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EFFECTS OF PREGNANCY AND DELIVERY IN THE NGF EXPRESSION IN URETHRA RATS.

Hypothesis / aims of study
Nerve growth factor (NGF) is a protein essential for the development and maintenance of function of the peripheral sympathetic nervous system and certain kinds of sensory neurons[1]. The aim of this study is evaluate the expression of NGF in the urethra of adult female rats during pregnancy and after vaginal delivery (four days and 12 weeks).

Study design, materials and methods
48 rats were divided into four groups. Group A – nuliparous rats; Group B – rats on the 20th day of pregnancy; Group C – rats on the 4th day of puerperium; Group D – rats sacrificed 12 weeks after vaginal delivery. After letal injection, the animals were sacrificed and the urethra and vagina were removed. By means of immune histochemistry using the primary mouse monoclonal antibody, subclass IgG, at the dilution of 1:600, specific for NGF. The immune expression was analyzed by optic microscopy (digital analysis of image system-Imagelab) with the magnification of 400x. The intensity of the dark brown color was used as the protein cytoplasmatic expression of the NGF and quantificated the percentile of epithelial and muscle cells that expressed this neurotrophin.

Results
We observed that in nuliparous rats and pregnant rats the NGF expression was the same in the epithelial and muscle layers. Evaluating four days after vaginal delivery, showed a significant diminished NGF expression in the epithelial layer. We observed an increase of NGF in the muscle layers in rats after 12 weeks of vaginal delivery. NGF was more expressed in the periurethral smooth muscle in all groups, except in pregnant rats. Statistical comparisons were made by Students' *t* test or analysis of variance. Tukey test was used to compaire the epithelial and muscle cells isolated in the groups. Differences were considered significant at the $p<0,05$ level.

Interpretation of results
Different of bladder, adrenergic nerves predominate over sensory nerves in the urethra. The results of this study further establish that NGF expression was regulated by different hormonal stimuli and interaction between sexual hormones and this neurotrophic factor and its neurotransmitters. The regional innervation differences may explain many symptons in different situations[2]. We have demonstrated no statistical differences between the NGF expression in the epithelial or muscle layer of urethra compared to the control group or pregnant group. After delivery the NGF expression in the epithelial layer diminished. This result confirms that NGF secretion is modulated by the hormonal state. The increase of NGF expression in the muscle layer 12 weeks postpartum suggests that at the urethra there may exist a mechanism to maintain and regulate this innervation mediated by NGF and its effects on neuronal survival and morphology. This neurotrophic mechanism may act in combination with strength and hormonal variation in this tissues and may suggest this effects in the lower urinary tract symptoms (LUTS) during pregnancy and puerperium[3].

Concluding message
There are quantitative differences in the genic expression of NGF, depending on the hormonal state, in the epithelium of urethra and periurethral smooth muscle.

References
1. International Review of Cytology, 1991; 128:109-33.
2. Neuroanatomy and neurophysiology: innervation of the lower urinary tract. In: Female Urology. W. B. Saunders Company 2nd ed 1996; 28-42.
3. BJU International 2002; 89:469-76.

Table 1: Comparison of NGF expression between the control group and others groups.

		mean	p value	95 % CI	
Epithelial layer	A = B	2,02	0,97	-4,82	8,87
	A = C	4,15	0,66	-3,73	12,02
	A = D	1,00	1,00	-6,28	8,27
Muscle layer	A = B	5,28	0,24	-1,62	12,17
	A = C	-1,35	1,00	-8,24	5,54
	A = D	-7,43	0,06	-15,12	0,26

A: nuliparous rats; B: pregnant rats; C: rats on 4th day pospartum; D: 12 weeks pospartum.

Table 2: Comparison of NGF expression between epithelial layer and periurethral smooth muscle.

	"t"	p value
Grupo A	-2,49	0,03
Grupo B	-1,30	0,22
Grupo C	-5,03	0,001
Grupo D	-4,44	0,002

A: nuliparous rats; B: pregnant rats; C: rats on 4th day pospartum; D: 12 weeks pospartum.

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Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**

Name of ethics committee	**Comitê de Ética em Pesquisa - UNIFESP - EPM**