·实验研究·

姿势性应力诱导椎间盘退变动物模型的实验研究

吕权[1,2] 高金金[3] 杨玉林[3] 王磊[2] 孙甫[1,2△]

[摘要] **目的**:分析椎间盘的退变机制,建立一种模仿人体姿势性应力诱发的椎间盘退变模型。**方法**:建立大鼠椎间盘纤维环穿刺及姿势性应力诱导椎间盘退变模型,将大鼠分为3组,每组10只。对照组不做处理,常规饲养;纤维环穿刺组使用16号针穿刺大鼠椎间盘纤维环建立模型;姿势性应力诱导椎间盘退变组将大鼠放置于大小适中的方形透明塑料管内,将管体倾斜60°放置,每日持续8 h,使大鼠在倾斜面上活动或休息,改变大鼠椎体应力,建立模型。分别在第6周和第12周观察大鼠苏木精-伊红(HE)染色、Masson染色和免疫组化结果。**结果**:建立了大鼠椎间盘纤维环穿刺及姿势性应力诱导椎间盘退变模型。HE染色结果显示:纤维环针刺组与姿势性应力诱导椎间盘退变组髓核细胞数量均较对照组减少,且随着时间的推移减少越发明显。Masson染色结果显示:与对照组相比,纤维环针刺组纤维环结构被破坏,纤维环与髓核边界不清;姿势性应力诱导椎间盘退变组纤维环结构紊乱,向两侧明显突出,髓核与纤维环边界不清。免疫组化结果显示:与对照组相比,第6周时,纤维环针刺组和姿势性应力诱导椎间盘退变组纤维环Ⅱ型胶原阳性细胞数量下降($P<0.05$,$P<0.01$);第12周时,纤维环针刺组和姿势性应力诱导椎间盘退变组仍表现出纤维环Ⅱ型胶原阳性细胞数量,姿势性应力诱导椎间盘退变组下降更为明显,但第6周与第12周对比,差异无统计学意义($P<0.05$,$P<0.01$)。椎间盘退变评分显示:与对照组相比,第6周时,纤维环针刺组和姿势性应力诱导椎间盘退变组椎间盘发生退变($P<0.05$,$P<0.001$);第12周时,纤维环针刺组和姿势性应力诱导椎间盘退变组椎间盘退变更加明显($P<0.01$,$P<0.001$)。**结论**:本实验通过无损伤姿势性应力诱导椎间盘退变成功建立椎间盘退变模型,该模型能够模仿人类直立行走的姿势,退变过程更接近人体结构,具有一定的合理性。

[关键词] 椎间盘退变;椎体失稳;髓核;髓核细胞;纤维环

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Experimental Study on Animal Model of Spontaneous Intervertebral Disc Degeneration Induced by Postural Stress

LÜ Quan[1,2] *GAO Jinjin*[3] *YANG Yulin*[3] *WANG Lei*[2] *SUN Fu*[1,2△]

[1] First Affiliated Hospital of Xi'an Medical College, Xi'an 710077, China;

[2] First Clinical School of Ningxia Medical University, Yinchuan 750004, China;

[3] Xi'an Medical College, Xi'an 710077, China.

Abstract **Objective**: To study the mechanism of intervertebral disc degeneration and establish an intervertebral disc degeneration model that mimics human postural stress induction. **Methods**: The rat models of intervertebral disc degeneration induced by fibrous ring puncture and postural stress were establish and divide into three groups, with 10 rats in each group. Control group: No treatment, conventional feeding. Annulus fibrosus puncture (AFP) group: A model was established by puncturing the fibrous ring of the intervertebral disc in rats using a 16th needle. Postural stress induced intervertebral disc degeneration group: Rats were placed in a moderately sized square transparent plastic tube, the tube was tilted at a 60° angle for 8 h a day, and the rats were

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[1] 西安医学院第一附属医院(西安,710077)

[2] 宁夏医科大学第一临床医学院

[3] 西安医学院

△通信作者 E-mail:sunfu20019@163.com

allowed to move or rest on the inclined surface to change the stress on the rat vertebral body and establish a model. The HE staining, Masson staining, and immunohistochemical results of rats were observed at 6 and 12 weeks, respectively. **Results**: The rat model of intervertebral disc degeneration induced by fibrous ring puncture and postural stress was established. HE staining results showed that the number of nucleus pulposus cells decreased in the fibrous ring puncture group and the postural stress induced intervertebral disc degeneration group compared to the control group, and the decrease became more pronounced over time. The Masson staining results showed that compared with the control group, the fibrous ring structure was disrupted in the fibrous ring acupuncture group, and the boundary between the fibrous ring and the nucleus pulposus was unclear; the fibrous ring structure in the intervertebral disc degeneration group induced by postural stress was disordered, protruded significantly to both sides, and the boundary between the nucleus pulposus and the fibrous ring was unclear. The immunohistochemical results showed that compared with the control group, the number of type Ⅱ collagen positive cells in the annulus fibrosus acupuncture group and the posture stress induced intervertebral disc degeneration group decreased at week 6 ($P<0.05$, $P<0.01$). At week 12, the annulus fibrosus acupuncture group and the posture stress induced intervertebral disc degeneration group still showed the number of type Ⅱ collagen positive cells, with a more significant decrease in the posture stress induced intervertebral disc degeneration group. However, there was no statistically significant difference between week 6 and week 12 ($P<0.05$, $P<0.01$). The intervertebral disc degeneration score showed that compared with the control group, the fibrous ring acupuncture group and the postural stress induced intervertebral disc degeneration group had intervertebral disc degeneration at week 6 ($P<0.05$, $P<0.001$); at week 12, the fibrous ring acupuncture group and the postural stress induced intervertebral disc degeneration group had more significant intervertebral disc degeneration ($P<0.01$, $P<0.001$). **Conclusion**: This experiment successfully established an intervertebral disc degeneration model through non-invasive postural stress. This model has a certain degree of innovation and can mimic the posture of human upright walking. The degeneration process is closer to the structure of the human body and has a certain degree of rationality.

Keywords: intervertebral disc degeneration; vertebral instability; nucleus pulposus; nucleus pulposus cells; fiber ring

腰痛是严重影响人们生活、生产的重要的疾病，椎间盘退变（Intervertebral Disc Degeneration，IDD）是腰痛的主要原因之一，并造成家庭及社会的较大的经济损失[1-4]。最近的研究明确了导致椎间盘退变发生发展的几个重要的风险因素，例如炎症、应力失衡和衰老、机械损伤、营养不良等[5-6]，但是椎间盘退变的具体病理机制目前还不完全明确。研究其退变机制用动物建立椎间盘退变模型是一种安全且经济适用的方式。目前，动物椎间盘退变模型的建立主要是通过穿刺纤维环、髓核抽吸、化学降解、基因敲除、脊柱失稳等方法[7-9]，各有其优缺点。但是由于人类直立行走的特殊性，目前仍缺乏理想的椎间盘退变动物模型。本实验主要通过姿势性应力诱导椎间盘退变来建立椎间盘退变模型，从组织学方面分析该建模方法的有效性。

1 材料和方法

1.1 实验动物

选取西安医学院基础与转化医学研究所动物中心（中国陕西）11 周龄 SD 大鼠 30 只，均为雄性 SD 大鼠，对照组（Ctl）和纤维环穿刺组（AFP）及姿势性应力诱导椎间盘退变组（PSI）大鼠分别在不同的笼中饲养，所有实验动物均饲养在恒温恒湿的洁净动物房内，具体饲养条件为：室温稳定在（24±2）℃，遵循 12 h/12 h昼夜原则，每日提供 12 h 光照时间，相对湿度为 30%～60%，所有大鼠均正常进食及饮水，每天增添饲料并更换垫料。所有动物实验由西安医学院动物伦理委员会批准。

1.2 建立椎间盘纤维环穿刺模型

通过吸入异氟烷诱导全身麻醉，麻醉后将大鼠放置在俯卧位，剃去背侧的毛，用记号笔标记两侧髂嵴连线，对应 $L_{5/6}$ 椎间盘大致位置，皮肤消毒后切开皮肤及筋膜，根据定位剥离 L_3、L_4、L_5、L_6 椎体附着肌肉，随后切断周围肌肉，找到横突后定位椎间盘位置，使用 16 号针穿刺 $L_{4/5}$ 及 $L_{5/6}$ 椎间盘，导致椎体失稳，消毒并缝合切口，放入笼中饲养（见图 1a）。

1.3 建立姿势性应力诱导椎间盘退变模型

随机选取 10 只大鼠饲养于大小适中的方形透明塑料管内，将塑料管倾斜大约 60°放置，每日 8 h，使大鼠在倾斜面活动或者休息，使动物由横向爬行改变为斜面爬行，从而改变大鼠脊柱应力，增加了大鼠椎间盘的垂直应力，使得椎间盘发生退变（见图 1b）。

1.4 苏木精-伊红染色和 Masson 染色

在第 6 周及第 12 周时，使用颈椎脱臼法每组随机处死 5 只大鼠，收集 3 组不同的实验大鼠椎间盘（$L_{5/6}$）。对收集到的组织标本进行固定、脱钙、脱水和石蜡包埋，在冠状位进行连续切片，厚度为 5 μm。脱蜡和水化后，进行苏木精-伊红（HE）染色和Masson

(a) 针刺纤维环模型 (b) 姿势性应力诱发椎间盘退变模型

图 1 大鼠穿刺建模及应力改变建模

染色，观察椎间盘退变程度。

1.5 免疫组化

将切片脱蜡并水化，放入柠檬酸溶液中进行抗原修复，然后在过氧化氢溶液中孵育 10 min，并在 37 ℃下封闭在 10%牛血清中 30 min。使用Ⅱ型胶原蛋白抗体（anti-collagen Ⅱ，Servicebio，货号为 GB11021-50）在 4 ℃下过夜，然后在室温下与二抗（Servicebio，货号为 G1213-100UL）孵育 1 h，DAB 显色，苏木精染色，并用光学显微镜（日本OLYMPUS公司）观察Ⅱ型胶原蛋白的表达。

1.6 椎间盘退变的评估

椎间盘退变评分根据评分方法计算[10]。依据椎间盘髓核细胞数量、纤维环结构完整度以及髓核与纤维环边界是否清晰评分，得分越高则退变越严重。

1.7 统计学方法

数据用 GraphPad Prism9.5 进行绘图和统计学分析，计量资料以 $\bar{x}\pm s$ 形式表示，两组样本均数的比较采用 t 检验，多组间的均数比较采用单因素方差分析（one-way ANOVA），$P<0.05$ 差异有统计学意义。

2 结果

2.1 HE 染色结果

实验第 6 周时，对照组髓核细胞排列整齐，数量正常；而纤维环穿刺组细胞排列紊乱，数量减少；姿势性应力诱导椎间盘退变组髓核细胞数量减少，排列不整齐，且随着时间增加。第 12 周时，与对照相比，纤维环穿刺组和姿势性应力诱导椎间盘退变组髓核细胞数量减少，排列不整齐，髓核细胞数量减少更明显，椎间盘退变加重（见图 2）。

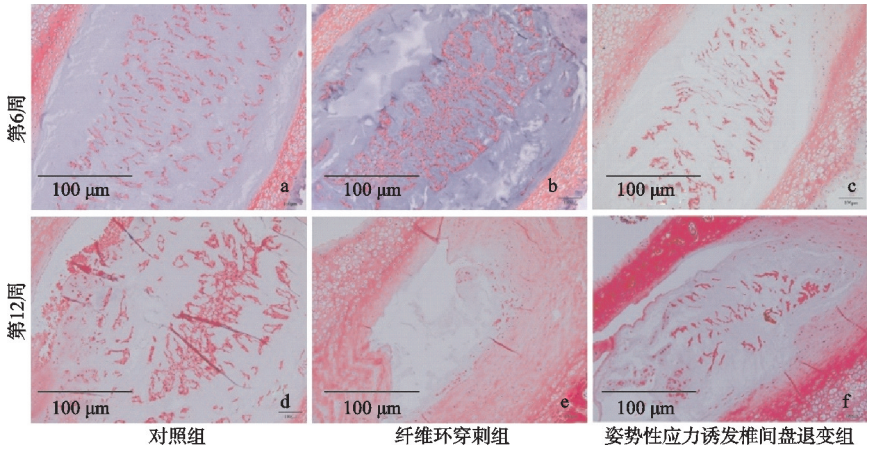

图 2 大鼠椎间盘髓核组织 HE 染色结果

2.2 Masson 染色结果

建模后第 6 周时，对照组椎间盘纤维环排列整齐，结构清晰，且与髓核边界清晰；纤维环穿刺组显示，纤维环结构排列紊乱，失去原有结构，层次不明，且与髓核组织界限不清；姿势性应力诱导椎间盘退变组显示，纤维环失去原有结构，髓核向外侧突出。建模后第 12 周时，与对照组相比，纤维环穿刺组和姿势性应力诱导椎间盘退变组的纤维环结构损伤加重，且与髓核边界分界不清，退变加重（见图 3）。

2.3 免疫组化染色结果

建模后第 6 周时，与对照组相比，纤维环穿刺组和姿势性应力诱导椎间盘退变组纤维环Ⅱ型胶原阳性细胞数量下降，但姿势性应力诱导椎间盘退变组下降更为明显。第 12 周时，与对照组相比，纤维环穿刺组和姿势性应力诱导椎间盘退变组仍表现出纤维环Ⅱ型胶原阳性细胞数量下降，但姿势性应力诱导椎间盘退变组下降更明显。第 6 周与第 12 周对比，差异无统计学意义（见图 4）。

2.4 椎间盘退变评分统计

从大鼠椎间盘髓核细胞数量、纤维环完整度及髓核与纤维环界限三个方面进行评分，从大鼠椎间盘退变评分图可以看出，第 6 周时，纤维环穿刺组与姿势性应力诱导椎间盘退变组退变程度比对照组加重；第 12 周时，纤维环穿刺组与姿势性应力诱导椎间盘退变组退变程度比对照组更明显（见图 5）。

3 讨论

椎间盘退变是腰椎间盘突出症的基础病理改变，是由于腰椎节段的椎间盘组织出现了退行性改变，纤

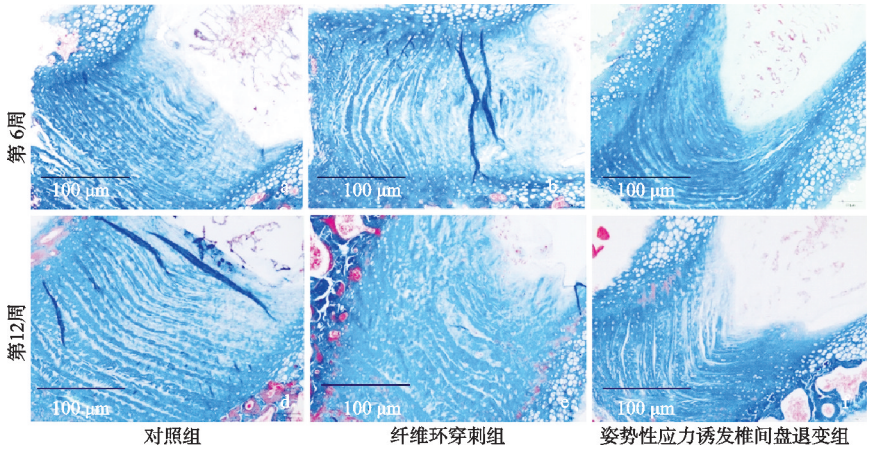

图 3 大鼠椎间盘纤维环及纤维环与髓核界限 Masson 染色结果

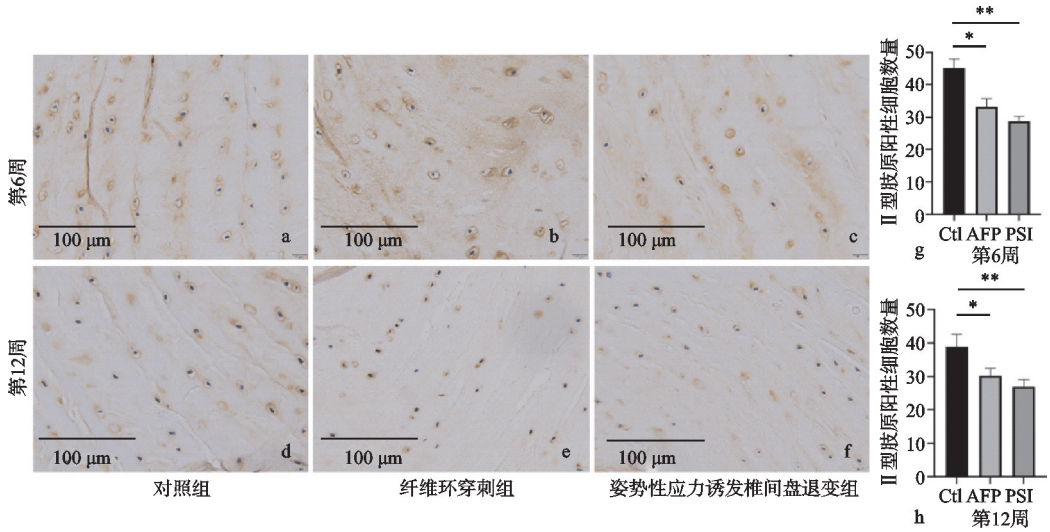

图 4 大鼠椎间盘免疫组化结果及Ⅱ型胶原阳性细胞数量比较(与 Ctl 组相比,$^{*}P<0.05$,$^{**}P<0.01$)

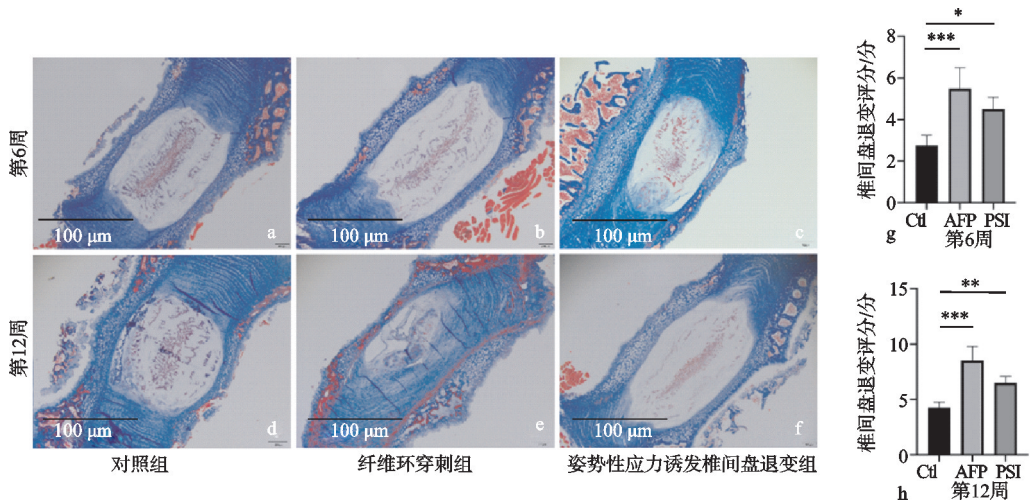

图 5 大鼠椎间盘形态比较及退变评分(与 Ctl 组相比,$^{*}P<0.05$,$^{**}P<0.01$)

维环发生部分撕裂或者破损后,髓核组织从纤维环的薄弱处或者撕裂部位突出,直接压迫或者炎性刺激周围的神经根、马尾神经,从而出现的腰痛、下肢放射痛等一系列症状或体征,俗称“腰突症”,是骨科临床的一种常见病、多发病,也是引起腰腿疼痛的主要原因[11-13]。正常情况下髓核良好的弹性有助于吸收脊柱负荷并传导到周围的组织,弹性胶原纤维围绕在髓核周围,软骨终板(CEP)调节营养物质的交换[4,14]。椎间盘所受的应力对维持椎间盘中基质的平衡很重要,急性机械损伤和累积的超负荷会诱发椎间盘退变[15-17]。椎间盘根据昼夜活动的变化承受内压的昼夜变化,在平卧位椎间盘负荷较低的压力,而在直立姿势和负重时承受高负荷,在高负荷下椎间盘变形,静水压增加[12,16,18],部分水分因压力变大被挤出,液体的流失

增加了蛋白聚糖浓度和固定电荷密度，并导致更高的渗透压和更低的 pH 值，在平卧位时压力骤减，液体被抽回椎间盘，以继续维持椎间盘的生理变化[19-20]。

正常人类由于其特殊的直立行走姿势，使其腰椎、颈椎均存在负荷，在此负荷作用下，加速了椎间盘退变的过程。Goff 等曾设计了一种双足鼠的动物模型，剪除小鼠的双前肢，使得小鼠腰椎所受负荷增加，2～6 周后观察发现小鼠腰椎间盘出现退变。然而由于伦理的原因，此类致残动物模型不被支持[21]。在动物种类方面，只有灵长类动物（如猴子、猩猩等）可以部分近似模拟直立行走的状态；但是这两种动物制作动物模型费用高，经济性差，并且有一定的伦理学障碍。在实验动物饲养过程中观察到，将小鼠或大鼠放置在一个倾斜平面的笼子中饲养时，实验动物会不自主地保持一种头高脚低位置。据此在本实验中提出这种姿势性应力诱导建立椎间盘退变模型的新方法。该模型建模中，将实验动物置于倾斜平面，同时实验动物可在该平面进行一定的活动或者休息、饮食、饮水，这在一定程度上模拟人体的活动体位或者其他对人体椎间盘有损伤的体位，具有一定的研究价值。本动物模型与常见的其他动物模型相比具有一定优势，其病理改变与人类椎间盘退变过程很接近[22-23]。

本研究中姿势性应力诱导椎间盘退变组大鼠表现出椎间盘形态学的改变，是一种进展型的变化，主要表现为髓核细胞数量和细胞外基质数量减少，纤维环结构紊乱，纤维环与髓核边界不清，该组实验动物模拟椎间盘应力改变导致椎间盘退行性改变，通过改变大鼠椎间盘的应力分布，导致发生椎间盘退行性改变，出现髓核、纤维环的退行性改变，与既往实验结果相似。

综上所述，姿势性应力诱导椎间盘退变可以在一定程度上模拟人腰椎间盘退行性改变的过程，然而人类发生椎间盘退变的复杂原因及退变过程中分子层面的变化尚未完全明确，因此还需要进一步通过更为实际且理想的动物模型来证实椎间盘退变的发病机制，以证实上述结论。

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参考文献

[1] LIANG T, GAO B, ZHOU J, et al. Constructing intervertebral disc degeneration animal model: a review of current models[J]. Front Surg, 2022, 9: 1089244.

[2] HUANG Y, WANG L, LUO B, et al. Associations of lumber disc degeneration with paraspinal muscles myosteatosis in discogenic low back pain[J]. Front Endocrinol (Lausanne), 2022, 13: 891088.

[3] PAGE M I, EASLEY J T, BONILLA A F, et al. Biomechanical evaluation of a novel repair strategy for intervertebral disc herniation in an ovine lumbar spine model[J]. Front Bioeng Biotechnol, 2022, 10: 1018257.

[4] KHALID S, EKRAM S, RAMZAN F, et al. Co-regulation of Sox9 and TGFβ1 transcription factors in mesenchymal stem cells regenerated the intervertebral disc degeneration[J]. Front Med (Lausanne), 2023, 10: 1127303.

[5] XIN J, WANG Y, ZHENG Z, et al. Treatment of intervertebral disc degeneration[J]. Orthop Surg, 2022, 14(7): 1271-1280.

[6] QIU S, SHI C, ANBAZHAGAN A N, et al. Absence of VEGFR-1/Flt-1 signaling pathway in mice results in insensitivity to discogenic low back pain in an established disc injury mouse model[J]. J Cell Physiol, 2020, 235(6): 5305-5317.

[7] GLAESER J D, TAWACKOLI W, JU D G, et al. Optimization of a rat lumbar IVD degeneration model for low back pain[J]. JOR Spine, 2020, 3(2): e1092.

[8] WANG Y, WU Y, DENG M, et al. Establishment of a rabbit intervertebral disc degeneration model by percutaneous posterolateral puncturing of lumbar discs under local anesthesia[J]. World Neurosurg, 2021, 154: e830-e837.

[9] VARDEN L J, TURNER E J, COON A T, et al. Establishing a through-puncture model for assessing post-injection leakage in the intervertebral disc[J]. Eur Spine J, 2022, 31(4): 865-873.

[10] TAM V, CHAN W C W, LEUNG V Y L, et al. Histological and reference system for the analysis of mouse intervertebral disc[J]. J Orthop Res, 2018, 36(1): 233-243.

[11] GULLBRAND S E, KIM D H, ASHINSKY B G, et al. Restoration of physiologic loading modulates engineered intervertebral disc structure and function in an in vivo model[J]. JOR Spine, 2020, 3(2): e1086.

[12] LEI M, ZHAO K, HUA W, et al. An in vivo study of the effect of c-Jun on intervertebral disc degeneration in rats[J]. Bioengineered, 2021, 12(1): 4320-4330.

[13] ZHANG C, GULLBRAND S E, SCHAER T P, et al. Inflammatory cytokine and catabolic enzyme expression in a goat model of intervertebral disc degeneration[J]. J Orthop Res, 2020, 38(11): 2521-2531.

[14] GUO J, YAN H, XIE Y, et al. A lumbar disc degeneration model established through an external compressive device for the study of microcirculation changes in bony endplates[J]. Heliyon, 2023, 9(5): e15633.

[15] WANG D L, JIANG S D, DAI L Y. Biologic response of the intervertebral disc to static and dynamic compression in vitro[J]. Spine (Phila Pa 1976), 2007, 32(23): 2521-2528.

（下转第 19 页）

Trauma,2007,21(10):S135-S160.
[7] 宫伟,李炳钻,王建嗣,等.新式垂直悬吊皮肤牵引治疗婴幼儿股骨干骨折 23 例[J].中国中医骨伤科杂志,2017,25(2):56-58.
[8] SANDERS R,REGAZZONI P. Treatment of subtrochanteric femur fractures using the dynamic condylar screw[J]. J Orthop Trauma,1989,3(3):206-213.
[9] ZHANG Y,XUE Y,ZHAO M,et al. Titanium elastic nails vs locking plate in pediatric subtrochanteric femur fractures:a systematic review and meta-analysis[J]. Front Pediatr,2023,11:1114265.
[10] GALAL S. External fixation of paediatric subtrochanteric fractures using calcar rather than neck pins[J]. Strat Traum Limb Recon,2016,11(2):99-104.
[11] KIM V,MILTON J,MASHRU R P,et al. Management of pediatric femur fracture using 90-90 traction[J]. J Orthop Trauma,2021,35(Suppl 2):S26-S27.
[12] CHURCHILL J L,KRONK T A,ADAMCZYK M J,et al. Waterproof hip spica casts for pediatric femur fractures[J]. J Pediatr Orthop,2024,44(1):20-24.
[13] SANJAY N,SEENAPPA H,SHANTHAPPA A H,et al. Functional outcome of pediatric subtrochanteric fractures treated with a titanium elastic nailing system (TENS) versus plating[J]. Cureus,2023,15(6):e40036.
[14] 文玉伟,朱丹江,王强,等.弹性髓内钉治疗学龄前儿童股骨干骨折的疗效及预后分析[J].中国骨与关节杂志,2023,12(11):806-811.
[15] MEMEO A,PANUCCIO E,D'AMATO R D,et al. Retrospective,multicenter evaluation of complications in the treatment of diaphyseal femur fractures in pediatric patients[J]. Injury,2019,50(Suppl 4):S60-S63.

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(上接第 14 页)

[16] SETTON L A,CHEN J. Cell mechanics and mechanobiology in the intervertebral disc[J]. Spine (Phila Pa 1976),2004,29(23):2710-2723.
[17] FU F,BAO R,YAO S,et al. Aberrant spinal mechanical loading stress triggers intervertebral disc degeneration by inducing pyroptosis and nerve ingrowth[J]. Sci Rep,2021,11(1):772.
[18] NEIDLINGER-WILKE C,W RTZ K,URBAN J P,et al. Regulation of gene expression in intervertebral disc cells by low and high hydrostatic pressure[J]. Eur Spine J,2006,15(Suppl 3):S372-S378.
[19] CHAN S C,FERGUSON S J,GANTENBEIN-RITTER B. The effects of dynamic loading on the intervertebral disc[J]. Eur Spine J,2011,20(11):1796-1812.
[20] SIVAN S,NEIDLINGER-WILKE C,WÜRTZ K,et al. Diurnal fluid expression and activity of intervertebral disc cells[J]. Biorheology,2006,43(3/4):283-291.
[21] GOFF C W,LANDMESSER W. Bipedal rats and mice;laboratory animals for orthopaedic research[J]. J Bone Joint Surg Am,1957,39-A(3):616-622.
[22] AO X,WANG L,SHAO Y,et al. Development and characterization of a novel bipedal standing mouse model of intervertebral disc and facet joint degeneration[J]. Clin Orthop Relat Res,2019,477(6):1492-1504.
[23] LI H,YIN J,HUANG Y,et al. Establishment of cervical dynamic and static imbalance models and preliminary study on the mechanism of cervical degeneration in rats[J]. Innate Immun,2020,26(2):107-116.

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