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"JÚLIO DE MESQUITA FILHO"
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INSTITUTO DE BIOCIÊNCIAS DE BOTUCATU

DESENVOLVIMENTO E PERFORMANCE REPRODUTIVA DA PROLE FEMININA DE RATAS WISTAR EXPOSTAS À SERTRALINA ASSOCIADA OU NÃO AO ESTRESSE NA GESTAÇÃO OU NA LACTAÇÃO

Mayara Silva Moura

Dissertação apresentada ao Instituto de Biociências, Campus de Botucatu, UNESP, para obtenção do título de Mestre no Programa de Pós-Graduação em Biologia Geral e Aplicada, Área de concentração *Biologia Celular Estrutural e Funcional.*

Orientadora: Profa. Dra. Wilma De Grava Kempinas

BOTUCATU – SP

2022

MAYARA SILVA MOURA

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DEVELOPMENT AND REPRODUCTIVE PERFORMANCE OF THE FEMALE OFFSPRING OF WISTAR RATS EXPOSED TO SERTRALINE ASSOCIATED OR NOT WITH STRESS IN PREGNANCY OR LACTATION

Dissertação apresentada ao Instituto de Biociências, Campus de Botucatu, UNESP, para obtenção do título de Mestre no Programa de Pós-Graduação em Biologia Geral e Aplicada, Área de concentração *Biologia Celular Estrutural e Funcional.*

Dissertation presented to the Biosciences Institute, Campus Botucatu, São Paulo State University (UNESP) to obtain the degree of Master in the General and Applied Biology Post-Graduate Program, in the Area of *Structural and Functional Cell Biology*

Orientadora: Prof. Dra. Wilma De Grava Kempinas

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Palavras-chave: Estresse materno; Gestação; Lactação; Sertralina; Toxicologia reprodutiva.

Resumo

Os inibidores seletivos de recaptação de serotonina (ISRSs) são os medicamentos de escolha para mulheres com quadros depressivos nos períodos gestacional e lactacional. No entanto, o uso desses antidepressivos nos períodos pré e pós-parto tem sido correlacionado com diversas alterações na prole em animais e humanos. A sertralina (ST) é um dos ISRSs mais usados na gestação e na lactação, mas pouco se sabe sobre o impacto da sua utilização nestes períodos, especialmente em relação à prole feminina. Assim, o objetivo do presente trabalho foi avaliar os efeitos da exposição gestacional (associada ou não ao estresse) ou lactacional à ST sobre o desenvolvimento somático, de reflexos, neucomportamental e reprodutivo da prole feminina de ratas Wistar. Na exposição gestacional, ratas Wistar prenhes receberam ST (20 mg/Kg/dia diluída em veículo) via gavagem associada ou não a estresse de contenção por 1 h/dia nos dias gestacionais 13 ao 20 (n=7-10). Na exposição lactacional, ratas Wistar lactantes foram tratadas com a ST (10mg/Kg/dia e 20mg/Kg/dia diluída em veículo) via gavagem durante toda a lactação (n=9/10). O peso corpóreo das mães e o consumo de ração no período de tratamento foram avaliados no experimento da exposição lactacional. Em ambos os experimentos, a prole feminina foi avaliada quanto ao peso corpóreo, ganho de peso e marcos do desenvolvimento somático e de reflexos. O teste do labirinto em cruz elevado foi realizado na prole jovem e adulta em ambos os experimentos. Foram avaliados os seguintes parâmetros do desenvolvimento e da função reprodutiva: distância ano-genital, instalação da puberdade, ciclo estral, peso de órgãos genitais, histomorfometria do útero e do ovário das ratas púberes e adultas, comportamento sexual e teste de fertilidade. Os resultados foram comparados entre os grupos pelos testes paramétricos ANOVA two-way (para o experimento realizado durante a gravidez) e ANOVA one-way (para o experimento realizado durante a lactação) seguidos pelos testes de Tukey, e pelos testes não paramétricos Kruskal-Wallis seguido pelo teste de Dunn ou teste do Chi-quadrado. As diferenças foram consideradas estatisticamente significativas quando $p \leq 0,05$. A exposição *in utero* à ST, independente da exposição ao estresse, reduziu o peso corpóreo ao nascimento, atrasou a erupção dos incisivos, aumentou o peso absoluto das tireoides no dia pós-natal (DPN) 80 e alterou o ciclo estral das ratas. A exposição à ST combinada ao estresse também provocou aumento no peso absoluto e relativo das tireoides no DPN 42, além de aumentar o peso relativo das tireoides, o peso absoluto do útero e a porcentagem de estruturas ovarianas DPN 80. No grupo ST, também foi observado peso corpóreo reduzido no DPN 21, menor ganho de peso no período pré-desmame, atraso no

aparecimento de pelos e aumento do comportamento relacionado à ansiedade na prole jovem. O tratamento com a ST durante a lactação não alterou o peso corpóreo materno ou o consumo de ração. A exposição lactacional à maior dose de ST reduziu o peso corpóreo no DPN 7, enquanto ambos os grupos tratados apresentam pesos corpóreos reduzidos no DPN 21. No grupo tratado com a maior dose de ST, também foram observadas adrenais e hipófises com maiores pesos relativos no DPN 21. O grupo tratado com a dose de 10 mg/Kg apresentou atraso na ocorrência do primeiro estro e redução no peso absoluto da hipófise no DPN 42. Ambos os grupos expostos à ST apresentaram redução na altura do endométrio no DPN 75 e aumento nos pesos relativos das tireoides no DPN 42, embora apenas o grupo tratado com a maior dose tenha apresentado tireoides aumentadas no DPN 75. Conclui-se que o tratamento com ST, nessas condições experimentais, teve repercussões em parâmetros reprodutivos, no crescimento inicial, no peso das tireoides, na maturação somática e no desenvolvimento neurocomportamental e de reflexos da prole feminina de ratas, o que levanta o questionamento em relação a segurança da utilização desse antidepressivo na clínica humana nos períodos gestacional e lactacional.

Abstract

The selective serotonin reuptake inhibitors (SSRIs) are the medications of choice for depressive women during gestation e lactation. However, the use of these antidepressants in the pre and postpartum periods has been correlated with several changes in offspring in animals and humans. Sertraline (ST) is one of the most used SSRIs in pregnancy and lactation, but little is known about the impact of its use in these periods, especially in relation to female offspring. Thus, the aim of the present study was to evaluate the effects of gestational (associated or not with stress) and lactational exposure to ST on the somatic, reflex, neurobehavioral and reproductive development of female offspring of Wistar rats. In gestational exposure, pregnant Wistar rats received ST (20 mg/Kg/day diluted in vehicle) by oral gavage associated or not with restraint stress for 1 h/day from gestational days 13 to 20 (n=7-10). In lactational exposure, lactating Wistar rats were treated with ST (10mg/Kg/day and 20mg/Kg/day diluted in vehicle) via gavage during lactation (n=9/10). Maternal body weight and chow consumption were assessed during treatment period in the experiment of lactational exposure. In both experiments, the female offspring were evaluated for body weight, body weight gain, and somatic and reflex developmental milestones. Elevated plus maze test was performed on juvenile and adult offspring in both experiments. The following parameters of development and reproductive function were evaluated: anogenital distance, onset of puberty, estrous cycle, weight of genital organs, ovarian and uterine histomorphometry at puberty and adulthood, sexual behavior and fertility testing. Results were compared among groups by parametric two-way ANOVA (for the experiment performed during pregnancy) and one-way ANOVA (for the experiment performed during lactation) tests followed by Tukey's test, and by the non-parametric Kruskal-Wallis test followed by Dunn's test or Chi-square test. Differences were considered statistically significant when $p \leq 0.05$. *In utero* exposure to ST, regardless of exposure to stress, reduced body weight at birth, delayed incisor eruption, increased absolute thyroid weight on postnatal day (PND) 80 and altered the estrous cycle of female rats. Exposure to ST combined with stress also increased the absolute and relative thyroid weight on PND 42, in addition to increasing the relative weight of the thyroids, the absolute weight of uterus and the percentage of ovarian structures on PND 80. In the ST group, reduced body weight on PND 21, reduced body weight gain in the preweaning period, delayed fur development and increased anxiety-related behavior in juvenile offspring were also observed. Treatment with ST during lactation did not change maternal body weight or chow consumption. Lactational exposure to the highest dose of ST reduced body

weight on PND 7, while both treated groups had reduced body weight on PND 21. In the group treated with the highest dose of ST, increased relative adrenal gland and pituitary weights were observed on PND 21. The group treated with the dose of 10 mg/kg had a delay in the occurrence of the first estrus and a reduction in the absolute weight of the pituitary gland in the PND 42. Both groups exposed to ST showed a reduction in endometrial height on PND 75 and an increase in relative thyroid weights on PND 42, although only the group treated with the highest dose had increased thyroids on PND 75. In conclusion, the treatment with ST, under these experimental conditions, had repercussions on reproductive parameters, initial growth, thyroid weight, somatic maturation, reflex and neurobehavioral development of female rat offspring, which raises questions regarding the safety of using this antidepressant in human clinical practice during pregnancy and lactation.

Sumário

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Lista de Abreviaturas e Siglas

5-HIAA - Ácido 5-Hidroxiindoleacético

5-HT – Serotonina

ACTH – Hormônio adrenocorticotrópico

AMH – Hormônio anti-Mulleriano

CGP – Célula germinativa primordial

CRH - Hormônio liberador de corticotropina

DG – Dia gestacional

DPN – Dia pós-natal

FDA – Food and Drug Administration

FSH – Hormônio folículo-estimulante

GnRH - Hormônio liberador de gonadotrofina

GnRHR – Receptor do hormônio liberador de gonadotrofina

HHA – Hipotálamo-Hipófise-Adrenal

HHG – Hipotálamo-Hipófise-Gônadas

ISRS – Inibidor seletivo de recaptação de serotonina

LH – Hormônio luteinizante

MAO-A – Monoamina oxidase A

OCT3 - Transportador de cátions orgânicos 3

SERT – Transportador de serotonina

TDAH – Transtorno do déficit de atenção com hiperatividade

TPH-1 – Triptofano hidroxilase 1

TRP – Triptofano

Introdução

Desenvolvimento e fisiologia do sistema reprodutor feminino

Em humanos e roedores, o sistema reprodutor feminino compreende os órgãos genitais internos (ovários, tubas uterinas, útero, cérvice e vagina) e os órgãos genitais externos (AMATO & YAO, 2021; CHUMDURI & TURCO, 2021). O desenvolvimento desses órgãos é um processo complexo que é iniciado no período pré-natal e estende-se até a vida pós-natal, envolvendo controle genético e hormonal (VUE *et al.*, 2018). Esse processo é iniciado pela determinação sexual, estabelecida na fertilização pelo sexo cromossômico (XX, fêmeas e XY, machos), e define a diferenciação da crista gonadal bipotencial em ovários ou testículos. A formação do ovário, a gônada feminina, depende da ausência do gene da região determinadora do sexo do cromossomo Y (Sry) e, consequentemente, da expressão de vias de sinalização específicas que impedem a formação da gônada masculina, incluindo os genes RSPO1, Wnt4/β-catenina e Foxl2 (SHE *et al.*, 2017).

O ovário serve como fonte de células germinativas e também como principal fornecedor de hormônios sexuais esteroides (RIMON-DAHARI *et al.*, 2016), que influenciam funções neurobiológicas reprodutivas e não reprodutivas (HANDA *et al*., 2009). No desenvolvimento embrionário de roedores, o modelo experimental mais utilizado em estudos na área da reprodução (MAEDA *et al*., 2000), as cristas gonadais bipotenciais são formadas a partir do espessamento do epitélio celômico que recobre as superfícies ventromediais do mesonefro por volta do dia gestacional (DG) 10 (SUZUKI *et al*., 2015; SVINGEN & KOOPMAN, 2013). As células germinativas primordiais (CGPs), que dão origem aos gametas femininos e masculinos, são originadas no epiblasto do embrião, adjacente à linha primitiva em formação, e migram para o endoderma (futuro intestino posterior) no DG 7.5 de camundongos (RICHARDSON & LEHMANN, 2010; KANAMORI *et al*., 2019). As CGPs migram através do endoderma para o mesoderma e, em seguida, migram bilateralmente para as cristas gonadais em desenvolvimento, alcançando-as aproximadamente no DG 10.5 (RICHARDSON & LEHMANN, 2010; LIU *et al.*, 2019), conforme esquematizado na figura 1.

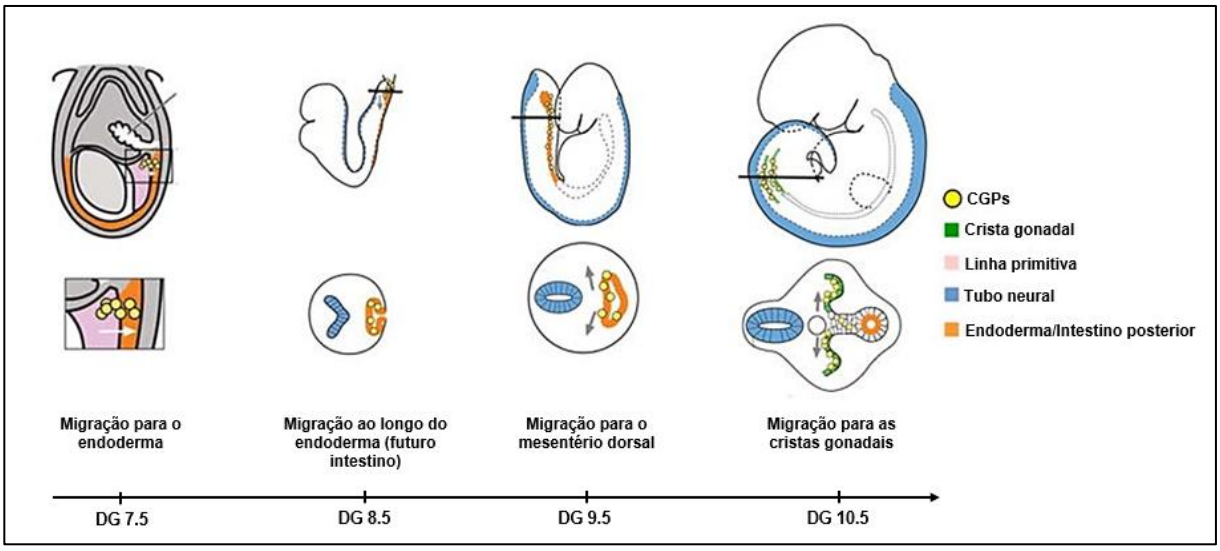

Figura 1. Modelo esquemático da migração das células germinativas primordiais (CGPs) até às cristas gonadais em desenvolvimento em roedores. Adaptado de RICHARDSON & LEHMANN (2010).

Após colonizarem as gônadas em formação, as CGPs diferenciam-se em ovogônias e passam por sucessivas divisões mitóticas (Figura 2), formandos cistos de células germinativas (WILSON & DAVIS, 2007; SARRAJ & DRUMMOND, 2012). Em ratos, isso ocorre por volta dos DGs 14 e 15 (WILSON & DAVIS, 2007). Em seguida, as ovogônias diferenciam-se em ovócitos primários e passam a se dividir por meiose (SARRAJ & DRUMMOND, 2012; SMITH *et al*., 2014). Um grande número de ovócitos sofre morte celular, enquanto os ovócitos restantes interrompem a divisão meiótica no estágio de prófase da meiose I e permanecem quiescentes nesta fase até a ovocitação na puberdade (EDSON *et al.*, 2009; ARRAJ & DRUMMOND, 2012; RICHARDS, 2018).

O epitélio celômico que recobre as gônadas continuamente sofre proliferação, invaginação e/ou expansão, induzindo a expansão da região cortical e levando à formação dos cordões ovarianos (SUZUKI *et al*., 2015; RODRÍGUEZ-GONZÁLEZ *et al.*, 2020). Estes cordões são constituídos de cistos de células germinativas circundados por células somáticas, onde pontes citoplasmáticas conectam os ovócitos uns aos outros (SUZUKI *et al*., 2015; LAMOTHE *et al.*, 2020). Os cordões ovarianos se fragmentam para formar os folículos primordiais nos primeiros dias após o nascimento (ZAMBRANO *et al.*, 2014), um processo acompanhado por apoptose de parte das células germinativas (RIMON-DAHARI *et al.*, 2016; HERNÁNDEZ-OCHOA *et al*., 2018). A morte programada das células dos cistos permite que os cistos se quebram em cistos menores, até que alguns ovócitos individuais permaneçam

(PEPLING, 2006). As células somáticas derivadas do epitélio celômico e as células do mesênquima da crista gonadal dão origem às células da granulosa e às células da teca, respectivamente, contribuindo para a formação dos folículos ovarianos (SCHOENWOLF *et al.*, 2016).

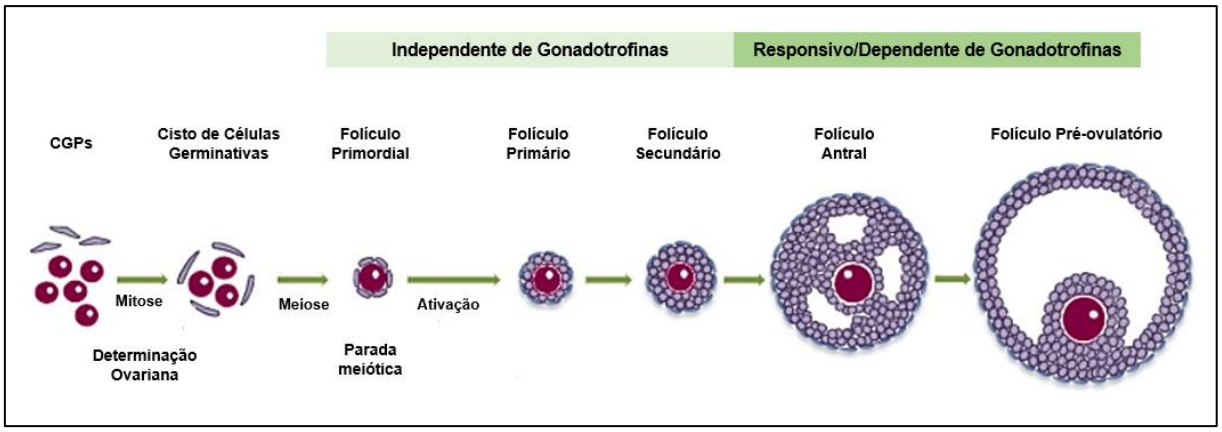

Figura 2. Modelo esquemático da formação e do desenvolvimento dos folículos ovarianos. Adaptado de SÁNCHEZ AND SMITZ (2012).

Os folículos primordiais se desenvolvem em folículos primários e, posteriormente, em secundários, por ação de fatores de crescimento parácrinos e autócrinos produzidos pelas células foliculares (PICUT *et al.*, 2015). Os folículos primordiais e primários consistem em um ovócito circundado por uma única camada de células da granulosa escamosa e cuboide, respectivamente, enquanto os folículos secundários consistem em ovócitos cercados por múltiplas camadas de células da granulosa e por células da teca (BREHM *et al.*, 2019). Por volta do dia pós-natal (DPN) 10, os ovários tornam-se responsivos à ação dos hormônios hipofisários FSH (hormônio folículo-estimulante) e LH (hormônio luteinizante). No entanto, nesse estágio, as gônadas femininas são pouco sensíveis à influência dos hormônios gonadotróficos, e tornam-se gradualmente mais sensíveis devido ao aumento de receptores para esses hormônios (PICUT *et al.*, 2015).

As tubas uterinas, o útero, a cérvice e a parte superior da vagina se desenvolvem a partir dos ductos paramesonéfricos ou ductos de Müller (Figura 3). Os ductos mesonéfricos (ou ductos de Wolff) e os ductos paramesonéfricos (que são formados entre os DGs 13.5 e 16.5) constituem o sistema de ductos sexuais indiferenciados (VUE *et al.*, 2018). Em fetos masculinos, os ductos mesonéfricos se desenvolvem por ação de andrógenos, como a testosterona, produzidos pelas células de Leydig do testículo fetal, e os ductos paramesonéfricos são eliminados pela ação do hormônio AMH (anti-Mülleriano), produzido pelas células de Sertoli. Como as células dos

ovários fetais não produzem AMH ou andrógenos, apenas os ductos paramesonéfricos se desenvolvem (MASSÉ *et al.*, 2009). Já a parte inferior da vagina se desenvolve a partir dos bulbos sinovaginais do seio urogenital (ROY & MATZUK, 2011).

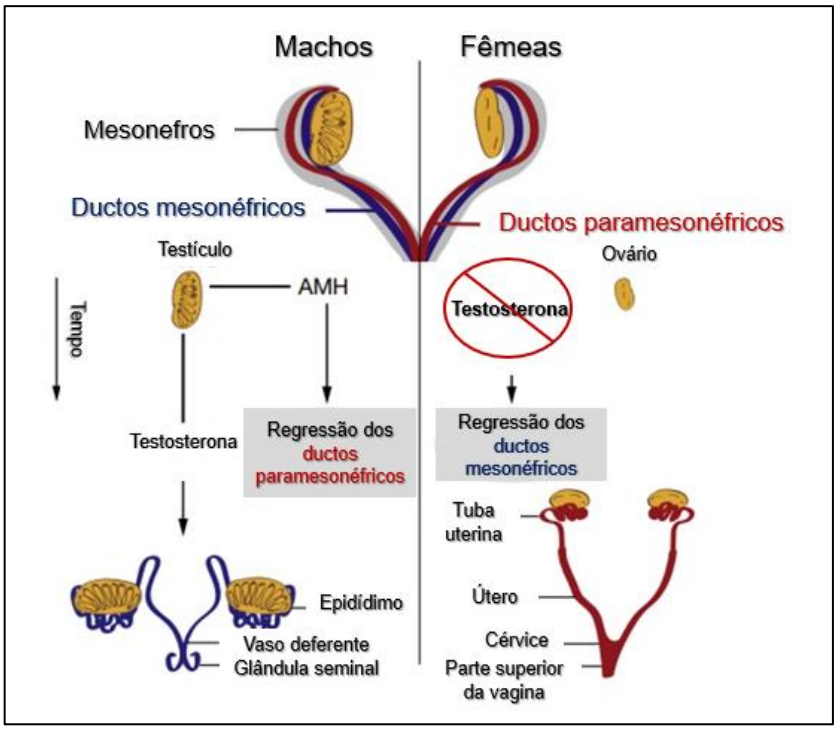

Figura 3. Modelo esquemático da formação dos órgãos dos sistemas genitais masculino e feminino de roedores a partir dos ductos mesonéfricos e paramesonéfricos. Adaptado de VUE *et al.* (2018).

Com o aumento da sensibilidade dos folículos ovarianos às gonadotrofinas, ocorre aumento na produção de hormônios esteroides, como o estradiol. Isso ocorre porque as células da teca são capazes de produzir andrógenos, que são convertidos em estrógenos pelas células da granulosa (RESENDE *et al.*, 2010). Entre os DPNs 30 e 40, ocorre a completa canalização da vagina (abertura vaginal), um marcador somático da instalação da puberdade desencadeado pelo aumento dos níveis de estradiol (VIDAL, 2016; JURASKA & WILLING, 2017). A puberdade corresponde ao período de aparecimento de características sexuais secundárias e início da maturidade sexual (ROSENFIELD *et al.*, 2014). Esse evento é marcado por alterações morfológicas, físicas, fisiológicas e comportamentais, e depende da maturação do eixo hipotálamo-hipófise-gônadas (HHG), da influência de neurotransmissores como a serotonina (5-HT) e de níveis adequados de estradiol (PICUT *et al.*, 2015, BARROS *et al.*, 2020).

O eixo HHG tem importante papel no controle neuroendócrino da fisiologia reprodutiva (BENTLEY *et al.*, 2010). O hormônio liberador de gonadotrofina (GnRH), sintetizado e

secretado por neurônios hipotalâmicos da área pré-óptica, conduz a síntese e secreção de LH e FSH através da ligação a receptores GnRH (GnRHRs) presentes na hipófise (BRUNTON, 2013; WHIRLEDGE & CIDLOWSKI, 2013). As gonadotrofinas, por sua vez, se ligam a receptores presentes nas gônadas, regulando a gametogênese e a esteroidogênese gonadal em ambos os sexos (MAGGI, *et al*., 2016; RIMON-DAHARI *et al.*, 2016). Os hormônios liberados nos ovários atuam na hipófise anterior e no hipotálamo para regular a produção de GnRH e LH/ FSH (CHAN *et al.*, 2014; OYOLA & HANDA, 2017). Como parte de um *feedback* clássico, ativinas, inibinas e hormônios esteroides ovarianos (principalmente estradiol e progesterona) se retroalimentam para regular a secreção de GnRH e das gonadotrofinas (WHIRLEDGE & CIDLOWSKI, 2013; VALSAMAKIS *et al.*, 2018).

Na pré-puberdade, a liberação de FSH e LH e, consequentemente, de estradiol é altamente estimulada pelo GnRH, o que ocasiona uma cascata de eventos que levam à liberação de ovócitos secundários em metáfase da meiose II (BOUÉ *et al.*, 1975; SCHOENWOLF *et al*., 2016). Além disso, o GnRH e os demais hormônios do eixo HHG regulam diversos eventos da fisiologia reprodutiva, como o comportamento sexual e o ciclo estral (ou ciclo menstrual, em primatas) (MAEDA *et al.*, 2007; EDSON *et al.*, 2009).

A cada ciclo estral, um número limitado de folículos é recrutado, a partir do qual um subconjunto é selecionado para dominância e ovocitação (EDSON *et al.*, 2009). Parte dos folículos entra em um processo denominado atresia e não é ovocitada (HOYER, 2004). Após a liberação do ovócito, as células da granulosa e da teca remanescentes nos ovários se unem para formar os corpos lúteos, que produzem progesterona e estrógeno para manter a gravidez caso o ovócito liberado seja fertilizado (PASK, 2015). O ciclo ovariano, por sua vez, impacta e é regulado por funções hipotalâmicas e hipofisárias, neurotransmissores e hormônios, além de direcionar funções específicas de vários órgãos, como o útero (BILLHAQ *et al.*, 2020).

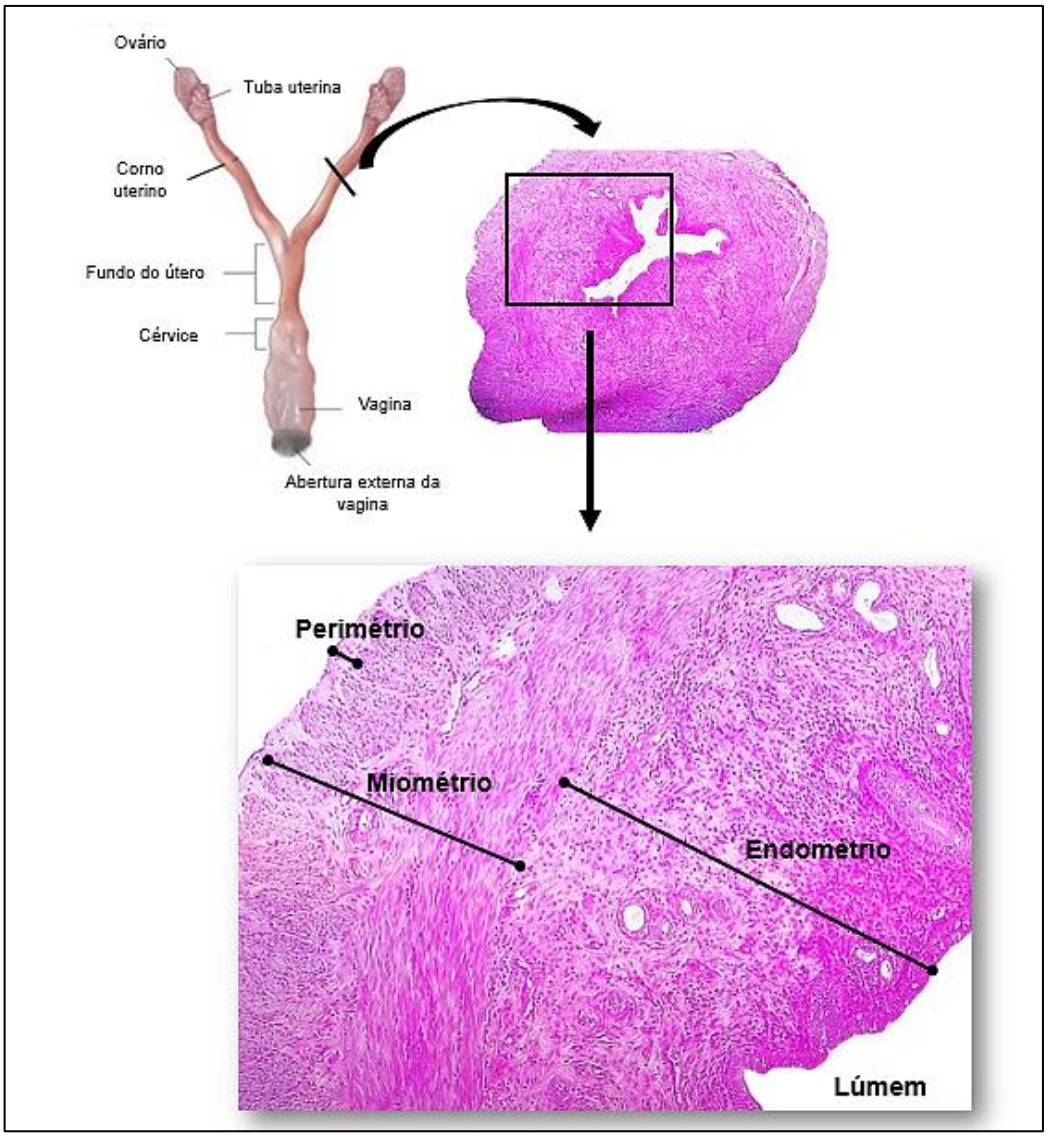

Figura 4. Aspecto histológico do útero de uma rata adulta em corte transversal corado com hematoxilina e eosina. Representações histológicas produzidas no Laboratório ReproTox. O modelo esquemático dos órgãos do sistema genital feminino foi traduzido de BOYD *et al.* (2017).

O útero (Figura 4) tem papel essencial na implantação e no desenvolvimento do embrião (VUE *et al.*, 2018). Roedores possuem um útero bicorno, com dois cornos uterinos que se unem distalmente em um único corpo (BOYD *et al.*, 2017). Histologicamente, o útero de fêmeas adultas possui dois compartimentos funcionais, o endométrio e o miométrio, que são circundados pelo perimétrio (VUE *et al.*, 2018). O endométrio possui um epitélio colunar simples que sofre alteração na altura durante o ciclo estral (MAYNARD & DOWNES, 2019), além de glândulas revestidas por epitélio glandular cuboide simples, que sintetizam e secretam substâncias com importante papel na gestação (COOKE *et al*., 2013). Já o miométrio é formado

por duas camadas de músculo liso: a camada circular interna e a camada longitudinal externa (MAYNARD & DOWNES, 2019).

Em roedores, apenas o epitélio luminal está diferenciado ao nascimento e a histoarquitetura deste órgão é estabelecida no período pós-natal (VUE *et al.*, 2018). A partir da puberdade, para adaptar o útero para uma possível gestação, a cada ciclo estral o endométrio uterino sofre crescimento, diferenciação e regeneração sob a influência dos hormônios estradiol e progesterona (GIBSON & SAUNDERS, 2012).

Programação do desenvolvimento

A exposição a adversidades, especialmente no início da vida, pode programar o desenvolvimento de órgãos e sistemas, alterando as trajetórias do desenvolvimento e aumentando a suscetibilidade a patologias ao longo da vida, incluindo anormalidades de crescimento e disfunção reprodutiva, metabólica, imunológica, comportamental ou cognitiva que pode persistir até mesmo nas gerações futuras. (PAWLUSKI *et al.,* 2012; REYNOLDS *et al.,* 2019).

De acordo com Zambrano e colaboradores (2014), programação pode ser definida como "uma resposta do organismo a um desafio específico durante um período crítico do desenvolvimento". Nesses períodos, os órgãos e sistemas em desenvolvimento são mais vulneráveis a perturbações endógenas e exógenas, como estresse e fármacos (ST-PIERRE *et al.,* 2016).

O prolongado período de formação e diferenciação do sistema reprodutor cria uma ampla janela de suscetibilidade a desafios endógenos e exógenos, de modo que diferentes agentes tem o potencial de perturbar a programação do desenvolvimento reprodutivo normal e o funcionamento desse sistema em diferentes janelas de exposição (HO *et al.,* 2017). Estudos em humanos e modelos animais fornecem evidências de que a exposição a adversidades no início da vida pode afetar parâmetros hormonais, moleculares e estruturais nos órgãos do sistema reprodutor feminino, impactando negativamente no fenótipo reprodutivo da prole feminina, podendo acarretar em consequências para a fertilidade e a capacidade reprodutiva na vida adulta (CHAN *et al.,* 2014; YAO *et al.,* 2021).

Transtornos mentais durante a gestação e a lactação

A ocorrência de sintomas depressivos, ansiedade, estresse e angústia no período gestacional é um problema que acomete muitas mulheres, sendo a depressão a morbidade psiquiátrica mais comum neste período (PAWLUSKI & GEMMEL, 2018; MARTÍNEZ-

PAREDES & JÁCOME-PEREZ, 2019). Os sintomas de ansiedade e depressão afetam entre 10% e 25% das gestantes e esse número tem crescido nos últimos anos em decorrência da pandemia da COVID-19 (LEBEL *et al*., 2020). Isso ocorre porque o período gestacional é considerado mais vulnerável ao desenvolvimento de sintomas depressivos ou exacerbação de sintomas preexistentes (MOORE *et al.*, 2015)

Em gestantes, a depressão está associada ao aumento do risco obstétrico, dificuldades na realização de atividades habituais, falha em procurar atendimento pré-natal, dieta inadequada e uso mais frequente de medicamentos, drogas, álcool e tabaco (SILVA *et al.*, 2010; GENTILE, 2017). Quando não tratada, pode levar ao desenvolvimento de hipertensão, pré-eclâmpsia e diabetes gestacional (MOORE *et al*., 2015; BECKER *et al.*, 2016).

Além de ser prejudicial à gestante, a depressão materna, assim como a ansiedade e o estresse, proporciona riscos à saúde do feto, estando associada a aborto espontâneo, restrição do crescimento fetal, parto prematuro, atrasos no desenvolvimento motor e mental e alterações metabólicas, reprodutivas e comportamentais (MACCARI & FLETCHER, 2007; LAZINSKI *et al.*, 2008; MANOJLOVIĆ-STOJANOSKI *et al.*, 2012; FAIRBROTHER *et al*., 2015; BECKER *et al.*, 2016; SHEN *et al.*, 2017). Como consequência dessas alterações, a exposição fetal a um ambiente adverso aumenta a susceptibilidade a doenças ao longo da vida, incluindo transtornos psiquiátricos (autismo, ansiedade, depressão e transtorno do déficit de atenção com hiperatividade (TDAH)), diabetes, hipertireoidismo, hipertensão e obesidade (MACCARI & FLETCHER, 2007; ST-PIERRE *et al.*, 2016).

A depressão pré-natal é um dos principais fatores de risco para depressão pós-parto, que é a complicação mais comum do parto (BECKER *et al.*, 2016; MILLARD *et al.*, 2017). De acordo com estimativas da Organização Mundial da Saúde (OMS), a depressão pós-parto atinge cerca de 10-15% das mulheres nos países desenvolvidos e 19% das mulheres nos países em desenvolvimento, como o Brasil. Contudo, um estudo que avaliou 23.894 mulheres de todo o Brasil entre 2011 e 2012 concluiu que a prevalência de mulheres com este transtorno no país é de cerca de 26,3% (THEME FILHA *et al.*, 2016).

No período pós-parto, a depressão tem impacto no comportamento materno e, consequentemente, no relacionamento mãe-filho, prejudicando, assim, o vínculo e o cuidado da criança (GENTILE, 2017). Resultados de estudos clínicos e de experimentação animal mostram que o comportamento materno alterado influencia negativamente no desenvolvimento neurológico, cognitivo, endócrino e motor da prole (MOTTA *et al*., 2005).

Inibidores seletivos de recaptura de serotonina (ISRSs)

Em resposta aos transtornos mentais maternos, um número significativo de mulheres recebe medicamentos antidepressivos durante a gestação e lactação (PAWLUSKI *et al*., 2012). Dentre as mulheres que utilizam esses medicamentos na gestação, cerca de 70% utilizam inibidores seletivos de recaptação de 5-HT (ISRSs) (BÉNARD-LARIBIÈRE *et al*., 2018). Essa classe de antidepressivos é considerada tratamento de escolha durante a gravidez e a lactação, pois apresenta menos efeitos colaterais e menor toxicidade em relação a outras classes de antidepressivos (MORRISON *et al.*, 2005; MACIAG *et al.,* 2006; ALWAN *et al*., 2007).

No entanto, esses medicamentos são passados ao feto por meio da placenta e são secretados no leite materno em diferentes concentrações detectáveis, podendo afetar a prole (ORNOY & KOREN, 2018; HORACKOVA *et al.*, 2021). De fato, o uso desses antidepressivos durante o período gestacional foi correlacionado com diversas alterações na prole em animais e em humanos, como parto prematuro, restrição do crescimento fetal, aumento do risco de desenvolvimento de malformações, atrasos no desenvolvimento (principalmente nas funções motoras e de linguagem), alterações reprodutivas, doenças metabólicas e cardiovasculares, hipertensão pulmonar e anormalidades comportamentais (incluindo déficits de atenção, distúrbios neuropsiquiátricos, e desordem do desenvolvimento neurológico) (MACIAG *et al.*, 2006; BÉRARD *et al.*, 2015; DOS SANTOS *et al.*, 2016; ORNOY, 2017; PAWLUSKI & GEMMEL, 2018; ARUMUGASAAMY *et al.*, 2019; KOTT *et al.*, 2019; HORACKOVA *et al.*, 2021).

Ainda que a transferência de ISRSs para o leite materno humano seja baixa na maioria dos casos, não está claro se esses fármacos são seguros durante a amamentação. Todos os antidepressivos utilizados na depressão pós-parto são detectáveis no leite materno e podem culminar em distúrbios na prole exposta (DA-SILVA *et al.,* 2021). Alguns estudos em humanos reportaram efeitos adversos como irritabilidade, redução do ganho de peso, diminuição da ingestão de alimentos e distúrbios do sono (EBERHARD-GRAN *et al*., 2006; BECKER *et al.,* 2016), enquanto estudos com animais de experimentação mostraram que a exposição a ISRSs durante a lactação promove alterações comportamentais, reprodutivas, endócrinas e neurais (ZUCKER, 2018).

Mecanismo de ação dos ISRSs

Há diversas evidências de que anormalidades dos sistemas de neurotransmissores monoaminérgicos, como o sistema serotoninérgico, estão envolvidas na fisiopatologia de transtornos depressivos (RESSLER & NEMEROFF, 2000). Os ISRSs bloqueiam o

transportador de serotonina (SERT), um transportador que permite o transporte da 5-HT do espaço extracelular para o compartimento intracelular (AGGARWAL E MORTENSEN, 2018). Desta forma, os ISRSs aumentam a disponibilidade desse neurotransmissor na fenda sináptica e, consequentemente, a transmissão serotoninérgica (TAYLOR *et al.*, 2005).

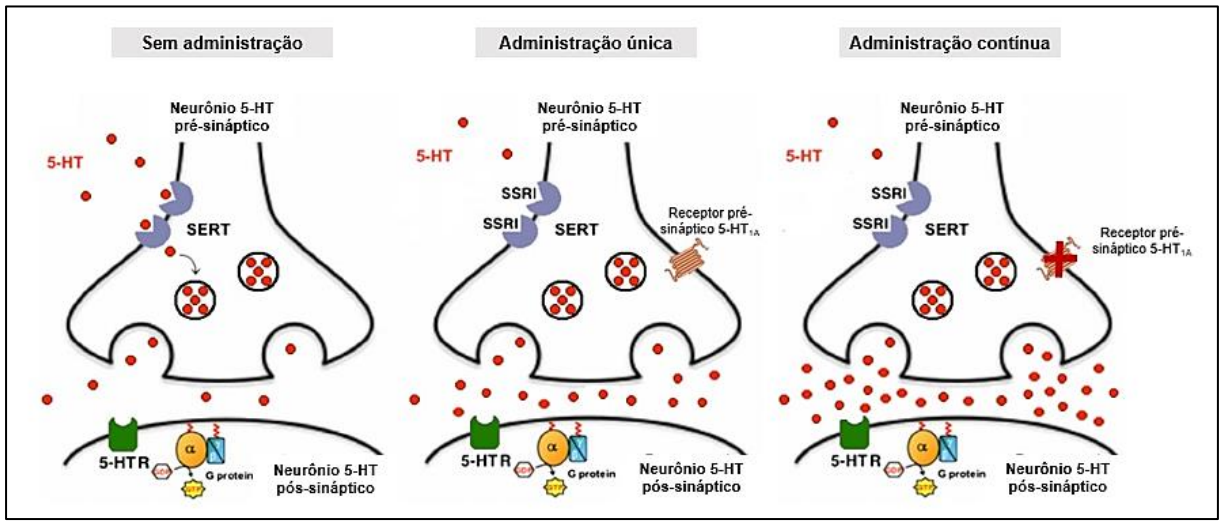

Figura 5: Modelo esquemático do mecanismo de ação dos ISRSs após administração única e contínua. Adaptado de FAKHOURY (2015).

Embora o mecanismo de ação dos ISRSs (Figura 5) envolva o bloqueio do SERT, o efeito antidepressivo ocorre por meio da ativação dos receptores de 5-HT pós-sinápticos. As moléculas desse neurotransmissor presentes na fenda sináptica ativam diferentes subtipos de receptores 5-HT, uma vez que os efeitos celulares da 5-HT são exercidos através da ativação de qualquer um dos 15 receptores pós-sinápticos presentes em 7 classes diferentes (5-HT_1 - 5-HT_7).

Nos primeiros dias de tratamento, a capacidade dos ISRSs de aumentar as concentrações extracelulares de 5-HT é limitada por autorreceptores somatodendríticos (5-HT_{1A}) e terminais (5-HT_{1B}), presentes em neurônios serotoninérgicos em núcleos da rafe. A ativação desses receptores, em particular o 5-HT_{1A}, pela 5-HT presente na fenda sináptica, retarda a atividade elétrica, a síntese e liberação de 5-HT. A administração crônica de um ISRS promove dessensibilização funcional desses autorreceptores, tornando possível obter uma resposta do tipo antidepressivo após uma semana de tratamento (DAVID & GARDIER, 2016).

Modelos animais de estresse materno

Além de anormalidades dos sistemas de neurotransmissores, diversas alterações neurobiológicas estão envolvidas na fisiopatologia da depressão, como aumento da atividade em redes cerebrais, neuroinflamação e desregulação do eixo hipotálamo-hipófise-adrenal (HHA), além de mecanismos genéticos, epigenéticos e fatores ambientais (CZÉH *et al.*, 2015; PLANCHEZ *et al.,* 2019; BECKER *et al.,* 2021).

O eixo HHA é um dos principais componentes envolvidos na resposta ao estresse (GOEL *et al*., 2014). Após um evento estressante, os neurônios do núcleo paraventricular do hipotálamo secretam o hormônio liberador de corticotropina (CRH) no sistema porta-hipofisário, estimulando a hipófise anterior a produzir o hormônio adrenocorticotrópico (ACTH). O ACTH, por sua vez, induz a síntese e secreção de glicocorticoides no córtex da glândula adrenal, a fim de promover o retorno à homeostase (GOEL et al., 2014; JOSEPH & WHIRLEDGE, 2017),

Considerando que a associação entre desregulação do eixo HHA e ocorrência de transtornos depressivos é bem estabelecida, muitos modelos animais usados no estudo da depressão e de terapias antidepressivas são baseados na aplicação de estresse (BECKER *et al*., 2021). O estresse por contenção é o modelo mais utilizado em experimentos animais em geral e estudos tem mostrado que esta metodologia induz alterações neurobiológicas similares às observadas em pacientes com depressão, como deficiências nas funções cognitivas, afetivas, neuroendócrinas e imunológicas (CZÉH *et al.*, 2015; FATIMA *et al.,* 2017; WEINSTOCK *et al.,* 2017, MO *et al*., 2019).

Evidências sugerem que a resposta do eixo HHA à depressão materna é a principal responsável pelos efeitos críticos na prole, o que torna o estresse uma importante ferramenta para avaliar os efeitos do uso pré e pós-natal de antidepressivos na prole (VALSAMAKIS *et al.,* 2019). A adversidade materna durante a gestação decorrente de estresse agudo/crônico, ansiedade e depressão promove aumento na exposição fetal e placentária aos hormônios glicocorticoides, o que impacta no desenvolvimento do feto (Figura 6) (GOEL *et al.,* 2014; FATIMA *et al*., 2017; MCGOWAN & MATTHEWS, 2018).

Os glicocorticoides possuem um papel importante na regulação do crescimento fetal de diversos órgãos e sistemas, incluindo a tireoide, rins, cérebro, hipófise e pulmão (BORGES *et al.,* 2017; MANOJLOVIĆ-STOJANOSKI *et al.,* 2012). Deste modo, a exposição excessiva a esses hormônios pode levar a resultados fisiológicos e fisiopatológicos de curto e longo prazo na prole (MCGOWAN & MATTHEWS, 2018). Além disso, outros hormônios relacionados ao

estresse, como o CRH, são transportados pela placenta, podendo afetar o desenvolvimento do feto (BECKER *et al.*, 2021).

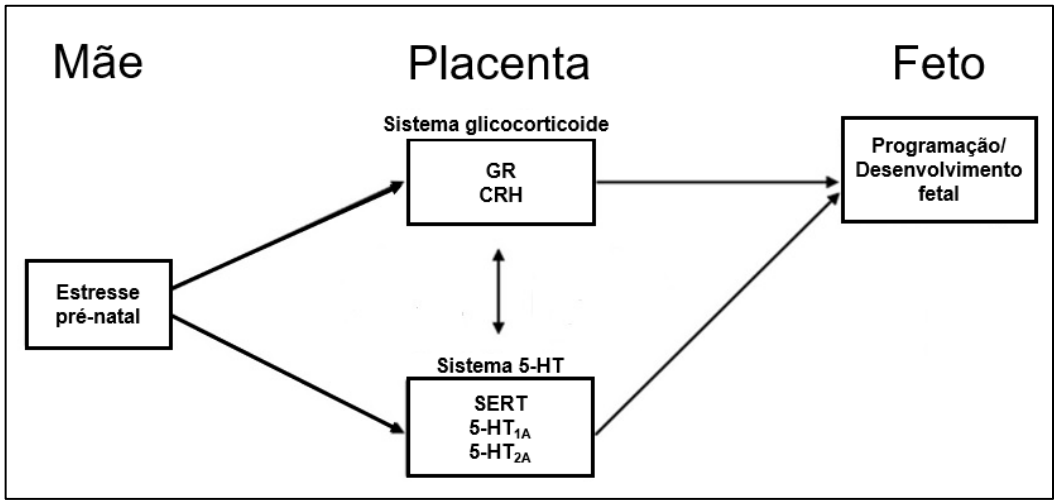

Figura 6. Mecanismos pelos quais o estresse pré-natal pode interferir com o desenvolvimento do feto. Adaptado de ST-PIERRE et al. (2016).

Além do eixo HHA, a aplicação de estresse em experimentos animais está relacionada à redução na neurotransmissão serotoninérgica, uma das primeiras alterações observadas na depressão. Sendo assim, o estresse também pode ser usado como modulador do sistema serotoninérgico para avaliar os efeitos de antidepressivos (ORNOY, 2017). Os efeitos da exposição ao estresse no início da vida (associado ou não a antidepressivos) no desenvolvimento da prole têm sido amplamente estudados em modelos animais, sendo o rato o animal mais empregado nesses estudos (GRAIGNIC-PHILIPPE, 2014; FATIMA *et al.*, 2017).

Uma vez que existem preocupações éticas e limitações em estudos com humanos, estes falham em isolar os efeitos da exposição à droga dos efeitos da saúde mental materna, o que dificulta avaliar a relação risco/benefício de fármacos antidepressivos (GEMMEL *et al.*, 2018; KOTT *et al.*, 2019). Em animais, temos a capacidade de estudar os efeitos do tratamento com antidepressivos sob o desenvolvimento durante uma gravidez saudável, isolando os efeitos desses fármacos, embora deva ser observado que o tratamento no contexto de estresse materno tem maior valor translacional (RAMSTEIJN *et al.*, 2020). Assim, modelos animais são muito úteis na avaliação dos efeitos da exposição gestacional a antidepressivos.

ISRSs: Efeitos no desenvolvimento da prole

A 5-HT é um neurotransmissor envolvido em diversas funções, como a regulação do humor, da atividade motora, da memória, da cognição, do apetite, do sono e da dor (RANZIL

et al., 2019). Também é fundamental para o desenvolvimento e função de dois principais sistemas de resposta ao estresse: os sistemas HHA e locus-coeruleus-norepinefrina (BRUMMELT *et al.*, 2017). No desenvolvimento pré-natal e pós-natal inicial, a 5-HT age como um fator de crescimento para seus próprios sistemas neurais e sistemas relacionados, regulando uma série de processos importantes do neurodesenvolvimento, como a proliferação celular, diferenciação neuronal, migração neural, mielinização e sinaptogênese (PEARLSTEIN, 2015; FAA *et al*., 2016; BRUMMELT *et al.,* 2017; RANZIL *et al.,* 2019). Além disso, influencia na formação do eixo direito/esquerdo, dos pulmões, do trato gastrointestinal, do eixo hipotálamo-hipófise, de estruturas craniofaciais e na morfogênese cardíaca (MOISEIWITSCH, 2000; THIBEAULT *et al.,* 2019; HORACKOVA *et al.*, 2021).

Em estágios iniciais do desenvolvimento, a placenta é a principal fonte de 5-HT para o neurodesenvolvimento fetal (Figura 7). Em roedores, entre os DG 10.5 e 15.5, a 5-HT é sintetizada por meio da enzima triptofano hidroxilase 1 (TPH-1) placentária, utilizando triptofano (TRF) materno, e degradada a ácido 5-hidroxiindoleacético (5-HIAA) pela monoamina oxidase A (MAO-A), também expressa na placenta (LAURENT *et al.*, 2017; RANZIL *et al.*, 2019). Porém, em estágios posteriores da gestação, o feto produz sua própria 5-HT a partir de TRF de origem materna e o suprimento placentário deixa de ser necessário (KARAHODA *et al.*, 2020).

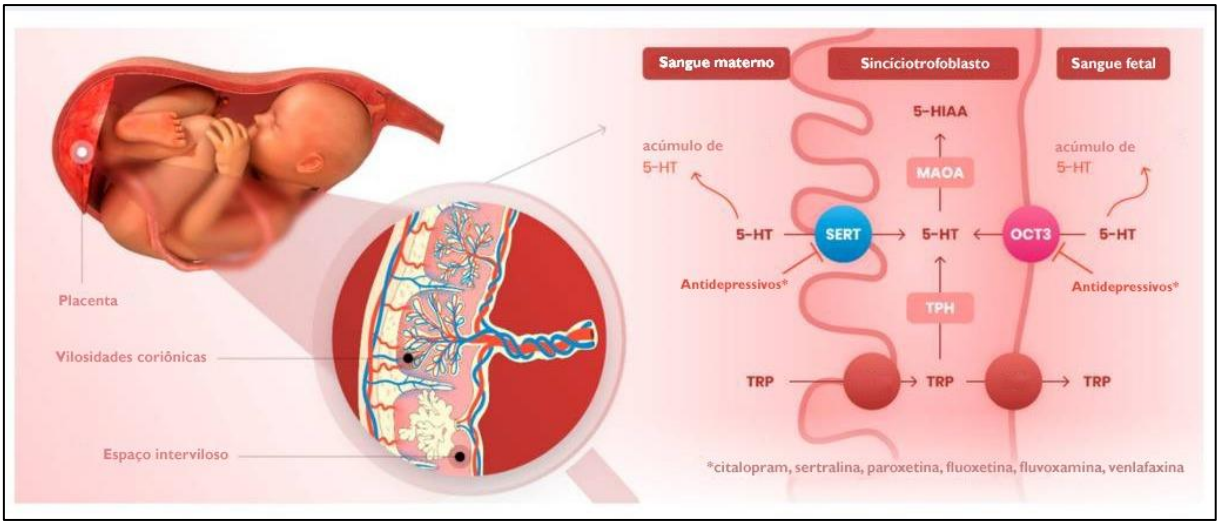

Figura 7. Modelo esquemático do transporte de 5-HT fetal. Adaptado de HORACKOVA *et al*. (2021)

A 5-HT é retirada da circulação materna pelo SERT, presente na membrana apical da placenta, enquanto o transportador de cátions orgânicos 3 (OCT3), que se encontra na membrana basal, retira a 5-HT da circulação fetal, para que seja posteriormente degradada pela

MAO-A. Em ratos e humanos, a interação destes transportadores oferece um mecanismo protetor contra a vasoconstrição local da vasculatura placentária induzida pelo excesso de 5-HT. No entanto, esse sistema pode ser comprometido por substâncias inibidoras do OCT3 e SERT, como os ISRSs, podendo afetar o desenvolvimento fetal (KARAHODA *et al*., 2020).

Em roedores, SERT é expresso no cérebro fetal no DG 12, enquanto neurônios serotoninérgicos surgem entre os DGs 12 e 14 (LAUDER & BLOOM, 1974; ST-PIERRE *et al.*, 2016). Cerca de um dia após seu aparecimento, esses neurônios são capazes de sintetizar 5-HT (BOOIJ *et al.*, 2015) e no DG 17, fibras de 5-HT projetam-se no telencéfalo e espalham-se por todo o córtex cerebral (MILLARD *et al.*, 2017). Uma vez que as fibras serotoninérgicas atingem seus alvos, essas fibras amadurecem e modificam os locais alvo, como o hipocampo e amígdala (GRIEB & RAGAN, 2019). Similarmente, em humanos, SERT é expresso a partir da 8ª semana de gestação, enquanto o aparecimento de neurônios produtores de 5-HT nos núcleos da rafe só ocorre na 15ª semana de gestação (ST-PIERRE *et al*., 2016; RANZIL *et al*., 2019).

No cérebro adulto, a 5-HT sintetizada perifericamente não pode cruzar a barreira hematoencefálica e, portanto, deve ser sintetizada nos núcleos da rafe. O cérebro fetal, no entanto, possui uma barreira hematoencefálica imatura, que permite que a 5-HT exógena passe para o cérebro. Várias classes de receptores 5-HT são expressas no cérebro em desenvolvimento, de modo que a exposição a ISRSs pode impactar diretamente no desenvolvimento deste órgão (BRUMMELT *et al.*, 2017; RANZIL *et al.*, 2019). Níveis anormais de 5-HT no cérebro durante o desenvolvimento foram associados à patogênese de vários distúrbios neurocognitivos em modelos de roedores, incluindo depressão, ansiedade, esquizofrenia e doença de Alzheimer (RANZIL *et al.*, 2019).

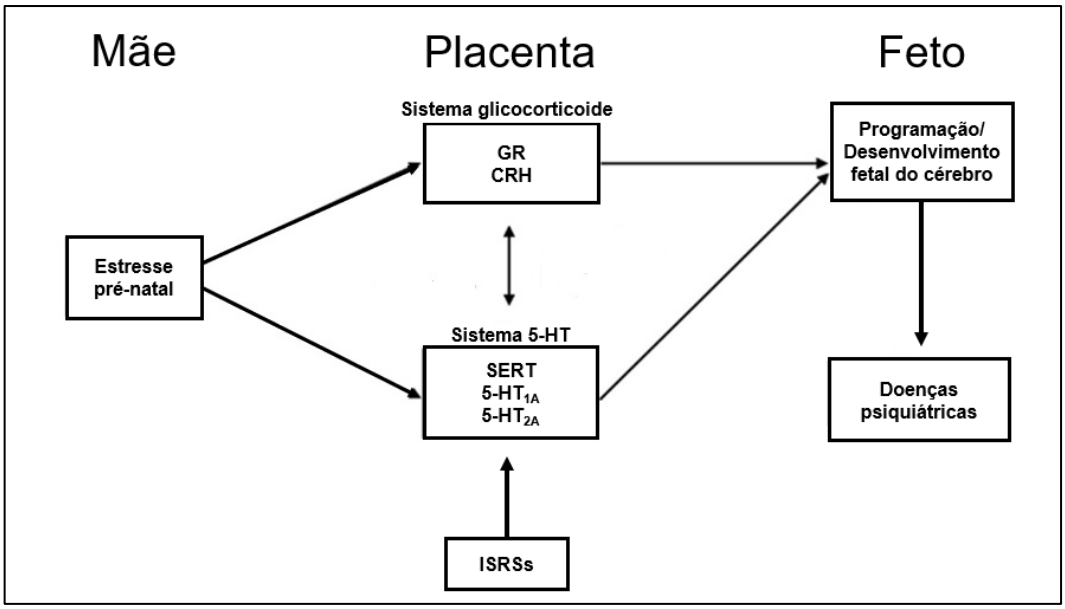

Figura 8. Mecanismos pelos quais a exposição pré-natal ao estresse e/ou aos ISRSs pode acarretar em doenças psiquiátricas ao longo da vida. Adaptado de ST-PIERRE et al. (2016).

SERT também é brevemente expresso em neurônios não serotoninérgicos, entre o DG 13 e o DPN 21, incluindo neurônios glutamatérgicos das vias tálamo-corticais, neurônios piramidais no córtex pré-frontal e hipocampo e neurônios de projeção dos sistemas somatossensorial e corticolímbico (ALTIERI *et al.*, 2015; MILLARD *et al.*, 2017). Em humanos, SERT também é expresso em neurônios não 5-HT entre a 12ª e 14ª semanas de gestação, porém pouco se sabe sobre marcos de desenvolvimento específicos no sistema 5-HT durante o segundo e terceiro trimestres da gravidez (BOOIJ *et al.*, 2015). Na vida adulta, a 5-HT é um fator importante para a neurogênese e manutenção neuronal no sistema nervoso central (DOS SANTOS *et al.*, 2016).

Considerando o papel do sistema 5-HT no desenvolvimento pré e pós-natal, alterações nos níveis deste neurotransmissor durante a gestação e/ou lactação podem comprometer o desenvolvimento do sistema serotoninérgico e de inúmeras regiões do cérebro que recebem estímulos deste sistema, afetando processos cruciais do desenvolvimento e o funcionamento de sistemas relacionados, como o sistema reprodutor (RAYEN *et al.*, 2014; PAWLUSKI & GEMMEL, 2018).

Efeitos dos ISRSs na reprodução

O sistema serotoninérgico atua no desenvolvimento e na modulação do sistema reprodutor. Sabe-se que neurônios hipotalâmicos produtores de GnRH recebem inervação serotoninérgica (figura 9) e a 5-HT é encontrada na hipófise, nos ovários e no útero (DOS SANTOS *et al.*, 2016; HEGSTAD *et al.*, 2020). Deste modo, a 5-HT pode influenciar o desenvolvimento e o funcionamento do eixo HHG (RAYEN *et al.*, 2013). A nível hipotalâmico, a 5-HT pode afetar a secreção de GnRH de forma direta, por ação nos neurônios GnRH, ou indireta, influenciando outros neurotransmissores como a noradrenalina (AYALA, 2009).

Além da 5-HT, receptores serotoninérgicos, TPH e SERT estão presentes nos ovários de mamíferos (MOORE *et al.*, 2015). Sendo assim, esse neurotransmissor pode regular a produção de hormônios esteroides nos ovários e mudanças no sistema serotoninérgico podem resultar em mudanças na síntese e/ou liberação desses hormônios. Os hormônios ovarianos também podem influenciar a atividade serotoninérgica, uma vez que interagem diretamente

com esse sistema por meio de receptores de estrógeno e progesterona localizados nos neurônios centrais serotoninérgicos (DOS SANTOS, 2016).

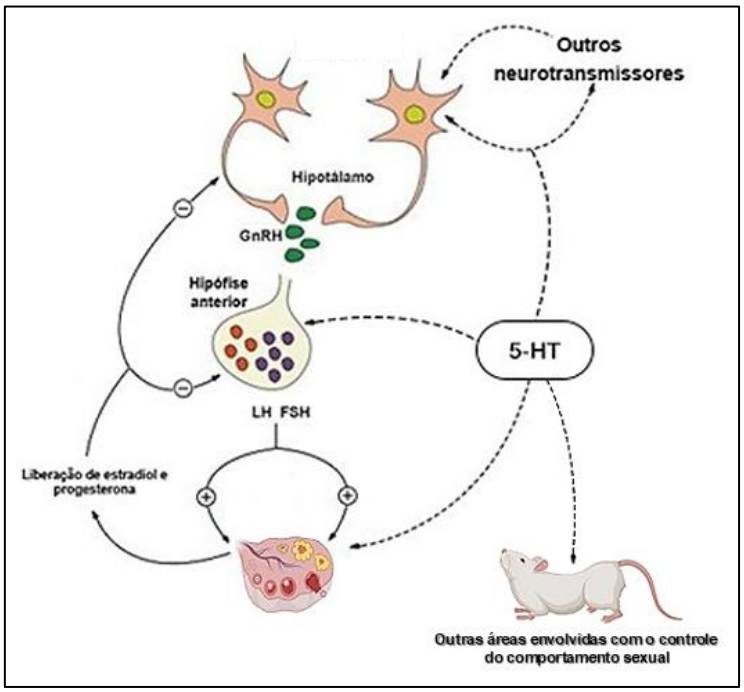

Figura 9. Modelo esquemático da relação entre a serotonina (5-HT) e a função reprodutiva em fêmeas. Adaptado de GERAGHTY & KAUFER (2015).

De acordo com isso, experimentos animais mostraram que o tratamento com ISRSs na vida adulta altera os níveis de FSH e LH, assim como a concentração de progesterona e estradiol (AYALA, 2009). Essas alterações hormonais estão associadas aos efeitos adversos reportados por muitos usuários de ISRSs, como disfunção sexual (diminuição da libido, da excitação e da intensidade do orgasmo) (MODELL *et al.*, 1997; GREGORIAN *et al.*, 2002).

Além disso, estudos apontam que a 5-HT tem papel na diferenciação sexual e é o principal neurotransmissor implicado no controle do comportamento sexual no adulto (RAYEN *et al.*, 2013). O sistema serotonérgico exerce uma ação dupla no comportamento sexual, facilitando-o ou inibindo-o por meio de diferentes vias de sinalização relacionadas à família de receptores serotonérgicos e ao SERT (MOLINA-JIMÉNEZ *et al*., 2018).

Levando em conta que a 5-HT está intimamente relacionada a vários aspectos da reprodução, a exposição pré-natal e/ou lactacional a ISRSs tem potencial de alterar o desenvolvimento e a função reprodutiva. Porém, estudos que focam nos impactos reprodutivos da exposição precoce aos ISRSs são escassos, principalmente em relação a prole feminina (RAMSTEIJN *et al.*, 2020). Os poucos estudos que investigaram esses efeitos em animais demonstraram que a exposição precoce a ISRSs pode ter impacto na função reprodutiva de

machos e fêmeas, alterando parâmetros como a idade da instalação da puberdade, o comportamento sexual e a fertilidade (MACIAG *et al*., 2006; RAYEN *et al.*, 2013, RAYEN *et al*., 2014; MOORE *et al.*, 2015; DOS SANTOS *et al*., 2016).

Em relação aos estudos em fêmeas, os resultados são conflitantes. Moore e colaboradores (2015) observaram que a exposição perinatal à fluoxetina altera o ciclo estral e o número de folículos nos ovários, mas não altera a idade de abertura da vagina. Já Dos Santos e colaboradores (2016) mostraram que a exposição ao mesmo fármaco na gestação e lactação retarda a instalação da puberdade, atrasando a abertura da vagina e a ocorrência do primeiro estro, mas não tem efeito no ciclo estral, na contagem de folículos ovarianos e de corpos lúteos e no comportamento sexual. Hegstad e colaboradores (2020) também observaram que exposição à Fluoxetina no mesmo período não teve consequências no comportamento sexual da prole feminina, mas os resultados de Rayen e colaboradores (2014) mostram que a exposição lactacional a Fluoxetina aumenta a receptividade das fêmeas no comportamento sexual.

ISRSs e glândula tireoide

Os hormônios produzidos pela glândula tireoide, tiroxina (T4) e triiodotironina (T3), regulam os processos metabólicos essenciais para o crescimento e desenvolvimento normais, assim como o metabolismo na vida adulta (MULLUR *et al.*, 2014). Os hormônios tireoidianos são liberados no lúmen folicular quando requisitados, processo que é estimulado pelo hormônio estimulante da tireoide (TSH) produzido na hipófise anterior, enquanto a síntese e a liberação de TSH é estimulada pela liberação do hormônio TRH (NUNES, 2003; OLIVEIRA, 2009). A tireoide secreta principalmente T4, que é convertido em T3 por enzimas desiodase específicas do tecido (FILIS *et al*., 2018).

Perturbações de longo prazo do eixo hipófise-tireoide predispõem o rato de laboratório a uma incidência maior de lesões proliferativas, já que a tireoide de ratos possui maior sensibilidade a alterações do que a de humanos. Isso ocorre devido à meia-vida plasmática mais curta do hormônio T4 (12-24 horas em ratos e 5-9 dias em humanos) e à ausência da proteína globulina de ligação à tiroxina (TBG), que se liga ao T4 circulante em humanos (BRÄNDLI-BAIOCCO *et al.*, 2018).

Os ISRSs parecem afetar negativamente a função da tireoide em pacientes depressivos e animais experimentais, embora a magnitude clinica desses achados não seja clara. Em humanos, alguns estudos relacionaram o tratamento com ISRSs com a redução dos hormônios T3 e T4 (CARVALHO *et al.*, 2009; CAYE *et al.*, 2020), ainda que, de acordo com o U.S. Food and Drug Administration (FDA), a incidência de hipotireoidismo em pacientes tratados com

sertralina seja baixa (< 2%). Um estudo *in vitro* de ESCUDERO et al. (2013) demonstrou potencial atividade anti-tireoidiana do cloridrato de sertralina, enquanto ratas que receberam sertralina (40mg/Kg) apresentaram aumento em adenomas foliculares da tireoide (FDA).

Níveis maternos adequados de hormônios tireoidianos são críticos para o crescimento fetal e neonatal e o desenvolvimento do cérebro (ENG & LAM, 2020), bem como o adequado desenvolvimento da tireoide da prole (KOREVAAR *et al*., 2016). Disfunções na tireoide materna na gestação e na lactação tem sido correlacionadas com alterações no desenvolvimento da tireoide da prole em estudos com animais de experimentação, como hiperplasia das células foliculares e desregulação do eixo hipotálamo-hipófise-tireoide (AHMED *et al.*, 2012; KOREVAAR *et al*., 2016). Deste modo, a tireoide da prole aparece como um potencial órgão alvo do tratamento materno com ISRSs como a sertralina.

Sertralina: Aspectos gerais

Entre os ISRSs, a sertralina é um dos medicamentos mais prescritos para tratamento da depressão na gestação, sendo a terapia recomendada pelas diretrizes de prática clínica devido a sua maior eficácia e tolerância em relação a outros fármacos de sua classe. A sertralina também é o antidepressivo mais indicado para tratamento de transtornos de humor no período pós-parto, devido aos baixos níveis de exposição em lactentes e poucos eventos adversos descritos em relatos de caso (FOND, 2015; LIANG *et al.,* 2019). Assim como os demais ISRSs, a sertralina potencializa a atividade serotoninérgica por meio da inibição da recaptação neuronal de 5-HT, com fraca afinidade na recaptação neuronal de outros neurotransmissores, como a norepinefrina e a dopamina. Juntamente com a paroxetina, é o mais potente inibidor da recaptação de 5-HT da sua classe (MORENO *et al.,* 1999).

O uso da sertralina é indicado para o tratamento de transtorno depressivo maior, transtorno obsessivo-compulsivo, transtorno do pânico, transtorno de estresse pós-traumático, transtorno de ansiedade social e transtorno disfórico pré-menstrual, de acordo com o FDA. Este fármaco foi classificado pelo FDA na categoria C de risco na gestação, na qual estudos em animais mostraram efeitos adversos no feto, mas não há estudos adequados e bem controlados em humanos (TRIFU *et al*., 2020). Seu principal metabólito, N-desmetil-sertralina, tem ação farmacológica modesta *in vivo* e *in vitro* (DEVANE *et al*., 2012).

As prescrições anuais de sertralina aumentaram drasticamente de 10,8 milhões em 2006 para 35,7 milhões em 2010 (HASKELL, *et al*, 2014). Em um estudo realizado em 2013, foi demonstrado que a sertralina foi o antidepressivo mais prescrito para adultos nos EUA (MOORE & MATTISON, 2017). Além disso, um estudo realizado na França em 2014 observou

que independentemente do antidepressivo usado no início da gestação, as trocas para sertralina foram as mais frequentes (BÉNARD-LARIBIÈRE *et al*., 2018). Embora seja muito utilizada, há poucos dados na literatura avaliando possíveis impactos de seu uso na gestação e na lactação.

Assim como observado com o uso dos demais ISRSs, o tratamento pré-natal com a sertralina tem sido correlacionado com alterações no desenvolvimento da prole, como redução do peso corpóreo no período lactacional (DE VASCONCELOS *et al.,* 2012), atraso no desenvolvimento somático e de reflexos da prole masculina de ratas (DE VASCONCELOS *et al.*, 2012; LOZANO *et al.*, 2021), malformações cardíacas e craniossinostose (BAKKER, 2010; BÉRARD *et al.*, 2015) e alterações no desenvolvimento de células β pancreáticas (DE LONG *et al*., 2015) e no desenvolvimento ósseo (FRAHER *et al.*, 2016). Resultados publicados pelo nosso grupo de pesquisa mostraram que o tratamento pré-natal com a sertralina em ratas também tem efeitos no desenvolvimento neurocomportamental da prole masculina (LOZANO *et al.*, 2021). Apesar da sertralina ser considerada segura durante a amamentação, tem sido associada a efeitos adversos em recém-nascidos (DA-SILVA *et al.*, 2021).

Justificativa

Este projeto justifica-se pela necessidade de se investigar experimentalmente possíveis impactos de curto e longo prazo da utilização do antidepressivo sertralina, um inibidor seletivo de recaptação de serotonina (ISRS), nos períodos gestacional e lactacional, sobre o desenvolvimento e a fisiologia do sistema reprodutor feminino. Diversos aspectos desse sistema podem ser influenciados pelo sistema serotoninérgico, assim como o desenvolvimento somático, de reflexos e neurocomportamental da prole cujas mães são expostas à sertralina, pois são processos altamente influenciados pelo desenvolvimento do sistema serotoninérgico. Estudos tem mostrado que a utilização de outros fármacos ISRSs nos períodos pré e pós-natal pode afetar o desenvolvimento de vários sistemas em machos e fêmeas, incluindo o sistema nervoso e reprodutor, mas estudos na área com a sertralina são escassos, especialmente em relação à prole feminina. Portanto, considerando-se que a sertralina é amplamente utilizada na clínica humana e que sua prescrição tem crescido nos últimos anos, é de grande importância investigar possíveis efeitos desse antidepressivo sobre o desenvolvimento e a performance reprodutiva da prole feminina, utilizando-se ratas Wistar como animais experimentais.

Objetivos

Objetivo geral

O presente trabalho teve como objetivo investigar possíveis efeitos da exposição gestacional, associada ou não ao estresse, ou lactacional à sertralina, sob parâmetros reprodutivos e de desenvolvimento somático, de reflexos e neurocomportamental da prole feminina de ratas Wistar.

Objetivos específicos:

Avaliar se a exposição à sertralina na gestação (associada ou não ao estresse) ou na lactação:

- Interfere no ganho de peso e/ou no consumo de ração das ratas Wistar lactantes;
- Tem efeitos sob marcos do desenvolvimento somático e de reflexos da prole feminina de ratas Wistar;
- Tem efeitos sob o desenvolvimento neurocomportamental da prole feminina jovem e/ou adulta;
- Impacta nos seguintes parâmetros de desenvolvimento e da função reprodutiva na prole feminina de ratas Wistar: a distância ano-genital, o número de mamilos, a morfofisiologia do útero e dos ovários, a maturação sexual, a regularidade do ciclo estral, o comportamento sexual e a fertilidade;
- Promove alterações no peso de órgãos potencialmente alvos em diferentes momentos da vida da prole.

Capítulo 1

O trabalho desenvolvido com a exposição gestacional de ratas Wistar ao estresse e/ou à sertralina deu origem ao manuscrito “Developmental and reproductive effects of gestational exposure to stress and/or sertraline on rat female offspring” a ser submetido para publicação no periódico *Reproductive Toxicology* (Fator de impacto: 3.143).

Developmental and reproductive effects of gestational exposure to stress and/or sertraline on rat female offspring

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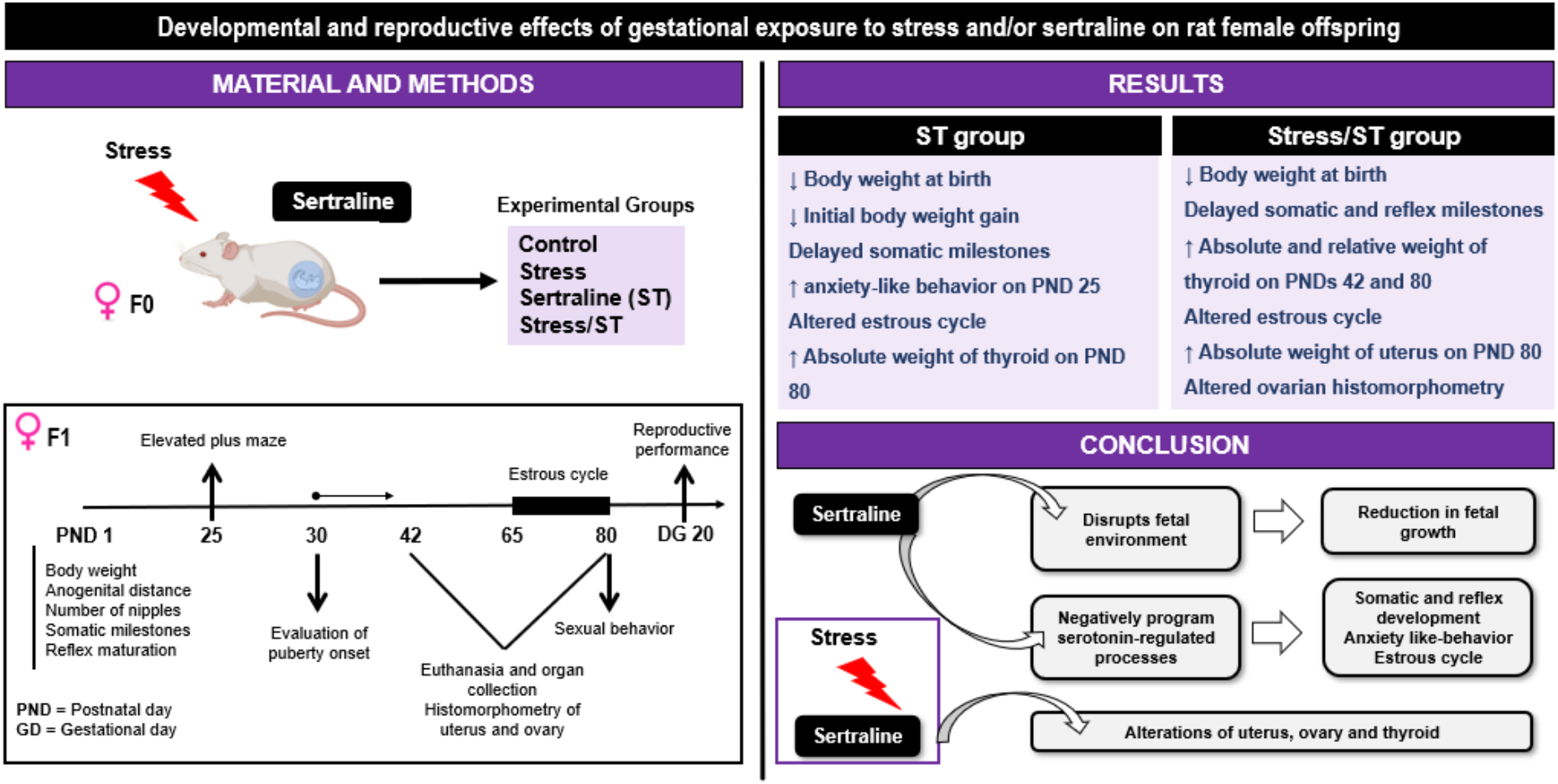

Figure 1. Graphical abstract.

HIGHLIGHTS

- Female rats were prenatally exposed to sertraline and/or stress
- Prenatal exposure to sertraline altered pre- and postnatal development of offspring
- Exposure only to sertraline increased anxiety-related behavior on juvenile offspring
- Sertraline associated with stress had effects on thyroid and morphology of ovary
- *In utero* exposure to sertraline affected serotonin-regulated processes

ABSTRACT

Selective serotonin reuptake inhibitors (SSRIs) are widely prescribed to pregnant women to treat mental illnesses. Among the drugs of this class, sertraline (ST) is the antidepressant therapy most recommended. Thus, the aim of this study was to evaluate the impact of prenatal treatment with ST on reproductive parameters of rat female offspring, as well as somatic, reflex and neurobehavioral development, in a model of maternal adversity based on the application of restraint stress. Pregnant Wistar rats received sertraline hydrochloride (20 mg/Kg/day diluted in vehicle) by oral gavage, associated or not with restraint stress, for 1 h/day from gestational days 13 to 20. Pups were evaluated on reproductive parameters, body weight and somatic and reflex development from postnatal day (PND) 1. On PND 25 and 72, elevated plus maze was performed. *In utero* exposure to ST, regardless of exposure to stress, reduced body weight at birth, delayed the incisor eruption and altered the estrous cycle. The absolute and relative thyroid weights on PND 42 were increased in Stress/ST group, as well as the relative thyroid weights, the absolute uterine weight and percentage of corpora lutea on PND 80. Prenatal exposure only to ST reduced body weight gain in the preweaning period, delayed fur development and increased anxiety-like behavior on PND 25. The present experimental study suggests that prenatal exposure to ST, combined or not with stress, disrupts fetal environment and can negatively program serotonin-regulated processes, such as somatic and neurobehavioral development and reproductive physiology. In addition, it impacts thyroid weight, especially when associated with stress.

Keywords: Sertraline, maternal depression, neurodevelopment, female rats, reproductive toxicology.

1. Introduction

The occurrence of depressive disorders and anxiety during pregnancy is a problem that affects many women, depression being the most common psychiatric morbidity in this period [1,2]. During pregnancy, about 7% to 20% of women are affected by symptoms of depression [3] and a recent study found that this number has been growing in the last years as a result of the COVID-19 pandemic [4].

In response to maternal mood disorders, a significant number of women receive antidepressant medication during pregnancy and lactation [5]. About 13% of pregnant women receive one or more antidepressants during all or part of pregnancy, of which about 70% use the class dos selective serotonin reuptake inhibitors (SSRIs) [6–8].

Several studies indicated a link between fetal exposure to both depressive disorders and SSRIs and negative effects on fetal development and programming in a sex-dependent way [9]. These negative effects include premature birth, fetal growth restriction, developmental delays and behavioral abnormalities [2,9–12]. Nevertheless, SSRIs are considered the treatment of choice during the gestational period and its antidepressant action occurs through blocking the serotonin transporter (SERT) [13,14].

In rats, SERT is expressed in the fetal brain on gestational day (GD) 12, while serotonergic neurons appear between GD 12 and 14 [12,15]. The peak of cell differentiation for this neurons occurs on GDs 15 and 16 [16]. In early development, serotonin [5-hydroxytryptamine (5-HT)] acts by regulating important neurodevelopmental processes, such as cell proliferation, neuronal differentiation, neural migration, myelination and synaptogenesis [17].

In the fetal brain, SERT is expressed much more diffusely than in the adult brain and the blood–brain barrier is not mature until after birth [18,19]. As SSRIs cross the placenta, there

are concerns about the potential short- and long-term effects of changing fetal 5-HT levels during critical periods of development, especially in neurodevelopment [17].

Studies in humans and animals have shown that early exposure to SSRIs can compromise the development of the serotonergic system and regions that are stimulated by this system, impacting on the activity of this neurotransmitter and on processes regulated by it throughout life [20]. According to this, Lozano et al. [21] found that prenatal exposure to SSRI sertraline (ST) compromise the somatic and neurobehavioral development of male rats. However, these parameters were not investigated in females.

The reproductive system is one of the systems regulated by 5-HT. It is known that GnRH-producing hypothalamic neurons receive serotoninergic innervation and this neurotransmitter is found in the pituitary, ovaries and uterus [22,23]. Thus, serotonin acts in the development and regulation of the hypothalamic-pituitary-gonadal (HPG) axis. Furthermore, 5-HT is also the main neurotransmitter involved in the control of sexual behavior [24].

Since 5-HT is closely related to several aspects of reproduction, SSRIs, such as ST, has the potential to affect reproductive function. In fact, one of the adverse effects reported by men and women using SSRIs is some form of sexual dysfunction. [25,26]. Among the SSRIs, ST is the drug with more sexual side effects [27], which raise a question regarding its effects on the reproductive system of offspring.

The few studies that have investigated reproductive impacts in animals have shown that developmental exposure to SSRIs can impact male and female reproductive function [13,28–32]. However, even though ST is the therapy recommended by clinical practice guidelines [33], there is no study in the literature evaluating the effects of gestational exposure to ST in the reproductive system of female offspring.

Considering the wide clinical use of this drug and the important role of 5-HT in intrauterine development and reproductive function, the aim of this study was to evaluate the

impact of gestational exposure to ST on reproductive parameters and somatic, reflex and neurobehavioral development of rat female offspring. In addition, to better model the clinical situation, we used a model of maternal adversity based on the application of restraint stress.

2. Material and Methods

2.1 Animals

Male and female Wistar rats (90 days old) from the Central Biotherium, São Paulo State University (UNESP), campus Botucatu/SP, Brazil, were kept in an environment of controlled temperature (average temperature of 23 °C) and light (12h light/12h darkness), with water and food *ad libitum.* The project was filed under number 1169 with the Ethics Committee on Animal Experimentation of the UNESP Institute of Biosciences, in Botucatu, in accordance with the Guidelines for the Care and Use of Laboratory Animals (National Institutes of Health).

During the dark period of the cycle, two females was placed in the male cage. After mating confirmation (determined by the presence of sperm in the vaginal smear), the females were housed in individual cages. The first 24 hours after the confirmation of the mating were considered as GD 0.

2.2 Experimental Design

Pregnant females were divided into four experimental groups and treated daily on GD 13 to 20, a critical period of development of serotonergic system in rats: Control group (n=10): Pregnant rats received vehicle (filtered water) by oral gavage; Stress group (n=8): Pregnant rats received vehicle by oral gavage and were restrained in an acrylic cylinder (with variable diameter) for 1 hour/day; ST group (n=8): Pregnant rats received ST Hydrochloride (20 mg/Kg/day diluted in vehicle) by oral gavage; and Stress/ST group (n=7): Pregnant rats received ST Hydrochloride (20 mg/Kg/day diluted in vehicle) by oral gavage and were restrained in an acrylic cylinder for 1 hour/day.

The methodology used is well established to induce maternal stress [34,35]. During the treatment, the removable restraining shield was adjusted to the tightest setting the expanding body size of the pregnant animals would allow. The dose of ST (purchased from Cruz

Vermelha, Botucatu, Brazil) used corresponds to a therapeutic dose in humans, according to the U.S. Food and Drug Administration (FDA) [36] and has been previously used in rats in other studies [11,37].

The day of birth of the offspring was considered postnatal day (PND) 0. On PND 1, pups were reduced in each litter (4 males and female pups/ per litter). The male offspring was evaluated in other study [21]. Pups were evaluated on parameters of development and reproductive function (anogenital distance (AGD) and number of nipples, age of puberty onset, genital organs weight, histomorphometry of uterus and ovary, evaluation of estrous cycle, sexual behavior and reproductive performance), body weight and somatic and reflex development from PND 1. On PND 25 and 72, elevated plus maze was performed. The Figure 2 describes the experimental procedures performed in this study.

2.3 Body weight, somatic and reflex development

Body weight was assessed weekly from PND 1 to 21. After this period, the body weight was assessed on PND 42 and 80. From PND 1, the somatic and reflex development of the female offspring were evaluated. The separation time of pups from dams during the evaluation of developmental milestones was restricted to less than 5 min to avoid stress. Somatic or physical maturation was assessed by recording the days when the following markers are observed: ear unfolding, incisor eruption, eye opening and fur development. Neurological reflexes acquisition was assessed by analyzing the following milestones: surface righting, negative geotaxis, palmar grasp and cliff aversion, as described by Coelho et al. [38]. Data were expressed as the mean of the litter and used to calculate the group mean.

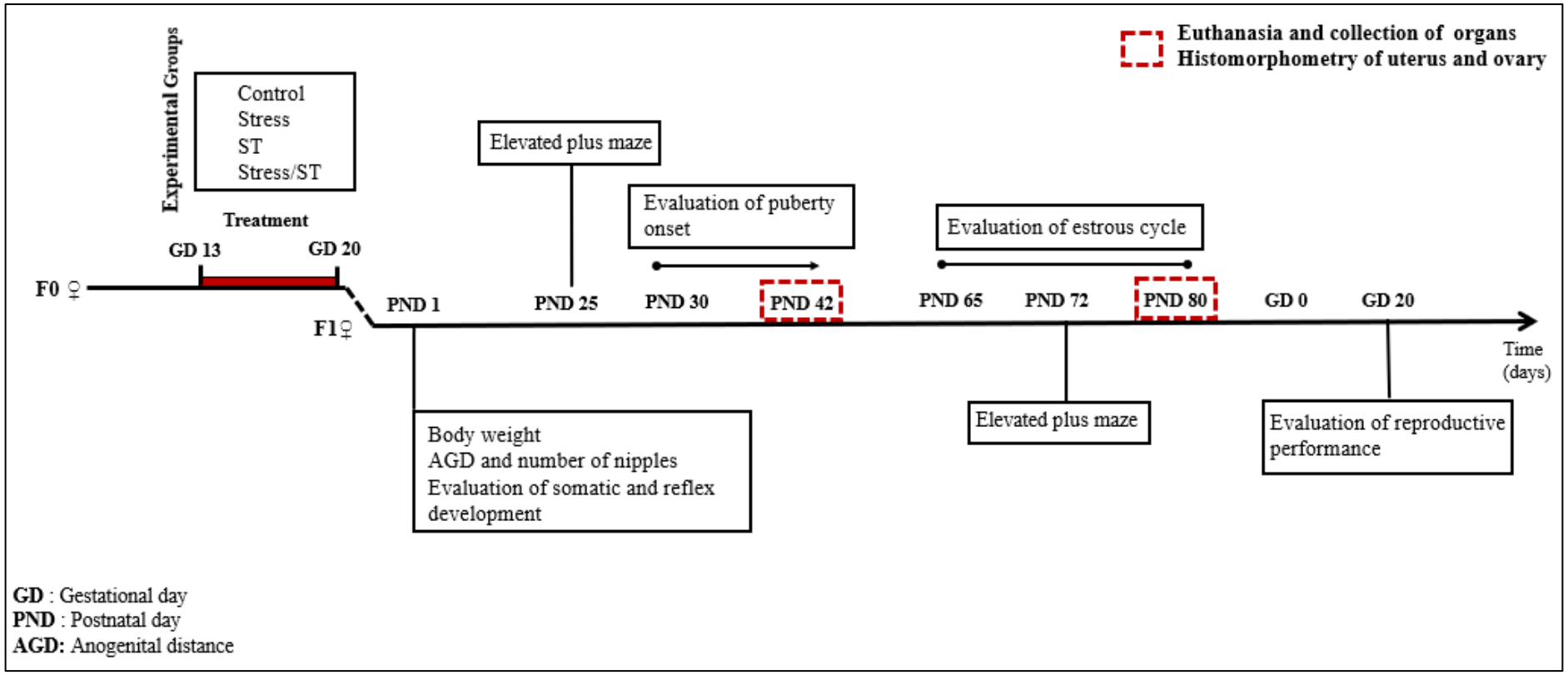

Figure 2. Experimental design of the study. ST=sertraline.

2.4 Anogenital distance (AGD) and number of nipples

The AGD (distance from the anus to the genital papilla) was measured on PND 1 and was normalized by its ratio by the cubic root of the animal body weight. The number of areolas was recorded on PND 13. Observations were scored based on the presence or absence of a nipple bud or a discoloration of the skin surrounding the nipple [39]. Litter data were used for statistical analysis.

2.5 Elevated plus maze

On PND 25 (juvenile offspring) and on PND 72 (adult offspring), the elevated plus maze test was performed in two females per litter to measure anxiety, in order to assess neurobehavioral development. In adult animals, the experiment was carried out in the first estrus from PND 72 onwards, since there is evidence that the estrous cycle influences the response of female rats in the elevated plus maze test [40]. The labyrinth consists of two walled arms (closed arms) and two open arms of equal length and width. The animals were placed in the center of the labyrinth facing one of the closed arms and their behavior was recorded for 5 min. The time spent (seconds) in the open arms and the number of entries (all four paws entering an arm) in the open and closed arms and were recorded [41]. After the test, the animal was removed from the device and cotton soaked in 5% ethyl alcohol was passed to clean the platform.

2.6 External physical signs of onset of puberty

Starting on PND 30, all females were evaluated for vaginal opening. From the observation of the complete vaginal opening, the estrous cycle was checked daily by a vaginal wash performed with the aid of a pipette containing 10 µl of saline solution into the vagina, and analyzed freshly with an optical microscope [42], until the detection of the first estrus

(predominance keratinized epithelial cells) [43]. Both procedures were performed to determine the age of puberty onset.

2.7 Reproductive cyclicity

Between PND 65 and 80, the estrous cycle was checked daily by means of a vaginal fluid performed with the aid of a pipette containing saline solution, and analyzed freshly with an optical microscope. The evaluation was made based on the cellular composition of vaginal fluid, as described by Marcondes et al. [43]: predominance of nucleated epithelial cells (proestrus); predominance of keratinized epithelial cells (estrus); presence of nucleated epithelial cells, keratinized epithelial cells and leukocytes (metestrus); abundant presence of leukocytes (diestrus). Data obtained during the 15 consecutive days of analysis were used to estimate the estrous cycle length (number of days from the first day of a cycle phase to the first day of the next phase), as well as the frequency of each phase of the cycle [44].

2.8 Euthanasia and collection of organs

The females were euthanized at different periods of life: right after the onset of puberty (PND 42, during estrus phase) and adulthood (PND 80, during estrus phase). The animals were weighed and euthanized by narcosis in CO_2. The genital (ovaries and uterus) and toxicological targets (brain, pituitary, thyroid, heart, liver, kidneys and adrenal glands) organs were collected and weighed. The uteri and ovaries were stored in a Bouin's fixative solution (75ml saturated picric acid solution, 25ml formaldehyde and 5ml acetic acid) until the time of the histological procedures. These materials were processed histologically for inclusion in Paraplast and, then, three sections of each organ were cut at a thickness of 5 µm and stained with hematoxylin and eosin (H&E) for histomorphometric analysis.

2.9 Histomorphometry of ovary and uterus

The number of ovarian follicles at different stages of maturation and corpus luteum was counted, classified according to Borgeest et al. [45] and Talsness et al. [46]: Primordial follicles and primary follicles were counted together and included oocytes surrounded by a single layer of either squamous or cuboidal epithelial cells. Secondary follicles were characterized by oocytes surrounded by more than one layer of granulosa cells. Tertiary follicles were indicated by antral formation. Characteristics of atretic follicles included pyknotic granulosa cells, disorganized granulosa cells, degenerating oocyte and detachment from the basement membrane.

In the uterine sections, the heights of perimetrium, myometrium, endometrium, glandular and luminal epithelium were measured, as described by Silva et al [42]. All analyzes were performed under light microscopy, with softwares Leica LASCore, version 4.12.0 and Image J 1.48.

2.10 Sexual Behavior and reproductive performance

At the first estrus after PND 80, one female per litter was used to assess sexual behavior. After detection of the estrous phase, female rats were put into cages of sexually experienced male rats, then allowed 10 mounts on the females while registering the presence of lordosis. The results were expressed as the lordosis quotient (number of lordosis/ten mounts x 100) [47]. This evaluation took place in the dark phase of the photoperiod and the lordosis were observed with the help of red lights.

After the end of sexual behavior test, the same female was maintained with the same male for additional 8h. The mating was confirmed by the presence of sperm in the vaginal smear and the females were weighed and housed in individual cages. The day when sperm was found in the vaginal smear was considered GD 0.

On GD 20, the naturally inseminated females were euthanized by CO2 inhalation followed by decapitation, to allow the assessment and calculation of fertility. The gestational rate (number of pregnant females/number of inseminated females x 100) was calculated to each group. After collecting the gravid uteri and ovaries, the numbers of corpus luteum, implantation sites, resorptions, live and dead fetuses were determined. From these results, the following parameters were calculated: fertility potential (number of implantation sites/corpus luteum × 100), rate of pre-implantation loss: (number of corpus luteum - number of implantations/number of corpus luteum) × 100, rate of post-implantation loss: (number of implantations - number of live fetuses / number of implantations) × 100 and sexual ratio: number of female fetuses / number of male fetuses [44,48]. In addition, fetal and placental weights were collected, as well as the final body weight and weight of the gravid uteri.

2.11 Statistical analysis

Data are presented as mean ± standard error of mean (S.E.M.), median and interquartile intervals or percentage. The results were compared among groups by Two-way ANOVA followed by Tukey's test, for parametric variables, and by Kruskal-Wallis followed by Dunn's test or Chi-Square test, for nonparametric variables. Differences were considered statistically significant when $p \leq 0.05$, trends were discussed if $p \leq 0.07$. Statistical analyses were performed using the software GraphPad Prism (version 8.0).

3. Results

3.1 Maternal data

Treatment with ST reduced the body weight of dams at the end of the treatment (GD 20) and promoted maternal transient vaginal bleeding between GDs 15 and 17. Furthermore, the percentage of litters with dead pups was higher in the groups treated with ST than in the control group. These data were published by Lozano et al. [21] in another work of our research group.

3.2 Body weight

In utero exposure to ST, combined or not with stress, reduced body weight on PND 1 ($p = 0.0031$) (Figure 3). On PND 7 and 14, there was no difference in body weight among groups, but body weight remained reduced in the ST group on PND 21 compared to the other groups. The body weight gain (Figure 3) of animals in the preweaning period (PND 1-21) was lower in the ST group (36.91 ± 1.52) when compared to the control (41.57 ± 0.79), but there was no statistical difference regarding body weight and body weight gain after this period.

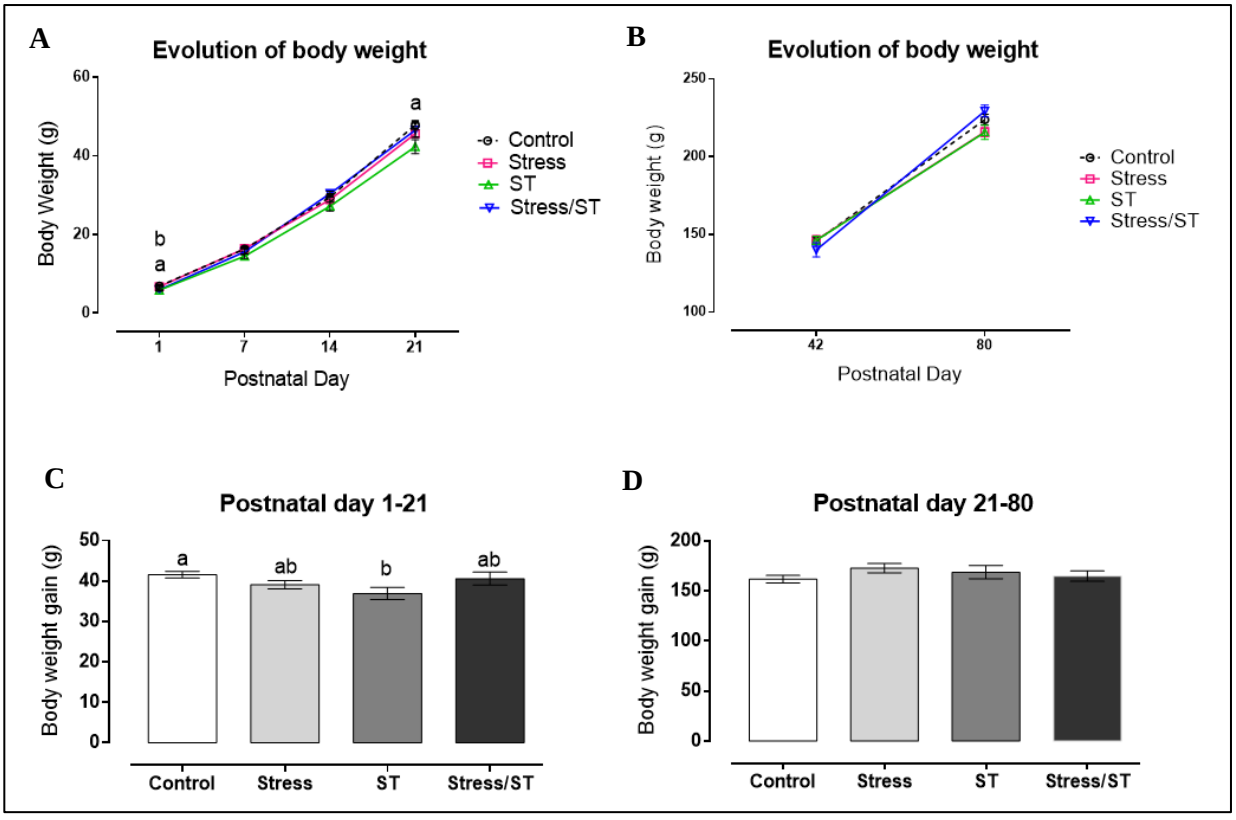

Figure 3. (A) Body weight on postnatal days 1, 7, 14 and 21 of prenatally exposed animals to sertraline (ST) and/or stress (n=7-10); a = $p \leq 0.05$ for control x ST, b = $p \leq 0.05$ for control x Stress/ST (B) Body weight of animals on postnatal days 42 and 80 (n=7-10). (C) Body weight gain during postnatal days 1-21 (n=7-10). (D) Body weight gain during postnatal days 21-80 (n=7-10). Values expressed as mean ± S.E.M. Two-way ANOVA followed by Tukey's test. Different letters (a and b) indicate $p \leq 0.05$. The litter mean was used a statistical unity in this analysis.

3.3 Anogenital distance and number of nipples

Normalized AGD of litters and number of nipples of female rats (Figure 4) showed no statistical differences among groups (p = 0.3881 and 0.2736, respectively).

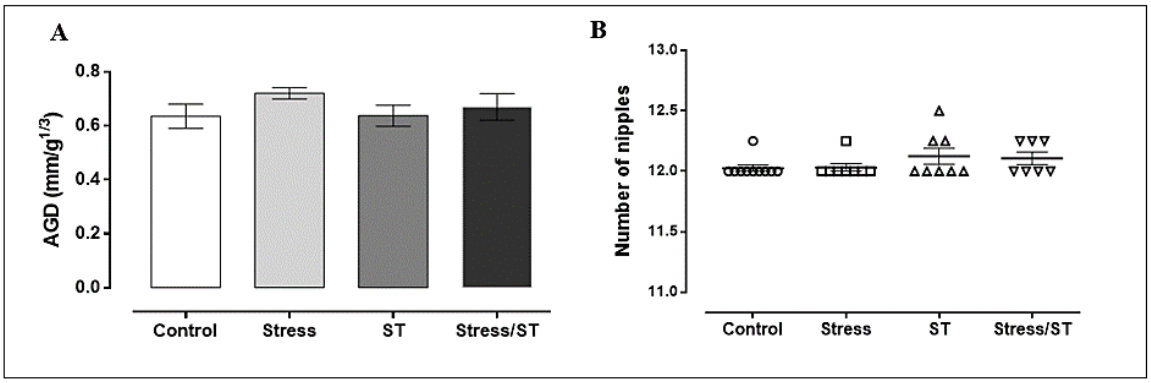

Figure 4. (A) Relative anogenital distance (AGD) on postnatal day 1 of the rat female offspring whose mothers were exposed to sertraline (ST) and/or stress (n=7-10). Values expressed as mean ± S.E.M. (B) Number of nipples on postnatal day 13 (n=7-10). Values expressed as mean ± S.E.M. Two-way ANOVA followed by Tukey's test, $p > 0.05$. The litter mean was used as a statistical unity in both analyses.

3.4 Somatic and reflex development

The fur development (Table 1) was delayed only in the ST group compared to the control group (p = 0.0497) and the eruption of the incisors occurred later in the ST and Stress/ST

groups, when compared to the other groups (p = 0.0001), while the days of ear unfolding and eye opening were similar among groups. The day of disappearance of palmar grasp (Table 1) was also delayed in the Stress/ST group (9.64 ± 0.83) compared to the control group (7.81 ± 0.28), although this increase was not statistical (p = 0.06). However, the day of appearance of other reflexes (righting reflex, negative geotaxis and cliff avoidance) were not changed by the treatments.

Table 1. Postnatal day of occurrence of somatic and reflex milestones in female rats whose mothers were exposed to sertraline (ST) and/or stress during pregnancy.

Parameters	Experimental groups			
	Control (n=10)	Stress (n=8)	ST (n=8)	Stress/ST (n=7)
Physical landmarks				
Ear unfolding	2.85 ± 0.14	2.75 ± 0.25	3.05 ± 0.15	2.68 ± 0.22
Fur development	7.08 ± 0.15 **a**	7.5 ± 0.19 **ab**	8.03 ± 0.27 **b**	7.72 ± 0.46 **ab**
Incisor eruption	10.04 ± 0.19 **a**	10.35 ± 0.21 **a**	11.25 ± 0.17 **b**	11.33 ± 0.24 **b**
Eye opening	14.46 ± 0.16	14.67 ± 0.12	14.48 ± 0.29	14.25 ± 0.14
Reflex maturation				
Righting reflex	1.91 ± 0.12	2.41 ± 0.30	2.67 ± 0.37	1.93 ± 0.34
Cliff avoidance	4.20 ± 0.18	3.69 ± 0.43	4.54 ± 0.50	4.24 ± 0.47
Negative geotaxis	6.08 ± 0.40	6.54 ± 0.68	6.78 ± 0.33	7.46 ± 0.62
Palmar grasp	7.81 ± 0.28	8.46 ± 0.40	9.55 ± 0.68	9.64 ± 0.83

Values are expressed as mean ± S.E.M. Two-way ANOVA followed by Tukey's test. Different letters (a and b) indicate $p \leq 0.05$. The litter mean was used as a statistical unity in this analysis.

3.5 Elevated plus maze

The results of the elevated plus maze test (Figure 5) on PND 25 show that gestational exposure only to ST promoted alterations in the neurobehavioral development of the female offspring. The animals in the ST group entered less into the open arms (in relation to the stress group) and closed arms (in relation to the other groups), as well as in both arms (when compared to the control and stress groups). In addition, ST group spent less time in open arms than the

control and stress groups (p = 0.001). In the female adult offspring (PND 72), the data did not show any significant difference between the groups in any of the parameters evaluated.

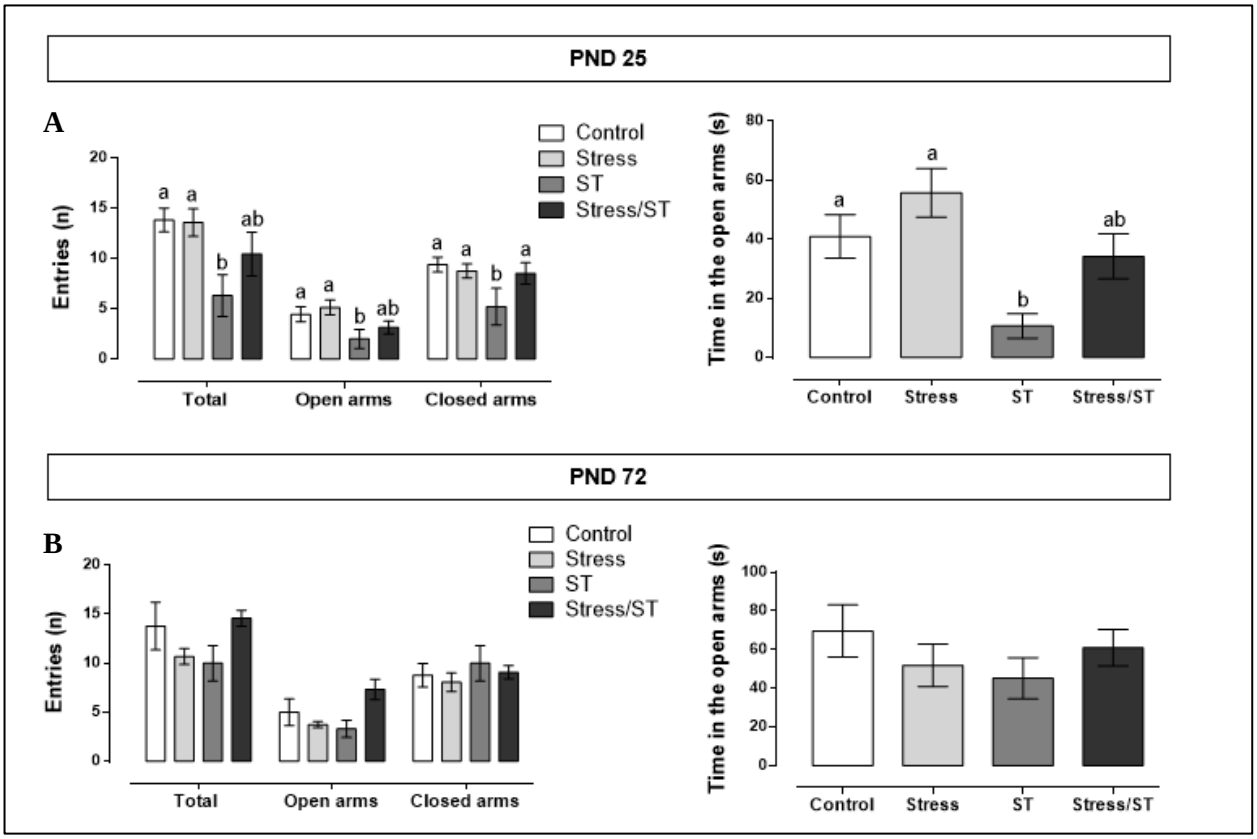

Figure 5. (A) Results of elevated plus maze on postnatal day 25 of female rats prenatally exposed to sertraline (ST) and/or stress (n=7-10). (B) Results of elevated plus maze on postnatal day 72 during estrus phase (n=7-10). Values expressed as mean ± S.E.M. Two-way ANOVA followed by Tukey's test. Different letters (a and b) indicate $p \leq 0.05$. The mean from two females per litter was used to calculate the group mean.

3.6 Puberty onset

The age of vaginal opening (Control = 35.97; Stress = 36.45; ST = 37.77; Stress/ST =37.64) and the first estrus (Control = 36.00; Stress = 36.87; ST = 37.95; Stress/ST = 38.05) on female offspring were similar among the experimental groups (p = 0.3249 and 0.4260, respectively), as shown in Figure 6.

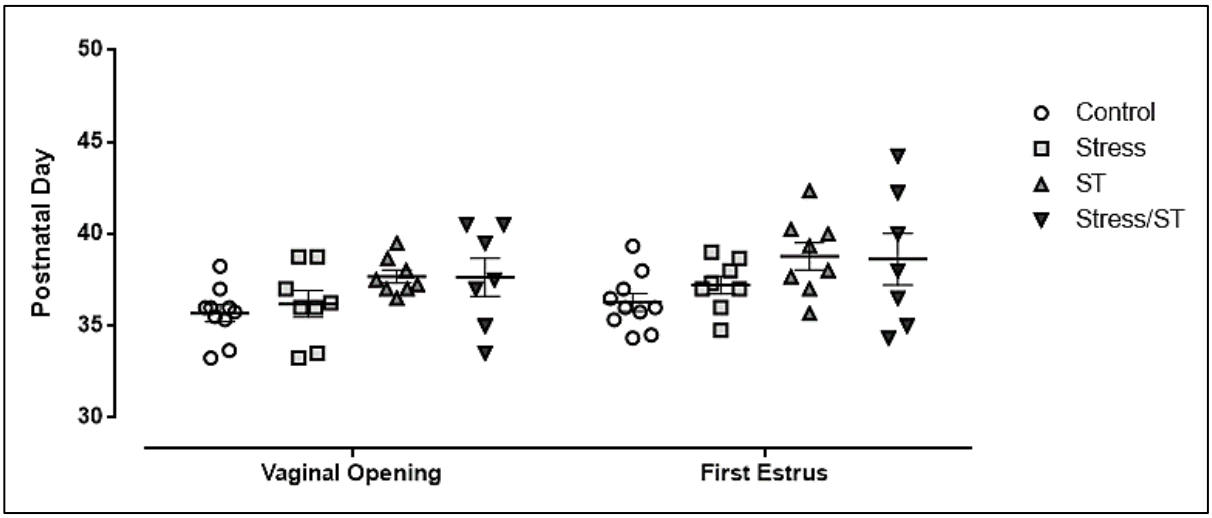

Figure 6. Age of the vaginal opening and occurrence of the first estrus in female rats whose mothers were exposed to sertraline (ST) and/or stress during pregnancy (n=7-10). Values are expressed as mean ± S.E.M and were analyzed by two-way ANOVA followed by Tukey's test, $p > 0.05$. The litter mean was used as a statistical unity in this analysis.

3.7 Body weight and organ weights

On PND 42 (Table 2), the absolute and relative weights of the thyroid were increased in Stress/ST group, while the absolute uterine weight was increased on PND 80 in this group (Table 3). The relative weights of genital organs (uterus with fluid and ovaries) of euthanized pubescent rats (PND 42) and adult rats (PND 80) were not different among groups (Table 2 and Table 3, respectively), but the ST and Stress/ST groups presented absolute thyroid weights increased on PND 80 in relation to the control and stress groups (Table 3).

Table 2. Body, absolute and relative organ weights on postnatal day 42 of female rats whose mothers were exposed to sertraline (ST) and/or stress during pregnancy.

	Postnatal day 42			
Parameters	**Control (n=10)**	**Stress (n=8)**	**ST (n=8)**	**Stress/ST (n=7)**
Body weight (g)	146.90 ± 3.61	148.80 ± 4.54	140.00 ± 5.31	147.80 ± 2.57
Absolute organ weights				
Uterus with fluid (mg)	237.60 ± 14.50	247.90 ± 21.35	247.30 ± 12.62	198.10 ± 14.06
Ovary (mg)	55.19 ± 2.85	62.68 ± 6.44	56.66 ± 6.07	58.48 ± 2.22
Brain (g)	1.70 ± 0.02	1.73 ± 0.04	1.73 ± 0.02	1.71 ± 0.03
Thyroid (mg)	12.37 ± 0.90 **a**	12.99 ± 0.90 **a**	14.23 ± 0.81 **ab**	17,97 ± 1,31 **b**
Pituitary (mg)	7.56 ± 0.57	6.43 ± 0.37	6.50 ± 0.37	6.82 ± 0.75
Liver (g)	7.36 ± 0.25	7.41 ± 0.25	7.97 ± 0.34	7.30 ± 0.19
Heart (mg)	703.80 ± 20.64	673.60 ± 22.79	733.70 ± 33.16	710.60 ± 25.03
Kidneys (g)	1.55 ± 0.03	1.54 ± 0.07	1.52 ± 0.09	1.56 ± 0.06
Adrenal glands (mg)	50.84 ± 1.47	48.26 ± 3.92	49.86 ± 3.01	52.07 ± 3.20
Lungs (g)	1.05 ± 0.04	1.07 ± 0.05	1.16 ± 0.06	1.07 ± 0.05
Relative organ weights				
Uterus with fluid (mg/100g)	176.40 ± 15.18	167.50 ± 10.05	173.10 ± 8.67	143.40 ± 12.63
Ovary (mg/100g)	37.48 ± 2.33	41.81 ± 3.57	38.79 ± 4.35	38.99 ± 1.28
Brain (g/100g)	1.15 ± 0.03	1.15 ± 0.04	1.21 ± 0.04	1.19 ± 0.03
Thyroid (mg/100g)	8.54 ± 0.55 **a**	8.78 ± 0.56 **a**	10.38 ± 0.46 **ab**	11.75 ± 0.67 **b**
Pituitary (mg/100g)	4.98 ± 0.32	4.87 ± 0.53	4.46 ± 0.45	4.77 ± 0.66
Liver (g/100g)	5.22 ± 0.12	5.15 ± 0.13	5.44 ± 0.16	5.12 ± 0.06
Heart (mg/100g)	502.60 ± 16.75	453.50 ± 12.50	503.80 ± 23.79	463.50 ± 7.98
Kidneys (g/100g)	1.06 ± 0.04	1.04 ± 0.03	1.07 ± 0.03	1.06 ± 0.05
Adrenal glands (mg/100g)	34.53 ± 1.37	32.32 ± 2.25	36.08 ± 2.11	35.72 ± 2.81
Lungs (mg/100g)	666.70 ± 29.40	726.70 ± 44.00	807.40 ± 40.60	720.60 ± 36.70

Values are expressed as mean ± S.E.M. Two-way ANOVA followed by Tukey's test. Different letters (a and b) indicate $p \leq 0.05$. One female per litter was used to calculate the group mean.

Table 3. Body, absolute and relative organ weights on postnatal day 80 of female rats whose mothers were exposed to sertraline (ST) and/or stress during pregnancy.

	Postnatal day 80			
Parameters	**Control (n=10)**	**Stress (n=8)**	**ST (n=8)**	**Stress/ST (n=7)**
Body weight (g)	216.50 ± 3.48	225.10 ± 5.28	231.40 ± 4.64	217.30 ± 5.40
Absolute organ weights				
Uterus with fluid (mg)	370.50 ± 12.32 **a**	377.50 ± 14.79 **a**	408.90 ± 9.18 **ab**	430.30 ± 23.39 **b**
Ovary (mg)	85.63 ± 4.15	96.63 ± 1.37	98.45 ± 7.07	94.13 ± 5.74
Brain (g)	1.83 ± 0.02	1.86 ± 0.03	1.85 ± 0.03	1.85 ± 0.04
Thyroid (mg)	14.39 ± 0.68 **a**	13.23 ± 1.01 **a**	18.72 ± 1.01 **b**	19.88 ± 1.16 **b**
Pituitary (mg)	10.16 ± 0.56	11.04 ± 0.46	11.08 ± 0.56	10.57 ± 0.65
Liver (g)	8.82 ± 0.24	9.12 ± 0.20	9.85 ± 0.29	8.60 ± 0.32
Heart (mg)	902.30 ± 17.69	904.00 ± 31.50	954.60 ± 54.80	913.00 ± 56.12
Kidneys (g)	1.90 ± 0.05	1.91 ± 0.05	1.97 ± 0.06	1.87 ± 0.07
Adrenals (mg)	87.94 ± 3.36	84.21 ± 5.03	93.31 ± 4.72	89.10 ± 6.58
Lungs (g)	1.38 ± 0.06	1.39 ± 0.08	1.29 ± 0.05	1.25 ± 0.06
Relative organ weights				
Uterus with fluid (mg/100g)	174.10 ± 5.05	170.90 ± 6.56	182.06 ± 9.62	198.90 ± 12.61
Ovary (mg/100g)	39.11 ± 2.04	43.02 ± 0.77	41.76 ± 2.50	44.00 ± 2.31
Brain (mg/100g)	846.40 ± 10.68	817.60 ± 23.90	796.20 ± 10.91	854.70 ± 19.55
Thyroid (m/100g)	6.12 ± 0.16 **a**	6.0 ± 0.44 **a**	8.14 ± 0.53 **ab**	8.39 ± 0.64 **b**
Pituitary (mg/100g)	4.34 ± 0.21	4.97 ± 0.20	4.71 ± 0.25	4.69 ± 0.23
Liver (g/100g)	3.97 ± 1.00	4.06 ± 1.01	4.15 ± 0.99	3.96 ± 1.27
Heart (mg/100g)	371.20 ± 41.20	403.60 ± 15.42	405.90 ± 19.91	418.30 ± 17.08
Kidneys (mg/100g)	826.70 ± 50.76	794.70 ± 61.94	838.80 ± 15.82	858.60 ± 20.74
Adrenal glands (mg/100g)	40.39 ± 1.46	37.50 ± 2.32	41.03 ± 2.63	40.90 ± 2.54
Lungs (mg/100g)	640.20 ± 30.53	618.10 ± 34.71	561.10 ± 17.94	595.80 ± 45.73

Values are expressed as mean ± S.E.M. Two-way ANOVA followed by Tukey's test. Different letters (a and b) indicate $p \leq 0.05$. One female per litter was used to calculate the group mean.

3.8 Estrous cycle

The female rats exposed to ST, regardless of exposure to stress, showed changes in reproductive cyclicity (Table 4), such as increased frequency of metestrus ($p = 0.0041$) in relation to control group, and increased duration of the estrous cycle ($p = 0.0381$) in relation to the other groups.

Table 4. Estrous cycle assessment over a period of 15 consecutive days of evaluation in the exposed rat female offspring to sertraline (ST) and/or stress starting at PND 65.

Parameters	Experimental Groups			
	Control (n=10)	Stress (n=8)	ST (n=8)	Stress/ST (n=7)
[1]Frequency of diestrus	21.88 (19.70 - 23.86)	20.51 (19.39 - 21.46)	20.02 (14.53 - 21.33)	17.50 (16.69 - 18.33)
[1]Frequency of proestrus	19.16 (18.53 - 20.02)	20.20 (18.65 - 21.25)	15.42 (14.23 - 18.14)	16.25 (15.28 - 19.81)
[1]Frequency of estrus	27.10 (24.08 - 31.09)	29.56 (24.76 - 30.63)	27.04 (26.23 - 29.85)	25.00 (23.38 - 27.62)
[1]Frequency of metestrus	29.82 (28.35 - 30.04) **a**	29.98 (29.16 - 30.72) **ab**	39.83 (36.79 - 40.34) **b**	40.00 (33.75 - 44.76) **b**
[2]Estrous cycle length (days)	4.07 ± 0.12 **a**	4.11 ± 0.07 **a**	4.45 ± 0.12 **b**	4.47 ± 0.14 **b**
[2]Number of estrous cycle	3.13 ± 0.13	3.13 ± 0.08	2.81 ± 0.16	2.75 ± 0.21

[1]Values expressed as median and interquartile intervals. Kruskal-Wallis test followed by Dunn's test. [2]Values expressed as mean ± S.E.M. Two-way ANOVA followed by Tukey's test. Different letters (a and b) indicate $p \leq 0.05$. The mean from two females per litter was used to calculate the group mean and median.

3.9 Sexual behavior and reproductive performance

Although it deregulated the estrous cycle and the count of ovarian structures, prenatal exposure to stress and/or ST had no impact on sexual behavior (Table 5) and in parameters evaluated in the analysis of reproductive performance (Table 5): gestational rate, fertility potential, sex ratio, fetal weight, placenta weight, pre-implantation loss, post-implantation loss and numbers of implantation sites, corpora lutea, fetus, and resorptions.

Table 5. Sexual behavior and reproductive performance of exposed rat female offspring to sertraline (ST) associated or not with stress.

Parameters	Experimental Groups			
	Control (n=8)	Stress (n=8)	ST (n=8)	Stress/ST (n=7)
[1]Lordosis quotient (%)	100.00 (97.50 - 100.00)	100 (92.50 - 100.00)	90.00 (62.50 - 100.00)	90.00 (70.00 - 100.00)
[2]Gestational rate (%)	100	100	87.5	100
[1]Fertility potential (%)	100.00 (91.96 - 100.00)	93.30 (83.65 - 100.00)	100.00 (87.50 - 100.00)	100.00 (72.73 - 100.00)
[3]Number of corpora lutea	13.00 ± 0.47	12.63 ± 0.73	13.00 ± 0.93	12.57 ± 0.57
[3]Number of implantations	12.50 ± 0.60	11.5 ± 0.63	13.00 ± 0.93	11.43 ± 0.81
[3]Number of fetuses	12.5 ± 0.59	10.71 ± 0.47	11.67 ± 1.02	10.80 ± 0.97
[3]Number of resorptions	0.00 ± 0.00	0.25 ± 0.16	0.00 ± 0.00	0.14 ± 0.14
[1]Pre-implantation loss (%)	0.00 (0.00 - 8.04)	7.14 (0.00 - 16.03)	0.00 (0.00 - 12.00)	4.17 (0.00 - 22.73)
[1]Post-implantation loss (%)	0.00 (0.00 - 0.00)	0.00 (0.00 - 8.33)	0.00 (0.00 - 0.00)	0.00 (0.00 - 6.67)
[3]Final body weight (g)	351.60 ± 9.46	357.7 ± 10.25	381.80 ± 18.16	341.40 ± 8.87
[3]Gravid uterus weight (g)	61.52 ± 2.96	58.17 ± 3.45	61.29 ± 5.55	58.76 ± 4.09
[3]Fetal weight (g)	2.87 ± 0.09	2.99 ± 0.06	2.76 ± 0.17	2.96 ± 0.08
[3]Placental weight (g)	0.50 ± 0.03	0.49 ± 0.05	0.45 ± 0.04	0.48 ± 0.07
[3]Sex ratio (F:M)	1.05 ± 0.14	1.16 ± 0.20	1.12 ± 0.13	0.85 ± 0.25

[1]Values expressed as median and interquartile intervals. Kruskal-Wallis test followed by Dunn's test, $p > 0,05$. [2]Values expressed as percentage. Chi-square test, $p > 0.05$. [3]Values expressed as mean ± S.E.M. Two-way ANOVA followed by Tukey's test, $p > 0.05$. One female per litter was used to calculate the group mean and median.

3.10 Ovarian and uterine histomorphometry

The percentage of structures in the ovary was not altered on PND 42 (Figure 7), but an increase in the percentage of corpora lutea and a reduction in the percentage of primordial and primary follicles were observed in the Stress/ST group on PND 80 (Figure 8). Histomorphometry of uterus was similar among groups on PND 42 (Figure 9) and 80 (Figure 10). Height of the perimetrium, myometrium, endometrial stroma, uterine luminal epithelium and glandular epithelium were similar among groups.

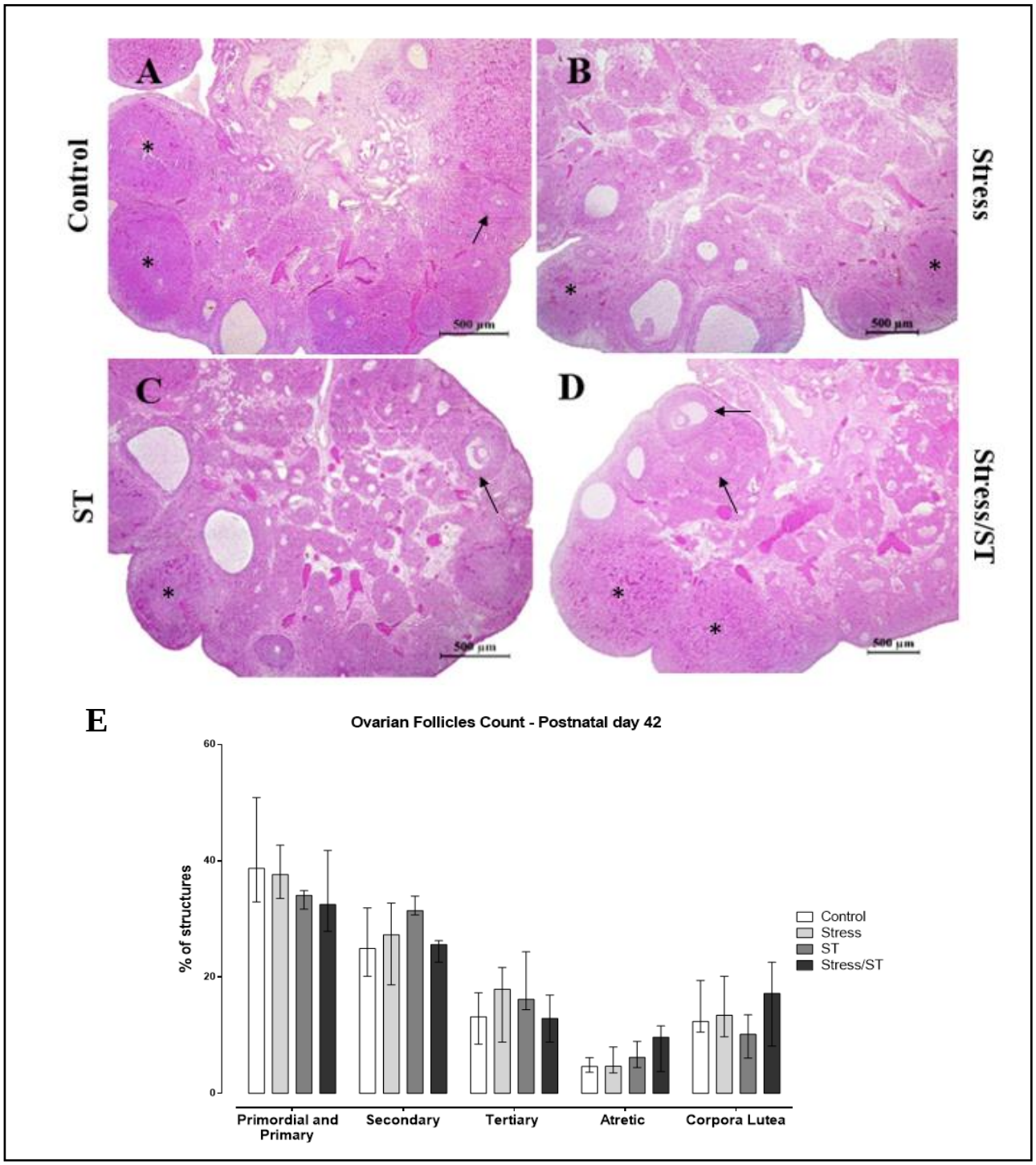

Figure 7. Histological evaluation of the ovaries, during the estrous phase on postnatal day 42 of female offspring whose mothers were exposed to sertraline (ST) associated or not with stress during pregnancy. (A-D) Representative histological aspect of ovaries. Arrows: Tertiary follicles; asterisks: corpora lutea. Hematoxylin and Eosin (HE). Scale bar = 500 µm. (E) Histomorphometric analysis of ovary (n=5-6/group). Values expressed as median ±

interquartilic range. Kruskal-Wallis test followed by Dunn's test, $p > 0.05$. One female per litter was used to calculate the group mean.

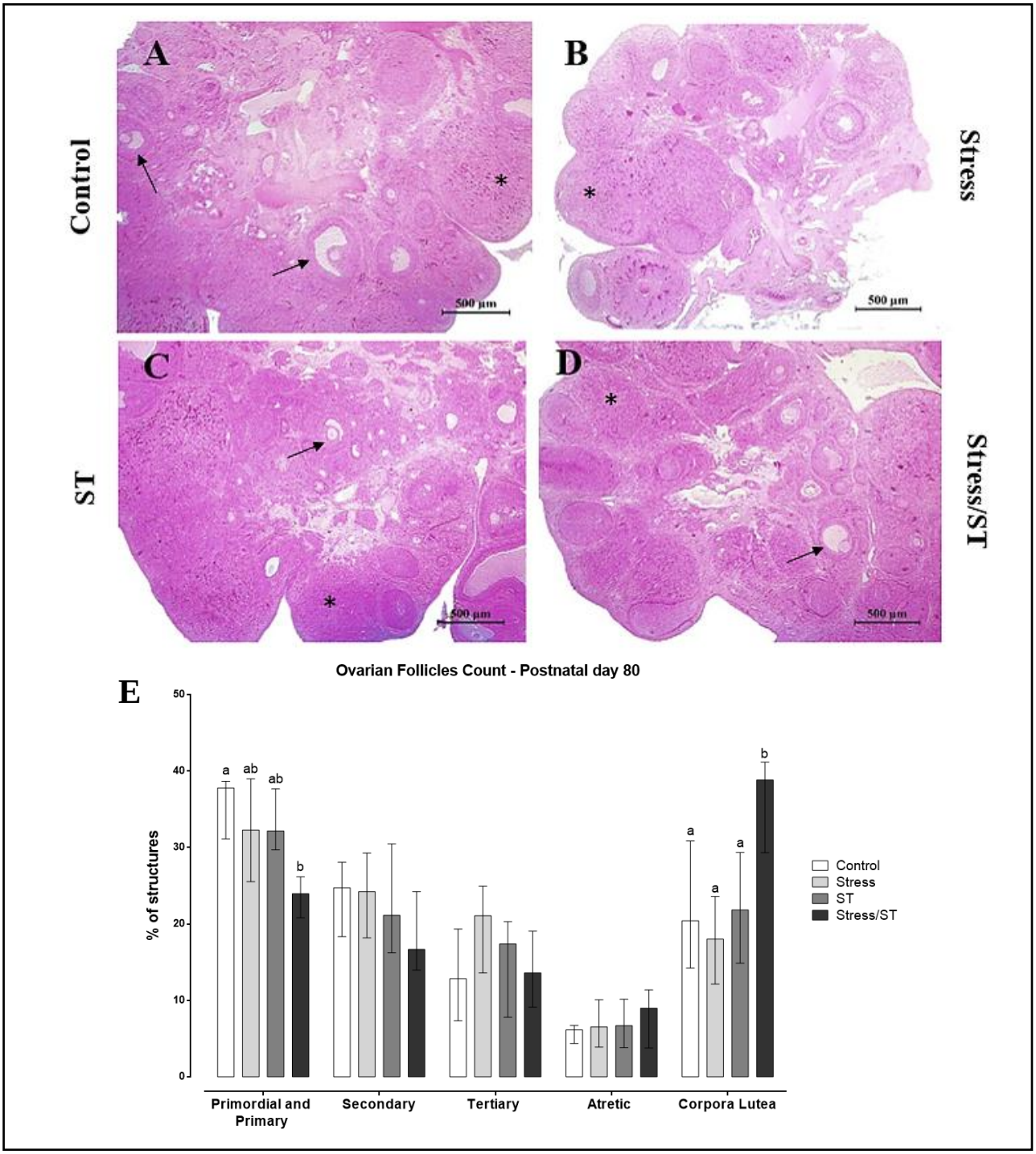

Figure 8. Histological evaluation of the ovaries, during the estrous phase on postnatal day 80 of female offspring whose mothers were exposed to sertraline (ST) associated or not with stress during pregnancy. (A-D) Representative histological aspect of ovaries. Arrows: Tertiary

follicles; asterisks: corpora lutea. Hematoxylin and Eosin (HE). Scale bar = 500 µm. (E) Histomorphometric analysis of ovary (n=5-6/group). Values expressed as median ± interquartilic range. Kruskal-Wallis test followed by Dunn's test, $p > 0.05$. One female per litter was used to calculate the group mean.

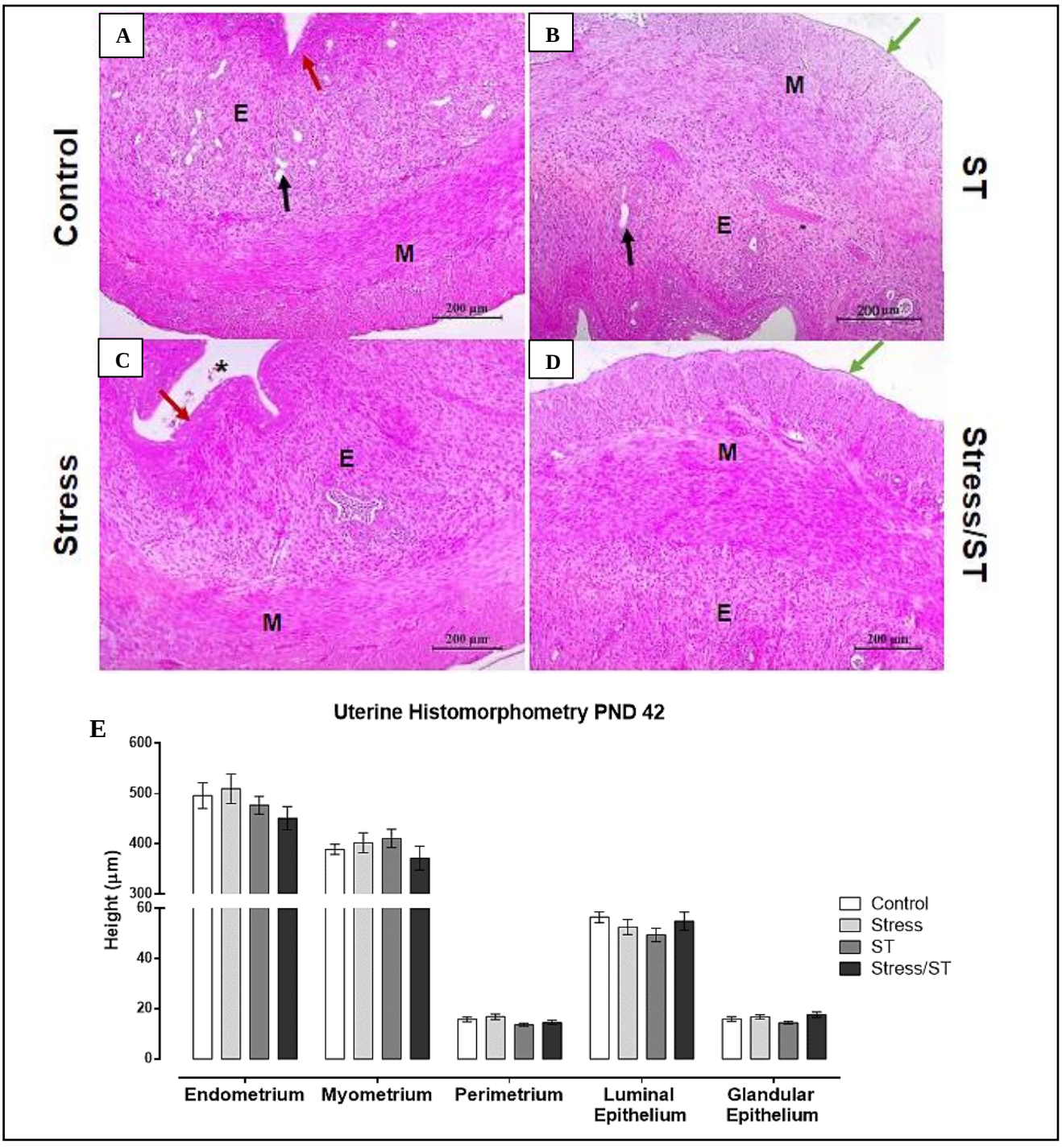

Figure 9. Histological evaluation of the uterus, during the estrous phase on postnatal day 42 of female offspring whose mothers were exposed to sertraline (ST) associated or not with stress during pregnancy. (A-D) Representative histological aspect of uterus. E: endometrium; M: myometrium; black arrows: uterine glands; red arrows: luminal epithelium; green arrows: perimetrium. Hematoxylin and Eosin (HE). Scale bar = 200 µm. (E) Histomorphometric analysis of uterus (n= 5-6/group). Values are expressed as mean ± S.E.M and were analyzed by two-way ANOVA followed by Tukey's test, $p > 0.05$.

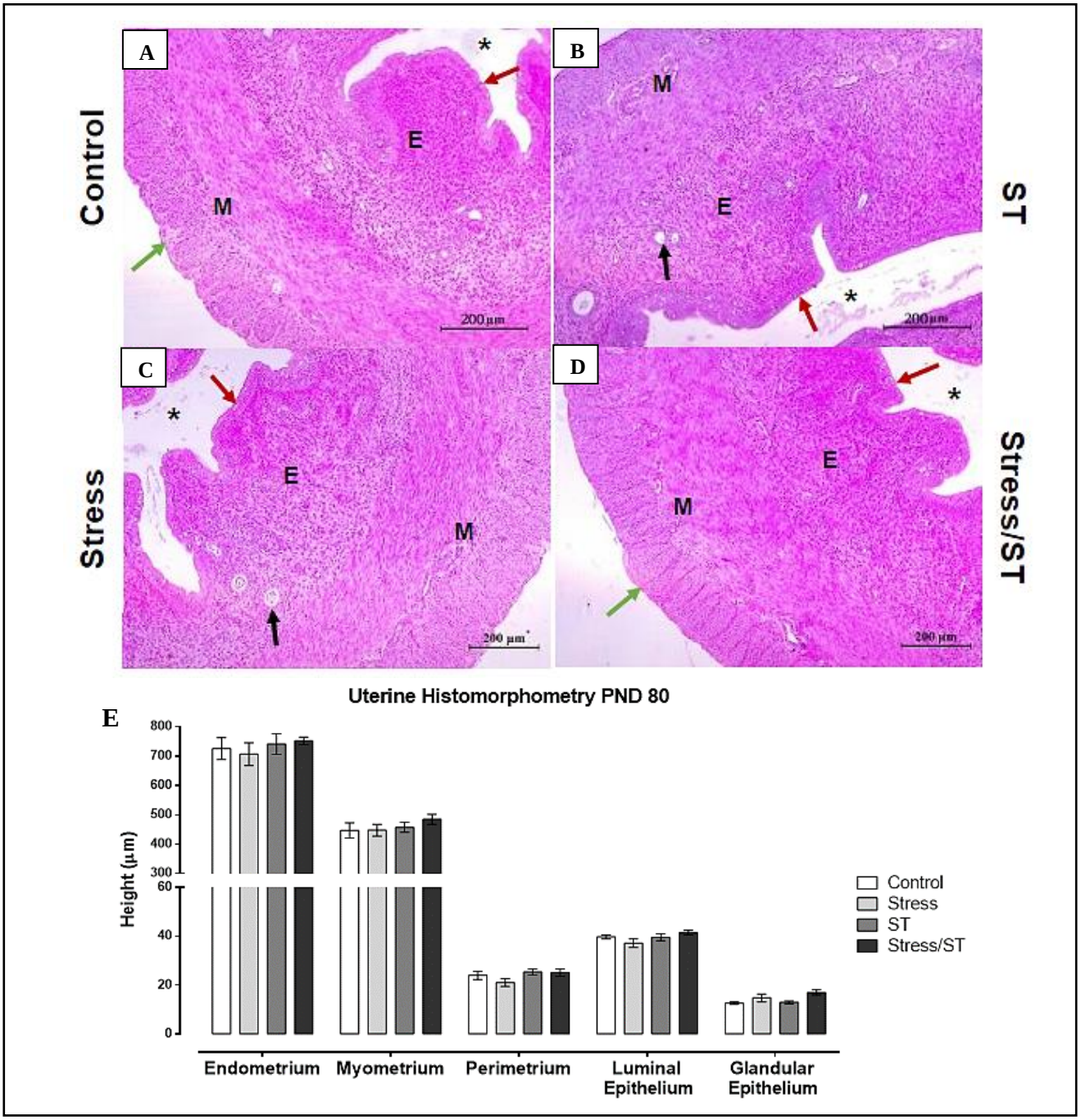

Figure 10. Histological evaluation of the uterus, during the estrous phase on postnatal day 80 of female offspring whose mothers were exposed to sertraline (ST) associated or not with stress during pregnancy. (A-D) Representative histological aspect of uterus. E: endometrium; M: myometrium; black arrows: uterine glands; red arrows: luminal epithelium; green arrows: perimetrium. Hematoxylin and Eosin (HE). Scale bar = 200 µm. (E) Histomorphometric analysis of uterus (n= 5-6/group). Values are expressed as mean ± S.E.M and were analyzed by two-way ANOVA followed by Tukey's test, $p > 0.05$.

4. Discussion

Considering that normal serotonergic system development is crucial for intrauterine development, especially for neurodevelopment [12,49], prenatal exposure to ST in critical periods of development can lead several consequences in the offspring. Our findings show that gestational exposure to ST (associated or not with stress) on GD 13-20, a critical period of development of serotonergic system in rats, disturbs the somatic and neurobehavioral development in early life, in addition to affecting the reproductive physiology of female offspring and potentially affect the morphology and/or function of the thyroid glands and the reflex maturation.

The application of stress in animals is related to the reduction in serotoninergic neurotransmission, one of the first alterations observed in depression. For this reason, stress is widely used in preclinical studies to investigate the effects of antidepressants [50]. The prenatal exposure to stress [51], as well as exposure to SSRIs [52] has been associated with intrauterine growth restriction, low birth weight and reduced body weight of offspring in the preweaning period (PND 1-21). In our experiment, we observed that treatment with ST, regardless of exposure to prenatal maternal stress, reduced the body weight of female offspring on PND 1. On PND 21, ST group continued with reduced body weight, but not the Stress/ST group, which may more precisely model the clinical situation [32].

Low birth weight reflects an inadequate intrauterine environment and is related to long-term negative consequences for development [53]. SERT is highly expressed in the placenta and it is likely that SSRIs may have high affinity binding sites in this organ [17]. By this way, ST can disrupt 5-HT homeostasis in the fetoplacental unit, which leads to a transient reduction in uterine blood flow and consequent reduction in fetal growth [9,54].

Both intrauterine and postnatal growths are markers correlated with fetal programming [59]. Body weight gain in the preweaning period was reduced in the ST group, which suggests

that the pups consumed less milk in this period, although this parameter has not been evaluated in the present experiment. This result can be due to an impact on serotonergic signaling, as 5-HT has a well-established role in controlling food consumption and following, body weight gain [60]. Accordingly, the mothers treated with ST had lower weight at the end of treatment. In addition, weight and feeding may also be altered by indirect mechanisms associated with developmental exposure to ST, including altered brain-gut relationship, metabolic consequences, and behavioral disturbances [54].

As well as depressive symptoms, symptoms of anxiety are mediated by dysregulation of the 5-HT system [57]. Although depression and anxiety does not occur in the same way in rats and humans, similar neuroanatomical pathways regulate these emotions in both species [20]. Furthermore, the sequence of brain developmental milestones is similar in rodents and humans [19]. Thus, animal models are important tools to understand how prenatal exposure to ST impacts on neurobehavioral development throughout life [20].

Elevated plus maze is one of the main tests used in experimental practice to assess anxiety in rats [58]. These animals usually prefer to stay in closed arms and avoid the exploration of open arms [59]. Thus, number of entries into open arms, time spent into the open arms and total number of entries can be used as a measure of anxiety or fear-induced inhibition of normal exploratory activity [60,61]. In this test, our results showed that modulation of the 5-HT system via developmental exposure to ST, no stress association, produce profile consistent with enhanced anxiety, as evident by the lower exploration of open and closed arms.

Most studies report that prenatal exposure to SSRIs in rats is related to the increased prevalence of anxiety and depression-like behaviors in juvenile and adulthood in a sex-dependent way [20,49,62,63]. The neurobehavioral effects after prenatal exposure to SSRIs are related to functional, structural or epigenetic changes in the development of neural circuits mediating behavior in the raphe nucleus, hippocampus, prefrontal cortex, amygdala and

hypothalamus [49,64]. For example, intrauterine exposure to SSRIs has been associated with the decrease of SERT expression of juvenile and adult animals in the raphe nucleus, hippocampus and medial prefrontal cortex [49,63].

On the other hand, no statistical difference was observed in elevated plus maze when we evaluated the adult offspring. Previous findings by our research group have shown that *in utero* exposure to ST (20 mg/Kg/day) on DG 13-20 decreases exploration in juvenile male offspring (anxious profile), but does not promote changes in the results of elevated plus maze in adult male offspring [21]. In the experiment by Cabrera-Vera and Battaglia [65], SERT densities was altered in the hippocampus, amygdala and hypothalamus of juvenile rats (PND 28) exposed to SSRI fluoxetine on the same exposure period as our experiment, but there was no difference in SERT densities in adult offspring (PND 70). Together, these results suggest that the neurobehavioral effects resulting from exposure to SSRIs in this period observed in the elevated plus maze are transient.

It is important to note that behavior in the elevated plus maze was altered only the ST group, and not the Stress/ST group, which demonstrates that presence of maternal stress interferes with the effects of ST on the female offspring. Data from animal studies have shown that stress during pregnancy also induces neurobehavioral changes in the offspring, increasing [66,67] or decreasing [11,68] anxiety related behaviors. Stress-related hormones such as corticotropin releasing hormone (CRH), catecholamines and glucocorticoids are transported across the placenta, so they can reach and affect the fetal brain development [69]. Interestingly, neurobehavioral anomalies observed in animals exposed to stress were restored following treatment with SSRIs, such as ST [70–73].

The assessment of reflex developmental milestones is used to assess the maturation of the nervous system [74]. Since 5-HT is essential in the regulation of neurodevelopment, an imbalance in fetal 5-HT levels may alter the normal progression of development of the central

nervous system and, consequently, delay in the maturation of neurodevelopmental reflexes [74,75]. In the current study, a delayed trend of disappearance of the palmar reflex, which assesses the integrity of the pyramidal pathway [76], was observed in the stress/ST group. According to this, results of Riccio et al. [77] indicate that excess of 5-HT in development decreases the migration speed of cortical pyramidal neurons. Disappearance of palm grasp reflex and the appearance of cliff avoidance, negative geotaxis and righting reflex were also delayed after early exposure to different doses of ST in other studies [78–80].

In addition to reflex development, our findings demonstrate that imbalance in fetal 5-HT levels can impact somatic development of rat female offspring, delaying fur development and incisor eruption. The 5-HT participates actively in development of craniofacial structures, including tooth development, and *in vitro* developmental toxicity studies show that ST may alter tooth development of offspring by interference with serotonergic regulation of epithelial-mesenchymal interactions important for normal craniofacial morphogenesis [81,82].

Exposure to SSRIs early in life also has the ability to negatively program the reproductive outcomes of female offspring, as demonstrated by other authors [22–24,32,83]. Reproductive programming can manifest as changes in various processes, such as the time of onset of puberty and menstrual/estrous cycles [84]. We showed, for the first time, that prenatal exposure to ST results in long-term changes in offspring reproductive function, manifest as impaired reproductive cycling in both groups treated with ST (ST and Stress/ST) and change in ovarian dynamics of rats exposed to stress combined with ST.

Monitoring the female's estrous cyclicity allows evaluating the integrity of the HPG axis and the impact of treatments on the reproductive function [39,85]. Prenatal exposure to SSRIs has the potential to promote changes in the HPG axis throughout life, as serotonergic system plays a key role in the development and function of the HPG axis and of systems that regulate this axis [23,31].

Moore et al. [83] also found prolonged estrous cycle after perinatal exposure to a SSRI. These authors suggested that altered estrous cycle was due to altered genes that regulate serotonin signaling and action in the ovary. Herein we observe altered follicle dynamics in the stress/ST group, but not in the ST group.

It is known that the components of serotonergic synthesis and signaling are located in the ovary and, therefore, 5-HT can modulate follicular development, luteinization and the production of ovarian sex steroids [30]. Thus, it is also possible that ST associated with stress had an impact on the percentage of follicles in the ovary through direct effects on this organ mediated by programmed changes in serotoninergic signaling or by other programmed changes related to the combination of stress and ST. Although we did not perform measurements of ovarian hormones, it is possible that the results of histomorphometry of ovary reflect altered ovarian hormone levels, which are the main responsible for the changes in the uterus [86]. Since we did not observe histomorphometric changes in the uterus on PNDs42 and 80, our hypothesis is that the increase in uterine weight was due to increased uterine luminal fluid.

The SSRIs have been claimed to negatively affect the thyroid function in depressive patients and experimental animals [87,88]. By interfering with the levels of hormones produced by the mother's thyroid, SSRI may affect offspring’s thyroid [89]. Additionally, gestational stress can affect thyroid development and functioning [90] and serotonergic system is implicated in these changes [91].

Changes in the thyroid weight may reflect endocrine perturbations and has good correlation to histopathologic findings [92,93]. Although these hormonal levels and histological outcomes were not measured in the present study, our findings suggest that prenatal exposure to ST, especially associated with stress, has effects on the thyroid of pubertal and adult offspring. The translation of this result must be done with care, as the thyroid of rats is more sensitive to changes triggered by drugs [94].

In conclusion, the present study, in rats, suggests that prenatal exposure to ST, regardless of exposure to stress, disrupts fetal environment and can negatively program serotonin-regulated processes, such as somatic, reflex and neurobehavioral development, and result in dysregulation of the reproductive function in adulthood. In addition, ST treatment, especially combined with stress, altered the thyroid weight of the female offspring, which appears to be due to endocrine dysregulation of the maternal thyroid. Although translation of data from animals to humans must be done with caution, our findings raise a question regarding the safety of ST in human clinic and future studies are encouraged.

5. Declaration of Interest

The authors declare that there is no conflict of interests.

6. Acknowledgments

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Capítulo 2

O trabalho desenvolvido com a exposição lactational de ratas Wistar à sertralina deu origem ao manuscrito “Postnatal development and reproductive parameters of rat female rats exposed to sertraline during lactation” a ser submetido para publicação no periódico Life Sciences (Fator de impacto: 5.037).

Postnatal development and reproductive parameters of female rats exposed to sertraline during lactation

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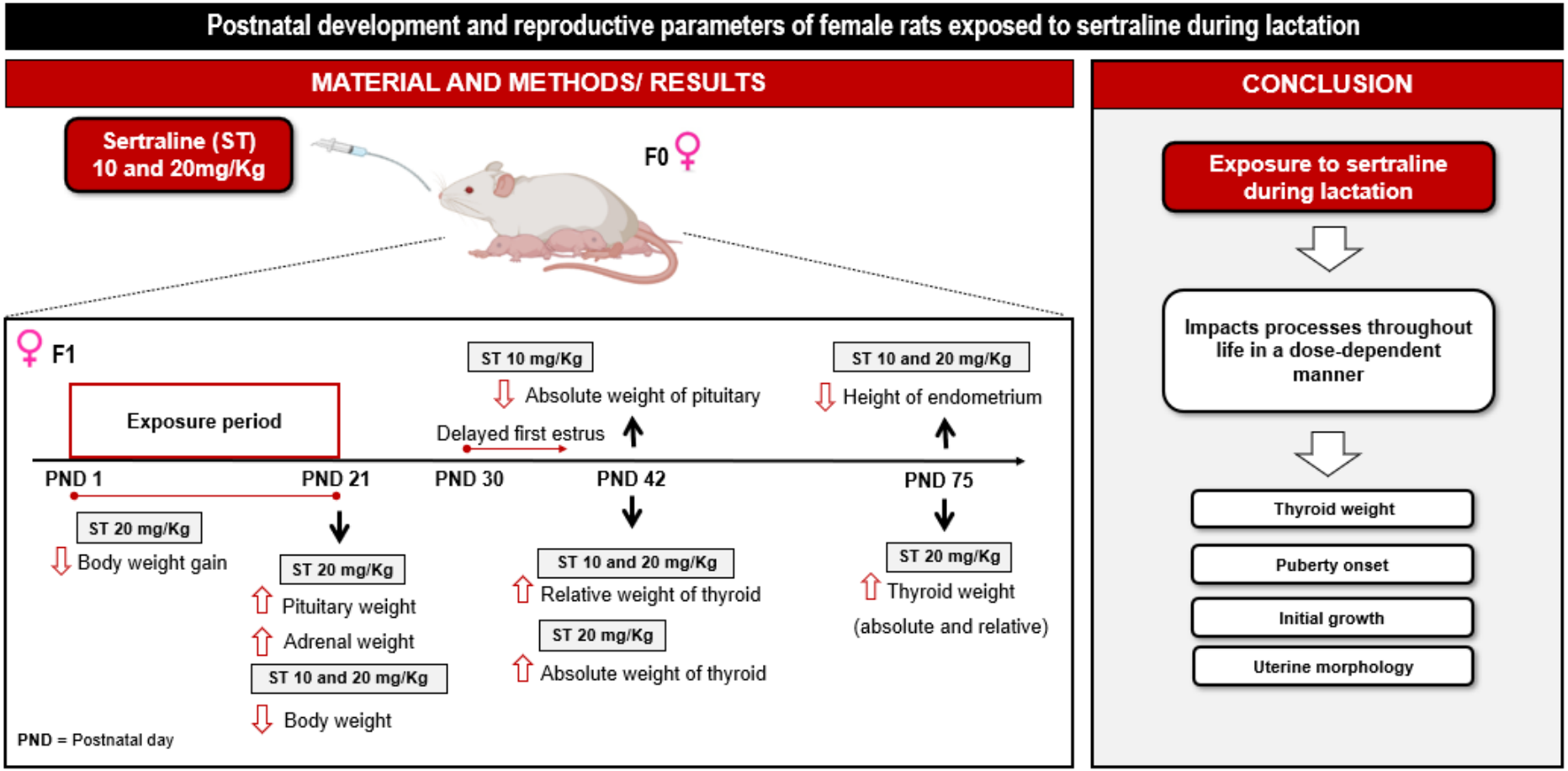


Figure 1. Graphical abstract.

HIGHLIGHTS

- Female rats were postnatally exposed to antidepressant sertraline (10 and 20 mg/Kg)
- Lactational exposure to both doses of sertraline affected initial growth
- Sertraline 20mgKg had effects on weight of adrenal and pituitary on prepuberty
- Sertraline 10mg/Kg delayed puberty onset and reduced pituitary weight at puberty
- Exposure to sertraline altered thyroid weight and adult uterine morphology

ABSTRACT

Aims: This study evaluated the impact of lactational exposure to sertraline (ST) on reproductive parameters of rat female offspring, as well as somatic, reflex and neurobehavioral development.

Main methods: Lactating Wistar rats received ST hydrochloride (10 mg/Kg/day and 20 mg/Kg/day diluted in vehicle) by oral gavage from postnatal day (PND) 1 to 21. Maternal body weight and chow consumption were recorded weekly during treatment. Pups were evaluated on body weight, somatic, reflex and neurobehavioral development from PND 2 and reproductive parameters were evaluated from PND 21.

Key findings: Lactational exposure to both doses of sertraline reduced body weight gain in preweaning period, increased the relative thyroid weights on PND 42 and reduced height of endometrium on PND 75. On PND 75, the relative thyroid weights only continuous increased in the ST 20 mg/Kg group. Female rats of ST 20 mg/Kg group showed increased relative weight of adrenal glands and pituitary on PND 21 and increased thyroid absolute weight on PND 42. In the ST 10mg/Kg group, the absolute weight of pituitary was reduced on PND 42 and the first estrus was delayed. Furthermore, the height of endometrium on PND 75 was reduced by both doses of ST.

Significance: Our findings show that lactational exposure to ST can affect growth, HPA axis, thyroid weight, and sexual development of rat female offspring, depending on the dose, and raise the question regarding the safety of sertraline in human clinic during breastfeeding.

Keywords: sertraline, lactation, postnatal development, female rats, reproductive toxicology.

1. Introduction

Pregnancy and postpartum periods are considered periods of relatively high risk for depressive episodes in women, particularly for those with pre-existing psychiatric illnesses [1]. Approximately one in seven women is treated for depression prior to pregnancy, during pregnancy or after delivery of a liveborn infant [2]. Postpartum depression, frequently in co-morbidity with anxiety symptoms, is the most common complication of childbirth and affects about 10-15% of women [3,4].

In the postpartum period, depression has an impact on maternal behavior and, consequently, on the mother-child relationship, thus damaging the bond and child care [5,6]. Results from studies in humans and animal models show that altered maternal behavior negatively influences the neurological, cognitive, endocrine and motor development of offspring [7,8].

Therefore, it may be necessary to start or continue pharmacological treatment for depression during the lactational period [1]. Serotonin reuptake inhibitors (SSRIs) are considered the antidepressants of choice in the treatment of post-partum affective disorders [9]. This class contains drugs such as fluoxetine, sertraline, paroxetine and citalopram [10], which work by selectively inhibiting reuptake of serotonin (5-HT), promoting an increase in the concentration of serotonin at the post-synaptic nerve terminal membrane [2].

Hundreds of thousands of babies are exposed to SSRIs during early development. However, these drugs and their metabolites cross into breast [9], which has raised questions regarding the potential effects of maternal SSRI treatment on offspring throughout life, since 5-HT is important for the regulation of postnatal development and maturation of the brain [11].

SERT is briefly expressed in non-serotonergic neurons between gestational day (GD) 13 and postnatal day (PND) 21, including glutamatergic neurons in the thalamocortical pathways, pyramidal neurons in the prefrontal cortex and hippocampus, and projection neurons

in the somatosensory and corticolimbic systems, which demonstrates the involvement of 5-HT in the development of several systems [4,12]. In early brain development, 5-HT acts as a trophic factor modulating several developmental processes such as neuronal division, differentiation, migration and synaptogenesis, which undergoes its most extensive development between PNDs 5-20 [13].

Considering that brain is very plastic in lactational period, changes in neurotransmitter level by SSRIs in this period have the potential to alter brain maturation and neurobehavioral development [14]. Furthermore, the early postnatal development of the reproductive system takes place under the control of several factors, which are potential targets of exposure SSRIs [15,16]. Accordingly, animal studies showed that exposure to this class of antidepressants during lactation can cause behavioral, reproductive, endocrine and neural changes [17].

These data demonstrate the need for further studies, which can help choose the best treatment for patients who are breastfeeding. Sertraline (ST) is a first-line drug for breastfeeding women [18], but few data are available about its use during lactation, especially in relation to female offspring. Hence, our objective was to evaluate developmental and reproductive effects of lactational exposure to ST on female offspring.

2. Material and Methods

2.1 Animals

Male and female Wistar rats (90 days old) from the Central Biotherium, São Paulo State University (UNESP), campus Botucatu/SP, Brazil were kept in an environment of controlled temperature (average temperature of 23 °C) and light (12h light/12h darkness), with water and food *ad libitum*. The animals were kept according to the Ethical Principles for Animal Experimentation, adopted by the Brazilian College of Animal Experimentation. The project was filed under protocol number CEUA 3548071220 with the Ethics Committee on Animal Experimentation of the UNESP Institute of Biosciences, in Botucatu.

During the dark period of the cycle, two females was placed in the male cage. After mating confirmation (determined by the presence of sperm in the vaginal smear), the females were weighed and housed in individual cages. The first 24 hours after the confirmation of the mating were considered as GD 0. The day of birth of the offspring was considered PND 0. On PND 1, pups were reduced in each litter (4 males and female pups/ per litter) and were individually marked.

2.2 Experimental design

After birth of the offspring, the dams were divided into three experimental groups (n=9-10/group) and treated orally, on PND 1 to 21, via gavage:

• Control group: The dams received vehicle (filtered water).

• ST 10 mg/Kg group: The dams received ST hydrochloride (10 mg/Kg/day* diluted in vehicle).

• ST 20 mg/Kg group: The dams received ST hydrochloride (20 mg/Kg/day* diluted in vehicle).

*Concentrations previously used in studies with rats [19–22] corresponding to recommended human doses, according with U.S. Food and Drug Administration (FDA) [23].

Maternal body weight and chow consumption were evaluated weekly from PND 1 to 21. Only female offspring were evaluated in the present study. All the male pups were used in a parallel study, to evaluate the effects of ST on the male reproductive system and development. Pups were evaluated on body weight, somatic and reflex development from PND 2. Parameters of reproductive development and function were evaluated from PND 21 (anogenital distance (AGD), puberty onset, genital organs weight, evaluation of estrous cyclicity, sexual behavior, reproductive performance and histomorphometry of ovary and uterus at puberty and adulthood). On PND 25 and 65, elevated plus maze (EPM) was performed to evaluate neurobehavioral development. The Figure 1 describes the experimental procedures performed in this study.

2.3 Body weight, somatic and reflex maturation

Body weight of dams and pups was assessed weekly from PND 1 to 21. The body weight of female offspring also was assessed on PNDs 28, 42 and 75. From PND 2, the somatic and reflex development of the female offspring were evaluated. The separation time of pups from dams during the evaluation of developmental milestones was restricted to less than 5 min to avoid stress. To evaluate somatic development, the following developmental markers are observed: ear unfolding, incisor eruption, eye opening and fur development. Reflex maturation was assessed by analyzing the following milestones: surface righting, negative geotaxis, palmar grasp and cliff aversion, following the methodology described by Coelho et al. [24]. Data were expressed as the mean of the litter and used to calculate the group mean and the reflex onset day was the first of three consecutive days of positive reflex. For the palmar grasp, the reflex disappearance day was evaluated.

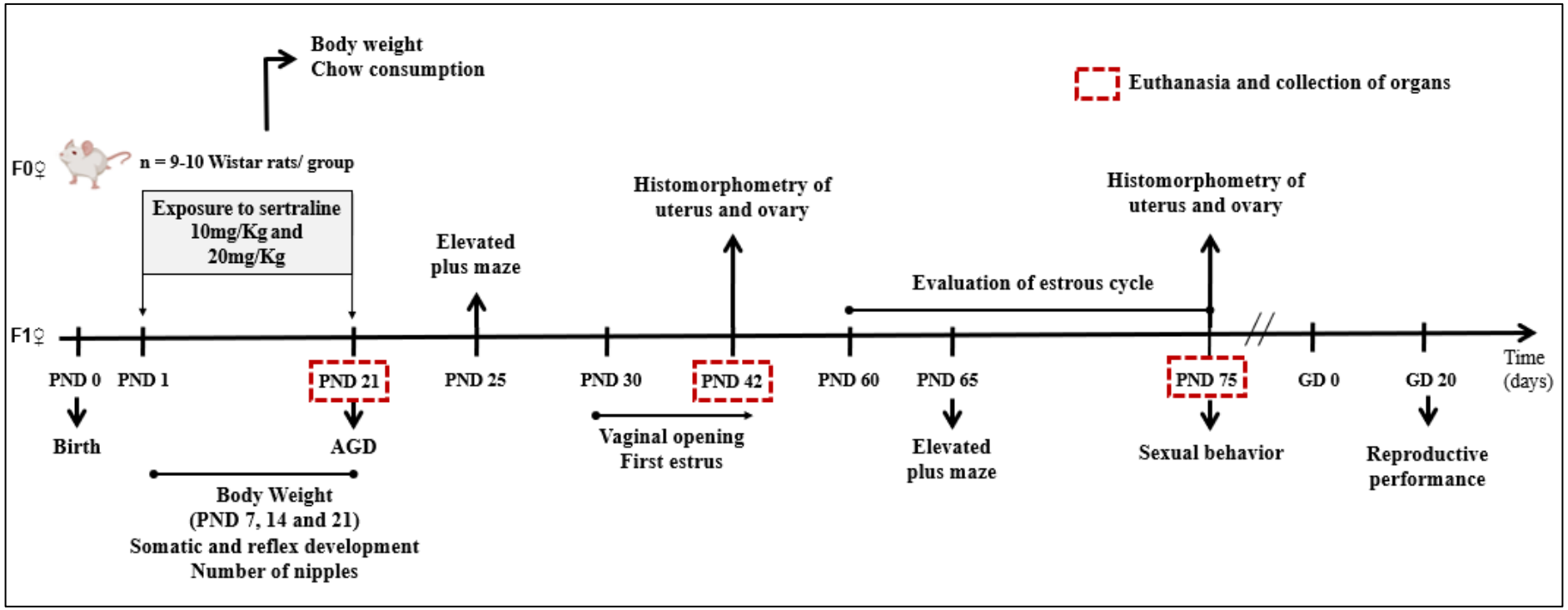


Figure 2. Experimental design of the study. GD: Gestational day; PND: Postnatal day; AGD: anogenital distance.

2.4 Chow consumption

The chow was weighed on PNDs 1, 7, 14 and 21 and the weekly consumption was calculated by subtracting the value corresponding to the rest of the chow from the total chow placed on the previous weighing day.

2.5 Anogenital distance (AGD)

The AGD (distance from the anus to the genital papilla) was measured at PND 21 and was normalized by its ratio by the cubic root of the animal body weight [25]. The number of areolas was recorded on PND 13. Observations were scored based on the presence or absence of a nipple bud or a discoloration of the skin surrounding the nipple [26]. Data were expressed as the mean of the litter and used to calculate the group mean.

2.6 Elevated plus maze

The elevated plus maze test was performed in two females per litter to measure anxiety on PND 25 and in the first estrus from PND 65. In adult animals, the experiment was carried out in the first estrus from PND 72 onwards, since there is evidence that the estrous cycle influences the response of female rats in the elevated plus-maze test [27]. The labyrinth consists of two walled arms (closed arms) and two open arms of equal length and width. The animals were placed in the center of the labyrinth facing one of the closed arms and their behavior was recorded for 5 min. The time spent (seconds) in the open arms and the number of entries (all four paws entering an arm) in the open and closed arms and were recorded [28]. This test has been the most extensively used task to assess anxiety and increase in open arm activity (duration and/or entries) reflects anti-anxiety behavior [29]. After the test, the animal was removed from the device and cotton soaked in 5% ethyl alcohol was passed to clean the platform.

2.7 External physical signs of onset of puberty

From PND 30, all females were evaluated for vaginal opening. From the observation of the complete vaginal opening, the estrous cycle was checked daily by a vaginal wash performed with the aid of a pipette containing saline solution, and analyzed freshly with an optical microscope, until the detection of the first estrus (predominance keratinized epithelial cells), as described by Marcondes [30]. Both methods are indicative of the onset of female puberty [31].

2.8 Reproductive cyclicity

Between PND 60 and 75 the estrous cycle was checked daily by means of a vaginal wash performed with the aid of a pipette containing saline solution, and analyzed freshly with an optical microscope. The evaluation was made based on the cellular composition of vaginal fluid, as described by Marcondes et al. [30]: predominance of nucleated epithelial cells (proestrus); predominance of keratinized epithelial cells (estrus); presence of nucleated epithelial cells, keratinized epithelial cells and leukocytes (metestrus); abundant presence of leukocytes (diestrus). Data obtained during the 15 consecutive days of analysis were used to estimate the estrous cycle length (number of days from the first day of a cycle phase to the first day of the next phase), as well as the frequency of each phase of the cycle [32].

2.9 Euthanasia of rats

The females were weighed and euthanized at different periods of life: at weaning (PND 21), at puberty (PND 42, during estrus phase) and at adulthood (PND 75, during estrus phase), by narcosis in CO_2 followed by decapitation. On PND 21, toxicological targets (brain, pituitary, heart, liver, kidneys and adrenal glands) organs were collected and weighed. On PND 42 and 75, toxicological targets (brain, pituitary, thyroid, heart, liver, kidneys and adrenal glands) and

genital (ovaries and uterus) organs were collected and weighed. The uterus and ovary were used for histological evaluation.

2.10 Histomorphometry of uterus and ovary

The ovary was collected and immersed in Bouin's fixative solution, histologically processed and enclosed in Paraplast®. Then, three sections of each organ were cut at a thickness of 5μm, and placed into slides and stained with hematoxylin and eosin (H&E). In the ovary, corpus luteum and follicles at different stages of maturation were counted, classified according to Talsness et al. [33] and Guerra et al. [34].

In the uterine sections, the heights of perimetrium, myometrium, endometrium, glandular and luminal epithelium were measured, as described by Silva et al [32]. Histological analyses were performed under light microscopy, with softwares Leica LASCore, version 4.12.0 and Image J 1.48.

2.11 Sexual behavior and reproductive performance

From the first estrus after PND 75, one female per litter was used to assess sexual behavior. After detection of the estrous phase, female rats were put into cages of sexually experienced male rats, then allowed 10 mounts on the females while registering the presence of lordosis. The results were expressed as the lordosis quotient (number of lordosis/ten mounts x 100) [35].

After the end of sexual behavior test, the same female was maintained with the same male for additional 8h. The mating was confirmed by the presence of sperm in the vaginal smear and the females were weighed and housed in individual cages. The day when sperm was found in the vaginal smear was considered GD 0.

On GD 20, the naturally inseminated females were euthanized by CO2 inhalation followed by decapitation, to allow the assessment and calculation of fertility. The gestational rate (number of pregnant females/number of inseminated females x 100) was calculated to each group. After collecting the gravid uteri and ovaries, the numbers of corpus luteum, implantation sites, resorptions, live and dead fetuses were determined. From these results, the following parameters were calculated: fertility potential (number of implantation sites/corpus luteum × 100), rate of pre-implantation loss: (number of corpus luteum - number of implantations/number of corpus luteum) × 100, rate of post-implantation loss: (number of implantations - number of live fetuses / number of implantations) × 100 and sexual ratio: number of male fetuses / number of female fetuses [32,34]. In addition, fetal and placental weights were collected, as well as the final body weight and weight of the gravid uteri.

2.12 Statistical analysis

Data are presented as mean ± standard error of mean (S.E.M.), median and interquartile intervals or percentage. The results were compared among groups by one-way analysis of variance (ANOVA) followed by Tukey's test, for parametric variables, and by Kruskal-Wallis followed by Dunn's test or Chi-Square test, for nonparametric variables. Differences were considered statistically significant when $p \leq 0.05$, trends were discussed if $p \leq 0.07$. Statistical analyses were performed using the software GraphPad Prism (version 8.0).

3. Results

3.1 Maternal data

Exposure to ST did not change the maternal body weight on PNDs 7, 14 and 21 or the body weight gain of dams during the treatment period (p = 0.1979) (Figure 3). Chow consumption also was similar among groups in the lactational period (Figure 3).

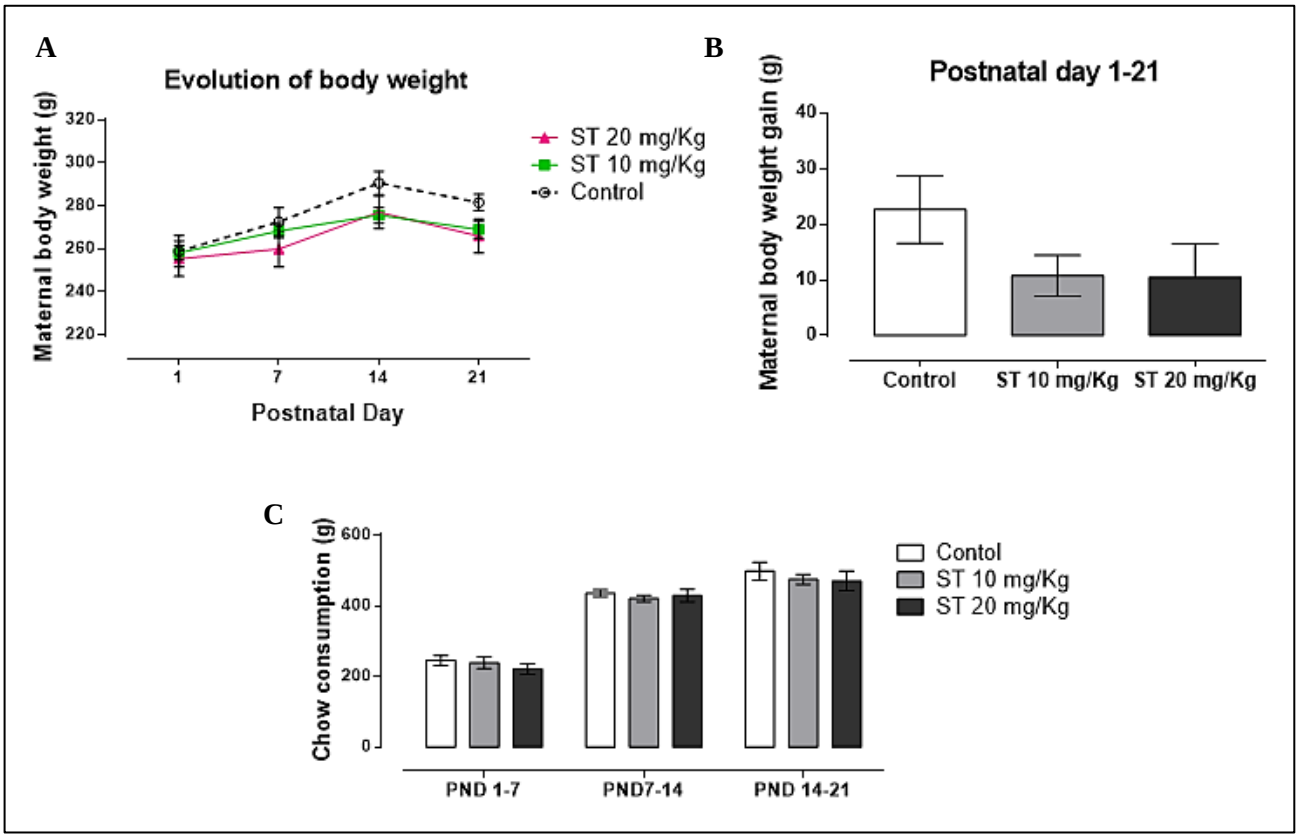


Figure 3. (A) Maternal body weight on postnatal days 1, 7, 14 and 21 during treatment with sertraline (ST) (n=9/10). (B) Maternal body weight gain during treatment period (postnatal days 1 to 21). (C) Weekly chow consumption during treatment period (n=9/10). Values are expressed as mean ± S.E.M. ANOVA followed by Tukey's test, p > 0.05.

3.2 Body weight

Exposure to highest dose of ST (20 mg/Kg) reduced body weight on PND 7 (p = 0.0419), while both doses (10 and 20 mg/Kg) reduced body weight on PND 21 (p = 0.0342) (Figure 4). The body weight gain was also reduced in both groups in preweaning period (Figure 4).

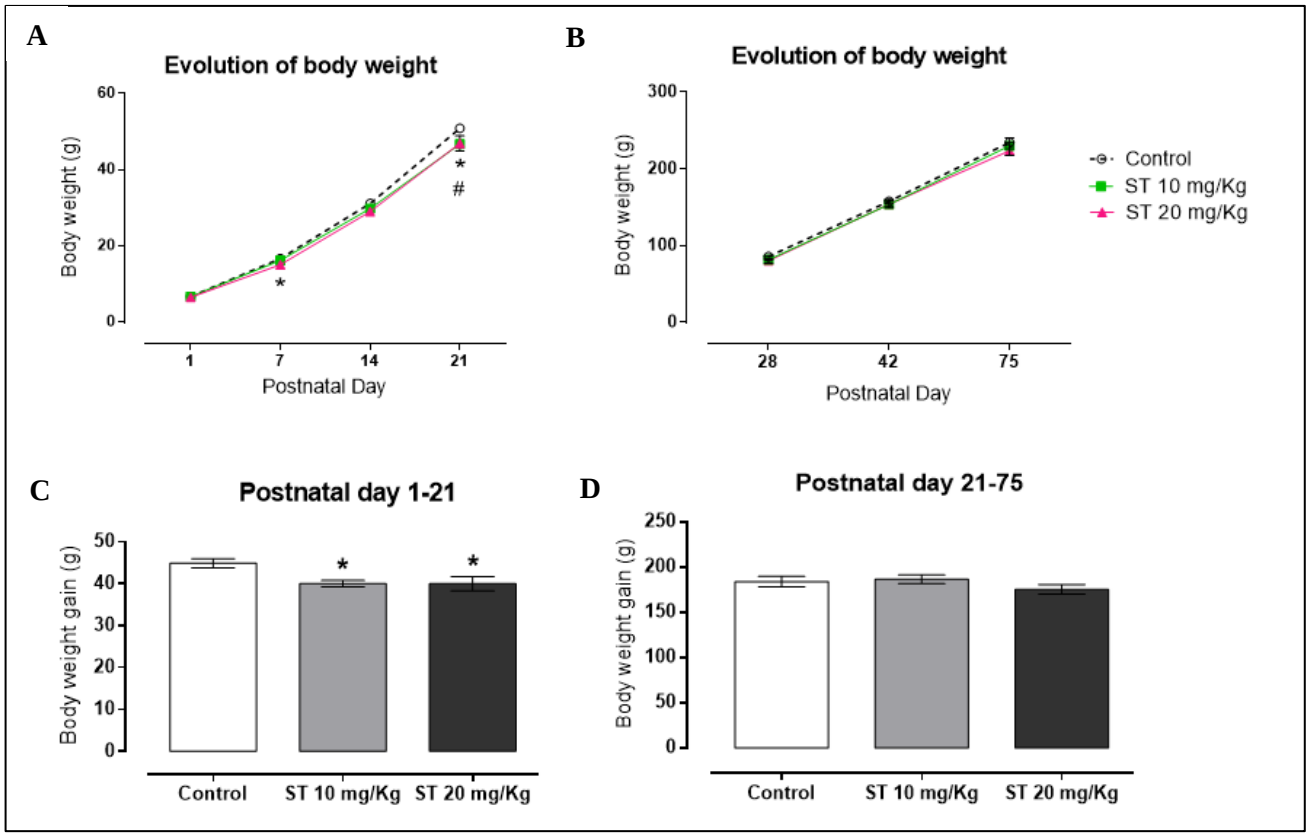


Figure 4. (A) Body weight on postnatal days 1, 7, 14 and 21 of female rats whose mothers were exposed to sertraline (ST) during lactation (n=9/10). (B) Body weight on postnatal days 28, 42 and 75 (n=9/10). (C) Body weight gain during lactational period (n=9/10). (D) Body weight gain on postnatal days 42 to 75 (n=9/10). Values are expressed as mean ± S.E.M. $^{*}p \leq 0.05$, for ST 10 mg/Kg vs. control (ANOVA followed by Tukey's test) $^{\#}p \leq 0.05$, for ST 20 mg/Kg vs. control (ANOVA followed by Tukey's test). The litter mean was used as a statistical unity in this analysis.

3.3 Somatic, reflex and neurobehavioral development

Postnatal day of occurrence of somatic (ear unfolding, fur development, incisor eruption and eye opening) and reflex (surface righting, palmar grasp, cliff avoidance and negative geotaxis) milestones, present in Table 1, were similar among the experimental groups, as well as the parameters evaluated in the elevated plus maze (Figure 5), used to assess neurobehavioral development.

Table 1. Postnatal day of occurrence of somatic and reflex milestones of rat female offspring whose mothers were exposed to sertraline (ST) during lactation.

Parameters	Experimental groups (n= 9/10)		
	Control	ST 10 mg/Kg	ST 20 mg/Kg
Physical landmarks			
Ear unfolding	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
Fur development	7.11 ± 0.06	7.13 ± 0.07	7.14 ± 0.09
Incisor eruption	10.41 ± 0.14	10.43 ± 0.13	10.50 ± 0.10
Eye opening	14.22 ± 0.07	14.22 ± 0.08	14.42 ± 0.11
Reflex maturation			
Surface righting	1.94 ± 0.07	2.23 ± 0.26	2.44 ± 0.19
Cliff avoidance	4.86 ± 0.16	5.11 ± 0.20	5.44 ± 0.44
Negative geotaxis	6.32 ± 0.31	6.37 ± 0.20	6.19 ± 0.31
Palmar grasp	7.17 ± 0.13	7.61 ± 0.15	7.25 ± 0.21

Values are expressed as mean ± S.E.M. ANOVA followed by Tukey's test, $p > 0.05$. The litter mean was used as a statistical unity in this analysis.

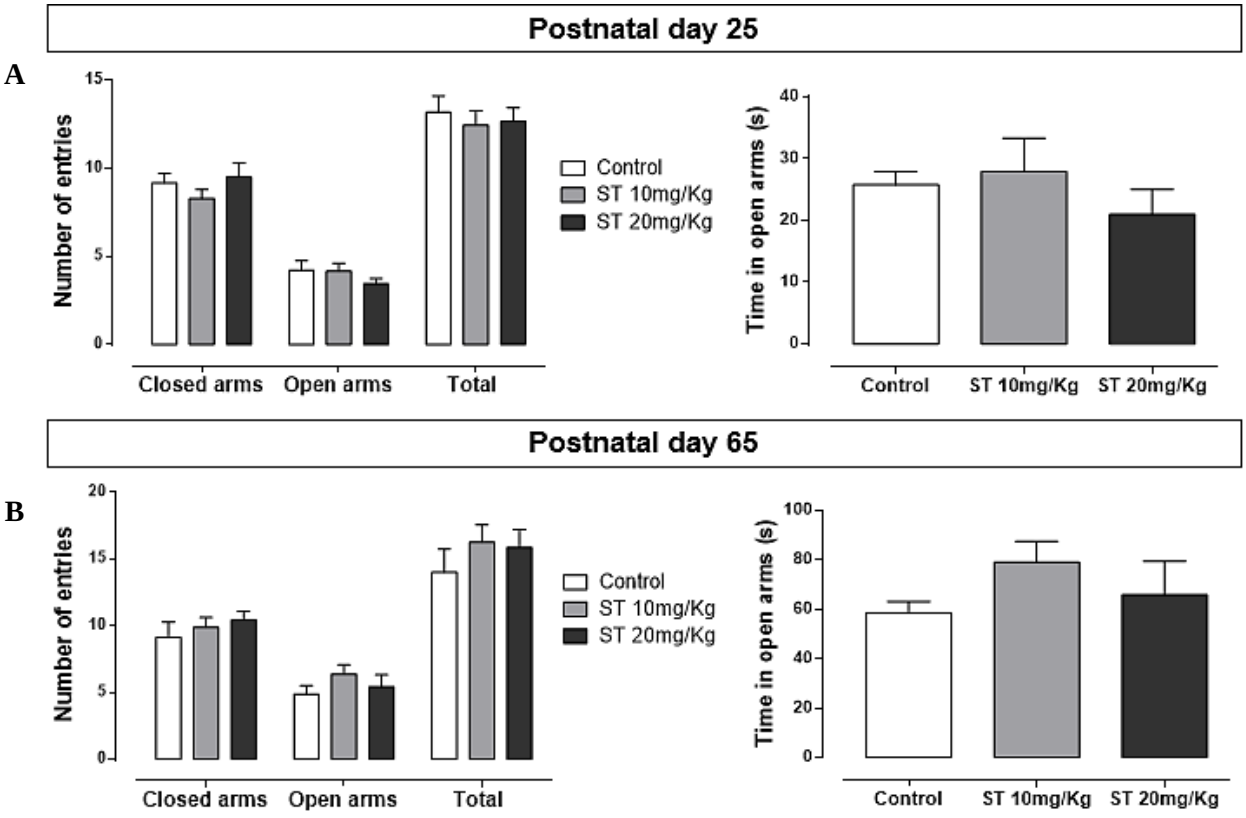


Figure 5. (A) Results of the elevated plus maze performed on postnatal day 25 of female rats postnatally exposed to sertraline (ST) (n=9/10). (B) Results of the elevated plus maze performed on postnatal day 65 during estrus phase (n=9/10). Values are expressed as mean +

S.E.M. ANOVA followed by Tukey's test, $p > 0.05$. Two females per litter were used to calculate the group mean.

3.4 Body weight and organ weights

Female rats postnatally exposed to ST 20 mg/Kg showed increased adrenal ($p = 0.021$) and pituitary weights ($p = 0.04$) on PND 21 (Table 2). Lactational exposure to both doses of ST increased the relative thyroid weights on PND 42 ($p = 0.0045$), while the absolute thyroid weight was increased only in the group exposed to higher dose (Table 3). On PND 75, the absolute and relative thyroid weights continuous increased in the ST 20mg/Kg group ($p = 0.0190$ and $p = 0.0134$, respectively) (Table 4). The other absolute and relative organ weights were not altered by exposure to ST.

Table 2. Body, absolute and relative organ weights on postnatal day 21 of female rats whose mothers were exposed to sertraline (ST) during lactation.

Parameters	Experimental groups (n= 9)		
	Control	ST 10 mg/Kg	ST 20 mg/Kg
Body weight (g)	50.24 ± 1.20	46.94 ± 1.15	44.48 ± 1.29 *
Absolute organ weights			
Brain (g)	1.41 ± 0.02	1.44 ± 0.02	1.36 ± 0.02
Pituitary (mg)	2.33 ± 0.16	2.26 ± 0.07	2.56 ± 0.13
Liver (g)	1.81 ± 0.04	1.69 ± 0.06	1.72 ± 0.09
Heart (mg)	271.00 ± 12.47	240.30 ± 10.74	232.70 ± 10.27
Kidneys (mg)	562.00 ± 15.96	525.40 ± 11.37	524.9 ± 21.64
Adrenal glands (mg)	16.67 ± 0.72	15.31 ± 0.56	16.93 ± 0.48
Lungs (mg)	455.30 ± 15.55	483.70 ± 23.12	442.40 ± 15.79
Relative organ weights			
Brain (g/100g)	2.81 ± 0.06	3.06 ± 0.07	2.98 ± 0.09
Pituitary (mg/100g)	4.65 ± 0.32	4.67 ± 0.17	5.62 ± 0.32 *
Liver (g/100g)	3.61 ± 0.08	3.59 ± 0.06	3.73 ± 0.1
Heart (mg/100g)	539.50 ± 29.80	511.50 ± 17.98	506.30 ± 11.64
Kidneys (g/100g)	1.12 ± 0.03	1.12 ± 0.02	1.14 ± 0.03
Adrenal glands (mg/100g)	33.10 ± 1.50	32.73 ± 1.30	38.16 ± 1.36 *
Lungs (g/100g)	0.92 ± 0.03	1.03 ± 0.04	0.97 ± 0.05

Values are expressed as mean ± S.E.M. ANOVA followed by Tukey's test. *$p \leq 0.05$ compared to control group. One female per litter was used to calculate the group mean.

Table 3. Body, absolute and relative organ weights on postnatal day 42 of female rats whose mothers were exposed to sertraline (ST) during lactation.

Parameters	Experimental groups (n= 9/10)		
	Control	ST 10 mg/Kg	ST 20 mg/Kg
Body weight (g)	163.60 ± 2.21	154.20 ± 2.66	156.30 ± 4.51
Absolute organ weights			
Uterus with fluid (mg)	271.30 ± 9.06	255.30 ± 11.61	279.10 ± 11.41
Ovary (mg)	59.86 ± 5.27	58.83 ± 4.43	61.43 ± 3.24
Brain (g)	1.71 ± 0.02	1.72 ± 0.03	1.74 ± 0.02
Pituitary (mg)	8.25 ± 0.37	6.76 ± 0.22 *	7.91 ± 0.57
Thyroid (mg)	10.19 ± 0.32	11.44 ± 0.72	13.20 ± 0.88 *
Liver (g)	7.52 ± 0.20	7.11 ± 0.23	7.57 ± 0.29
Heart (mg)	742.40 ± 22.77	690.10 ± 18.82	748.90 ± 23.19
Kidneys (g)	1.64 ± 0.04	1.54 ± 0.04	1.64 ± 0.06
Adrenal glands (mg)	58.87 ± 2.18	55.69 ± 2.04	55.54 ± 2.11
Lungs (g)	1.05 ± 0.03	1.00 ± 0.04	1.03 ± 0.07
Relative organ weights			
Uterus with fluid (mg/100g)	165.80 ± 4.90	165.01 ± 5.56	183.10 ± 6.77
Ovary (mg/100g)	42.78 ± 3.29	38.22 ± 2.97	40.00 ± 2.43
Brain (g/100g)	1.05 ± 0.02	1.12 ± 0.02	1.10 ± 0.03
Pituitary (mg/100g)	4.86 ± 0.27	4.38 ± 0.22	4.69 ± 0.27
Thyroid (mg/100g)	6.22 ± 0.19	7.40 ± 0.43 *	7.95 ± 0.36 **
Liver (g/100g)	4.60 ± 0.14	4.61 ± 0.12	4.83 ± 0.09
Heart (mg/100g)	453.80 ± 13.13	447.30 ± 9.06	479.80 ± 10.46
Kidneys (g/100g)	1.00 ± 0.03	1.00 ± 0.01	1.05 ± 0.02
Adrenal glands (mg/100g)	36.07 ± 1.54	36.07 ± 1.05	35.55 ± 0.97
Lungs (mg/100g)	646.60 ± 17.86	644.90 ± 30.83	657.60 ± 35.88

Values are expressed as mean ± S.E.M. ANOVA followed by Tukey's test. *$p \leq 0.05$, **$p < 0.01$ compared to control group. One female per litter was used to calculate the group mean.

Table 4. Body, absolute and relative organ weights on postnatal day 75 of female rats whose mothers were exposed to sertraline (ST) during lactation.

Parameters	Experimental groups (n= 8/9)		
	Control	ST 10 mg/Kg	ST 20 mg/Kg
Body weight (g)	234.30 ± 6.78	231.10 ± 5.31	223.50 ± 6.18
Absolute organ weights			
Uterus with fluid (mg)	398.40 ± 23.94	415.40 ± 16.5	383.10 ± 13.14
Ovary (mg)	89.54 ± 5.02	82.20 ± 3.72	77.81 ± 4.30
Brain (mg)	1.81 ± 0.02	1.87 ± 0.03	1.83 ± 0.03
Pituitary (mg)	9.37 ± 0.49	8.82 ± 0.54	8.43 ± 0.86
Thyroid (mg)	13.61 ± 0.71	13.02 ± 0.59	15.76 ± 0.68*
Liver (g)	8.56 ± 0.23	8.55 ± 0.34	8.37 ± 0.42
Heart (mg)	867.7 ± 37.42	872.6 ± 25.98	877.00 ± 24.48
Kidneys (g)	1.87 ± 0.43	1.82 ± 0.40	1.79 ± 0.60
Adrenal glands (mg)	87.20 ± 2.57	87.04 ± 2.60	77.54 ± 5.15
Lungs (mg)	1.42 ± 0.09	1.41 ± 0.09	1.25 ± 0.09
Relative organ weights			
Uterus with fluid (mg/100g)	157.60 ± 4.63	180.20 ± 7.38	171.30 ± 3.19
Ovary (mg/100g)	38.37 ± 2.25	35.63 ± 1.56	34.72 ± 1.31
Brain (mg/100g)	782.70 ± 17.19	801.80 ± 10.75	820.80 ± 17.05
Pituitary (mg/100g)	4.00 ± 0.17	3.80 ± 0.25	3.78 ± 0.40
Thyroid (mg/100g)	5.85 ± 0.37	5.60 ± 0.25	7.10 ± 0.42*
Liver (g/100g)	3.67 ± 0.08	3.69 ± 0.08	4.24 ± 0.1
Heart (mg/100g)	372.6 ± 17.15	378.0 ± 9.49	392.9 ± 8.43
Kidneys (mg/100g)	801.70 ± 16.42	787.70 ± 15.46	800.90 ± 20.04
Adrenal glands (mg/100g)	37.28 ± 0.88	37.74 ± 1.06	34.53 ± 1.67
Lungs (mg/100g)	608.00 ± 37.38	612.30 ± 40.81	563.90 ± 42.93

Values are expressed as mean ± S.E.M. ANOVA followed by Tukey's test. *$p \leq 0.05$ compared to control group. One female per litter was used to calculate the group mean.

3.5 AGD, number of nipples and puberty onset

The AGD and the number of nipples (Figure 6) did not present statistical difference among groups. The age of the vaginal opening was not altered in the present experiment, but the age of occurrence of the first estrus was delayed in the ST 10 mg/Kg group compared to control group; p = 0.0452 (Figure 7).

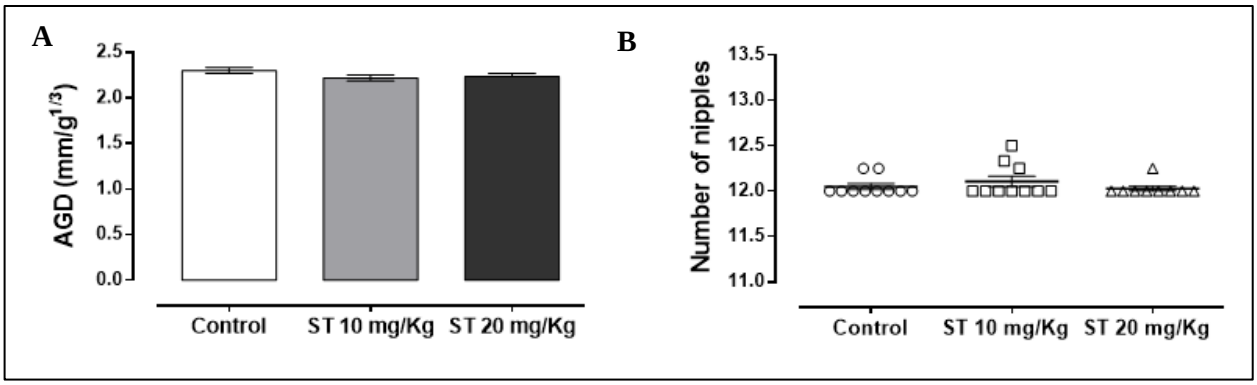


Figure 6. (A) Relative anogenital distance (AGD) on postnatal day 21 of female rats whose mothers were exposed to sertraline (ST) during lactation (n=9/10). (B) Number of nipples on PND 13 (n=9/10). Values are expressed as mean ± S.E.M. ANOVA followed by Tukey's test, p > 0.05. The litter mean was used as a statistical unity in both analyses.

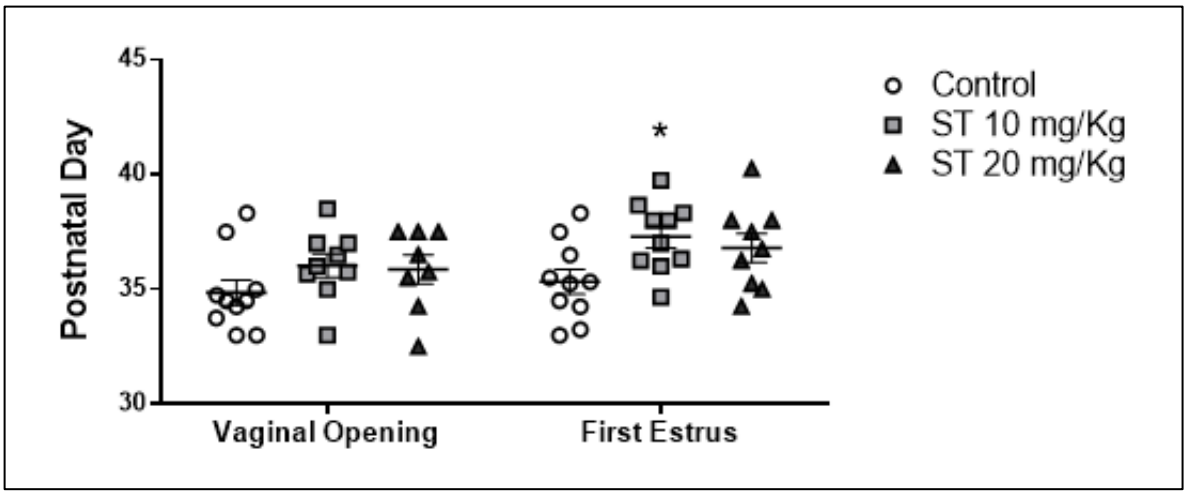


Figure 7. Postnatal day of the vaginal opening and occurrence of the first estrus of rat female offspring whose mothers were exposed to sertraline (ST) during lactation (n=9/10). Values are expressed as mean ± S.E.M. ANOVA followed by Tukey's test, *p ≤ 0.05. The litter was used to calculate the group mean.

3.6 Estrous cycle, sexual behavior and reproductive performance

The estrous cyclicity (Table 5) was similar among experimental groups, as well as the sexual behavior (measured by lordosis quotients present in Table 6) and the parameters used to evaluate the reproductive performance (Table 6) (gestational rate, fertility potential, sex ratio, fetal weight, placenta weight, pre-implantation loss, post-implantation loss and numbers of implantation sites, corpora lutea, fetus, and resorptions).

Table 5. Estrous cycle assessment over a period of 15 consecutive days of evaluation of exposed rat female offspring to sertraline (ST) starting at PND 60.

	Experimental Groups (n=9/10)		
	Controle	**ST 10 mg/Kg**	**ST 20 mg/Kg**
[1]Frequency of diestrus	25.00 (22.05 - 25.73)	19.87 (18.37 - 23.84)	23.57 (15.54 - 24.82)
[1]Frequency of proestrus	21.76 (17.99 - 23.13)	21.03 (16.72 - 23.56)	22.50 (20.99 - 27.44)
[1]Frequency of estrus	29.58 (26.72 - 31.92)	32.05 (25.35 - 37.18)	29.64 (25.60 - 32.80)
[1]Frequency of metestrus	25.42 (23.12 - 26.61)	25.17 (24.04 - 30.13)	25.87 (21.43 - 29.17)
[2]Estrous cycle length	4.44 ± 0.13	4.46 ± 0.13	4.40 ± 0.15
[2]Number of estrous cycle	3.06 ± 0.06	3.00 ± 0.12	3.06 ± 0.15

[1]Values are expressed as median and interquartile intervals. Kruskal-Wallis test followed by Dunn's test, $p > 0.05$. [2]Values are expressed as mean ± S.E.M. One-way ANOVA test followed by Tukey's test, $p > 0.05$. Data from two females per litter were used to calculate the group mean and median.

Table 6. Sexual behavior and reproductive performance of female rats whose mothers were exposed to sertraline (ST) during lactation.

Parameters	Experimental groups (n=7/8)		
	Control	**ST 10 mg/Kg**	**ST 20 mg/Kg**
[1]Sexual behavior	100.00 (100.00 -100.00)	100.00 (100.00 -100.00)	100.00 (80.00 -100.00)
[2]Gestational rate (%)	100	87.5	100
[1]Fertility potential (%)	100.00 (91.96 - 100.0)	93.30 (83.65 - 100.00)	100.00 (72.73 - 100.00)
[3]Number of corpora lutea	12.83 ± 0.87	12.36 ± 0.34	12.86 ± 0.67
[3]Number of implantations	12.33 ± 0.80	12.14 ± 0.40	12.86 ± 0.67
[3]Number of fetuses	12.00 ± 0.73	12.14 ± 0.40	12.57 ± 0.57
[3]Number of resorptions	0.29 ± 0.18	0.00 ± 0.00	0.29 ± 0.18
[1]Pre-implantation loss (%)	0.00 (0.00 - 7.14)	0.00 (0.00 - 15.38)	0.00 (0.00 - 0.00)
[1]Post-implantation loss (%)	0.00 (0.00 - 7.14)	0.00 (0.00 - 0.00)	0.00 (0.00 - 6.67)
[3]Final body weight (g)	367.20 ± 12.73	375.50 ± 10.81	387.00 ± 21.82
[3]Gravid uterus weight (g)	73.54 ± 3.46	78.38 ± 2.26	82.74 ± 3.69
[3]Fetal weight (g)	4.31 ± 0.07	4.40 ± 0.13	4.42 ± 0.06
[3]Placental weight (g)	0.64 ± 0.02	0.60 ± 0.02	0.64 ± 0.02
[3]Sex ratio (F:M)	0.85 ± 0.17	0.76 ± 0.11	0.88 ± 0.20

[1]Values expressed as median and interquartile intervals. Kruskal-Wallis test followed by Dunn's test. [2]Values expressed as percentage. Chi-square test, $p > 0.05$. [3]Values expressed as mean ± S.E.M. ANOVA followed by Tukey's test, $p > 0.05$. One female per litter was used to calculate the group mean and median.

3.7 Histomorphometry of uterus and ovary

The histomorphometry of ovary on PNDs 42 and 75 (Figure 8 and 9, respectively) did not present statistical difference among groups. The histomorphometry of uterus was similar among groups on PND 42, but the height of endometrium was reduced in both treated groups on PND 75 (Figure 10).

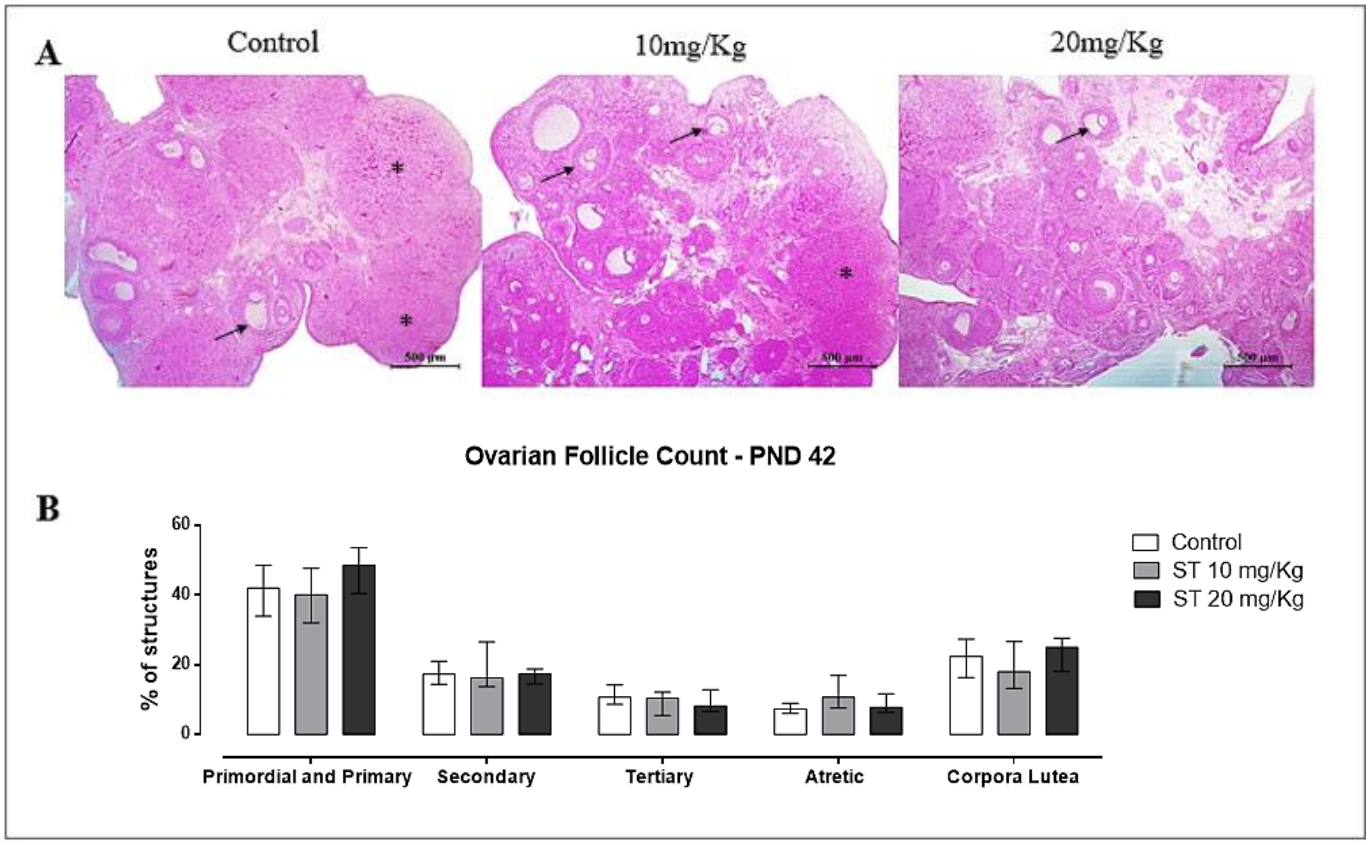


Figure 8. Histological evaluation of ovary, during the estrus phase on postnatal day 42 of female rats whose mothers were exposed to sertraline (ST) during lactation. (A-C) Representative histological aspect of ovaries. Arrows: Tertiary follicles; asterisks: corpora lutea. Hematoxylin and Eosin (HE). Scale bar = 500 µm. (D) Histomorphometric analysis of ovary (n= 6/7). Values expressed as median and interquartile range. Kruskal-Wallis test, followed by Dunn's test. $p > 0.05$. One female per litter was used to calculate the group median.

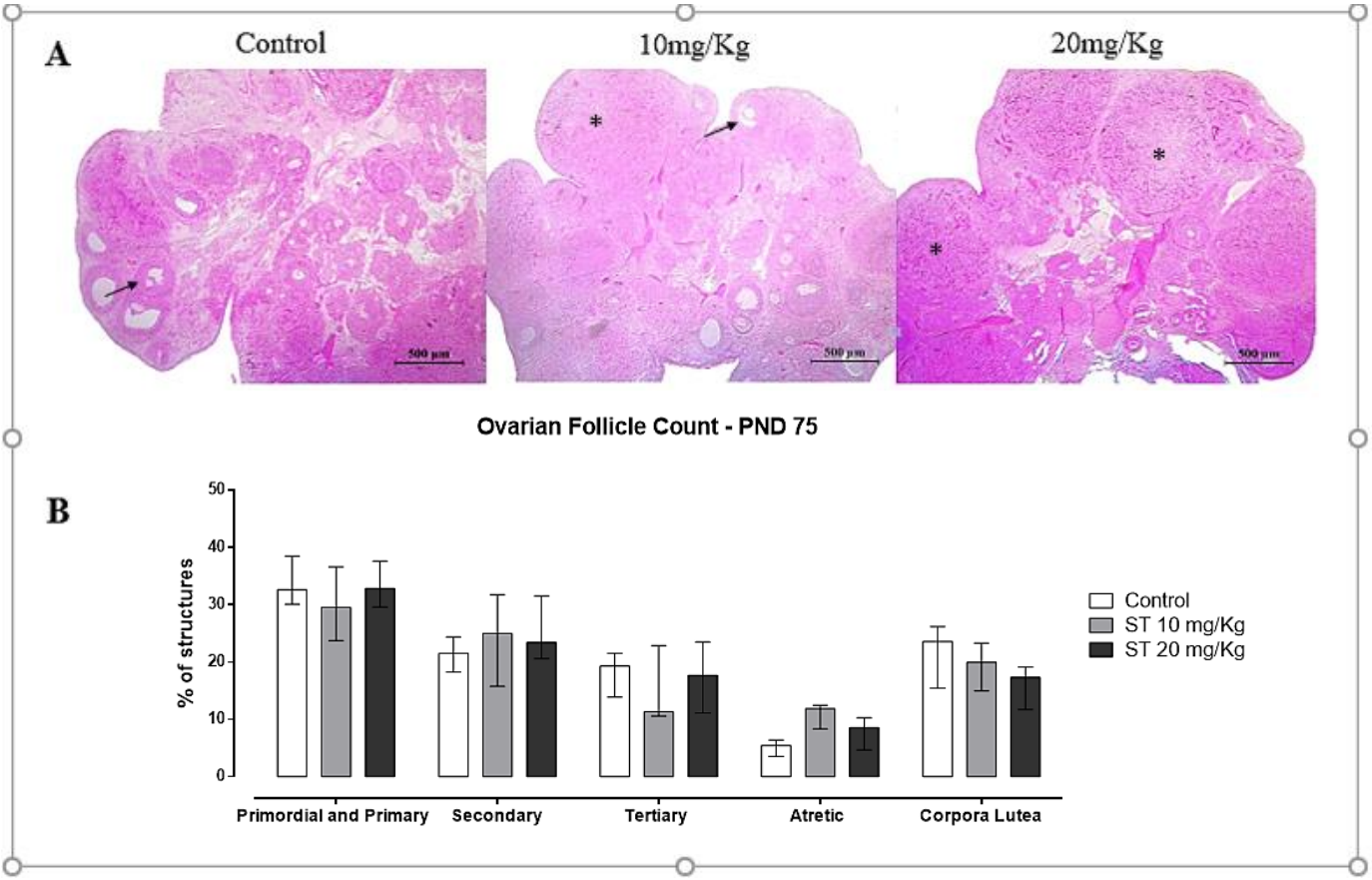
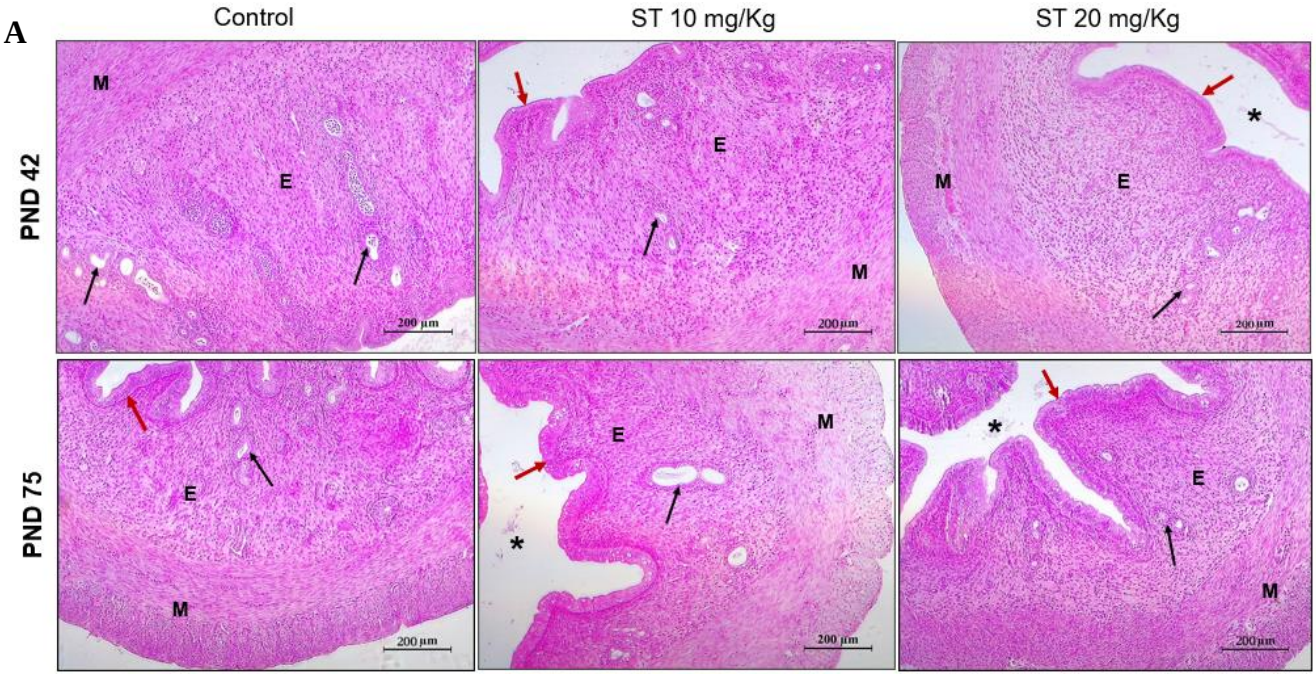
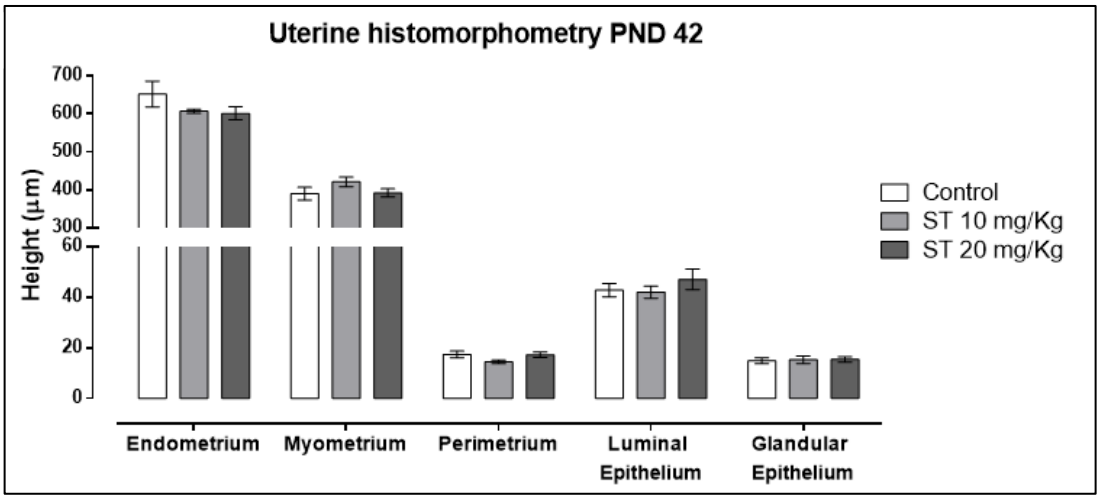


Figure 9. Histological evaluation of the ovaries, during the estrous phase on postnatal day 42 of female offspring whose mothers were exposed to sertraline (ST) during lactation. (A) Representative histological aspect of ovaries. Arrows: Tertiary follicles; asterisks: corpora lutea. Hematoxylin and Eosin (HE). Scale bar = 500 µm. (B) Histomorphometric analysis of ovary (n=5-6/group). Values expressed as median ± interquartilic range. Kruskal-Wallis test followed by Dunn's test, $p > 0.05$. One female per litter was used to calculate the group mean.


A
Control
ST 10 mg/Kg
ST 20 mg/Kg
PND 42
PND 75
M
E
*
200 µm


B
Uterine histomorphometry PND 42
Height (µm)
700
600
500
400
300
60
40
20
0
Control
ST 10 mg/Kg
ST 20 mg/Kg
Endometrium
Myometrium
Perimetrium
Luminal Epithelium
Glandular Epithelium

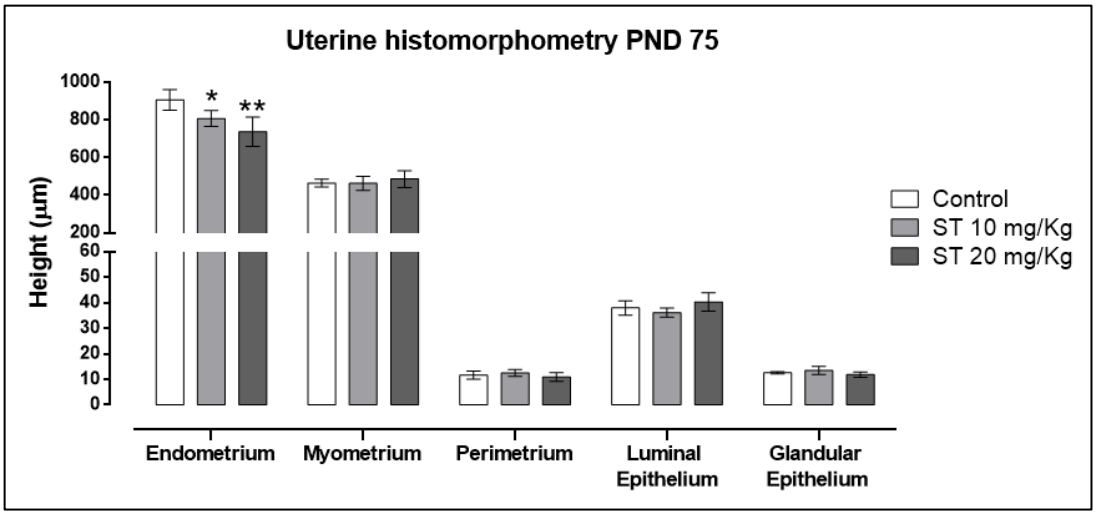

C
Uterine histomorphometry PND 75
Height (µm)
1000
800
600
400
200
60
50
40
30
20
10
0
*
**
Control
ST 10 mg/Kg
ST 20 mg/Kg
Endometrium
Myometrium
Perimetrium
Luminal Epithelium
Glandular Epithelium

Figure 10. Histological evaluation of the uterus, during the estrous phase on postnatal days 42 and 80 of female offspring whose mothers were exposed to sertraline (ST) during lactational period. (A) Representative histological aspect of uterus on PNDs 42 and 75. E: endometrium; M: myometrium; black arrows: uterine glands; red arrows: luminal epithelium. Hematoxylin and Eosin (HE). Scale bar = 200 µm. (G) Histomorphometric analysis of uterus on PND 42 (n= 6/group). (H) Histomorphometric analysis of uterus on PND 75 (n= 5-6/group). Values are expressed as mean ± S.E.M and were analyzed by two-way ANOVA followed by Tukey's test, $p > 0.05$.

Discussion

Although the transfer of ST to human breast milk is low in most cases, it is unclear whether this antidepressant is safe during lactation [36]. Experiments by Capello et al. [37] show that, even in the absence of detectable parent compounds, SSRIs are capable of producing some magnitude of SERT occupation in the central nervous system. Moreover, due to the lower capacity of newborns to metabolize and excrete drugs and metabolites, the presence of this drug in breast milk, even if present in small concentrations, may lead to negative effects in the offspring [38]. Based on this, the present study evaluated the effects of lactational exposure to ST on somatic, reflex, neurobehavioral and reproductive development of female offspring.

It is very important to assess the potential effects of postnatal exposure to ST on body weight, given the well-established role of the serotonergic system in controlling food intake and body weight [39]. Although ST treatment did not change maternal body weight or body weight gain during treatment period, the lactational exposure to both doses of ST (10 and 20 mg/Kg/day) reduced body weight on PND 21, but the body weight was reduced on PND 7 only in the ST 20 mg/Kg group. The lactational exposure to both doses of ST also reduced body weight gain in the preweaning period (PND 1-21), which is in agreement with previous studies [19,40].

Furthermore, the 5-HT system is expressed in the mammary gland and acts as a homeostatic regulator of lactation in a variety of species, including rodents and humans [41,42] and, thus, milk yield can be affected by treatment with ST. In fact, in vitro and in vivo experiments demonstrate that the administration of SSRIs decreases milk yield [41]. Therefore, the reduction in weight gain in this period may have been due to the role of 5-HT in body weight gain, reduced milk production or both mechanisms.

On PND 21, increased relative weight of the adrenal glands and pituitary in the ST 20 mg/Kg group was observed, suggesting chances in hypothalamic-pituitary-adrenal axis (HPA)

axis activity in this period. The 5-HT system is highly involved in regulating HPA axis and adrenocortical secretion of cortisol [43] and changes in serotonergic transmission during critical periods of its development can impact the activity of this axis [44].

Accordingly, animal studies have demonstrated that pre- and postnatal exposure to SSRIs may alter central and peripheral HPA signaling [45,46]. Pawluski et al. [47] showed that exposure fluoxetine during lactation decreases circulating levels of corticosterone and reduces the expression of the glucocorticoid receptor in the hippocampus of adolescent rats. On the other hand, results from Gobinath et al. [48] show that postnatal exposure to fluoxetine on PNDs 2-23 impaired HPA axis negative feedback in adult male, but not female, offspring.

Thyroid and pituitary weight alterations may reflect endocrine perturbations [49]. In addition to the increase in the weight of the adrenal glands, our results demonstrate that exposure to ST by breastfeeding has effects on the thyroid weight at the highest and lowest doses and these effects persisted into adulthood only in rats exposed to the highest dose.

The normal development of the offspring's thyroid is highly influenced by maternal thyroid functioning [50], which may be altered by treatment with ST. There is evidence that SSRIs decrease maternal thyroid function, but the results of these studies are controversial [51,52]. In humans, treatment with ST was correlated with changes in TSH, T3 and T4 levels, although the clinical magnitude of such effect is yet nuclear [51,53]. Therefore, the developmental exposure to ST may affect the thyroid through endocrine disruption of maternal thyroid, and further studies focusing on investigating these impacts are encouraged.

In female rats, external signs of puberty, such as vaginal opening and epithelial cell cornification (first estrus) are external markers of puberty onset [54]. To the puberty establishment, the activation and coordination of hypothalamic-pituitary-gonadal (HPG) axis is highly required [55].

The activation of the HPG axis in prepuberty causes a cascade of events that lead to the onset of puberty. Gonadotropin releasing hormone (GnRH) stimulates the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and, consequently, estradiol [56]. This increase in circulating levels of estradiol promotes the opening of the vaginal canal and then female rodents begin to show estrous cyclicity. The 5-HT system can affect GnRH secretion directly, by acting on GnRH neurons, or indirectly, influencing other neurotransmitters such as norepinephrine [57]. Thus, the delay in sexual maturity may be related to altered hormonal secretion of HPG axis or hormonal signaling, resulting from changes in serotonergic signaling after developmental exposure to ST.

The relative weight of the pituitary also was reduced in the pubertal rats of the ST 10 mg/Kg group, which reinforces the hypothesis that the dysregulation of neuroendocrine control of reproduction after lactational exposure to ST was responsible for the delay in the occurrence of the first estrus. Prenatal and postnatal exposure to SSRIs also delayed the onset of puberty in male and female offspring in other studies [58,59]. Dos Santos et al. [55] suggest that the delay in puberty onset may be due to the delay in estradiol release, which occurred due to changes in the GnRH release after neuroadaptations resulting from repeated exposure to fluoxetine.

Several experimental studies have shown that exposure to SSRIs can lead to adverse endocrine and reproductive effects in different species [60–66]. In rats, the uteri consist of a simple epithelium supported by undifferentiated mesenchyme at birth and this organ develops mainly during the first two postnatal weeks [67]. Thus, exposure to agents that can interfere with the development of the uterus during this period can lead to harmful changes in uterine morphology.

Accordingly, the results of some authors demonstrate that exposure to SSRIs can affect uterine morphology and/or physiology. A clinical study found endometrial hyperplasia in

children exposed to fluoxetine, while *in vivo* and *in vitro* studies showed estrogenic and antiestrogenic activity of fluoxetine and ST [60,68]. Endometrial growth and differentiation can interfere with the implantation of the embryo into the uterus and successful pregnancy and parturition [69,70], but treatment with ST did not impact the fertility of female offspring in the present experiment.

Only the group exposed to the lowest dose of ST has an impact on sexual maturity, which is agreement with the results of other studies. Findings of Munkboel et al. [71] indicate that ST has the capacity to disrupt steroidogenesis especially in the lowest exposure group (1.25 mg/kg). Harris et al. [72] also observed that maximum effects on sexual behavior were achieved with the intermediate dose of 10 mg/kg of SSRI citalopram and not the highest dose (20 mg/kg). Together, these results demonstrate that the effects of SSRIs on reproductive function are likely not dose-dependent.

Serotonergic activity in the developing brain is the highest in rats during the neonatal period up to PND 21 [73]. The assessment of reflex developmental milestones in this period was used to assess the maturation of the nervous system [74], as the manipulation of the serotonergic system by SSRIs during this period has the potential to alter the neurodevelopment and lead to disturbances in brain function and behavior [4,75]. Despite that, no alterations were observed in the reflex maturation and neurobehavioral test (elevated plus maze) in the present study. This is similar to data observed after developmental treatment with other SSRIs [73,76,77].

3. Conclusion

Our findings show that lactational exposure to therapeutically relevant doses of ST, in these experimental conditions, can affect sexual development of rat female offspring, possibly through by interference with the functioning of the HPG axis. In addition, changes were observed on initial growth, HPA axis, thyroid gland and adult morphology of uterus, depending on the dose. These results demonstrate that treatment with sertraline during lactation has effects on developmental and reproductive parameters of female rat offspring at different periods of life and raise the question regarding the safety of ST in human clinic during breastfeeding and additional studies are encouraged to better characterize its mode of action.

4. Declaration of Interest

The authors declare that there is no conflict of interests.

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Conclusão

Com base nos resultados obtidos em ratas, o tratamento com a sertralina na gestação e na lactação tem repercussões em parâmetros reprodutivos da prole feminina, o que pode ter ocorrido por interferência na atividade do eixo HHG e demonstra a necessidade de estudos que investiguem suas implicações para a saúde reprodutiva do ser humano. A exposição à sertralina em ambos os períodos também teve efeitos no crescimento inicial e no peso da tireoide da prole púbere e adulta, o que sugere que a exposição precoce à sertralina tem efeitos deletérios neste órgão, possivelmente devido a alterações promovidas por esse fármaco na tireoide materna. Além disso, a exposição gestacional a este antidepressivo, associada ou não ao estresse, teve efeitos negativos no desenvolvimento somático, de reflexos e neurocomportamental da prole feminina, provavelmente devido à desregulação dos níveis de serotonina em um período crítico do desenvolvimento serotoninérgico. Nossos achados, portanto, levantam o questionamento em relação a segurança da utilização da sertralina na clínica humana nos períodos pré e pós-natal.

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Anexos

Anexo I – Certificado de aprovação da pesquisa pela Comissão de Bioética.

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Botucatu

Instituto de Biociências de Botucatu - Unesp

Certificado

Certificamos que o projeto intitulado "Exposição gestacional ao estresse e ao antidepressivo Sertralina: impacto sobre o desenvolvimento sexual e trato genital da prole masculina de ratos, com ênfase à diferenciação das células de Leydig", Protocolo nº **1169-CEUA**, sob a responsabilidade de **Wilma De Grava Kempinas**, 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) – encontra-se de acordo com os preceitos da Lei nº 11.794, de 9 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela ***COMISSÃO DE ÉTICA NO USO DE ANIMAIS*** (CEUA), nesta data.

Finalidade:	( ) Ensino	(x) Pesquisa Científica
Vigência do Projeto:	Início: 1/8/2019	Término: 30/6/2022
Espécie/linhagem:	*Rato Wistar*	
Nº de animais:	*280*	
Peso:	*300-500g*	Idade: 90 dias
Sexo:	*Macho e fêmea*	
Origem	*Biotério Central da Unesp-Câmpus de Botucatu/SP.*	

Botucatu, 14 de junho de 2019.

Comissão de Ética no Uso de Animais IBB-Unesp

Prof. Assoc. Wellerson Rodrigo Scarano
Coordenador da CEUA

Comissão de Ética no Uso de Animais - CEUA
Instituto de Biociências da Unesp – Câmpus de Botucatu
Rua Prof. Dr. Antonio Celso Wagner Zanin, 250 - Distrito de Rubião Júnior - CEP 18618-689 Botucatu/SP
Tel 14 3880 0851 mail: secdta@ibb.unesp.br

Anexo II - Certificado de aprovação da pesquisa pela Comissão de Bioética.

unesp
UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

Comissão de Ética no Uso de Animais
Instituto de Biociências de Botucatu

CERTIFICADO

Certificamos que a proposta intitulada "Desenvolvimento pós-natal e performance reprodutiva das proles masculina e feminina de ratas Wistar expostas à Sertralina durante a lactação ", protocolada sob o CEUA nº 3548071220 (ID 000158), sob a responsabilidade de **Wilma de Grava Kempinas** *e equipe; Mayara Silva Moura; Ana Flávia Quiarato Lozano* - 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 21/12/2020.

We certify that the proposal "Postnatal development and reproductive performance of male and female offspring of Wistar rats exposed to Sertraline during lactation", utilizing 320 Heterogenics rats (males and females), protocol number CEUA 3548071220 (ID 000158), under the responsibility of **Wilma de Grava Kempinas** *and team; Mayara Silva Moura; Ana Flávia Quiarato Lozano* - 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 12/21/2020.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 01/2021 a 06/2021 Área: Ciências Biológicas

Origem:	Biotério Central da UNESP						
Espécie:	Ratos heterogênicos	sexo:	Machos e Fêmeas	idade:	1 a 120 dias	N:	320
Linhagem:	Wistar			Peso:	3 a 450 g		

Local do experimento: Depto. de Biologia Estrutural e Funcional (Setor de Morfologia), do Instituto de Biociências de Botucatu (IBB - UNESP).

Botucatu, 04 de janeiro de 2021

Prof. Dr. Wellerson Rodrigo Scarano
Coordenador da Comissão de Ética no Uso de Animais
Universidade Estadual Paulista

Prof. Dr. Bruno César Schimming
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade Estadual Paulista

Rua Prof. Dr. Antônio Celso Wagner Zanin, 250 - Distrito de Rubião Junior - Botucatu/SP - CEP 18618-689 - tel: 55 (14) 3880-0851
Horário de atendimento: 2ª a 6ª das 8h às 11h30 - 14h ás 18h : e-mail: ceua.ibb@unesp.br
CEUA N 3548071220