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UNIVERSIDADE ESTADUAL PAULISTA  
“JÚLIO DE MESQUITA FILHO”

UNIVERSIDADE ESTADUAL PAULISTA “JÚLIO DE MESQUITA FILHO”

INSTITUTO DE BIOCÊNCIAS DE BOTUCATU

PROGRAMA DE PÓS-GRADUAÇÃO EM FARMACOLOGIA E BIOTECNOLOGIA

**SUYANE DA SILVA MOREIRA**

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

**Botucatu – SP**

**2021**

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

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preparação para compreender o que há mais além."*

*(Hipátia)*

*Resumo*

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

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

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

*Abstract*

---

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

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

## **LISTA DE ABREVIATURAS E SIGLAS**

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

## LISTA DE SÍMBOLOS

Kg            Kilograma

Mg           Miligrama

## *Sumário*

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## *Capítulo 1*

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

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## **Abbreviations**

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

## **Symbols**

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

## Highlights

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

## **ABSTRACT**

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

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

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