

· 述 评 ·

处在十字路口的男科学

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1 一个观点

我们的共同事业——男科学,目前仍然处在虽雏形初成但尚未完全定型的初级阶段。在医学界,有关男科学的定义,范畴与学术地位一直处在争论与质疑之中,这种学科发展不确定的局面对于男科学的健康发展和男科学的学术梯队建设极为不利。一句话,当前的男科学正处于十字路口,我们必须认真思索男科学的未来发展方向和可持续发展之路,尽快取得共识,使男科学跨上一个新台阶。

2 二个挑战

2.1 挑战一

男科学究竟是一门什么样的学科?男科学在医疗卫生服务中究竟能发挥什么作用?

传统的男科学主要聚焦于男性不育,男性性功能障碍及男性生殖内分泌紊乱三大临床问题,学术界不少人认为这算不上一门独立的学科,只是生殖医学、性医学及生殖内分泌学的一个分支内容而已。俗话说“名不正,言不顺”。如果我们自己也说不清男科学的定义与范畴,理不清与相关学科的关系,那么男科学的地位就岌岌可危了。

2.2 挑战二

当前男科学工作者大部分来自泌尿科、内分泌科、生殖生物学及生殖医学等专业,在当前医疗卫生体系中,男科学还未形成自己独特的行业标准与单独的人才体系。资深的男科学工作者还能依赖于自己的原有学科专业背景与基础,但一大批年青的男科学工作者必然会出现彷徨迷惘与前景黯淡的困惑,因为“有位才能有为”。男科学学科的明确定位及人才体系的建立与完善已势在必行,迫在眉睫。

3 三个建议

3.1 建议一

尽快在男科学的定义与范畴上取得共识,这是一门学科得以独立生存和人才梯队赖以发展的基础。近年来,国内、外男科学工作者已逐步形成以下共识:①男科学是一门研究维护与促进男性健康的综合性医学学科,应当从以疾病为主导走向以健康为主导,提出了“Male Health —— Nature and Nurture”的理念,男科学要在整合医学(Holistic Integrative Medicine)的战略框架下,制订学科发展规划;②男科学不仅要关心成年男性的生殖健康,应当着眼于维护与促进男性生命全程的健康,即从胚胎期、儿童期、青春期、成年期、直至更年期与老年期,男子一生健康促进的概念,提出了“From Men's Sexual and Reproductive Health to Male Whole Life-Span Health”的口号,并提出四维(三维立体加上时间维)的纵向研究战略;③从传统的男性不育、男性性功能障碍及男性生殖内分泌紊乱的研究重点,外延到其他与男性生命全程健康的诸多热点,尤其是:a)男子一生的健康需求及男子生命各阶段的特殊健康问题;b)影响男性健康的诸因素,包括遗传与表观遗传、行为、饮食与生活方式,以及自然与社会环境因素等,同时要分析这些因素之间的相互关系;c)男性生殖系统疾病(除男性不育、性功能障碍及男性生殖内分泌紊乱外,还应包括生殖道感染, HIV 感染及前列腺疾病)的病因、发病机理、预防,预测和最佳临床诊疗路径;d)男性慢性病、损伤、心理障碍及老年退化性疾病的特征,预测、预防及干预;e)有关促进男性健康的公共卫生政策以及如何加强公众理解、认知与主动参与。

3.2 建议二

在男科学研究重点领域中,要加强学科交叉合作,采用转化医学研究战略,争取早日取得突破,尤其是重视以下几个热点问题的转化医学研究:①男性不育从精液质量诊断走向病因及发生机制诊断,从经验型治疗走向以循证医学为基础的治疗规范;②实施ICSI时,确定精子的选择标准以及研究精子质量与ART结局的关系,建立新的无损伤性精子功能检测技术;③ED治疗的新途径与ED的个体化综合干预与康复新理念;④前列腺疾病(CP, BPH及PCa)的发病机理与临床分型,预防、预测和最佳诊疗临床路径,如何避免过度诊断与过度治疗;⑤中国男性更年期健康的基线调查、影响因素分析及人群综合干预方案,如何预防与衰老相关疾病的发生(糖尿病、高血压、肿瘤及老年痴呆症等);⑥男性节育的新思路与新方法,包括精子发生与精子成熟中的新靶点、新环节以及免疫避孕疫苗的研制等;⑦环境污

染及生活方式与行为对男性健康的影响,例如性早熟,性发育异常和子代发育异常等;⑧促进男性生命全程健康干预的公共卫生综合方案。

3.3 建议三

树立男科学良好的学术与公众形象:当前世界上男科学有3个中心,即北美、欧洲与亚太地区。中国的男科学工作者除了积极参与国内学术界的学术交流与合作外,还应当不断提高中国男科学家在亚太地区与国际男科学领域的发言权,例如2014年在澳大利亚Newcastle召开的第12届International Conference on Spermatology以及同年11月份在上海召开的第六届International Conference on Epididymis Research。

此外,应当加强与政府决策部门和公众的联系沟通,积极参与“男性健康日”的公益活动,让政府、学术界及公众认识到男科学的重要作用,树立男科学良好的公众形象,不断为男科学可持续发展输送“正能量”。

• 实验研究 •

受激活调节正常T细胞表达和分泌因子 (RANTES)受体 CCR1 和 CCR5 在人附睾中的表达

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【摘要】目的: 探讨受激活调节正常T细胞表达和分泌因子(RANTES)受体 CCR1、CCR5 在成人附睾中的表达和定位。方法: 采用 RT-PCR 检测 CCR1 和 CCR5 mRNA 在成人附睾中的表达, 免疫组织化学法观察 CCR1 和 CCR5 在人附睾中的细胞定位, 免疫荧光双标染色分别检测 RANTES 与 CCR1 及 CCR5 的共定位情况。结果: 在人附睾组织中获得了 RANTES 受体 CCR1、CCR5 的 cDNA 片段, 免疫组织化学显示 CCR1 表达于输出小管的纤毛细胞, 附睾管的顶细胞和基细胞; CCR5 表达于附睾输出小管的纤毛细胞以及全部附睾管上皮细胞。免疫荧光双标显示 RANTES 分别与 CCR1 和 CCR5 的阳性信号在输出小管的纤毛细胞、附睾管的顶细胞和基细胞共存。结论: CCR1 和 CCR5 在附睾上皮有表达, 且与 RANTES 共定位, 推测 RANTES 可能通过其受体在附睾中起作用, 从而为精子成熟和储存提供适宜的微环境。

关键词: 成人附睾; 受激活调节正常T细胞表达和分泌因子(RANTES); CCR1; CCR5;
附睾管腔微环境

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• 实验研究 •

Expression of Regulated upon Activation Normal T Cell Expressed and Secreted (RANTES) Receptors CCR1 and CCR5 in Human Epididymis

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【ABSTRACT】Objective: To explore the expression and the cell localization of regulated upon activation normal T cell expressed and secreted (RANTES) receptors CCR1 and CCR5 in human epididymis. **Methods:** RT-PCR was used to detect the mRNA expression of CCR1 and CCR5 in human epididymis. Immunohistochemical staining was employed to display the distribution of CCR1 and CCR5 in human epididymis. The colocalization of RANTES and CCR1 or CCR5 in epididymis was observed by immunofluorescence double staining method. **Results:** The RT-PCR products of the predicted sizes for CCR1 and CCR5 were acquired from human epididymis. Immunohistochemical study demonstrated that CCR1-positive signal was identified in ciliated cells of the efferent ducts, apical and basal cells of the epididymal ducts, whereas CCR5 was located in ciliated cells of the efferent ducts and all epithelial cells of epididymal ducts. Double-labelling analysis indicated that immunofluorescence of RANTES coincided with CCR1 or CCR5 in ciliated cells, apical and basal cells of epididymis. **Conclusion:** CCR1 and CCR5 are expressed in the human epididymal epithelium and coincide with RANTES. According to the experimental results, it is assumed that RANTES may play a role in epididymis via receptors CCR1 and CCR5, thereby providing a suitable environment where sperm cell can be matured and stored.

Key words: adult epididymis; regulated upon activation normal T cell expressed and secreted (RANTES); CCR1; CCR5; luminal microenvironment

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*MEST*基因在正常人前列腺移行带和外周带 基质成纤维细胞中的mRNA表达差异 及其与DNA甲基化水平的关系

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【摘要】目的: 探讨正常人的前列腺移行带(PZ)和外周带(TZ)基质成纤维细胞中 *MEST*(mesoderm specific transcript)基因的甲基化水平变化和表达差异, 及DNA甲基化抑制剂(5-Aza-CdR)对其表达水平的影响。方法: 用硫化测序PCR(bisulfite sequencing PCR, BSP)和实时荧光定量PCR方法分别检测5-Aza-CdR处理前、后在前列腺PZ和TZ原代成纤维细胞中 *MEST*基因的甲基化水平和相应的mRNA表达。结果: *MEST*基因在PZ成纤维细胞中为低甲基化高表达, 在TZ成纤维细胞中为高甲基化低表达。加入5-Aza-CdR后, *MEST*在PZ和TZ成纤维细胞中都为去甲基化, 而表达水平上升, 但是TZ成纤维细胞表达水平的改变比PZ成纤维细胞大。结论: *MEST*基因的DNA甲基化差异可能是前列腺外周带和移行带成纤维细胞生物学行为差异的分子基础。

关键词: 前列腺; 基质细胞; *MEST* 基因; DNA 甲基化

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Methylation and mRNA Expression of *MEST* Gene in Normal Prostate Peripheral Zone and Transitional Zone Fibroblast Cells

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【ABSTRACT】 Objective: To investigate the methylation and the expression level of mesoderm specific transcript (*MEST*) gene between the prostate peripheral zone (PZ) and transitional zone (TZ) fibroblast cells of human normal prostate, and to explore the effect of DNA methylation inhibitor on its expression. **Methods:** Bisulfite sequencing PCR (BSP) and Real-time PCR were used to detect *MEST* methylation and mRNA expression respectively in PZ and TZ fibroblast cells before and after treatment with DNA methylation inhibitor (5-Aza-CdR). **Results:** The methylation level of *MEST* was lower and the mRNA level of *MEST* was higher in PZ fibroblast cells, compared with those in TZ fibroblast cells. After being treated with 5-Aza-CdR, both cells showed hypomethylation and up-regulated expression of *MEST* gene. However, the *MEST* mRNA expression in TZ fibroblast cells was significantly higher than that in PZ fibroblast cells. **Conclusion:** DNA methylation of *MEST* gene may be the molecular basis of different biological behaviors of fibroblast cells in prostate PZ and TZ.

Key words: prostate; stroma cells; mesoderm specific transcript (*MEST*); DNA methylation

组织培养观察胎牛血清和血清替代物 ——Knockout™ SR对大鼠未成熟睾丸 生殖细胞发育的影响

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【摘要】目的: 比较胎牛血清(FBS)和血清替代物——Knockout™ SR(KSR)对睾丸组织生长发育的影响。方法: 未成熟大鼠睾丸组织分别采用FBS和KSR连续培养5周, 观测培养睾丸组织大体面积, 苏木素伊红(HE)染色观察睾丸组织学形态及生殖细胞发育, 并测量曲细精管直径。TUNEL法凋亡实验观察培养5周睾丸组织中的凋亡细胞, RT-PCR检测精子发生不同阶段的标准化标志基因*Kit*、*Sycp3*、*Crisp1*。结果: KSR组睾丸组织体外持续生长, 曲细精管逐渐增大, 培养5周观察到精原细胞、初级精母细胞、次级精母细胞、圆形精子细胞, RT-PCR检测到*Kit*、*Sycp3*、*Crisp1*的表达。FBS组睾丸组织随着培养时间延长逐渐萎缩, 培养5周曲细精管出现明显坏死, 培养睾丸组织中*Sycp3*、*Crisp1*表达不稳定。结论: KSR更有利于未成熟睾丸组织培养中生殖细胞从精原细胞发育成精子细胞, 并能长期维持生殖细胞发育及睾丸组织生长。

关键词: 睾丸; 组织培养; 精子发育; 大鼠

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Influence of Fetal Bovine Serum and Knockout™ SR on Germ Cell Development by Rat Immature Testicular Tissue Culture

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【ABSTRACT】 Objective: To evaluate the effect of fetal bovine serum (FBS) and serum replacement —— Knockout™ SR (KSR) on germ cell development. **Methods:** The FBS and the KSR were used as supplement of culture medium, the testicular tissues were cultured for 5 weeks. Area of the cultured tissues was measured. The testicular histology, the development of the germ cell and the diameter of seminiferous tubules were observed by HE staining. The apoptotic cells were observed in the testis cultured for 5 weeks by apoptosis experiments. The marker genes of spermatogenesis in various stages, such as *Kit*, *Sycp3* and *Crisp1*, were identified by RT-PCR. **Results:** In KSR group, the cultured tissues could sustain growth and the seminiferous tubules gradually increased in the period of culture. The spermatogonia, primary spermatocytes, secondary spermatocytes and round spermatids were observed in the cultured tissues after cultured for 5 weeks, and their marker genes *Kit*, *Sycp3* and *Crisp1* were identified. In FBS group, the testicular organization dwindled in the period of culture; the seminiferous tubules appeared obviously necrosis and the expression of the *Sycp3* and *Crisp1* were instability after cultured for 5 weeks. **Conclusion:** KSR has more advantages for spermatogonial cells to develop into sperm cells in immature tissue culture, and can maintain the germ cell development and the cultured tissues growth development for a long time.

Key words: testis; tissue culture; spermatogenesis; rat

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• 临床研究 •

非梗阻性无精子症和隐匿精子症与睾丸体积、血FSH和AZF基因微缺失的相关性分析

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【摘要】目的: 探讨非梗阻性无精子症和隐匿精子症与睾丸体积、血FSH和AZF基因微缺失的相关性。方法: 161例男性不育患者分为非梗阻性无精子症(A组, $n=86$)、隐匿精子症(B组, $n=49$)、严重少精子症(C组, $n=13$)和其它患者(D组, $n=13$, 包括死精子症4例, 梗阻性无精子症3例, 正常精子1例, 其他少弱精子症5例), 进行睾丸体积、血FSH水平和AZF基因微缺失检测, AZF基因微缺失检测位点包括AZFa(sY84、sY86)、AZFb(sY127、sY134)、AZFc(sY254、sY255、sY157、sY145和sY152)。结果: 4组睾丸体积 <12 ml的比例分别为73.26%(63/86)、34.69%(17/49)、7.69%(1/13)、30.77%(4/13); 4组FSH水平升高1倍以上的比例分别为67.44%(58/86)、32.65%(16/49)、15.38%(2/13)和0.00%(0/13), 组间均有极显著统计学差异($P<0.001$); 4组AZF基因微缺失率分别为15.12%(13/86)、18.37%(9/49)、0.00%(0/13)和0.00%(0/13), 组间均无统计学差异($P>0.05$), 其中A组AZFa基因缺失1例, AZF(b+c+d)缺失5例, AZF(c+d)基因缺失7例, B组9例均为AZFc和(或)AZFd基因缺失, C组和D组均未见AZF基因缺失。结论: 随着生精功能障碍程度的加重, 血FSH水平升高和睾丸体积缩小趋势明显, 而与AZF缺失几率无高度相关性; 但在非梗阻性无精子症和隐匿精子症组中AZF基因缺失几率仍然偏高, AZFa和AZFb缺失大多出现在非梗阻性无精子症组中, 而隐匿精子症组以AZFc和AZFd缺失为主。

关键词: 非梗阻性无精子症(NOA); 隐匿精子症; 严重少精子症; 睾丸体积; 血FSH; AZF基因; 微缺失

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• 临床研究 •

Relationship of Testicular Volume, Follicle-stimulating Hormone (FSH) and Azoospermia Factor (AZF) Gene Microdeletion in Patients with Non-obstructive Azoospermia (NOA) and Cryptozoospermia

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【ABSTRACT】 Objective: To explore the relationship of testicular volume, follicle-stimulating hormone (FSH) and azoospermia factor (AZF) deletion gene microdeletion in patients with non-obstructive azoospermia (NOA) and cryptozoospermia. **Methods:** Totally 161 male infertility patients were drawn, and testicular volumes, FSH level and AZF microdeletion were recorded. The examination of AZF microdeletion includes AZFa (sY84, sY86), AZFb (sY127, sY134), AZFc (sY254, sY255, sY157), AZFd (sY145, sY152). All patients were divided into four groups. Group A: 86 patients were NOA; group B: 49 patients were cryptozoospermia; group C: 13 patients were severe oligozoospermia patients; group D: 13 cases were enrolled, including 4 necrospermia patients, 3 obstructive azoospermia patients, 1 normal sperm patient and 5 oligoasthenozoospermia patients. **Results:** The proportions of the testicular volume less than 12 ml were 73.26% (63/86) in group A, 34.69% (17/49) in group B, 7.69% (1/13) in group C, 30.77% (4/13) in group D with significant differences ($P<0.001$); the proportions of increasing FSH level with value increased more than 1 fold in 4 groups were 67.44% (58/86), 32.65% (16/49), 15.38% (2/13) and 0.00% (0/13), respectively, with significant differences ($P<0.001$); the AZF gene microdeletion of four groups were 15.12% (13/86), 18.37% (9/49), 0.00% (0/13) and 0.00% (0/13), respectively ($P>0.05$). In group A, one patient was AZFa gene microdeletion, 5 were AZF(b+c+d) gene microdeletion, 7 were AZF(c+d) gene microdeletion and in group B, 9 patients were AZFc and/or AZFd gene microdeletion. **Conclusion:** With development of spermatogenesis dysfunction, there was a significant higher level of FSH and lower volume of testes, additionally, no difference was found when comparing the incidence of AZF gene microdeletion. However, incidence of AZF gene microdeletion was still high in patients with NOA or cryptozoospermia. Moreover, AZFa and AZFb gene microdeletion were common in patients with NOA, while AZFc and AZFd gene microdeletion were common in cryptozoospermia.

Key words: non-obstructive azoospermia (NOA); cryptozoospermia; severe oligozoospermia; testicular volume; follicle-stimulating hormone (FSH); azoospermia factor (AZF); microdeletion

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17例AZFc区缺失患者家系研究 及缺失断点序列分析

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【摘要】目的: 探讨在自然生育条件下Y染色体AZFc区缺失来源, 同时进行断点序列比对来分析断点的位置。方法: 通过对AZF区22个序列标签位点(STS)多重PCR扩增, 检测家系样本缺失情况。结果: 17例AZFc区缺失家系中, 13例为新生突变, 突变率为76.47%; 4例为垂直传递, 遗传率为23.53%。新生突变和垂直传递患者在年龄、生殖激素水平及睾丸体积组间差异无统计学意义($P < 0.05$)。近端缺失断点多集中于sY1197、sY1191, 远端多集中于sY157、sY1054。结论: AZFc区缺失患者绝大多数为新生突变, 且新生突变与垂直传递患者无明显差别; AZFc区缺失近、远端缺失断点多集中于复制子b2和b4。

关键词: 家系研究; AZFc区微缺失; 断点序列分析

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An Investigation of 17 Yq AZFc Microdeletion Pedigrees and Sequence Analysis of Breakpoint

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【ABSTRACT】 Objective: To study the patrilineal line of infertile men with Y chromosome microdeletions and to describe the breakpoint of AZFc microdeletions after naturally conceived. **Methods:** AZF microdeletions were detected by PCR of 22 sequencetagged sites (STSs) within Yq emphasizing the AZFa, b and c regions. **Results:** The de novo mutation rate of AZFc microdeletions was 76.47% (13/17), the vertical transmission of Yq AZFc microdeletion was detected in 4 cases of 17 investigated families (23.53%, 4/17), and there were no significant differences between the two groups regarding age, reproductive hormone levels and testicular volume. The proximal breakpoints were focused on sY1197, sY1191 and the distal breakpoints were focused on sY157, sY1054. **Conclusion:** The vast majority of patient' Yq AZFc deletions were de nove mutations and there were no obviously statistical differences between the denovo mutation patients and vertical transmission patients; the proximal and distant breakpoints mostly located at the replicons b2 and b4, respectively.

Key words: pedigree study; Yq AZFc microdeletion; breakpoint sequence analysis

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精液细胞DNA倍体分析结合生精细胞检查 鉴别梗阻性与非梗阻性无精子症

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【摘要】 目的: 拟通过比较梗阻(OA)和非梗阻性无精子(NOA)症患者精液细胞DNA倍体分析与细胞学检查结果的特点, 以期建立一种无创的诊疗方案以鉴别OA与NOA。方法: NOA患者20例和OA患者10例, 按照WHO方法进行精液分析诊断, 获取其精液标本, 液化后离心取沉淀物, 1份采用体积分数70%冰乙醇重悬精液沉淀, 4℃固定后用PI染色, 采用流式细胞仪进行DNA倍体分析。同时, 取另1份精液沉淀涂片, 进行Diff-Quick染色和免疫细胞化学染色。结果: DNA倍体分析结果表明, 20例NOA患者精液中均存在单倍体(1N)、二倍体(2N)和四倍体(4N)细胞, 其中2N细胞含量最高, 约占 $71.25 \pm 8.73\%$ 。1N细胞的百分比含量为 $5.46 \pm 2.93\%$, 4N细胞含量为 $3.28 \pm 2.54\%$ 。10例OA患者精液中有8例只存在2N细胞, 含量为 $71.67 \pm 13.09\%$, 未检测到1N和4N, 2例未检测到各种倍体细胞。精液细胞学检查结果表明, 20例NOA患者中有15例患者精液中可检测出生精细胞, 5例患者精液中未检出生精细胞。10例OA患者精液中均未检出生精细胞, 其中2例没有任何细胞。结论: 精液细胞DNA倍体分析与细胞学检查作为2种无创的检查方法, 均可作为评估患者生精功能的辅助诊疗手段, 两者结合可作为鉴别OA和NOA的辅助诊断指标。

关键词: 倍体分析; 精液; 生精细胞检查; 梗阻性无精子症(OA); 非梗阻性无精子症(NOA)

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Identification of Obstructive and Non-obstructive Azoospermia with Seminal Cell DNA Content Analysis and Seminal Spermatogenic Cells Examination

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【ABSTRACT】 Objective: To establish a non-invasive approach to identify obstructive azoospermia (OA) and non-obstructive azoospermia (NOA) by comparing the results of seminal cell DNA content analysis and seminal spermatogenic cells examination. **Methods:** Semen samples from 20 NOA and 10 OA patients were examined according to the WHO 5 Guidelines. After liquefaction, the samples were centrifuged to collect spermatogenic cells. For DNA content analysis, the pellet was resuspended with 70% (v/v) ice ethanol and incubated with propidium iodide (PI). Meanwhile, the pellet smears were performed Diff-Quick staining and immunocytochemistry. **Results:** The results of DNA ploidy analysis revealed the presence of haploid (1N), diploid (2N) and tetraploid (4N) cells, of which the highest percentage of 2N cells, accounting for $71.25 \pm 8.73\%$. The percentage of 1N cells and 4N cells were $5.46 \pm 2.93\%$ and $3.28 \pm 2.54\%$, respectively. Among 10 samples of OA patients, only 8 samples had 2N cells ($71.67 \pm 13.09\%$) and no cells were detected in 2 patients. Semen cytology results showed that spermatogenic cells could be detected in 15 cases of 20 NOA patients and no spermatogenic cells could be observed in 5 samples of NOA patients. No spermatogenic cells could be detected in samples of OA patients. **Conclusion:** The results of seminal cell DNA content analysis and seminal spermatogenic cells examination showed that 1N, 2N and 4N cells could be detected in semen of NOA patients and spermatogenic cells could be observed in most semen of NOA patients; meanwhile, only 2N cells or no cells could be detected in semen of OA patients and no spermatogenic cells could be observed in semen of OA patients. Seminal cell DNA content analysis and cytology could be applied for evaluating the status of spermatogenesis as two kinds of non-invasive examination methods. The combination of above methods could be used as diagnostic approach for identification of OA and NOA.

Key words: DNA content analysis; seminal spermatogenic cells; obstructive azoospermia (OA); non-obstructive azoospermia (NOA)

固相透明质酸法优选精子的效果观察

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【摘要】目的: 对比研究高通量的固相透明质酸(HA)分离新技术较传统密度梯度法在精子优化分离效果方面的优势性。方法: 收集新鲜精液标本31份, 每份标本液化后被均分成2部分, 一部分精液以固相HA法分离精子, 即将精液加入到底部表面包被有HA的培养皿中, 洗脱掉未结合的精子, 分离、收集与HA结合的精子; 另外一部分精液以传统的密度梯度法优化分离精子, 观察2种方法提取后精子各项研究指标的差异性, 以及相对变化幅度。结果: 与密度梯度法组相比, 固相HA法组处理后精子的正常形态率、获能2 h 酪氨酸磷酸化(TP)、 Δ TP(精子获能2 h TP率 - 未获能精子TP率)、诱发顶体反应(AR)和 Δ AR(诱发AR率 - 自发AR率)等指标明显增高($P < 0.001$), 增长幅度依次为: 1.55倍(95%CI=1.04~2.81)、1.81倍(95%CI=0.89~6.11)、3.35倍(95%CI=1.04~10.32)、1.37倍(95%CI=0.96~2.71)和1.88倍(95%CI=1.09~4.71); 而固相HA法组自发AR、核蛋白不成熟度和DNA碎片率显著降低($P < 0.001$), 分别是密度梯度法组的68%(95%CI=19%~102%)、47%(95%CI=2%~103%)和21%(95%CI=0%~70%); 2种方法处理后精子的前向运动率和活动率无统计学差异($P > 0.05$)。结论: 基于成熟精子头部具有HA受体可与外源性HA特异性结合原理的精子-固相HA分离技术, 与传统的密度梯度法相比, 分离后的精子具有更优的受精潜能和成熟度, 可显著提高分离精子的整体质量, 有望进一步改善辅助生殖结局。

关键词: 固相; 透明质酸(HA); 精子分离; 精子功能

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Pilot Evaluation of Sperm Selection Procedure Based on Solid State Hyaluronic Acid Binding Ability on Improving Sperm Qualities

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【ABSTRACT】Objective: To explore the advantages of solid state hyaluronic acid (HA) selection procedure for high throughput sperm separation compared with traditional gradient centrifugation (GC) method in the aspect of separated sperm qualities. **Methods:** Totally 31 fresh semen samples were collected. Every sample was divided into 2 parts. One part was applied to the bottom surface of Petri dishes which pre-coated by immobilized HA. The unbound sperm were rinsed gently, and the HA-bound sperm were removed and collected from dish surface. Another part of semen was separated synchronically using GC method. The qualities of separated sperm were compared between the two groups, and calculation the extent of function indexes were promoted of selected sperm were compared by solid state HA technique. **Results:** In the HA-bound sperm fractions compared with GC-selected sperm fractions, the proportions of normal morphology, protein tyrosine phosphorylation (TP) after capacitated for 2 h (2 h TP), TP score ("2 h TP" minus "sperm TP without capacitation"), A23187-induced acrosome reaction (AR) and AR score ("induced AR" minus "spontaneous AR") were higher ($P<0.001$), and were increased to 1.55-fold (1.04–2.81), 1.81-fold (0.89–6.11), 3.35-fold (1.04–10.32), 1.37-fold (0.96–2.71) and 1.88-fold (1.09–4.71), respectively. Conversely, the levels of spontaneous AR, immature nucleoprotein and DNA fragment were lower by solid state HA technique than by GC method ($P<0.001$), and were reduced to 68% (19%–102%), 47% (2%–103%) and 21% (0%–70%), respectively. However, there was no significant difference in percentages of sperm progressive motility and total motility between the two methods ($P>0.05$). **Conclusion:** The solid state HA sperm selection technique, which is based on a specific binding capacity between sperm receptors for zona pellucida and exogenous HA, will likely increase the ability of spermatozoan fertilization and reduce immaturity, and will be promising in improving the outcome of assisted reproduction.

Key words: solid state; hyaluronic acid (HA); sperm preparation; sperm function

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98%无头精子一例并文献复习

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【摘要】目的: 报道98%无头精子1例并探讨其发生机制, 旨为该类患者的诊治提供实验室依据。方法: 对此例无头精子患者的睾丸曲细精管病理结构和精子超微结构进行分析; 将20%、15%无头精子患者设为对照组, 分别对3例无头精子患者的精子进行精子染色质扩散实验(sperm nucleus DNA integrity kit, SCD)合并精子荧光原位杂交技术(fluorescence *in situ* hybridization, FISH)检测。结果: 无头精子患者睾丸曲细精管严重病变; 在透射电子显微镜下可见到精子顶体、线粒体、轴丝等程度不同的结构异常; 无头精子患者的精子染色体非整倍体率与精子核DNA损伤程度成正比, 且随无头精子比例增多而升高。结论: 无头精子有病理意义, 其发生与精子DNA损伤、精子染色体异常以及编码头尾连接段的基因缺陷有关。

关键词: 无头精子; 男性不育; 精子核DNA损伤; 基因缺陷

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98% Acephalic Sperm —— A Case Report and Review of Literature

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【ABSTRACT】 Objective: To report a case of 98% acephalic sperm and explore its mechanism, in order to provide a laboratory evidence to those who want to have a treatment with acephalic sperm. **Methods:** Transemission electronic microscopic study of the semen sample from testicular seminiferous tubules was done and ultrastructural study of acephalic sperm was done; with 20%, 15% acephalic sperm as controls, the sperm nucleus DNA integrity kit combined with sperm fluorescence *in situ* hybridization (FISH) were used to detect the 3 cases. **Results:** The testicular seminiferous tubules had severe lesions in patients with acephalic sperm; under transmission electron microscopy (TEM), different levels of structural abnormalities in acephalic sperm, such as acephalic sperm, with abnormalities in mitochondria, axoneme could be observed. Sperm chromosome aneuploidy rate had proportional to the degree of sperm nucleus DNA damage in the patients with acephalic sperm, and increased with the proportion of acephalic sperm. **Conclusion:** Acephalic sperm have a pathological significance, its occurrence is associated with sperm nucleus DNA damage, sperm chromosomal abnormalities and gene defect which encodes the connection of the sperm head and tail segments.

Key words: acephalic sperm; male infertility; sperm nucleus DNA damage; gene defect

· 综述 ·

GC/cGMP信号转导途径在雄性生殖作用的研究进展

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【摘要】 鸟苷酸环化酶(guanylate cyclases, GCs)通过催化细胞内第二信使环磷酸鸟苷(cyclic guanosine monophosphate, cGMP)的生成来转导信号; cGMP主要激活其下游效应分子cGMP依赖性蛋白激酶(PKG)、环核苷酸门控离子通道(CNG)和磷酸二酯酶(PDE),从而介导多种生物学效应。近年来大量研究证实,GC/cGMP信号转导体系与雄性生殖过程密切相关,多种介质通过此信号通路影响精子活力、获能和顶体反应,参与精子发生、运输、成熟及阴茎勃起等过程。

关键词: 鸟苷酸环化酶(GC); 环磷酸鸟苷(cGMP); 雄性生殖; 利钠肽; 一氧化氮(NO); 一氧化碳(CO)

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• 综述 •

Roles of GC/cGMP Signal Transduction System in Male Reproduction

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【ABSTRACT】 Guanylate cyclases (GCs) are important enzymes that signal via the production of the intracellular second messenger cyclic guanosine monophosphate (cGMP). cGMP, mainly through activation of its downstream effectors cGMP-dependent protein kinases (PKG), phosphodiesterases (PDE) and cyclic nucleotide-gated ion channels (CNG), has been implicated in mediating a variety of physiological effects. In recent years, a large number of studies have demonstrated specific relevance of the GC/cGMP system in male reproductive processes because many substances can influence sperm motility, capacitation and acrosome reaction, and are involved in spermatogenesis, sperm transport, maturation and penile erection. This review will focus on the GC/cGMP signal transduction system and advances of its roles in the regulation of male reproductive processes.

Key words: guanylate cyclases (GCs); cyclic guanosine monophosphate (cGMP); male reproduction; natriuretic peptides; nitrogenmonoxide (NO); carbon monoxide (CO)

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曲细精管内移植多能干干细胞的研究

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【摘要】近年来, 干细胞体内分化的研究得到了广泛的开展, 其中干细胞曲细精管内移植与分化的相关研究取得了令人瞩目的进展, 常用的曲细精管内移植的方法有曲细精管注射法、睾丸输出管注射法和睾丸网注射法, 各有优缺点。所移植的细胞可以为精原干细胞、体外分化的胚胎干细胞和诱导多能性干细胞及成体干细胞, 从而为研究睾丸细胞功能、生物学特性及更好地保存个体基因组和治疗某些疾病所引起的少精子症、无精子症提供强而有力的生物学工具。

关键词: 曲细精管; 干细胞; 移植; 体内分化

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Transplantation of Pluripotent Stem Cells in Seminiferous Tubules

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【ABSTRACT】 Stem cells are pluripotent and self-renewing cells, which can differentiate into specific tissue cell types under certain conditions. In recent years, the studies of stem cells differentiation *in vivo* have been widely carried out. Moreover, transplantation and differentiation of stem cells in the seminiferous tubules have made great progress, which may provide a novel clinical treatment for male infertility. In this review, we discussed the development of seminiferous tubules transplantation techniques and recent progresses of stem cells differentiation within the seminiferous tubules, and prospected the potential application in this field as well.

Key words: seminiferous tubule; stem cell; transplantation; differentiation *in vivo*

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Survivin在男性泌尿系统肿瘤中的临床意义

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【摘要】 泌尿系统肿瘤是威胁男性健康的最常见的恶性肿瘤。肿瘤的发生发展是多种基因参与的细胞增殖与凋亡平衡的过程。Survivin 是凋亡抑制蛋白家族(inhibitor of apoptosis proteins, IAP)的成员之一,它的主要功能是抑制细胞凋亡和促进细胞增殖,具有在正常组织中低表达,在恶性肿瘤组织中高表达的特异性。Survivin可作为泌尿系统肿瘤的早期诊断和预后判断指标,也可作为肿瘤治疗的新靶点。

关键词: Survivin; 细胞凋亡; 男性泌尿系统肿瘤

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Clinical Significance of Survivin in Male Urinary System Tumor

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【ABSTRACT】 Male urinary system tumor is the most common malignancies of threats man's health. The occurrence and progress of tumor is a balance process between cell proliferation and apoptosis, which involves multiple genes to participate in. Survivin is a member of inhibitor of apoptosis proteins (IAP), which regulates two essential cellular processes, it inhibits apoptosis and promotes cell proliferation. Survivin is rarely expressed in normal differentiated tissues but is unregulated in the majority of malignancies. Survivin may be a judgment index for diagnosis and prognosis of malignant tumors and a new target for cancer therapy. This review presents recent advances on molecular structure, biological functions, mechanism of gene actions and the research in male urinary system tumors of Survivin.

Key words: Survivin; cell apoptosis; male urinary system tumor

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精液与妊娠

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【摘要】自首例试管婴儿诞生以来, 辅助生殖技术不断取得进步, 显微操作技术的应用以及序贯培养基的使用使成功率得到了提高, 而与自然妊娠相比, 精液的存在与否是其不同之处。精液在形成受精卵、为精子供能方面的作用外是否还有更重要的作用, 一直为广大生殖医学学者所研究。本文就精液与妊娠、精液与母体免疫、精液与孕体共同抗原、精液引起的反应等方面进行综述。

关键词: 精液; 妊娠; 转化生长因子- β (TGF- β); 粒细胞-巨噬细胞集落刺激因子(GM-CSF)

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Semen and Pregnancy

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【ABSTRACT】 From the birthday of the first baby of *in vitro* fertilization-embryo transfer (IVF-ET), assisted reproductive technology (ART) continues to make progress. The success rate was improved by the application of micromanipulation technique and sequential medium, but when compared with the normal pregnancy, the existence of semen or not is the difference between them. Pregnancy cannot occur in absence of sperm. Seminal plasma promotes sperm survival, transport. Is there any potential function between semen and female tissues? This question gained many experts' attention. This article review the relationship of semen and pregnancy, semen and maternal immune, antigens shared by semen and conceptus, reproductive related cytokines in semen.

Key words: semen; pregnancy; TGF- β ; GM-CSF

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