symptoms of toxicity were observed, however it was able to change the relative weight of the spleen. An increase in the volume of this organ is usually followed by hematological alterations, causing changes in normal levels of platelets, erythrocytes, hematocrit, among others [47,48]. Although the hematological evaluation presented here indicates a tendency towards an increase in the platelets number in the same groups in which the change in spleen weight occurred, the values did not represent statistical differences in relation to the control group.

Previous studies show that exposure to Fluoxetine, a known SSRI, leads to a significant increase in urea and uric acid levels as a result of liver toxicity [49,50]. It is also known that there is a relationship between the use of VEN and hepatotoxicity [51,52], indicating that the exposure in the present study was able to cause a degree of toxicity,

despite other parameters indicative of hepatoxicity, such as ALT, AST and GGT have not been altered.

Although the use of drugs such as antipsychotics and antidepressants during pregnancy and lactation was able to alter the maternal behavior in rats in previous studies [53,54], treatment with VEN under the conditions presented in this study was not enough to cause disturbances in the parameters evaluated, ruling out the possibility of influence of maternal behavior on late changes observed in the offspring.

VEN exposure during the second neonatal testosterone peak was not sufficient to alter the AGD of the pups exposed via milk at any of the ages evaluated. However, at PND 13, animals exposed to the lowest dose of VEN showed an increase in body weight. Weight changes are related to VEN exposure in the literature [55–57], but the results presented in this study demonstrate that this change was normalized at PND 22 in these animals.

Play behavior is a facilitator for social, cognitive, emotional, and motor development during preadolescence. Deprivation of this behavior in rats can generate negative consequences for brain development and social interactions [58,59]. Serotonin is an important modulator of social behavior, and increased availability of this neurotransmitter in the developing brain inhibit or reduce this behavior [59]. This relationship is well established in the literature [58,60–62], corroborating with the findings in the present study. In general, males exhibit the play behavior twice as often as females, due of structural and chemical differences in the brain, and these characteristics are susceptible to change through hormonal influences [63–66].

Lactational exposure to venlafaxine compromised the weight of the vas deferens and kidneys of exposed males. A recent toxicity study observed significant renal changes in rats after treatment with VEN [67]. Another group studied the impacts of exposure to Fluoxetine during gestation in rats and found adverse effects on offspring kidney

development [68]. Such findings support the hypothesis that VEN and similar class drugs may negatively impact kidney function. There is also a relationship between VEN, or similar class drugs, and the vas deferens [69–71], however others investigations are necessary to better understand the impact on these organs.

The use of antidepressants with pharmacological targeting on serotonin reuptake is associated with impaired sperm quality, and can compromise parameters such as sperm count, morphology, and motility [72–74]. These data corroborate with the findings in this study, in which VEN exposure impaired sperm maturation and reflected in reduced progressive sperm and proportionally increased motile ones. Such changes may result from hormonal deregulation caused by the use of this drug class, as pointed out by studies conducted in rats [75–77]. Concentrations of the hormones LH, FSH, testosterone, and estrogen are determinants for spermatogenesis to happen properly, with emphasis on testosterone, which also contributes to sperm maturation, that takes place in the epididymis [78–80]. Therefore, compromised sex hormone production can impair the maturation process in the epididymis, and result in reduced sperm motility [81,82]. Progressive sperm motility is vital for oocyte fertilization in the female tract, so this type of alteration is related to fertility problems [83,84].

Studies have evidenced sperm DNA alterations and the generation of oxidative stress in testicular tissue after administration of SSRI-class drugs, indicating a genotoxic potential for male germ cells, and contributing to human infertility [85–87]. Although this sperm alteration was not able to compromise the fertility potential, a change in the proportion of males and females were observed in this study. Since the determination of genetic sex depends on the sperm [88], these data may be linked to changes in sperm capacitation and lifespan.

Serotonin has a clear role in the regulation of male sexual behavior and its main action is the inhibition of male sexual behavior [89]. Evidence shows that increasing this

neurotransmitter inhibits male sexual behavior, therefore, central serotonin levels are inversely related to sexual behavior [11,90,91]. However, studies by Mos et al. [92] and Vieira et al. [93] demonstrated that experimental exposure to a series of SSRIs at therapeutic doses did not cause relevant changes in sexual behavior parameters in male rats. These data corroborate the findings of the present study, because under the conditions of exposure performed, the sexual performance of male rats was not significantly altered, although there was a tendency to increase the time for first breeding and for first intrusion.

The hypothalamus sexual differentiation process depends on hormonal actions and in males it is induced by testosterone, which will act in the rodent brain through two different signaling pathways, the estrogenic pathway, mediated by the conversion of testosterone to estradiol by the enzyme P450 aromatase, and the androgenic pathway, mediated by the androgen receptor (AR). However, the role of the androgenic pathway is poorly understood in the literature [94,95].

The expression of AR in the hypothalamic regions differs between sexes, that is, some regions have greater expression of AR in males than in females and this expression can be reduced by external factors [96,97]. Studies show that the action of testosterone through the AR is related to the number of neurons in different sexually dimorphic nuclei of the CNS, thus contributing to the masculinization of the brain. It is also been showed that AR actions on the brain is necessary for the of typically male behaviors and the maintenance of the gonadotropic axis [98–100], and this regulation can affect sexual preference.

Bodo & Rissman [101] conducted a study demonstrating that the application of dihydrotestosterone in the neonatal life of female mice induced a preference for other females, showing results similar to the control males in the experiment. This result indicates the importance of AR activation for the maintenance of this behavior. The

reduction in AR expression in the present study was dose dependent and may have impaired the masculinization of the brain and impacted sexual preference of these rats, although the difference was not statistically significant.

The process of male sexual differentiation of the hypothalamus requires peaks of testosterone in the brain, which in rats occur between the 18-19 gestational day and a few hours after birth [17,102,103]. Thus, the critical period for hypothalamic sexual differentiation is between embryonic day 18 and postnatal day 10 [104] which makes this period susceptible to the action of endogenous and exogenous substances. However, a third testosterone peak occurs early in peripuberty and may mask possible changes in brain organization that have occurred previously [105]. In the present study, the results of behavioral tests may be related to the action of the third testosterone peak in animals, masking possible negative effects of venlafaxine.

In conclusion, the exposure of lactating rats to venlafaxine caused mild maternal toxicity for females, but did not change the maternal behavior. Regarding the male offspring, lactational exposure was able to alter play behavior and sperm quality, besides altering the expression of androgen receptors in hypothalamic neurons. However, these changes were not enough to significantly alter important behavioral parameters, such as male and female sexual behavior and sexual preference. Therefore, it is possible to infer that the VEN exposure compromised the development of the male offspring's reproductive system, and further studies are needed to understand the potential mechanisms by which these changes occurred.

5. Conflict of interests

The authors declare that there are no conflicts of interest.

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Figure and tables

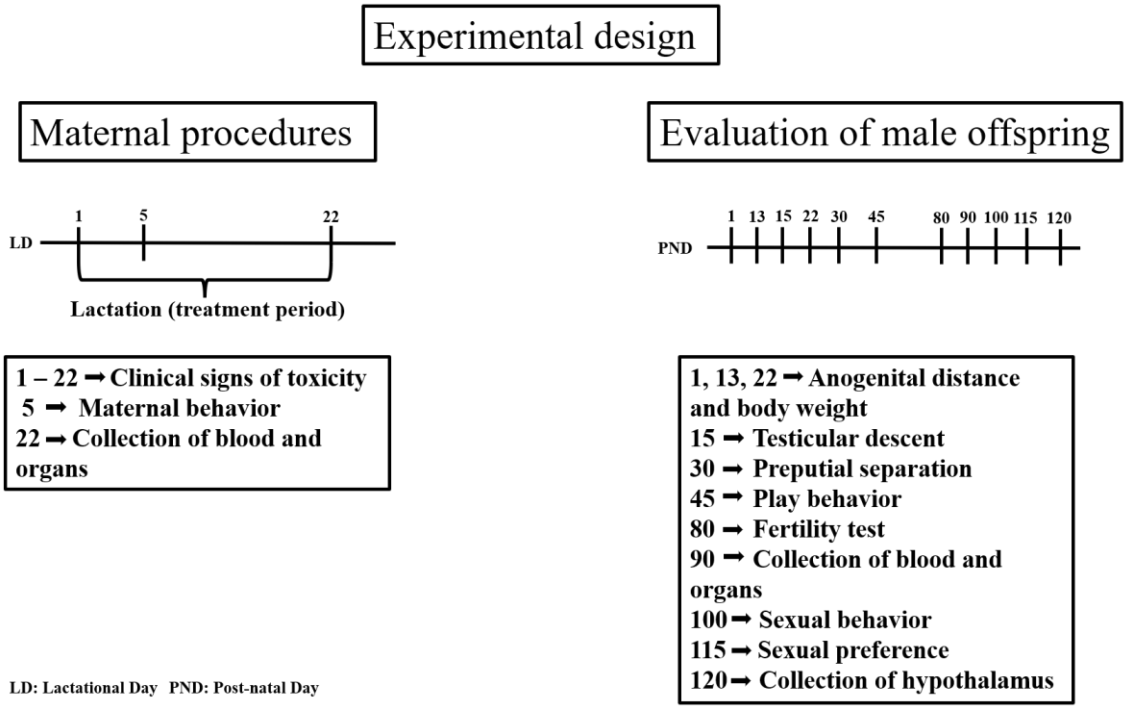


Figure 1: Experimental design

Table 1. Body weight and water and food intake of lactating females treated with venlafaxine.

Parameters	Experimental group			
	Control	3.85 mg/kg	7.7 mg/kg	15.4 mg/kg
Initial weight (g)	255.2 ± 8.05	256.14 ± 5.54	254.69 ± 5.02	253.31 ± 6.51
Final weight (g)	295.86 ± 10.36	295.22 ± 5.83	298.10 ± 5.11	283.72 ± 8.47
Weight gain (%)	14.22 ± 2.46	15.43 ± 1.92	20.32 ± 3.28	11.12 ± 0.50
Food intake (g/day)	48.28 ± 2.51	48.50 ± 2.03	52.70 ± 2.22	51.23 ± 0.84
Water intake (mL/day)	78.26 ± 4.52	76.77 ± 4.10	81.41 ± 2.71	86.21 ± 4.18

Values expressed in mean ± SEM. n=9/group. ANOVA with posterior test in Dunnett. $p>0.05$.

Table 2. Maternal behavior of lactating females treated with venlafaxine.

Parameters	Experimental groups			
	Control	**3.85 mg/kg**	**7.7 mg/kg**	**15.4 mg/kg**
Grouping (s)	619.33 ± 190.07	688.00 ± 203.65	883.33 ± 255.78	844.11 ± 267.41
Latency to grouping (s)	32.13 ± 15.20	37.75 ± 13.86	22.11 ± 8.77	27.78 ± 12.19
Licking (s)	216.00 ± 58.58	229.89 ± 72.46	216.11 ± 62.16	170.44 ± 50.31
No interaction (s)	1000.56 ± 143.30	1171.89 ± 163.36	799.00 ± 186.69	1135.89 ± 165.03
#Nest	2.00 (1.00 – 3.00)	0.00 (0.00 – 2.00)	1.00 (0.00 – 2.00)	2.00 (0.00 – 3.00)

Values expressed in mean ± SEM. n=9/group. ANOVA with posterior test in Dunnett. #Value expressed as median (Q1-Q3). Kruskal-Wallis/Dunn test. (s) time in seconds. $p>0.05$.

Table 3. Relative organ weight (g/100g body weight) of lactating rats treated with venlafaxine.

Parameters	Experimental groups			
	Control	**3.85 mg/kg**	**7.7 mg/kg**	**15.4 mg/kg**
Body weight (g)	269.11 ± 11.83	266.60 ± 6.29	271.06 ± 6.94	252.95 ± 4.92
Liver (g)	4.43 ± 0.25	4.21 ± 0.26	5.06 ± 0.23	5.11 ± 0.26
Kidney (g)	0.39 ± 0.01	0.37 ± 0.01	0.41 ± 0.01	0.40 ± 0.01
Adrenal gland (mg)	16.73 ± 0.68	16.45 ± 0.75	16.99 ± 1.14	16.56 ± 0.52
Spleen (g)	0.22 ± 0.01	0.29 ± 0.02**	0.25 ± 0.01	0.29 ± 0.01**
Heart (g)	0.39 ± 0.02	0.37 ± 0.01	0.40 ± 0.01	0.39 ± 0.01
Lung (g)	0.50 ± 0.03	0.56 ± 0.02	0.52 ± 0.03	0.56 ± 0.01
Total ovary (mg)	32.52 ± 1.94	33.31 ± 1.59	33.28 ± 3.09	37.91 ± 3.98
Uterus (g)	0.15 ± 0.01	0.19 ± 0.02	0.15 ± 0.02	0.18 ± 0.01

Values expressed in mean ± SEM. n=9/group. ANOVA with posterior test in Dunnett. **$p<0.01$.

Table 4. Hematological and biochemical parameters of lactating rats treated with venlafaxine.

Parameters	Experimental groups			
	Control	**3.85 mg/kg**	**7.7 mg/kg**	**15.4 mg/kg**
Hematological				
Platelets (10^3/µL)	11.66±5.00	13.86±1.61	12.13±1.38	13.75±1.19
Erythrocytes (10^6/µL)	5.65±0.40	5.84±0.40	5.95±0.23	6.41±0.24
Hematocrit (%)	46.90±0.67	45.25±1.79	46.44±1.22	48.13±1.03
Leukocytes (10^3/µL)	4.26±0.23	3.69±0.27	4.11±0.40	3.91±0.28
Hemoglobin	17.10±0.51	17.64±0.57	19.19±0.95	18.53±0.73
MCV (fL)	86.53±5.77	80.39±6.94	75.95±1.98	77.41±3.72
MCH (pg)	33.15±2.32	33.10±3.12	31.55±1.52	29.33±1.91
MCHC (%)	36.63±1.16	39.31±1.27	43.87±3.20	40.51±1.17
Neutrophils (%)	19.30±2.44	12.62±1.61	17.25±2.48	17.00±1.93
Lymphocytes (%)	75.10±3.09	82.88±2.01	78.00±3.16	79.25±2.37
Monocytes (%)	4.10±1.10	2.00±0.57	3.89±0.66	2.88±0.69
Eosinophils (%)	1.2±0.51	2.13±1.00	0.89±0.51	0.50±0.27
Basophils (%)	0.3±0.15	0.38±0.26	0.44±0.24	0.38±0.18
Biochemical				
Aspartate aminotransferase	30.78±6.14	44.38±10.61	49.88±6.67	54.89±12.39
Alanine aminotransferase	20.67±5.33	22.50±3.30	20.63±3.58	26.00±5.94
#Gamma glutamyl	0.00(0.00 – 2.00)	0	0.00(0.00-2.00)	0
#Total protein (g/dL)	6.70(6.30-8.10)	6.50(5.30-6.90)	7.00(6.40-7.40)	6.85(6.30-7.30)
Albumin (g/dL)	3.22±0.30	2.61±0.29	2.69±0.25	2.86±0.14
Urea (mg/dL)	65.75±3.38	71.86±2.30	81.13±4.65*	73.38±4.29
#Creatinine (mg/dL)	1.10(1.10-1.20)	1.00(0.80-1.20)	1.20(1.00-1.40)	1.10(0.80=1.40)
Cholesterol (mg/dL)	64.25±3.78	76.50±6.76	82.13±8.85	80.00±3.32
#Uric Acid	2.30(1.80-3.30)	2.80(2.30-8.10)	3.00(1.30-4.90)	4.60(2.30-12.10)*
Alkaline phosphatase (U/L)	338.44±28.20	294.88±47.18	387.78±65.82	319.33±42.28

MCV – mean corpuscular volume; MCH - mean corpuscular hemoglobin; MCHC- mean corpuscular hemoglobin concentration.
Values expressed in mean ± SEM. n=9/group. ANOVA with posterior test in Dunnett.
#Values expressed as median (Q1-Q3). Kruskal-Wallis/Dunn test. *p<0.05.

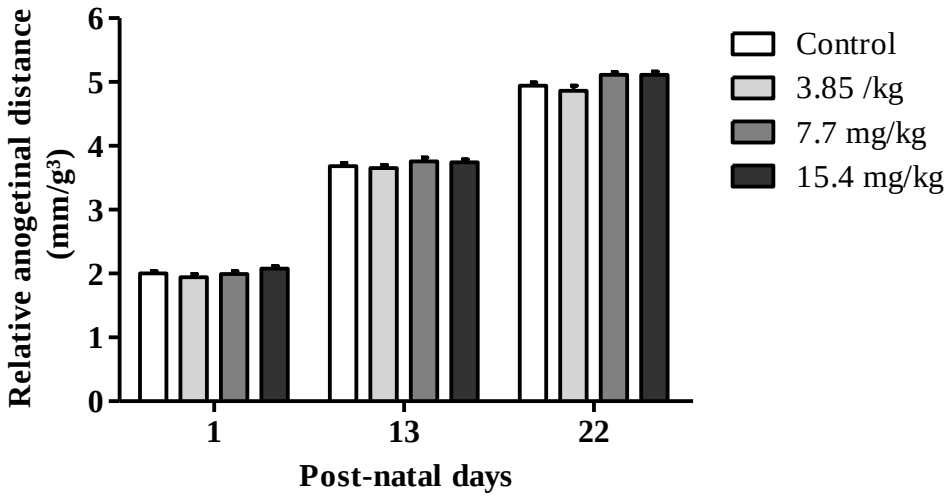


Figure 2. Relative anogenital distance of the male offspring exposed to venlafaxine in the lactational period. Values expressed in mean ± standart error mean (SEM). n=9 litter/group. ANOVA with posterior test in Dunnett. p>0.05.

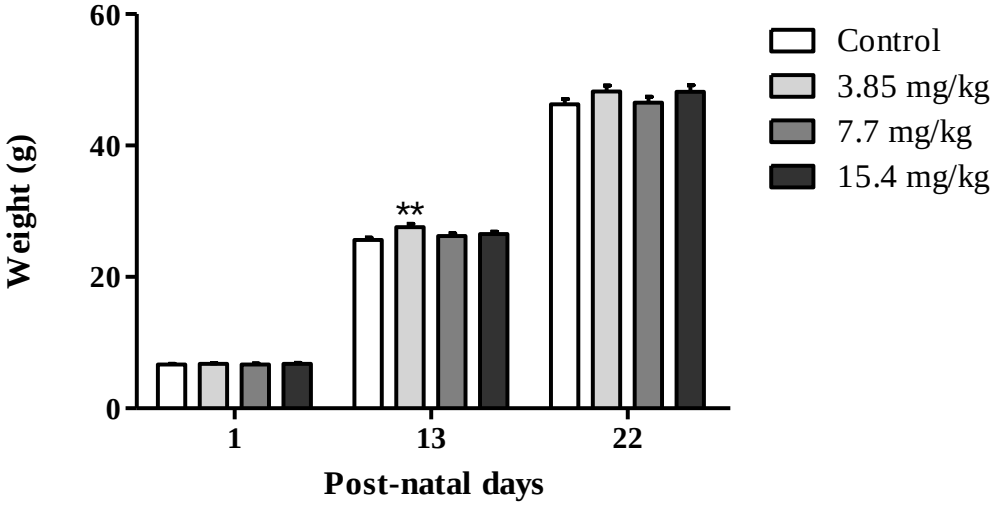


Figure 3. Weight of male offspring exposed to venlafaxine in the lactational period on relative anogenital distance measurement day. Values expressed in mean ± SEM. n=9 litter/group. ANOVA with posterior test in Dunnett. **p<0.01.

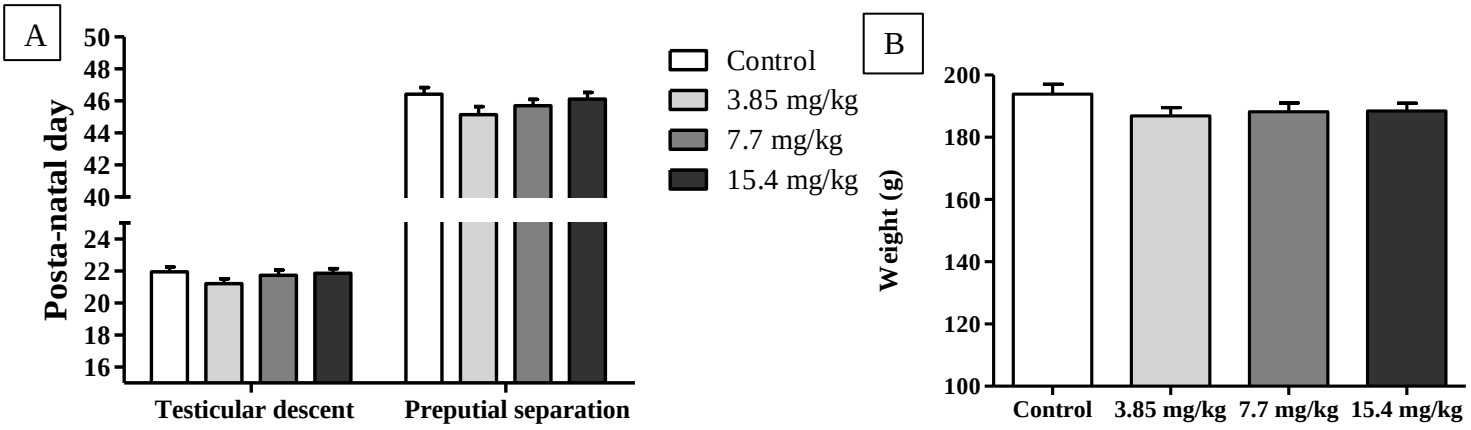


Figure 4. A. Evaluation of early sexual development and onset of puberty and **B.** Evaluation of body weight at the moment of prepuce separation of male pups exposed to venlafaxine during lactation. Values expressed in mean ± SEM. n=9 litter/group. ANOVA with posterior test in Dunnett. p>0.05.

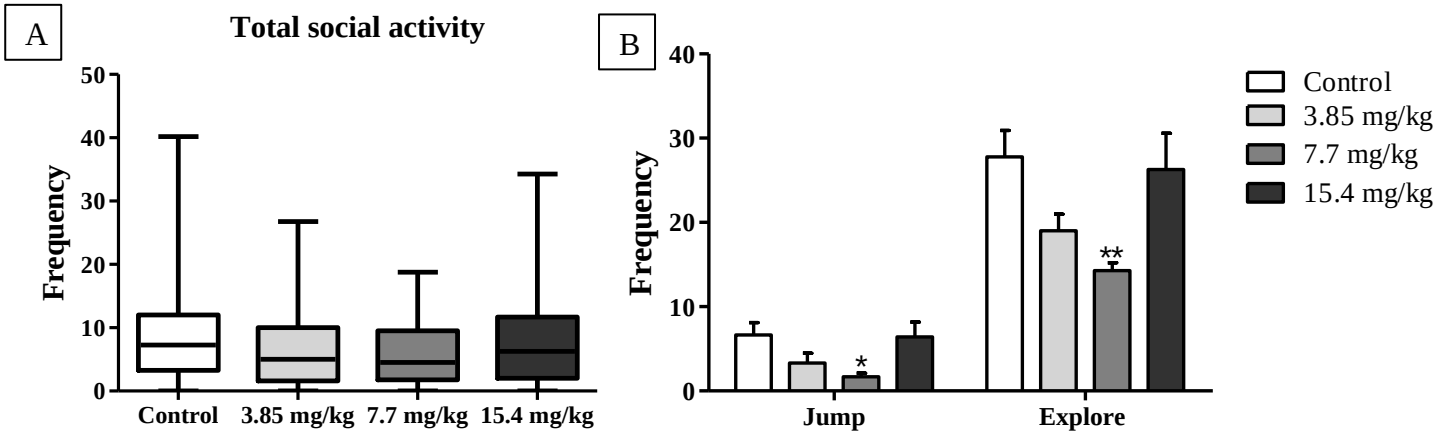


Figure 5. Evaluation of the play behavior of male offspring exposed to venlafaxine. **A.** Total social activity assessed. Values expressed as median (Q1-Q3). Kruskal-Wallis/Dunn test. **B.** Behavioral parameters of Jumping and Exploring the environment. Values expressed in mean ± SEM. n=9 litter/group. ANOVA with posterior test in Dunnett. *p<0.05 **p<0.01.

Table 5. Relative organ weight (g/100g body weight) of the male offspring exposed to venlafaxine in the lactational period on the PND 90.

Parameters	Experimental groups			
	Control	**3.85 mg/kg**	**7.7 mg/kg**	**15.4 mg/kg**
Body weight (g)	403.76 ± 6.42	402.54 ± 14.36	388.42 ± 14.48	380.74 ± 11.30
Liver (g)	3.92 ± 0.07	3.10 ± 0.10	3.91 ± 0.10	4.22 ± 0.10
Kidney (mg)	385.04 ± 3.83	396.09 ± 5.06	394.06 ± 7.60	417.69 ± 12.88*
Adrenal gland (mg)	8.02 ± 0.41	9.16 ± 0.85	7.99 ± 0.60	8.30 ± 0. 69
Spleen (g)	0.18 ± 0.01	0.18 ± 0.01	0.20 ± 0.01	0.19 ± 0.01
Heart (g)	0.35 ± 0.01	0.33 ± 0.01	0.33 ± 0.01	0.37 ± 0.01
Lung (g)	0.44 ± 0.02	0.45 ± 0.02	0.45 ± 0.02	0.45 ± 0.01
Thyroid (mg)	4.98 ± 0.48	4.57 ± 0.43	4.99 ± 0.58	4.73 ± 0.62
Testicles (g)	0.42 ± 0.01	0.40 ± 0.01	0.43 ± 0.01	0.43 ± 0.01
Epididymis (mg)	141.93 ± 4.09	137.40 ± 5.04	143.93 ± 3.37	147.86 ± 4.67
Vas deferens (mg)	19.30 ± 1.48	20.22 ± 0.87	23.90 ± 1.43*	22.94 ± 0.79
Seminal vesicle (g)	0.33 ± 0.02	0.30 ± 0.01	0.37 ± 0.03	0.38 ± 0.03
Prostate (mg)	83.13 ± 7.66	72.57 ± 2.97	77.83 ± 9.62	76.70 ± 5.23

Values expressed in mean ± SEM. n=9/group. ANOVA with posterior test in Dunnett.
*$p<0.05$

Table 6. Number and sperm count of adult male rats exposed to venlafaxine in the lactational period.

Parameters	Experimental groups			
	Control	**3.85 mg/kg**	**7.7mg/kg**	**15.4mg/kg**
Testis				
Mature spermatids number ($x10^6$/testis)	167.25 ± 7.63	147.26 ± 10.39	158.82 ± 8.67	138.49 ± 7.60
Relative mature spermatids number ($x10^6$/g/testis)	112.52 ± 5.18	102.93 ± 5.93	108.02 ± 5.27	95.65 ± 5.34
Daily sperm production ($x10^6$/testis/day)	27.42 ± 1.25	24.14 ± 1.70	26.04 ± 1.42	22.70 ± 1.25
Relative daily sperm production ($x10^6$/testis/g/day)	18.45 ± 0.85	16.87 ± 0.97	17.71 ± 0.86	15.68 ± 0.88
Epididymis (caput/corpus)				
Sperm number ($x10^6$/organ)	72.89 ± 2.55	73.22 ± 2.91	72.04 ± 2.70	68.63 ± 2.69
Relative sperm number ($x10^6$/g/organ)	235.00 ± 4.36	234.17 ± 6.03	241.53 ± 3.41	221.25 ± 7.84
Sperm transit time (days)	2.72 ± 0.18	3.15 ± 0.23	2.84 ± 0.19	3.09 ± 0.19
Epididymis (cauda)				
Sperm number ($x10^6$/organ)	128.57 ± 8.44	130.15 ± 5.15	135.56 ± 3.77	140.07 ± 8.07
Relative sperm number ($x10^6$/g/organ)	751.00 ± 27.86	759.44± 22.20	775.56 ± 24.86	729.38 ± 16.88
Sperm transit time (days)	4.79 ± 0.41	5.65 ± 0.54	5.35 ± 0.37	6.39 ± 0.63

Values expressed in mean ± SEM. n=9/group. ANOVA with posterior test in Dunnett. $p>0.05$.

Table 7. Sperm morphology of sperm from the tail of the epididymis of adult male rats exposed to venlafaxine in the lactational period.

Parameters (%)	Experimental groups			
	Control	**3.85 mg/kg**	**7.7mg/kg**	**15.4mg/kg**
Normal sperm	97.00 (84.50 – 99.50)	97.00 (93.00 – 99.00)	97.50 (84.00 – 99.50)	98.50 (93.50 – 99.50)
#Straight head sperm	0.00 (0.00 – 1.00)	1.00 (0.00 – 3.00)	0.00 (0.00 – 1.00)	0.00 (0.00 – 1.00)
#Isolated head sperm	5.00 (0 – 8.00)	4.00 (1.00 – 12.00)	4.00 (0 – 31.00)	2.00 (1.00 – 12.00)
Curled Tail Sperm	0	0	0	0
#Broken Tail Sperm	0.00 (0.00 – 2.00)	0.50 (0.00 – 1.00)	0.00 (0.00 – 3.00)	0.00 (0.00 – 2.00)
Cytoplasmatic droplet	11.00 (4.50 – 19.50)	10.00 (0.00 – 15.00)	12.50 (7.00 – 20.00)	8.00 (1.00 – 19.00)

Values expressed by the median - interquartile range (Q1-Q3). n=9/group. Kruskal-Wallis with posterior test in Dunn. $p>0.05$.

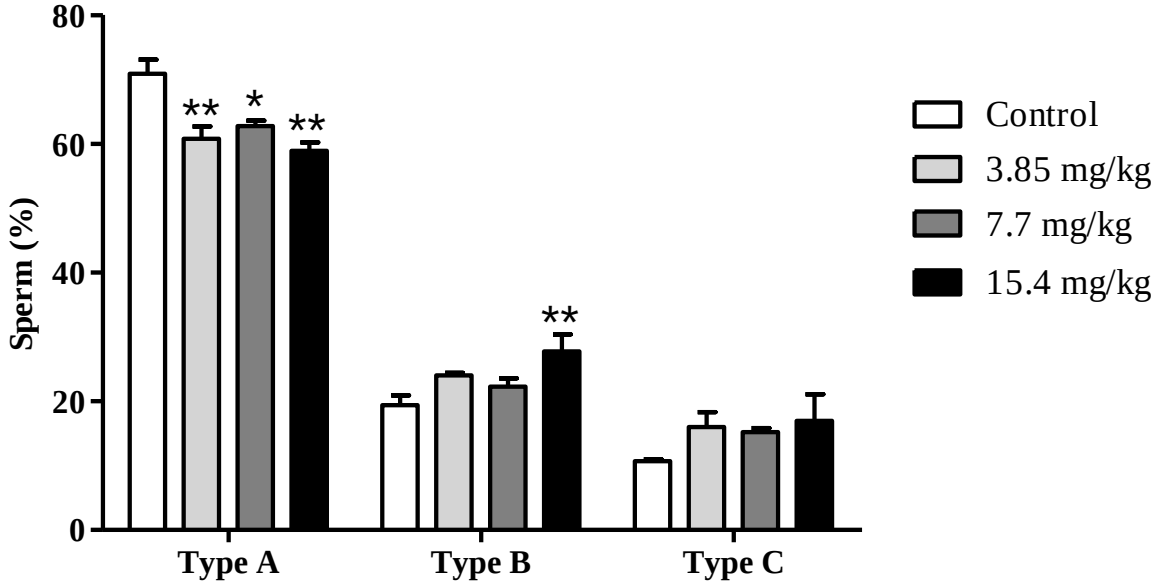


Figure 6. Sperm motility of adult male rats exposed to venlafaxine in the lactational period. Type A – motile with progressive movement; Type B – motile without progressive movement; Type C – Static; Values expressed in mean ± SEM. n=9/group. ANOVA with posterior test in Dunnett. *p<0.05 **p<0.01.

Table 8. Fertility parameters of female rats mated with adult male rats exposed to venlafaxine in the lactational period.

Parameters	Experimental groups			
	Control	**3.85 mg/kg**	**7.7 mg/kg**	**15.4 mg/kg**
Fertility potential (%)	87.94 ± 4.11	83.89 ± 6.64	90.67 ± 6.53	96.25 ± 1.77
Uterus + fetal weight (g)	81.71 ± 4.79	70.97 ± 6.57	73.58 ± 12.94	69.19 ± 6.29
Placenta weight (g)	0.61 ± 0.03	0.59 ± 0.02	0.60 ± 0.01	0.57 ± 0.04
Placenta efficency	6.99 ± 0.51	7.19 ± 0.38	7.20 ± 0.21	6.50 ± 0.76
Number of live fetuses	13.25 ± 0.77	12.00 ± 1.13	14.20 ± 0.37	13.60 ± 0.40
Number of implantations	13.63 ± 0.75	12.43 ± 1.09	14.40 ± 0.51	13.80 ± 0.49
Number of corpora lutea	15.63 ± 0.89	14.86 ± 0.74	17.17 ± 1.40	13.50 ± 0.92
Number of resorptions	0.38 ± 0.18	0.43 ± 0.20	0.00 ± 0.00	0.00 ± 0.00
Pre-implantation loss (%)	12.06 ± 4.11	10.47 ± 4.13	9.33 ± 6.53	3.75 ± 1.77
Post-implantation loss (%)	2.86 ± 1.45	3.59 ± 1.70	0	0
#Sex ratio (M:F)	1.31 (0.88 – 3.00	0.63 (0.56 – 0.75)	1.80 (0.44 – 2.50)	1.75 (0.44 – 2.75)
Masculinization index	0.59 ± 0.04	0.36 ± 0.03*	0.62 ± 0.10	0.55 ± 0.08

Values expressed as mean ± SEM. n=9/group. ANOVA/Dunnett test. #Values expressed as median (Q1-Q3). Kruskal-Wallis/Dunn test. *p<0.05.

Table 9. Male sexual behavior of adult male rats exposed to venlafaxine in the lactational period.

Parameters	Experimental groups			
	Control	**3.85 mg/kg**	**7.7 mg/kg**	**15.4 mg/kg**
#Latency to the first mount (s)	11.50(1.00-25.00)	9.00(7.00-33.00)	34.50(7.00-62.00)	22.00(7.00-59.00)
Number of mounts	7.10 ± 1.70	4.25 ± 1.25	4.11 ± 0.75	6.00 ± 1.20
#Latency of the first intromission (s)	16.00(3.00-43.00)	29.00(7.00-106.00)	35.50(11.00-90.00)	35.00(23.00-188.00)
Number of intromissions	15.30 ± 1.18	15.50 ± 2.67	12.13 ± 1.34	12.22 ± 1.55
#Latency to the first ejaculation (s)	620.00(202.00-1032.00)	474.00(91.00-1212.00)	322.00(223.00-459.00)	459.50(298.00-1436.00)
Latency of the first post-ejaculatory mount (s)	299.10 ± 45.95	296.63 ± 20.86	358.86 ± 16.11	290.00 ± 51.89
Number of post-ejaculatory mounts	3.60 ± 0.79	3.13 ± 0.85	3.25 ± 1.13	2.57 ± 0.57
Latency of the first post-ejaculatory intromission (s)	340.22 ± 34.51	303.75 ± 20.47	360.14 ± 15.87	340.13 ± 60.22
#Number of post-ejaculatory intromissions	7.50(0.00-17.00)	8.00(4.00-13.00)	7.50(2.00-14.00)	3.50(1.00-20.00)
#Total number of ejaculations	3.00(1.00-3.00)	3.50(1.00-5.00)	3.00(2.00-4.00)	1.50(1.00-3.00)

Values expressed as mean ± SEM. n=9/group. ANOVA/Dunnett test. #Values expressed as median (Q1-Q3). Kruskal-Wallis/Dunn test. $p>0.05$.

Table 10. Sexual preference behavior evaluation of adult male rats exposed to venlafaxine in the lactational period.

Parameters	Experimental groups			
	Control	**3.85 mg/kg**	**7.7 mg/kg**	**15.4 mg/kg**
Time spent in male zone (s)	237.80 ± 40.21	254.25 ± 65.96	251.22 ± 50.44	384.11 ± 70.53
Time spent in female zone (s)	427.30 ± 77.57	421.38 ± 56.16	432.44 ± 57.50	353.77 ± 89.62
Number of visits in male zone	18.7 ± 2.67	15.75 ± 2.70	15.78 ± 2.03	21.67 ± 2.14
Number of visits in female zone	23.56 ± 2.93	24.13 ± 2.18	20.00 ± 2.00	21.22 ± 3.07
Preference score	0.61 ± 0.06	0.64 ± 0.07	0.64 ± 0.06	0.46 ± 0.09

Values expressed in mean ± SEM. n=9/group. ANOVA with posterior test in Dunnett. $p>0.05$.

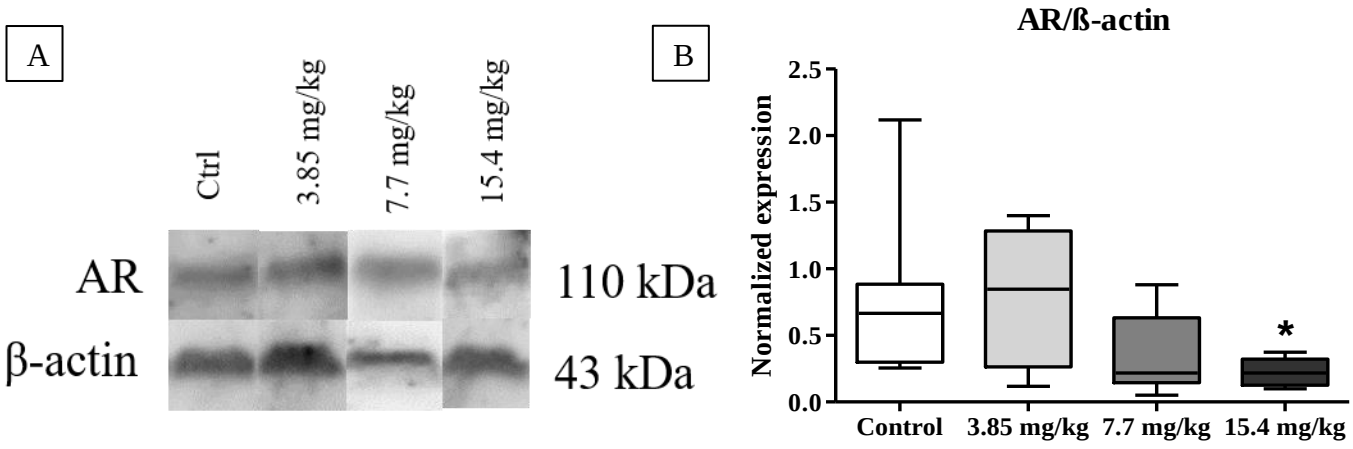


Figure 7. Androgen receptor (AR) expression in hypothalamic neurons of rats exposed to venlafaxine in the lactational period (image (**A**) and graph (**B**)). Values expressed as median (Q1-Q3). n=8/group. Kruskal-Wallis/Dunn test. *p<0.05.

Conclusão

De acordo com os resultados apresentados, a exposição ao antidepressivo venlafaxina durante o período lactacional foi levemente tóxica para as mães expostas e prejudicial para o desenvolvimento da prole masculina, afetando parâmetros espermáticos, comportamentais e possivelmente a masculinização do cérebro, indicando que o uso desse medicamento durante a fase crítica para a diferenciação sexual hipotalâmica pode ser prejudicial.