



利用Transwell膜孔筛选高质量精子及其性能评估*

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【摘要】目的 基于Transwell膜孔的分子筛作用,探讨利用Transwell优选人类高质量精子的可行性。**方法** 收集2023年11月-2024年5月广州中医药大学第一附属医院男科新鲜精液标本162份。分析经5 μm和8 μm孔径Transwell精子优选的差异并经透射电子显微镜(transmission electron microscope, TEM)观察微观结构差异,比较Transwell优选精子30 min、60 min、80 min后精液参数的变化,比较Transwell与密度梯度离心法(density gradient centrifugation, DGC)和上游法(swim-up methods, SU)对不同质量精子优化后精液参数的差异。本研究将精子前向运动(progressive motility, PR)百分率设定为主要结局指标,精子活力、正常精子形态率(normal sperm morphology rate, NSMR)、DNA碎片指数(DNA fragmentation index, DFI)、精子浓度及回收率设定为次要结局指标。**结果** 当孔径为5 μm、优选时间为60 min时,Transwell优选精子效果更佳。在正常精液组中,与DGC法相比,Transwell优选后的精子PR百分率中位数从77.50%升至92.05%($P<0.05$),NSMR中位数从9.00%升至13.25%($P<0.01$),DFI中位数从2.16%降至0.25%($P<0.01$);与SU法相比,Transwell优选后的精子浓度中位数从 $4.70\times 10^6\text{ mL}^{-1}$ 升至 $14.15\times 10^6\text{ mL}^{-1}$ ($P<0.01$),DFI中位数从0.47%降至0.25%($P<0.01$),回收率中位数从2.50%升至6.48%($P<0.01$),但精子活力、PR百分率和NSMR差异无统计学意义。在异常精液组中,与DGC法相比,Transwell优选后的精子PR百分率中位数由37.20%升至88.75%($P<0.01$),精子活力中位数由53.00%升至95.00%,DFI中位数由12.49%降至0.92%($P<0.01$);与SU法相比,Transwell优选后的精子PR百分率中位数由86.30%升至88.75%($P<0.05$),DFI中位数由1.63%降至0.92%($P<0.01$)。**结论** Transwell优选方法在获得足够数量、活力高、形态良好以及DNA完整性较佳的精子方面表现出较好性能。这种特性使其有望成为辅助生殖技术中精选高质量精子的简便实用方法。

【关键词】 辅助生殖技术 精子 精子活力 DNA碎片 Transwell

Screening of High-quality Spermatozoa Using Transwell Membrane Pores and Assessment of Their Performance

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This study was supported by the National Natural Science Foundation of China (No. 82360311), the Guangdong Basic and Applied Basic Research Foundation (No. 2021A1515010308), and the Guangdong Medical Scientific Research Foundation (No. A2023401).

[Abstract] Objective The feasibility of using Transwell, based on the molecular sieve effect of its membrane pores, to select high-quality human sperm was explored. **Methods** A total of 162 fresh semen samples were collected from the Department of Andrology at the First Affiliated Hospital of Guangzhou University of Chinese Medicine between November 2023 and May 2024. Differences in sperm selection using 5 μm and 8 μm pore Transwell filters were analyzed,

* 国家自然科学基金(No. 82360311)、广东省基础与应用基础研究基金(No. 2021A1515010308)和广东省医学科研基金(No. A2023401)资助

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and microscopic structural differences were observed by transmission electron microscopy (TEM). Changes in semen parameters after 30, 60, and 80 minutes of Transwell sperm selection were compared. Differences in semen parameters after optimization of sperm of varying qualities by Transwell, density gradient centrifugation (DGC), and swim-up (SU) methods were also compared. In this study, the percentage of progressive motility (PR) of sperm was set as the primary outcome measure, while sperm motility, normal sperm morphology rate (NSMR), DNA fragmentation index (DFI), sperm concentration, and recovery rate were set as secondary outcome measures. **Results** When the aperture is 5 μm and the preferred time is 60 minutes, the Transwell method yields better results for sperm selection. In the normal semen group, compared with the DGC method, the median percentage of PR in sperm after Transwell selection increased from 77.50% to 92.05% ($P < 0.05$), the median NSMR increased from 9.00% to 13.25% ($P < 0.01$), and the median DFI decreased from 2.16% to 0.25% ($P < 0.01$). Compared with the SU method, the median sperm concentration after Transwell selection increased from $4.70 \times 10^6 \text{ mL}^{-1}$ to $14.15 \times 10^6 \text{ mL}^{-1}$ ($P < 0.01$), the median DFI decreased from 0.47% to 0.25% ($P < 0.01$), and the median recovery rate increased from 2.50% to 6.48% ($P < 0.01$). but there were no statistically significant differences in sperm motility, PR percentage, or NSMR. In the abnormal semen group, compared with the DGC method, the median PR percentage of sperm after Transwell selection increased from 37.20% to 88.75% ($P < 0.01$), the median sperm motility increased from 53.00% to 95.00%, and the median DFI decreased from 12.49% to 0.92% ($P < 0.01$). Compared with the SU method, the median PR percentage of sperm after Transwell selection increased from 86.30% to 88.75% ($P < 0.05$), and the median DFI decreased from 1.63% to 0.92% ($P < 0.05$). **Conclusion** The Transwell preferred method demonstrates excellent performance in obtaining sufficient sperm with high viability, good morphology, and good DNA integrity. This makes it a promising, simple, and practical method for selecting high-quality sperm in assisted reproductive technologies.

[Key words] Assisted reproductive technology Spermatozoa Sperm motility DNA fragmentation
Transwell

精液优化处理技术作为辅助生殖技术的关键环节,其目的是将非精子细胞成分进行有效剔除,并回收高质量精子,为后续受精环节提供足够数量的优质精子,进而改善临床结局^[1]。密度梯度离心法(density gradient centrifugation, DGC)和上游法(swim-up methods, SU)是临床常用的精子优选方法,但前者因多次离心可能导致机械损伤和氧化应激增加,影响精子质量,后者回收率较低,限制了其应用^[2]。新型方法如磁性活化细胞分选虽可改善精子活力和DNA完整性,但操作繁琐且存在外源污染风险^[3];微流控分选具精子质量提升优势,但回收率低、成本高,难以广泛应用^[4];浮游器上游法操作简便,但对低浓度或差质精液效果不佳^[5]。因此,亟需研发更简便、高效的精子优选方法以满足临床需求。

Transwell培养皿是一种常用的细胞过滤装置,由上下腔室和多孔膜组成,可选择不同孔径(如0.4 μm 、5.0 μm 、8.0 μm)用于多种实验^[6]。其主要应用包括:①细胞迁移和侵袭研究,通过多孔膜观察细胞迁移特性^[7];②药物筛选和细胞间相互作用研究,用于测试药物渗透、毒性及细胞相互作用^[8];③分泌物研究,用于收集细胞分泌因子或蛋白质^[9]。Transwell因其灵活性被广泛用于体外细胞实验,其膜筛可以模拟人体生理多孔跨膜环境,并保持细胞形态和功能完整^[10]。正常人类成熟精子头部直径约为

2~3 μm ,当选用膜孔径大于3 μm 时,人类精子可自由穿透。目前尚未见有Transwell用于优选高质量精子的相关报道,本次研究依据Transwell的结构特点,将精子置于小室下层,利用精子的运动特性穿过隔离膜聚集于上层,从而实现高质量精子的优选。

1 资料与方法

1.1 研究对象

收集2023年11月–2024年5月在广州中医药大学第一附属医院男科实验室做精液检查的男性新鲜精液162份。样本根据研究目的分为3组:32份正常精液样本用于不同孔径比较,30份正常精液样本用于不同时长比较,100份(正常精液50份,异常精液50份)用于3种方法比较。纳入条件:患者年龄22~45岁,禁欲时间2~7 d,采用手淫法收集精液样本于无菌无毒的广口杯内,精液量 $\geq 2 \text{ mL}$,浓度 $\geq 10 \times 10^6 \text{ mL}^{-1}$,活力 $\geq 10\%$,精液液化时间正常。分组:根据WHO第5版标准^[11],①正常精液组,精液体积 $\geq 1.5 \text{ mL}$,精子浓度 $\geq 15 \times 10^6 \text{ mL}^{-1}$ 或精子总数 $\geq 39 \times 10^6$,精子前向运动(progressive motility, PR)百分率 $\geq 32\%$,正常精子形态率(normal sperm morphology rate, NSMR) $\geq 4\%$;②异常精液组,符合以下任一条件者纳入异常精液组:精子浓度 $< 15 \times 10^6 \text{ mL}^{-1}$,PR百分率 $< 32\%$,

NSMR<4%。本次研究中方法比较组的50份异常精液样本中,有43份(86%)PR百分率<32%,有42份(84%)NSMR<4%,同时包含PR百分率<32%和NSMR<4%有36份。排除标准:存在排精障碍。本研究已通过广州中医药大学第一附属医院伦理委员会批准(批件号NO.K-2023-014),所有研究对象均已获得知情同意,知情同意书包括研究目的、方法、可能的风险和受益、隐私保护措施以及自愿参与和随时退出的权利说明。

1.2 试剂和仪器

梯度离心培养液(ART-1005,美国Sage公司);G-IVF Plus(10136,瑞典Vitrolife公司);Transwell培养皿(14331/14342,北京兰杰柯科技有限公司),经精子存活实验检测合格后使用;Diff-Quik染液和吡啶橙(OA)染色液试剂盒(深圳华康生物医学工程有限公司)。

计算机辅助精液分析系统(CASA,西班牙Microptic公司);CX41相差生物显微镜(OLYMPUS公司);CytoPOC流式细胞仪(北京指真生物科技有限公司)。

1.3 实验方法

1.3.1 不同孔径Transwell对优选高质量精子的影响

32份正常精液用于不同孔径比较。每份精液样本分成2份,每孔取1 mL完全液化的精液加入Transwell培养孔下室,分别放入5 μm 和8 μm 孔径的内嵌上室,并在上室中加入300 μL G-IVF Plus, 37 $^{\circ}\text{C}$ 、体积分数为5%CO₂培养箱中静置30 min,回收上室悬液,检测精液浓度、精子活力、PR百分率、NSMR和DFI等精液参数。所有样本设置3个重复孔取平均值。

1.3.2 优选时长对Transwell优选高质量精子的影响

30份正常精液用于不同时长比较。每份精液样本分成3份,每孔取1 mL完全液化的精液加入Transwell培养孔下室,然后放入5 μm 孔径的内嵌上室,并在上室中加入300 μL G-IVF Plus, 于37 $^{\circ}\text{C}$ 、体积分数为5%CO₂培养箱中静置,分别在30 min、60 min、80 min时回收上室悬液,检测精液浓度、精子活力、PR百分率、NSMR和DFI等精液参数。所有样本设置3个重复孔取平均值。

1.3.3 三种不同处理方法优选精子的效果比较

100份精液样本(正常精液样本50份,异常精液样本50份)精液量 ≥ 3 mL/份,每份精液样本完全液化后分成3份,分别用DGC、SU和Transwell优选精子。

DGC: 在15 mL锥形离心管中,沿壁缓慢依次加入1 mL 80%和40%梯度离心液,以及1 mL完全液化且混匀的精液样本。300 $\times$ g离心20 min,弃上清后,将沉淀加入2 mL G-IVF Plus重悬,300 $\times$ g离心5 min,如此洗涤两次,弃上清,加0.5 mL G-IVF Plus重悬,混匀后用于后续检测。

SU: 在15 mL圆底试管中加入1.5 mL G-IVF Plus, 37 $^{\circ}\text{C}$ 预热。待精液完全液化后,将1 mL充分混匀的精液缓慢加入试管底部,倾斜45 $^{\circ}$ 置于37 $^{\circ}\text{C}$ 培养箱60 min。吸取云雾状上层液至另一支试管,加入2 mL G-IVF Plus混匀,300 $\times$ g离心5 min,弃上清,加入0.3 mL G-IVF Plus重悬后用于后续检测。

Transwell: 精液加入Transwell培养孔下室,然后放入5 μm 孔径的内嵌上室,并在上室中加入300 μL G-IVF Plus, 于37 $^{\circ}\text{C}$ 、体积分数为5%CO₂培养箱中静置,60 min回收上室悬液并检测精子质量相关参数。

1.3.4 精液浓度与精子活力检测

按WHO第5版要求^[1],应用计算机辅助精液分析系统对精液进行分析,包括精液浓度、精子活力。精子活力(%)= PR百分率+非前向运动(non- progressive motility, NP)百分率。

1.3.5 精子形态分析

按WHO第5版要求^[1],取10 μL 精液均匀涂于玻片上,晾干后用Diff-Quik快速染色试剂盒染色,CX41相差生物显微镜观察,每份标本分析200个以上精子形态,并统计NSMR。

1.3.6 DNA碎片指数检测

参照OA染色液试剂盒说明书操作,用OA对精子进行染色后,通过流式细胞仪对精子悬液进行检测,收集红色和绿色荧光的数据并进行公式转换,计算其DNA碎片指数(DNA fragmentation index, DFI)。

1.3.7 精子超微结构的电镜检测

为从亚细胞层面验证不同孔径Transwell的筛选机制,随机抽取处理前组、5 μm 组和8 μm 组样本进行透射电镜观察。精子优选后2 h内经2.5%戊二醛固定1 h, PBS缓冲液清洗固定液后采用1%醋酸铅进行渗透,增加膜的对对比度。30%~100%乙醇梯度脱水,将脱水后的精子样本在环氧树脂中包埋,经过加热聚合,使样本变硬。使用超薄切片机(超薄切片器)切取样本,切片厚约50~100 nm,经广州华银医学检验中心进行电镜观察与分析。随机选取5个视野,每组从这5个视野中共计数200个精子,统计头部畸形、顶体异常、线粒体异常、轴丝结构异常等的发生率。

1.4 结局指标定义

主要结局指标: 基于PR百分率在评估精子穿透能力和受精潜能方面的核心作用,故将其作为主要结局指标。

次要结局指标: 精子活力、NSMR、DFI、精液浓度及回收率。回收率(%)=(优选后精子总数/优选前精子总数) $\times 100\%$ 。次要结局指标作为探索性分析指标。

1.5 统计学方法

数据处理采用SPSS 22.0软件分析。差异性比较前对数据进行正态分布检测,若均符合正态分布,采用 $\bar{x}\pm s$ 表示,否则用中位数(四分位间距)表示。对于配对设计的多组间比较,正态分布且方差齐性的资料采用重复测量方差分析,非正态分布或方差不齐的资料采用Friedman检验,事后两两比较采用Bonferroni校正的Wilcoxon符号秩检验,以控制 I 类错误。多重比较校正: 鉴于本研究涉及多个结局指标的比较,主要结局指标(PR百分率)的检验水准为 $\alpha=0.05$,次要结局指标(精子活力、NSMR、DFI、精液浓度、回收率)经Bonferroni校正后检验水准为 $\alpha'=0.05/5=0.01$ 。超微结构异常率的组间比较采用卡方检验或Fisher确切概率法,检验水准为 $\alpha=0.05$,该分析作

为机制验证性研究,不纳入主要/次要结局指标的多重比较校正框架。

2 结果

2.1 不同孔径Transwell优选精子的效果比较

本组实验共纳入32例样本,平均年龄(31.56 ± 4.09)岁(24~38岁)。与处理前组比较,5 μm 组和8 μm 组各精液参数的差异均有统计学意义($P<0.01$)。主要结局指标: 5 μm 组与8 μm 组相比,PR百分率差异无统计学意义($P>0.05$)。次要结局指标: 5 μm 组与8 μm 组相比,DFI更低($P<0.01$),精子活力、NSMR和精液浓度差异均无统计学意义($P>0.01$)。结果提示5 μm 孔径Transwell在降低DFI方面效果更佳。见表1和图1。

表 1 不同孔径Transwell优选后各精液参数的比较 [M (Q_R)]

Table 1 Comparison of various semen parameters after Transwell selection with different pore sizes (M [Q_R])

Parameter	Pre-treatment (n = 32)	5 μm group (n = 32)	8 μm group (n = 32)	χ^2	P
Concentration/ 10^6 mL^{-1}	51.80 (39.90)	4.50 (7.90)**	6.35 (11.30)**	66.49	< 0.001
Motility/%	73.10 (14.90)	96.20 (5.00)**	94.85 (6.90)**	71.35	< 0.001
PR/%	46.10 (16.90)	87.40 (11.20)**	85.65 (14.20)**	181.84	< 0.001
NSMR/%	4.00 (2.50)	11.88 (9.80)**	10.88 (6.50)**	107.93	< 0.001
DFI/%	8.35 (4.50)	0.31 (0.60)**	0.81 (1.54)**,#	69.36	< 0.001

PR: percentage of progressive motility; NSMR: normal sperm morphology rate; DFI: DNA fragmentation index; M: median; Q_R: interquartile range. Overall comparison using Friedman test for repeated measures; pairwise comparisons using Wilcoxon signed-rank test with Bonferroni correction. ** $P<0.01$, vs. pre-treatment group; # $P<0.01$, vs. 5 μm group.

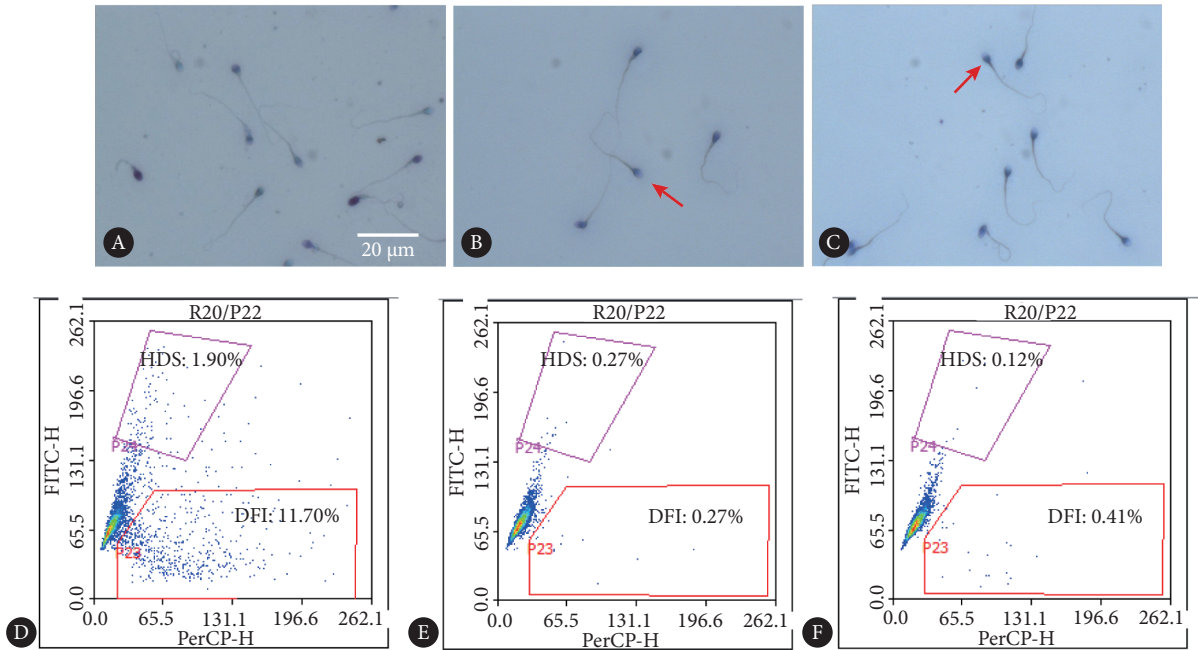


图 1 不同孔径Transwell优选前后的精子形态和DFI

Fig 1 Sperm morphology and DFI before and after selection with different pore diameters in the Transwell assay

HDS: high DNA stainability; DFI: DNA fragmentation index. A-C, Morphology in the pre-treatment group (A), 5 μm group (B), and 8 μm group (C); D-F, DFI in the pre-treatment group (D), 5 μm group (E), and 8 μm group (F). Red arrows indicate morphologically normal spermatozoa (Diff-Quik staining $\times 1000$).

2.2 不同孔径Transwell优选精子的超微结构分析

随机抽取处理前组、5 μm 组与8 μm 组样本进行精子超微结构的电镜分析, 随机选取5个视野下的200个精子进行结构分析, 统计其中异常比例。结果显示, 与处理前组相比, Transwell选择后异常精子形态率有所改善, 5 μm 孔径选择组异常精子形态率最低, 具体表现为5 μm 组头部畸形率、顶体结构异常率、细胞核发育不良率、线粒体异常率、轴丝异常率、微管/ODF异常率和末

端轴丝异常率均低于处理前组($P < 0.05$); 8 μm 组头部畸形率、线粒体异常率、轴丝异常率、微管/ODF异常率和末端轴丝异常率低于处理前组($P < 0.05$), 但顶体结构异常率和细胞核发育不良率与处理前组差异无统计学意义($P > 0.05$)。5 μm 组线粒体异常率、细胞核发育不良率和末端轴丝异常率均低于8 μm 组($P < 0.05$)。综上, 5 μm 孔径Transwell在降低精子超微结构异常率方面表现最佳。见表2。

表 2 不同孔径Transwell优选后精子的电镜超微结构检测

Table 2 Electron microscopic ultrastructural examination of preferentially selected sperm with varying pore diameters using the Transwell system

Group	Head		Cell	Mid-piece		Principal piece	End piece
	Deformity rate/%	Acrosomal abnormality/%	Nuclear dysplasia/%	Mitochondrial abnormality/%	9 + 2 axonemal abnormality/%	Microtubule/ODF abnormality/%	Axonemal abnormality/%
Pre-treatment	98	79	41	83	47	46	26
5 μm	70 ^{***}	55 ^{***}	23 ^{**}	45 ^{***}	25 ^{**}	20 ^{***}	4 ^{***}
8 μm	81 ^{***}	68	38 [#]	65 ^{**, #}	26 ^{**}	27 ^{**}	14 ^{*, #}

ODF: outer dense fiber. A total of 200 spermatozoa per group were counted from 5 random fields to assess ultrastructural abnormality rates. Statistical comparisons were performed using Chi-square test or Fisher's exact test. Ultrastructural analysis was exploratory with $\alpha = 0.05$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, vs. pre-treatment group; # $P < 0.05$, ** $P < 0.01$, vs. 5 μm group.

2.3 5 μm 孔径Transwell优选精子时长对精子质量的影响

本组实验共纳入30例样本, 平均年龄(31.50 $\pm$ 4.60)岁(25 ~ 39岁)。与处理前组比较, 30 min组、60 min组和80 min组各精液参数的差异均有统计学意义($P < 0.01$)。主要结局指标: PR百分率在60 min组达到最高值(中位数91.60%), 高于30 min组和80 min组, 差异有统计学意义($P < 0.05$)。次要结局指标: 精子活力在3组间差异无统计

学意义($P > 0.01$); NSMR在60 min组达到最高值(中位数13.95%), 高于30 min组($P < 0.01$), 与80 min组比较差异无统计学意义($P > 0.01$); DFI在60 min组(中位数0.35%)与30 min组相比差异无统计学意义($P > 0.01$), 但低于80 min组($P < 0.01$); 精液浓度随着优选时间的延长逐渐上升($P < 0.01$)。以上结果提示60 min是Transwell优选精子的最佳时间。见表3和图2。

表 3 5 μm 孔径的Transwell优选不同时间后各精液参数的比较 [M (Q_k)]

Table 3 Comparison of semen parameters at different times after using a 5 μm pore size Transwell (M [Q_k])

Parameter	Pre-treatment (n = 30)	30 min group (n = 30)	60 min group (n = 30)	80 min group (n = 30)	χ^2	P
Concentration/ 10^6 mL^{-1}	48.20 (43.33)	4.20 (5.08) ^{**}	11.30 (9.25) ^{**, #}	15.20 (15.43) ^{**, #, ▲}	32.85	< 0.001
Motility/%	69.30 (21.43)	95.70 (7.17) ^{**}	96.85 (4.10) ^{**}	94.25 (8.73) ^{**}	95.71	< 0.001
PR/%	40.70 (12.05)	86.20 (8.25) ^{**}	91.60 (8.90) ^{**, #}	86.45 (10.53) ^{**, ▲}	203.82	< 0.001
NSMR/%	4.50 (3.00)	12.00 (9.56) ^{**}	13.95 (7.75) ^{**, #}	12.45 (11.15) ^{**}	22.95	< 0.001
DFI/%	8.75 (5.43)	0.45 (0.60) ^{**}	0.35 (0.69) ^{**}	0.69 (1.19) ^{**, ▲}	71.48	< 0.001

The abbreviations are explained in the note to Table 1. Overall comparison using Friedman test for repeated measures; pairwise comparisons using Wilcoxon signed-rank test with Bonferroni correction. ** $P < 0.01$, vs. pre-treatment group; # $P < 0.05$, ** $P < 0.01$, vs. 30 min group; ▲ $P < 0.05$, ▲▲ $P < 0.01$, vs. 60 min group.

2.4 3种不同处理方法优选精子的比较

本组实验共纳入100例样本。其中, 正常精液标本50例, 平均年龄(32.34 $\pm$ 4.32)岁(24 ~ 41岁), 异常精液标本50例, 平均年龄(32.96 $\pm$ 5.01)岁(23 ~ 42岁)。

正常精液组中, 与处理前组比较, 不同处理方法组各精液参数的差异均有统计学意义($P < 0.01$)(除外DGC组

的精子活力)。主要结局指标: 与DGC组(中位数77.50%)相比, SU组(中位数92.60%)和Transwell组PR百分率(中位数92.05%)升高($P < 0.05$), SU组与Transwell组间PR百分率差异无统计学意义($P > 0.05$)。次要结局指标: 3组间精子活力的差异无统计学意义($P > 0.01$); 与DGC组比较, SU组和Transwell组NSMR升高($P < 0.01$), DFI、精液浓度

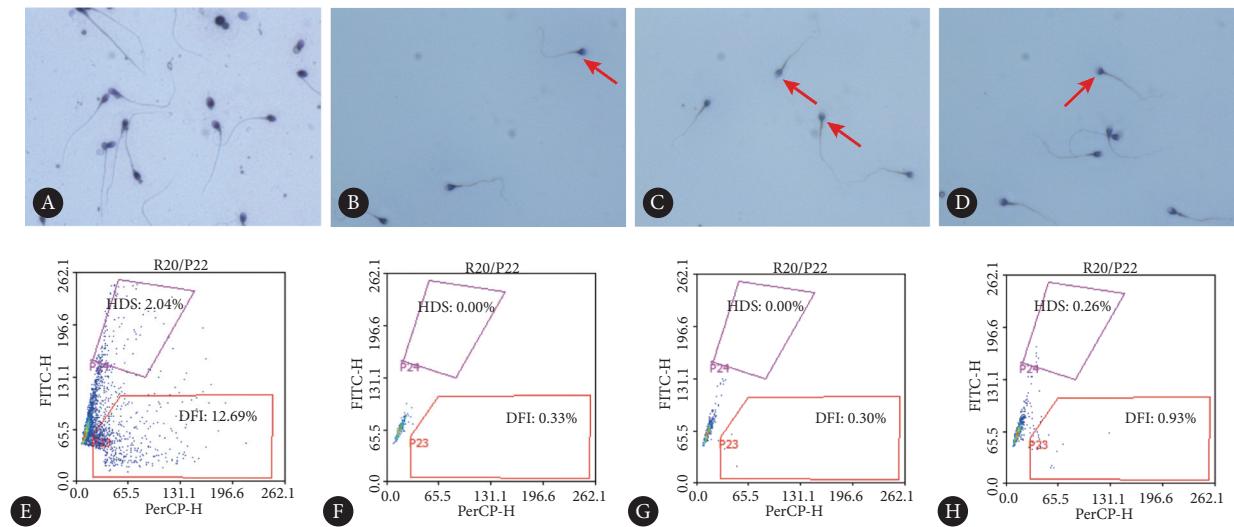


图 2 5 μm 孔径的 Transwell 不同时间优选前后的精子形态和 DFI

Fig 2 Sperm morphology and DFI before and after optimal selection at different times using a 5 μm pore size in Transwell

HDS: high DNA stainability; DFI: DNA fragmentation index. A-D, Morphology in the pre-treatment group (A), 30-minute group (B), 60-minute group (C), and 80-minute group (D); E-H, DFI in the pre-treatment group (E), 30-minute group (F), 60-minute group (G), and 80-minute group (H). Red arrows indicate morphologically normal spermatozoa (Diff-Quik staining $\times 1000$).

和回收率降低 ($P < 0.01$); 与 SU 组相比, Transwell 组 NSMR 无明显变化 ($P > 0.01$), 但 DFI 更低 ($P < 0.01$), 精液浓度和回收率更高 ($P < 0.01$)。

异常精液组中, 与处理前组比较, 不同处理方法组各精液参数的差异均有统计学意义 ($P < 0.01$) (除外 DGC 组的 DFI)。主要结局指标: 与 DGC 组 (中位数 37.20%) 相比, SU 组 (中位数 86.30%) 和 Transwell 组 PR 百分率 (中位数 88.75%) 升高 ($P < 0.05$), Transwell 组 PR 百分率较 SU 组升

高更明显 ($P < 0.05$)。次要结局指标: 与 DGC 组相比, SU 组和 Transwell 组精子活力和 NSMR 升高 ($P < 0.01$), DFI、精液浓度和回收率降低 ($P < 0.01$); 与 SU 组相比, Transwell 组 DFI 更低 ($P < 0.01$), 精液浓度和回收率更高 ($P < 0.01$)。

综上, 无论是质量正常还是质量较差的精液样本, Transwell 法在改善主要结局指标 (PR 百分率) 及次要结局指标 (DFI) 方面较 DGC 法和 SU 法更具优势。见表 4 和图 3。

表 4 3 种不同方法精液优选后各精液参数的比较 [M (Q_k)]

Table 4 Comparison of various semen parameters after selecting the top three semen processing methods (M [Q_k])

Parameter	Normal semen group (n = 50)				F/χ^2	P	Abnormal semen group (n = 50)				χ^2	P
	Pre-treatment	DGC	SU	Transwell			Pre-treatment	DGC	SU	Transwell		
Concentration/ 10 ⁶ mL ⁻¹	54.55 (45.53)	34.00 (40.73)**	4.70 (12.58)**,**	14.15 (16.83)**,**,▲▲	67.56	< 0.001	20.50 (18.08)	13.90 (11.98)**	1.55 (1.95)**,**	3.20 (5.73)**,**,▲▲	53.04	< 0.001
Motility/%	77.80 (18.63)	89.55 (6.80)	97.00 (4.67)**	97.15 (2.70)**	67.48	< 0.001	39.95 (17.63)	53.00 (34.60)**	94.00 (8.00)**,**	95.00 (6.63)**,**	107.84	< 0.001
PR/%	49.85 (19.75)	77.50 (14.32)**	92.60 (8.55)**,**	92.05 (4.95)**,*	173.67	< 0.001	21.35 (13.65)	37.20 (38.33)**	86.30 (13.60)**,**	88.75 (11.27)**,**,▲	132.20	< 0.001
NSMR/%	4.50 (2.06)	9.00 (5.13)**	12.50 (5.25)**,**	13.25 (5.90)**,**	63.80	< 0.001	2.50 (1.63)	3.95 (3.77)**	6.20 (4.45)**,**	7.50 (6.15)**,**	39.71	< 0.001
DFI/%	7.43 (6.03)	2.16 (4.50)**	0.47 (0.86)**,**	0.25 (0.32)**,**,▲▲	53.30	< 0.001	13.33 (13.28)	12.49 (15.53)	1.63 (7.60)**,**	0.92 (2.66)**,**,▲▲	12.77	< 0.01
Recovery rate/%	—	30.42 (13.75)	2.50 (3.68)**	6.48 (4.47)**,**,▲▲	235.46	< 0.001	—	30.75 (12.52)	1.78 (1.32)**	4.36 (4.95)**,**,▲▲	254.52	< 0.001

DGC: density gradient centrifugation; SU: swim-up. The abbreviations are explained in the note to Table 1. Within each group, overall comparison using Friedman test for repeated measures; pairwise comparisons using Wilcoxon signed-rank test with Bonferroni correction. ** $P < 0.01$, vs. pre-treatment group; * $P < 0.05$, ** $P < 0.01$, vs. DGC group; ▲ $P < 0.05$, ▲▲ $P < 0.01$, vs. SU group.

3 讨论

Transwell 是一种由带微孔薄膜相隔为上下两层的特殊培养皿, 薄膜材质为聚碳酸酯 (PC) 薄膜, 采用纯医用级

聚苯乙烯材料, 经严格的工艺控制做到无菌、无热原、无内毒素、无 DNase 和 RNase, 最大限度地保证培养细胞的安全性^[12]。其常用于共培养^[13]、细胞趋化^[14]、细胞迁移和细胞侵袭^[15]等方面的体外研究, 但目前尚未见将 Transwell

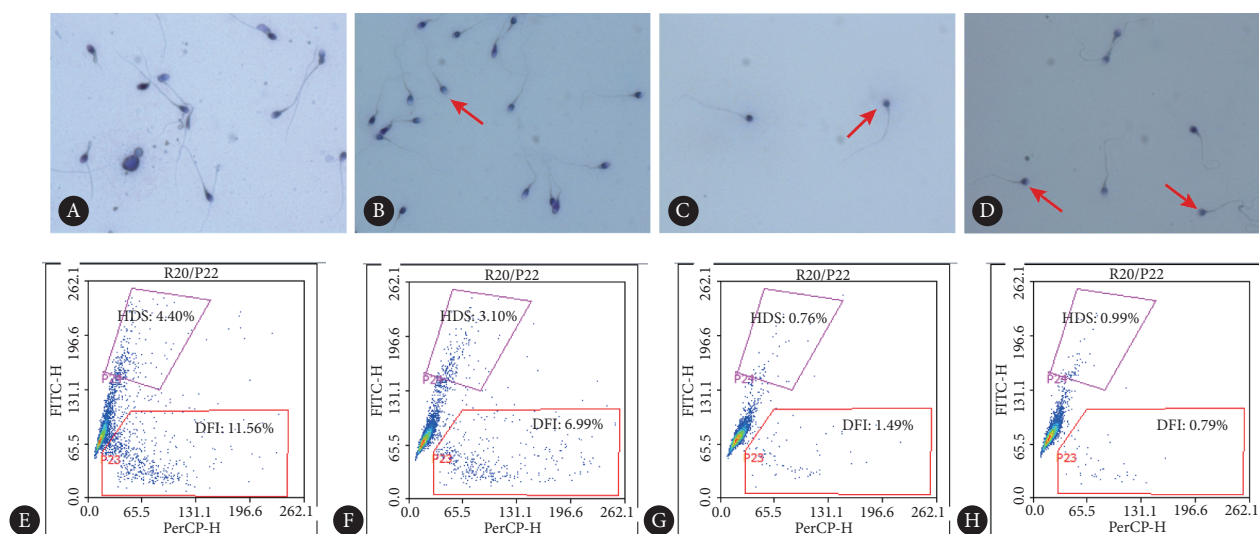


图3 3种不同方法精液优选前后的精子形态和DFI (正常精液组)

Fig 3 Three different methods for evaluating sperm morphology and DFI before and after sperm selection (normal semen group)

HDS: high DNA stainability; DFI: DNA fragmentation index. A-D, Morphology in the pre-treatment group (A), density gradient centrifugation group (B), Swim-up group (C), and Transwell group (D); E-H, DFI in the pre-treatment group (E), density gradient centrifugation group (F), Swim-up group (G), and Transwell group (H). Red arrows indicate morphologically normal spermatozoa (Diff-Quik staining $\times 1000$).

用于优选高质量精子的相关报道。

本研究基于精子穿过膜上的微孔从而实现高质量精子优选的物理学原理,发现微孔孔径大小和优选时长是高质量精子优选的重要影响因素^[16]。本研究比较了5 μm 和8 μm 孔径Transwell对精子的优选效果。结果显示,正常精子经过Transwell优选后,能有效筛选出高质量精子。电镜检测进一步证实,5 μm 孔径Transwell优选后的精子在所有超微结构指标上均显著优于处理前,包括头部、顶体、细胞核、线粒体及轴丝等多个亚细胞结构。相比之下,8 μm 孔径虽然在部分指标(头部畸形、线粒体异常、轴丝、微管/ODF异常及末端轴丝异常)上优于处理前组,但在顶体结构和细胞核发育方面未显示出筛选优势。两组间比较显示,5 μm 孔径Transwell在降低线粒体异常率方面的优势最为显著,提示小孔径可能对精子质量筛选具有更好的选择性。结合5 μm 孔径Transwell在降低DFI和多项超微结构异常率方面的表现,提示较小孔径不仅在DNA完整性筛选上更具优势,在精子亚细胞结构质量控制方面也表现出更强的选择性。关于优选时长,本研究发现60 min的回收时间能够在精子数量和质量之间达到最佳平衡。

本研究进一步分析了Transwell法、DGC法和SU法对正常精液和异常精液标本优选结果的比较。在正常精液组中,与DGC法相比,Transwell法PR百分率明显升高,DFI明显降低;与SU法相比,Transwell法在精液浓度和回收率方面具有优势,但PR百分率差异无统计学意义。在

异常精液组中,Transwell法相较DGC法,PR百分率明显升高,DFI明显降低;与SU法相比,Transwell法PR百分率更高,DFI更低,精液浓度和回收率更高。

与传统精子优选方法相比,Transwell法展现出独特优势。DGC法虽然精子回收率较高,但其优选原理基于精子密度差异,而高质量精子与质量较差精子之间并无明显密度分界线^[17]。另外,多次离心操作会对精子造成不可逆的机械性损伤^[18]。离心力所产生的惯性力使精子与精子之间造成碰撞和摩擦,导致精子质膜和顶体外膜破裂或损伤^[18]。同时,产生的剪切力可使精子不同部分之间会受到不同速度和方向的拉力,导致精子内部细胞器结构的扭曲和变形,进而影响精子的形态、功能及结局^[19-20],这可能是无法优选出高质量精子的原因之一。与DGC法的机械损伤不同,Transwell法和SU法均依靠精子自身活力进行优选,避免了机械力造成的潜在损伤。然而,SU法缺乏严格的分界区,无法有效隔离低质量精子^[21]。Transwell的膜筛不仅能过滤大分子精浆杂质(如白细胞、免疫活性细胞、死精子和细胞碎片等),减少氧化应激,还能有效分离高质量精子。这种物理屏障机制使得Transwell在处理异常精液标本时表现尤为突出,能够获得质量和数量均满足辅助生殖技术要求的精子。

本研究存在以下局限性:①本研究涉及多个结局指标的比较,尽管采用Bonferroni法校正检验水准,仍可能存在假阳性风险,次要结局指标的结果应仅作为探索性分析解读;②本研究为单中心研究,且样本量相对有限,

同时未排除患者生活习惯、基础疾病等潜在混杂因素,可能影响结果的外推性;③仅聚焦于精子质量参数(如活力、形态、DNA完整性等)的评估,未能进一步观察Transwell优选精子对胚胎发育潜能及临床妊娠结局的影响,其临床应用价值仍需后续研究验证。

综上所述,Transwell小室性价比高,且能够快捷优选活力、形态和DNA完整性较好且数量足够的精子,尤其是在低质量精液样本上更具优势。因此,本研究为精子优选技术,尤其是卵胞质内单精子注射(ICSI)技术中高质量精子的获得提供了一种简单实用的参考方法,证明Transwell有改进为更适合辅助生殖技术优选精子的产品的价值。但用Transwell优选精子改善胚胎发育和临床结局的可能性仍有待进一步临床研究证实。

* * *

作者贡献声明 黎允诗负责论文构思、调查研究、研究方法和初稿写作,刘永祥负责数据审编、调查研究和可视化,张淑婷负责正式分析、软件和可视化,黄雪坤和周少虎负责研究项目管理和提供资源,翁治委负责调查研究和提供资源,刘志辉负责研究方法、监督指导、验证和审读与编辑写作,李瑞岐负责论文构思、经费获取、验证和审读与编辑写作。所有作者已经同意将文章提交给本刊,且对将要发表的版本进行最终定稿,并同意对工作的所有方面负责。

Author Contribution LI Yunshi is responsible for conceptualization, investigation, methodology, and writing--original draft. LIU Yongxiang is responsible for data curation, investigation, and visualization. ZHANG Shuting is responsible for formal analysis, software, and visualization. HUANG Xuekun and ZHOU Shaohu are responsible for project administration and resources. WENG Zhiwei is responsible for investigation and resources. LIU Zhihui is responsible for methodology, supervision, validation, and writing--review and editing. LI Ruiqi is responsible for conceptualization, funding acquisition, validation, and writing--review and editing. All authors consented to the submission of the article to the Journal. All authors approved the final version to be published and agreed to take responsibility for all aspects of the work.

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Declaration of Conflicting Interests All authors declare no competing interests.

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(2025-05-08收稿, 2026-02-04修回)

编辑 余琳



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Editorial Office of *Journal of Sichuan University (Medical Sciences)*