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AUTOLOGOUS MYOBLAST AND FIBROBLAST CELL CULTURES FROM PORCINE BIOPSIES

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INTRODUCTION & OBJECTIVES: Different technologies have been developed in the past few years to generate skeletal muscle cell cultures as a potentially limitless source for tissue engineering and transplantation medicine. In the present study we wanted to demonstrate that fibroblasts as well as self-renewing clones of autologous myoblasts can be prepared from small muscle biopsies of porcine skeletal muscles.

MATERIAL & METHODS: Muscle biopsies were obtained from 24 pigs. Each biopsy was enzymatically dissociated according to a modified procedure of a cell dispersion technique first described by Blau and Webster (1981). Single myoblasts in suspension were manually collected with a micropipette under microscopic control and transferred to the cell culture wells of 95-well plates. The cells were cultured in a growth medium and maintained in a proliferating state for several weeks. Desmin was used as a marker to identify clones of autologous myoblasts. Fusion competence and calcium channels were investigated in all myoblast cultures. In addition, fibroblast cultures were obtained from all 24 biopsies.

RESULTS: Autologous porcine and human myoblasts as well as fibroblasts could be grown without problems. When cultured in differentiation medium, desmin-positive mononucleated myoblasts fused into multinucleated myotubes. After one week spontaneous contractions of the myotubes was observed. L-type Ca^{2+} channels could be detected in all myoblast cultures. Pure porcine cultures of self-renewing myoblasts could be grown for long periods. The yield of myoblasts did not decrease until passage number 15. In addition, the influence of different calcium channel blockers on the calcium currents of the myotubes was evaluated.

CONCLUSIONS: Autologous fibroblasts and clones of myoblasts can be grown routinely from porcine biopsies. The cells can be used for electrophysiological and pharmacological studies. Furthermore, autologous myoblasts as well as fibroblasts serve as ideal source for preclinical studies investigating new tissue engineering applications of the lower urinary tract.

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OFFSPRING OF FEMALE RATS SUBMITTED TO MALNUTRITION DURING LACTATION HAVE ALTERED VESICAL EXTRACELLULAR MATRIX COMPOSITION

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INTRODUCTION & OBJECTIVES: It is well established that experimental malnutrition of lactating females has a prolonged effect on their offspring even when the latter are normally fed after weaning. It is not known, however, how this malnutrition model affects the urinary tract, especially the structural components of its wall. Here we addressed this issue by analyzing the biochemical composition of the vesical extracellular matrix (ECM) using the undernourished lactating female rat model.

MATERIAL & METHODS: Whole bladders were obtained from three groups of female Wistar rats whose mothers had been submitted to proteic malnutrition (PM), caloric malnutrition (CM), and normal nutrition (control group) during lactation. After weaning all pups were normally fed, and at 40 days of age their bladders were removed, fixed in acetone, delipidated, and dried. Glycosaminoglycans (GAG) were isolated from bladder tissue by papain digestion and precipitations in cetylpyridinium chloride and ethanol. Total GAG was estimated as hexuronic acid and expressed as µg hexuronic acid per mg dry tissue. Agarose gel electrophoresis was used to identify and quantitate the relative contents of the different sulfated GAG species. Collagen concentration was determined by a hydroxyproline assay and expressed as µg hydroxyproline per mg dry tissue.

RESULTS: Whole bladder dry weight decreased by 20% ($p<0.02$) in the PM group (9.74 ± 1.00 mg, n=5) when compared to the control group (12.20 ± 1.35 mg, n=5), but in the CM group (11.08 ± 0.70 mg, n=5) no significant difference was noticed. Vesical GAG concentration did not differ among the three groups, but vesical collagen concentration decreased by 20% ($p=0.005$) in the PM group (37.04 ± 2.16) and by 10% ($p=0.005$) in the CM group (42.03 ± 0.95) when compared to the control group (46.74 ± 2.37). The relative concentration of heparan sulfate in the CM group, compared to the control group, increased by 20% (16.02% ± 0.77, n=5, vs. 13.26% ± 2.33, n=5, $p<0.04$) whereas that of dermatan sulfate decreased by 3% (83.98% ± 0.77, n=5, vs. 86.7% ± 2.33, n=5, $p<0.04$). However, in the PM group proportions of these GAG did not differ significantly compared to control group.

CONCLUSIONS: Both proteic and caloric malnutrition of female rats have a long lasting effect on the vesical ECM of their offspring. These effects vary according to type of malnutrition and include alterations in GAG and/or collagen contents, in addition to overall vesical weight, which may adversely affect bladder function of adult animals.

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OPTIMAL LENGTH OF A PRESSURE DEVICE TO ENSURE URINARY CONTINENCE COMPRESSING THE URETHRA, SHOWN ON A PIG URETHRA MODEL

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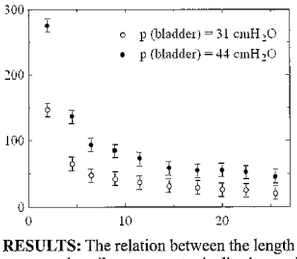
INTRODUCTION & OBJECTIVES: Surgical treatment of severe sphincter deficiency includes the artificial sphincter and sling procedures that compress the bulbar urethra. To prevent urethral atrophy by pressure on the one hand and ensure continence on the other hand, the question arises of the optimal length of the urethral pressure device in dependence on the pressure on the urethra.
We developed a model to examine the relation between the length of a pressure device and the urethral leak point pressure in case of static state (no water flowing) with a given bladder pressure of 31 and 44 cmH2O. The urethral leak point pressure produces a loss of water (first drop) by reducing the urethral outflow resistance pressure applied by the device on the urethra.

MATERIAL & METHODS: We simulate the bladder by a cylindrical tank. The water level determines the bladder pressure inside of the urethra. The pressure is directed to a connected pig urethra. The circular pressure on the urethra is released by special devices of different lengths. On the urethrae of 8 pigs, measurements of the urethral leak point pressure and length of the pressure device were carried out at a bladder pressure of 31 and 44 cmH2O.



RESULTS: The relation between the length of the pressure device and the applied urethral leak point pressure describes a monotonically decreasing curve. The urethral leak point pressure proves to be extremely high for shorter lengths of a pressure device and decreases asymptotically to the bladder pressure for longer pressure devices. For long pressure devices, the force to deform the urethra at the edge of the pressure device becomes less and less important.

CONCLUSIONS: The optimal length of the urethral pressure device proves to be in the range of an almost constant urethral leak point pressure, i.e., at the length, where the urethral leak point pressure does not decrease substantially for longer pressure devices any more. According to the measurements, the optimal length of the pressure device is approximately 15 mm, at a bladder pressure of 31 and 44 cmH2O in the static state. Ongoing studies evaluate the dynamic state, i.e. sudden pressure increase.

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THE NECESSITY OF THE SECOND CUFF IN THE AMS 800 DOUBLE CUFF SYSTEM TO PROVIDE SATISFACTORY CONTINENCE - STUDY ON A STATIC MODEL

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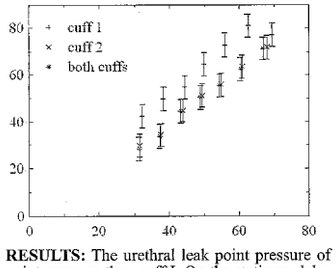
INTRODUCTION & OBJECTIVES: The indication to implant a double cuff system is recommended in patients with artificial urinary sphincter suffering from persistent urinary incontinence, and patients with significant stress urinary incontinence after radical prostatectomy. We developed a model to examine the necessity of a second cuff to ensure continence in a state of no urine flow.

MATERIAL & METHODS: We simulate the bladder by a cylindrical tank. The water level determines the bladder pressure. The pressure is directed to a connected pig urethra. For compression, 2 AMS 800 cuffs, size 4.5 cm, are placed, one after the other, at 2 and 4 cm distance to the proximal end of the urethra. In a first step, the urethra is occluded with cuff I (2 cm), in a second step with cuff II (4 cm), and then with both cuffs, simultaneously. Both cuffs are connected by a tube to the same water tank that, held up, causes urethral outflow resistance pressure. We measure the urethral leak point pressure of cuff I and II individually, then of both cuffs simultaneously. The urethral leak point pressure causes water loss (first drop), when urethral outflow resistance pressure of the cuffs on the urethra is reduced.



RESULTS: The urethral leak point pressure of cuff II shows a by 15 cmH2O lower urethral leak point pressure than cuff I. On the static model, urethral leak point pressure of both cuffs is the same as of cuff II, occluding before cuff I.

CONCLUSIONS: Patients with significant stress urinary incontinence: Looking at the static state, measurements show no difference in implanting one or two cuffs to ensure continence, provided that both cuffs have the same urethral leak point pressure. The reason for the difference lies in the inhomogeneity of the tissue alongside the urethra.
Patients with artificial urinary sphincter suffering from persistent urinary incontinence: According to the measurements, there is no reason, in case of static state, to leave the first cuff in place, when the second, newly implanted cuff provides a lower urethral leak point pressure. Ongoing studies evaluate the dynamic state, e.g. coughing attack.