Hypothesis / aims of study
Urinary incontinence and pelvic organ prolapse derive from the loss of connective tissue support, which occur as a consequence of trauma (pregnancy and delivery, obesity and chronically elevated abdominal pressure), ageing and hypoestrogenism. The extracellular matrix contributes to a substantial part of connective tissue volume and is responsible for absorbing and resisting most of the mechanical stress the tissue is subjected to (Falconer, 1994). Due to its role on tissues resistance to mechanical trauma, GAG metabolism could possibly be involved in the pathogenesis of urinary incontinence and prolapse. The objective of this study is to test this hypothesis by analysing the impact of vaginal trauma (pregnancy, delivery and simulated delivery) at the concentration of sulfated glycosaminoglycans (GAGs) in suburethral vaginal tissues in rats.

Study design, materials and methods
One hundred and ten female Wistar rats were used for this experimental and divided into ten intervention groups and one control group, as follows:
- Group A: 17 nulligravid rats (control group) sacrificed at ages five (Group A1) and eight (Group A2) months after the simulated trauma, which corresponded to the ages of sacrifice of the other groups subjects.
- Group B: 17 nulligravid rats with simulated delivery trauma sacrificed four days (Group B1) and 12 weeks (Group B2) after the simulated trauma. All the rats were sacrificed at the estrogenic phase of the cycle, which was induced by the presence of a vasectomized rat into the cage.
- Group C: 17 rats submitted to cesarian delivery at the 20th day of pregnancy and sacrificed at four days and 12 weeks after delivery.
- Group D: 12 rats submitted to cesarian delivery at the 20th day of pregnancy, followed by the simulated delivery trauma, and sacrificed at four days (Group D1) and 12 weeks (Group D2) after delivery.
- Group E: 16 rats sacrificed four days (Group E1) and 12 weeks (Group E2) after natural vaginal delivery.
- Group F: seven pregnant rats sacrificed on the 20th days of pregnancy.

The simulated delivery trauma was performed with a 10Fr. Foley balloon introduced into the vagina and inflated with 5ml of saline, after fixation with a stitch on the introitus. The anesthesized rats were kept for 3 hours with a 100g weight tied to the catheter and the pelvis hanging at the edge of the table, granting continuous tension on the vagina (figure). Female rats were sacrificed by lethal injection of anesthetics and exsanguinated. The urinary bladder, urethra and vagina were removed en bloc through an abdominal longitudinal incision. After organ removal, the distal half of the vagina was identified and cut into sections. After exeresis, the specimens were washed with a phosphate buffered solution (PBS) for removal of blood residues and fixed in 2% formalin solution. Vaginal tissues were fragmented in a ketone solution (100%) at room temperature and then were dehydrated and delipidated with 10 volumes of the ketone solution and incubated for 24 hours at 4ºC. The dry material (ketone resin) was submitted to proteolysis with maxatase. Methanol was added for GAG precipitation and centrifuged. The precipitate containing GAGs was resuspended in a 0.05 M sodium acetate buffer solution, 0.02 M MgCl₂, pH 5.0 with desoxiribonuclease I (Sigma Chemical CO, St Louis, MO, US) for 18 hours at 30ºC for degradation of the contaminating DNA remaining in the specimen. GAGs were then identified through electrophoresis in agarose gel and measured by densitometry. Each sulfate GAG was identified by comparing the electrophoretic migration of each specimen with known purified standards. The measure of each compound in densitometric units was then converted into μg/mg of GAGs in dry tissue based on baseline weight of ketone resin. The comparison of the amounts of total GAGs between groups was performed using Kruskal-Wallis Statistics to check for differences of medians between the groups studied both at time point 1 and time point 2. Mann-Whitney’s test was used to compare medians between two groups.

Results
Comparison between groups at T1 (four days after intervention), with Kruskal-Wallis statistics, showed:
- Total GAGs content was significantly lower in groups E1 (natural delivery) and F (pregnant rats), when compared to group A1 (nulligravid controls) (p.<.0001);
- Total GAGs content was significantly lower in groups E1 (natural delivery), when compared to groups B1 (nulligravid with simulated trauma) and C1 (cesarian delivery with simulated trauma) (p.<.0001);
- DS content was significantly lower in groups E1 (natural delivery), when compared to group A1 (nulligravid controls) (p.<.0001);
- DS content was significantly lower in groups E1 (natural delivery), when compared to groups B1 (nulligravid with simulated trauma) and C1 (cesarian delivery) (p.<.0001);
- No difference in heparan sulfate (HS) was observed between (p=1.233);

Comparison between groups at T2 (12 weeks after intervention), with Kruskal-Wallis statistics, showed:
- Total GAGs content was significantly higher in groups B2 (nulligravid with simulated trauma), D2 (cesarian delivery with simulated trauma) and E2 (natural delivery), when compared to group A2 (nulligravid control) (p.<.0001);
- DS content was significantly higher in groups B2 (nulligravid with simulated trauma), D2 (cesarian delivery with simulated trauma) and E2 (natural delivery), when compared to group A2 (nulligravid control) (p.<.0001);
• HS content was significantly higher in groups B2 (nulligravid with simulated trauma), C2 (cesarian delivery), D2 (cesarian delivery with simulated trauma) and E2 (natural delivery), when compared to group A2 (nulligravid control) (p=.0003);

Comparison between times T1 and T2, with Mann-Whitney's test, showed:
• Total GAGs content was significantly higher in T2 in comparison with T1 in groups B (nulligravid with simulated trauma), C (cesarian delivery), D (cesarian delivery with simulated trauma) and E2 (natural delivery) (p<.0001); in group A (nulligravid control), no difference in total GAGs concentration between T1 and T2;
• DS content was significantly higher in T2 in comparison with T1 in groups B (nulligravid with simulated trauma), C (cesarian delivery), D (cesarian delivery with simulated trauma) and E2 (natural delivery) (p<.0001); in group A (nulligravid control), no difference in total GAGs concentration between T1 and T2;
• HS content was significantly higher in T2 in comparison with T1 in groups B (nulligravid with simulated trauma), C (cesarian delivery), D (cesarian delivery with simulated trauma) and E2 (natural delivery) (p<.0001); in group A (nulligravid control), no difference in total GAGs concentration between T1 and T2;

Interpretation of results
Summarizing, DS was the predominant glycosaminoglycan in all groups. In the evaluation four days after the procedures, results showed a significant decrease in total GAGs and DS in groups B1, C1, D1, E1 and in group F when compared to A1. This means that pregnancy seems to have an effect at the extracellular matrix, leading to a decrease, mainly in DS content, regardless of the way of delivery or the occurrence of vaginal trauma.
The evaluation 12 weeks after the procedures indicated a significant increase in total GAGs in all groups, except for controls. This was observed also in group C, which showed no difference in DS or total GAGs concentration, when compared to controls, meaning that this increase in GAGs content is probably the natural regeneration process, when vaginal trauma is not present.
The evaluation 12 weeks after the procedures indicated a significant increase in total GAGs in the groups where vaginal trauma (natural or simulated) was not present, meaning that trauma may break the balance in the regeneration process, leading to and exaggerated DS concentration.

Concluding message
On groups where vaginal trauma was present (natural or simulated), a decrease of total GAGs and DS was observed at four days after delivery, while an increase of total GAGs and DS was observed with ageing (12 weeks after vaginal trauma), probably as a healing mecanism. These data indicate that vaginal trauma lead to an imbalance in DS and GAGs metabolism. This imbalance may be involved at the pathogenesis of prolapse and incontinence as a consequence of vaginal delivery.

References

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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  Comite de Etica em Pesquisa em Animais - UNIFESP