卵泡液中神经营养因子4及卵丘细胞TrkB受体与卵子发育潜能的关系

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摘　要：【目的】探索人卵泡液中神经营养因子4(NT-4)及卵丘颗粒细胞中TrkB受体的表达与卵子发育潜能的关系。【方法】收集2020年5月至2020年11月在中山大学附属第六医院生殖中心，因男方因素行卵胞浆内单精子注射(ICSI)治疗的63例患者的卵泡液和卵丘颗粒细胞，用ELISA检测卵泡液中NT-4水平，实时荧光定量PCR检测卵丘颗粒细胞中TrkB受体两种不同亚型TrkB-fl和TrkB-t1的表达水平，分析与卵子成熟、受精和胚胎发育的关系。【结果】卵泡液中NT-4水平与正常受精数(r_s=0.250，P=0.048)、可利用胚胎数(r_s=0.320，P=0.011)、优质胚胎数(r_s=0.327，P=0.009)和优质囊胚数(r_s=0.303，P=0.029)呈正相关。卵丘颗粒细胞中TrkB-t1在高囊胚形成率组(≥60%)和高优质囊胚率组(≥50%)中的表达均较低[0.86 (0.60，1.85) *vs.* 2.29 (1.09，3.44)，P=0.008；0.84(0.64，1.45) *vs.* 1.73 (0.96，3.14)，P=0.031]。多重线性回归分析结果示优质胚胎数受获卵数(P=0.001)、卵泡液中NT-4水平(P=0.005)和卵丘颗粒细胞TrkB-t1表达水平(P=0.049)影响。【结论】人卵泡液中NT-4水平与ICSI患者卵子发育潜能正相关，其可能在卵子发育过程中发挥着重要作用。卵丘颗粒细胞中TrkB-t1的高表达与卵子发育潜能受损有关。

关键词：神经营养因子-4；酪氨酸相关激酶B受体；卵泡液；卵丘颗粒细胞；卵子质量
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Relationship between Follicular-fluid Neurotrophin-4, TrkB Receptors in Cumulus Cells and Oocyte Developmental Competence

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Abstract:【Objective】To investigate whether the neurotrophin-4 (NT-4) in human follicular fluid (FF) and the expression of tropomyosin-related kinase B (TrkB) receptors in cumulus cells (CCs) have any correlation with the developmental competence of oocytes.【Method】FF and CCs were collected from 63 patients undergoing intracytoplasmic sperm injection (ICSI) in the Reproductive Medicine Center from May to November 2020. NT-4 level in FF was measured by enzyme-linked immunosorbent assay (ELISA) method. The expression levels of two major TrkB isoforms, the full-length receptor (TrkB-fl) and the truncated receptor (TrkB-t1), were quantified in CCs by real-time PCR. The correlation of oocyte maturation, fertilization and embryo development with NT-4 level in FF, TrkB-fl and TrkB-t1 expression in CCs were analyzed.【Results】NT-4 level in human FF was positively correlated with the number of normally fertilized oocytes (r_s=0.250, P=0.048), available embryos (r_s=0.320, P=0.011), top-quality embryos (r_s=0.327, P=0.009) and top-quality blastocysts (r_s=0.303, P=0.029). The expression level of TrkB-t1 gene in CCs was negatively associated with a higher blastocyst formation rate (≥60%) and top-quality blastocyst rate (≥50%) [0.86 (0.60, 1.85) *vs.* 2.29 (1.09, 3.44), P=

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0.008; 0.84 (0.64, 1.45) *vs.* 1.73 (0.96, 3.14), P=0.031]. Multiple linear regression analysis showed that the number of top-quality embryos was affected by the number of oocytes retrieved (P=0.001), FF NT-4 level (P=0.005) and the expression of TrkB-t1 gene in CCs (P=0.049).【Conclusion】FF NT-4 level was positively associated with oocyte developmental competence, and it might play an important role in the development of oocytes. Over-expressed TrkB-t1 in CCs might be related to compromised oocyte developmental potential.

Key words: neurotrophin-4 (NT-4); tropomyosin-related kinase B (TrkB) receptor; follicular fluid (FF); cumulus cells (CCs); oocyte competence

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在体外受精/卵胞浆内单精子注射(in vitro fertilization /intracytoplasmic sperm injection , IVF/ICSI)治疗中,获得具有受精和胚胎发育潜能的优质卵子对妊娠结局至关重要。良好的卵泡微环境是保障卵子获得充分发育潜能的必要条件。神经营养因子(neurotrophins, NTs)家族是一类分泌性多肽类生长因子,最早发现于神经系统中,能够调控神经元和神经胶质细胞的生长、分化和凋亡等[1]。近年来众多研究表明NTs在卵子发育和卵泡微环境中同样发挥着重要作用[2]。神经营养因子4(neurotrophin-4, NT-4)是NTs家族的成员之一,在多种哺乳动物和人的卵巢中均有表达,并且参与卵泡募集、卵泡发育和卵子成熟的过程[3]。小鼠卵巢中NT-4的水平随着卵泡募集的进程而增加,NT-4基因敲除小鼠的初级和次级卵泡发育明显受阻[4]。在体外培养的人胎儿卵巢组织中,NT-4能够促进卵泡募集[5]。Seifer等[6]发现NT-4能提高小鼠体外成熟卵子的成熟率。除了对卵子的作用外,NT-4还能上调小鼠颗粒细胞中FSHR基因表达和促进cyclin D2的合成,从而促进颗粒细胞增殖[7]。NTs主要通过与两类受体结合发挥作用,分别是特异性的高亲和力酪氨酸相关激酶(tropomyosin-related kinase, Trk)受体和非特异性的低亲和力p75神经营养因子受体(p75 neurotrophin receptor, p75NTR)。Trk受体家族包括TrkA, TrkB和TrkC三种受体,其中TrkB受体是NT-4的特异性受体。TrkB敲除小鼠原始卵泡和次级卵泡数量减少[4,7],颗粒细胞的增殖抑制[8]。TrkB受体主要有全长型(full-length TrkB receptor, TrkB-fl)和截短型(truncated TrkB receptor, TrkB-t1)两种不同亚型[9],它们在结构和功能上存在差异。在小鼠和人类卵巢中均发现了TrkB-fl和TrkB-t1的表达[10-11]。NT-4及其受体TrkB在卵泡发育过程中发挥着重要作用,然而既往研究主要针对NT-4在早期卵泡发育中的作用,仅Seifer等[6]的动物实验发现NT-4能够促进卵子成熟。NT-4是否会影响人卵子成熟和发育潜能,及不同亚型TrkB受体在其中是否发挥不同作用,目前尚无相关研究。因此本研究拟探索人卵泡液中NT-4水平及卵丘颗粒细胞中TrkB-fl和TrkB-t1表达量与卵子发育潜能的关系,为进一步了解影响卵子发育的机制,及探索其在IVF/ICSI治疗中的应用提供研究基础。

1 材料与方法

1.1 研究对象

纳入2020年5月至2020年11月期间,在中山大学附属第六医院生殖医学中心接受ICSI治疗的患者共63例。纳入标准为:年龄<40岁;因男方因素行ICSI治疗;获卵数≥5个且抗苗勒氏管激素(anti-müllerian hormone, AMH)≥1.1 ng/mL。排除标准为:钙激活周期;卵子经体外成熟(in vitro maturation, IVM);胚胎移植前遗传学筛查/移植前基因诊断(preimplantation genetic screening/preimplantation genetic diagnosis, PGS/PGD)周期;多囊卵巢综合征,子宫内膜异位症和子宫因素相关的不孕。本研究通过中山大学附属第六医院伦理委员会审查(批件编号:2020ZSLYEC-277),并获得所有研究对象知情同意。

1.2 控制性卵巢刺激和ICSI受精

根据患者具体情况选择长方案或拮抗剂方案促排卵。长方案于黄体中期注射促性腺激素释放激素激动剂(gonadotropin-releasing hormone agonist, GnRH-a;醋酸曲普瑞林,达菲林,辉凌制药)进行垂体降调节。14 d后行阴道B超及性激素检查,如达到降调标准:雌激素(estrogen, E_2)<50 pg/mL、

黄体生成素（luteinizing hormone，LH）≤5 U/L、子宫内膜厚度≤5 mm，予促性腺激素（gonadotropin，Gn）启动促排卵。拮抗剂方案于月经周期第2天予Gn启动促排卵，当优势卵泡平均直径达12~14 mm时，每天皮下注射GnRH拮抗剂（加尼瑞克，默沙东）0.25 mg直到扳机日。每2~4 d通过阴道B超及血激素水平监测卵泡，根据卵巢反应调整Gn剂量。当至少1个卵泡直径≥18 mm或3个卵泡直径≥17 mm，肌注5 000~10 000 U人绒毛膜促性腺激素（hCG，珠海丽珠医药）或皮下注射重组人绒促性素（艾泽，默克雪兰诺）250 μg扳机。36~38 h后在超声引导下行经阴道穿刺取卵术。

将卵丘卵母细胞复合体（cumulus oocyte complexes，COCs）于37 ℃、体积分数6%CO_2培养箱培养3~4 h，80 U/mL透明质酸酶（vitrolife，瑞典）脱去卵丘颗粒细胞，于倒置显微镜下挑选出M Ⅱ期卵子置于IVF液（vitrolife，瑞典）中，按照ICSI标准流程进行受精。

1.3　胚胎培养和移植

ICSI后16 ~ 18 h评估卵子受精情况，出现两个原核判断为正常受精。正常受精卵子置于胚胎培养液（Sage，美国）中继续培养。D3胚胎按照Racowsky标准[12]进行形态学评分，可利用胚胎标准为4细胞1级或5个及以上细胞1~2级胚胎，优质胚胎标准为卵裂球6~9的1~2级胚胎。根据胚胎情况和患者意愿选择1~2个D3优质胚胎新鲜移植或冷冻，其余胚胎进行囊胚培养或冷冻。囊胚依据囊胚扩张程度、内细胞团和滋养层细胞，采用Gardner′s评分系统[13]评价，D5评分≥3BB定义为优质囊胚。取卵后第3或5天新鲜胚胎移植，移植后予常规黄体支持。胚胎移植14 d后查血清β-hCG，35~42 d后B超下观察到宫内孕囊及胎心搏动确定为临床妊娠.

1.4　卵泡液收集和NT-4检测

取卵过程中收集患者第一管无血污染的卵泡液，每管卵泡液以2 000 r/min，4 ℃离心10 min（r = 18 cm）去除细胞沉淀和杂质，取上清保存于-80 ℃。使用人NT-4酶联免疫吸附测定试剂盒（Human NT-4 ELISA Kit，伊莱瑞特，E-EL-H6094）检测卵泡液中NT-4浓度，检测范围为31.25 ~ 2 000 pg/mL，灵敏度为18.75 pg/mL，板间及板内变异系数<10%。

1.5　卵丘颗粒细胞总RNA提取及实时荧光定量PCR

收集每个患者全部COCs拆卵后分离的卵丘颗粒细胞，1 500 r/min，4 ℃离心5min（r=6 cm），弃上清，用磷酸盐缓冲液清洗2次后保存于-80 ℃。使用Trizol（Invitrogen，15596026）提取卵丘颗粒细胞中总RNA，NanoDrop2000测定RNA浓度。用逆转录试剂盒PrimeScript RT Reagent Kit（Takara，RR047A）合成cDNA，按照SYBR Green qRCR Master Mix（Genstar，A311）说明书在Racho Lightcycler480中进行实时荧光定量PCR。PCR扩增引物序列为：TrkB-fl sense：5′- CCCCAGGAGGTGTATGA-3’和antisense：5’-CAGCCCGTCTGAGGAGTA-3’；TrkB-t1 sense：5’-TAGATGTGGGCGGTGTTT-3’和antisense：5’-ATGGGATTTCATTTCAGTTT-3’；GAPDH sense：5’-GGAGCGAGATCCCTCCAAAAT-3’和antisense：5’-GGCTGTTGTCATACTTCTCATGG-3’。以GAPDH为内参基因，确定本研究中一位患者的基因表达水平为对照，采用$2^{-\Delta\Delta Ct}$法计算目的基因相对表达量。

1.6　统计学分析

使用SPSS 25.0统计软件进行统计学分析。符合正态分布计量资料用均数±标准差（$\bar{x} \pm s$）表示，组间比较用Student’s t检验。不符合正态分布计量资料用中位数和四分位数M（P_{25}，P_{75}）表示，组间比较用非参数Mann-Whitney U检验。相关性采用Spearman秩相关进行分析。多重线性回归分析以优质胚胎数为因变量，逐步法纳入年龄、AMH、获卵数等相关因素。双侧检验，P<0.05认为差异存在统计学意义。

2　结 果

2.1　患者的基本资料和促排情况

患者的基本资料和促排情况见表1。本研究纳入的患者63.49%（40/63）为原发性不孕，36.51%（23/63）为继发性不孕。17.46%（11/63）的患者使用拮抗剂方案，82.54%（52/63）的患者使用长方案促排卵。其中52例患者进行了囊胚培养，45例患者行新鲜胚胎移植，其中43例移植D3胚胎，2例移植D5囊胚，临床妊娠率为53.55%（24/45）。

2.2　卵泡液中NT-4水平与促排卵结局相关性分析

卵泡液中NT-4水平与促排卵结局的相关性分析如图1所示。卵泡液NT-4与正常受精数（r_s=0.250，P=0.048）、可利用胚胎数（r_s=0.320，P=0.011）、优质胚胎数（r_s=0.327，P=0.009）和优质囊胚数（r_s=0.303，P=0.029）正相关。获卵数和成熟卵

表1 患者的基本资料和促排情况

Table 1 Baseline and treatment characteristics of patients

Items	($\bar{x} \pm s$)
Age/years	31 ± 3.9
Duration of infertility/years	4.4 ± 3.1
Body mass index/(kg/m^2)	22 ± 2.9
Basal FSH/(U/L)	6.1 ± 1.9
AMH/(ng/mL)	3.6 ± 2.2
Antral follicle count/*n*	14 ± 5.2
Dose of gonadotropins/U	2 200 ± 730
Gn stimulation duration/days	11 ± 1.7
Retrieved oocytes/*n*	13± 4.4
MII oocytes/*n*	10 ± 3.9
Mature oocyte rate/%	76 ± 17
Normally fertilized oocytes/*n*	7.1 ± 3.4
Normal fertilization rate/%	71 ± 20
Available embryos/*n*	5.0 ± 3.1
Top-quality embryos/*n*	4.1 ± 2.8
Blastocysts/*n*	2.7 ± 2.4
Blastocyst formation rate/%	55 ± 35
Top-quality blastocysts/*n*	1.7 ± 2.1

FSH: follicle-stimulating hormone; AMH: anti-Müllerian hormone; Gn: gonadotrophin; MII: metaphase II.

子数与卵泡液中NT-4水平无相关性。

2.3 不同卵子质量间卵丘颗粒细胞TrkB受体表达水平比较

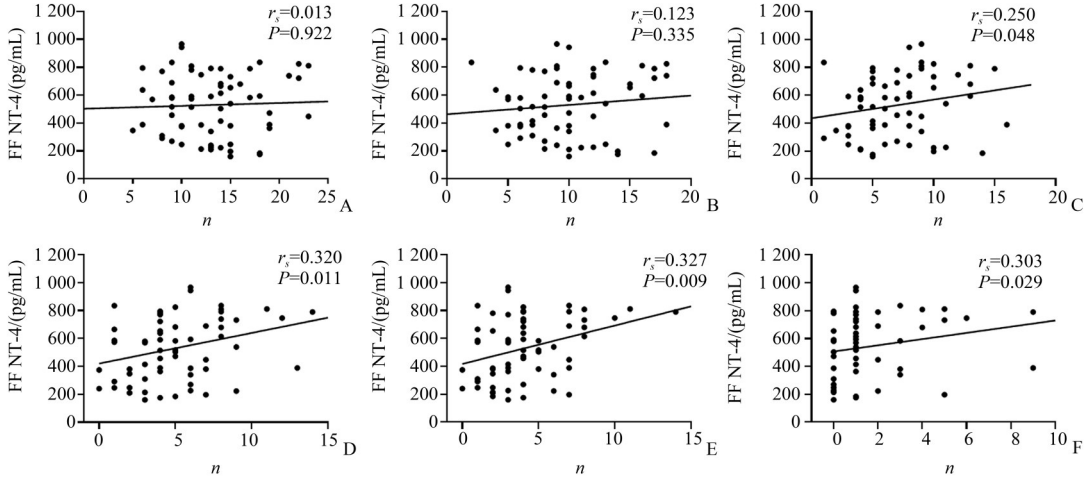
不同卵子质量间卵丘颗粒细胞中TrkB-fl和TrkB-t1表达水平比较结果如表2和图2、3所示。TrkB-fl和TrkB-t1在不同卵子成熟率(≥75%和<75%)、受精率(≥70%和<70%)及优质胚胎率(≥50%和<50%)组间表达无差异。TrkB-t1在高囊胚形成率组(≥60%)中的表达显著低于低囊胚形成率组(<60%)[0.86(0.60, 1.85) *vs.* 2.29(1.09, 3.44), P=0.008],在高优质囊胚率组(≥50%)的表达低于低优质囊胚率组(<50%)[0.84(0.64, 1.45) *vs.* 1.73(0.96, 3.14), P=0.031]。*TrkB-fl*基因在不同囊胚形成率和优质囊胚率组间的表达无差异。

2.4 优质胚胎数相关因素多重线性回归分析

以优质胚胎数为因变量,纳入年龄、AMH、获卵数、卵泡液NT-4和卵丘颗粒细胞中TrkB-t1表达水平,通过逐步法筛选自变量,进行多重线性回归分析,结果见表3。优质胚胎数受获卵数(P=0.001)、卵泡液中NT-4水平(P=0.005)和卵丘颗粒细胞中TrkB-t1表达水平影响(P=0.049)。

2.5 不同临床妊娠结局间比较

不同临床妊娠结局患者间的比较如表4所示。临床妊娠组年龄低于非妊娠组(30±3.8 *vs.* 33±3.4, P=0.015),不孕年限也明显短于非妊娠组(3.3±1.8



The correlation between NT-4 level in FF and A: the number of oocytes retrieved; B: the number of mature oocytes; C: the number of normally fertilized oocytes; D: the number of available embryos; E: the number of top-quality embryos; F: the number of top-quality blastocysts. FF: follicular fluid; NT-4: Neurotrophin-4; r_s: Spearman's rank correlation coefficient.

图1 卵泡液中NT-4水平与促排卵结局相关性分析

Fig.1 The corrlation between NT-4 level in follicular fluid and outcomes of ovary stimulation

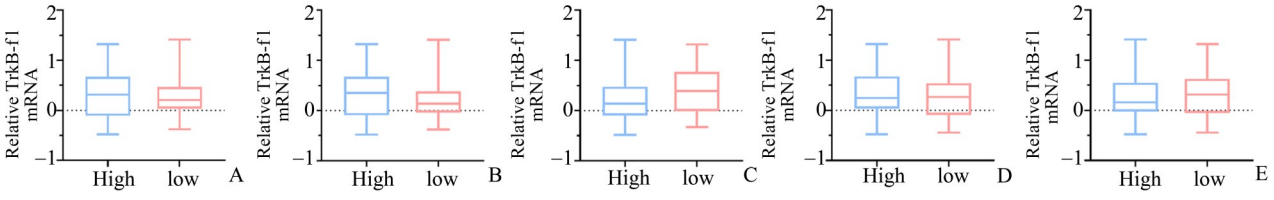
表2　不同卵子质量间卵丘颗粒细胞TrkB-fl和TrkB-t1表达水平比较

Table 2　Comparison of relative TrkB-fl and TrkB-t1 expression level in groups with different oocyte competence

[$M(P_{25},P_{75})$]

Items	n	TrkB-fl	TrkB-t1
Oocyte maturation rate			
High group (≥75%)	36	2.07 (0.78, 4.71)	1.39 (0.84, 3.00)
Low group (<75%)	27	1.60 (1.07, 2.95)	1.49 (0.58, 2.36)
Z		−0.042	−0.750
P		0.967	0.453
Normal fertilization rate			
High group (≥70%)	36	2.26 (0.81, 4.71)	1.37 (0.91, 2.42)
Low group (<70%)	27	1.14 (0.66, 2.76)	1.49 (0.82, 3.40)
Z		−1.042	0.298
P		−0.972	0.331
Top-quality embryo rate			
High group (≥50%)	42	1.39 (0.80, 3.02)	1.00 (0.67, 2.40)
Low group (<50%)	21	2.44 (0.98, 5.91)	1.66 (1.17, 3.43)
Z		−1.589	−1.808
P		0.112	0.071
Blastocyst formation rate			
High group (≥60%)	28	1.79 (1.07, 4.74)	0.86 (0.60, 1.85)
Low group (<60%)	24	1.85 (0.81, 3.46)	2.29 (1.09, 3.44)
Z		−0.624	−2.662
P		0.533	0.008[2)]
Top-quality blastocyst rate			
High group (≥50%)	18	1.45 (0.94, 3.61)	0.84 (0.64, 1.45)
Low group (<50%)	34	2.07 (0.89, 4.24)	1.73 (0.96, 3.14)
Z		−0.289	−2.154
P		0.773	0.031[1)]

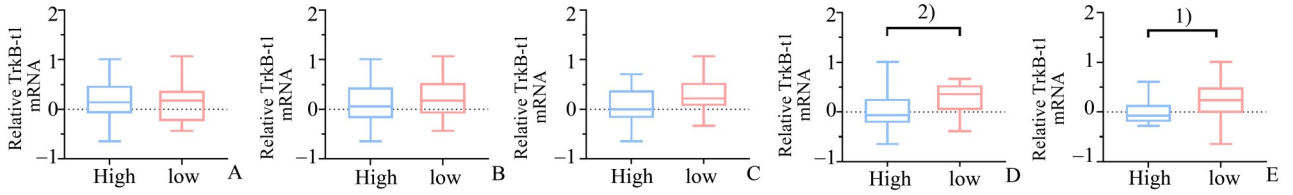
TrkB-fl: full-length tropomyosin-related kinase B receptor, TrkB-t1: truncated tropomyosin-related kinase B receptor; [1)] $P<0.05$, [2)] $P<0.01$.



Relative TrkB-fl mRNA level in groups with different. The ordinate value has been taken as log10. A: oocyte maturation rate. B: normal fertilization rate. C: top-quality embryo rate. D: blastocyst formation rate. E: top-quality blastocyst rate. TrkB-fl: full-length tropomyosin-related kinase B receptor. The box plots represent medians and interquartile intervals and the error bars represent the minimum and maximum value.

图2　不同卵子质量间卵丘颗粒细胞TrkB-fl表达水平比较

Fig.2　Comparison of relative TrkB-fl expression level in groups with different oocyte competence



Relative TrkB-t1 mRNA level in groups with different. The ordinate value has been taken as log10. A: oocyte maturation rate. B: normal fertilization rate. C: top-quality embryo rate. D: blastocyst formation rate. E: top-quality blastocyst rate. 1) $P<0.05$, 2) $P<0.01$. TrkB-t1: truncated tropomyosin-related kinase B receptor. The box plots represent medians and interquartile intervals and the error bars represent the minimum and maximum value.

图3 不同卵子质量间卵丘颗粒细胞TrkB-t1表达水平比较

Fig.3 Comparison of relative TrkB-t1 expression level in groups with different oocyte competence

表3 优质胚胎数相关因素多重线性回归分析

Table 3 Multiple regression analysis of potential variables related to the number of top-quality embryos

Variables	Unstandardized coefficients		t	P	Standardized coefficients
	b	Sb			
Constant	−0.631	1.184	−0.533	0.596	
The number of oocytes retrieved/n	0.247	0.067	3.656	0.001[2)]	0.396
NT-4 level in FF/(pg/mL)	0.004	0.001	2.937	0.005[2)]	0.318
Relative TrkB-t1 mRNA	−0.292	0.145	−2.011	0.049[1)]	−0.218
Age/years			−0.178	0.859	excluded
AMH/(ng/mL)			−1.357	0.180	excluded

NT-4: Neurotrophin-4, FF: follicular fluid, TrkB-t1: truncated tropomyosin-related kinase B receptor, AMH: anti-Müllerian hormone. [1)] $P<0.05$, [2)] $P<0.01$.

vs. 5.2±3.1, $P=0.017$)。卵泡液中NT-4及卵丘颗粒细胞中TrkB-fl和TrkB-t1表达水平无显著差异。

3 讨论

卵子的发育潜能受全身和局部多种因素影响,卵泡微环境中卵泡液和卵丘颗粒细胞与卵子间有着丰富的物质信息交流,共同支持卵子在发育过程中获得充分的发育潜能[14]。卵泡微环境中多种NTs及其受体的水平已被发现与卵子质量和卵巢功能相关。如多囊卵巢综合征患者卵泡液中神经生长因子(nerve growth factor, NGF)水平显著升高,动物实验也证实高浓度NGF会抑制卵子成熟[15]。而卵泡液中脑源性神经营养因子(brain-derived neurotrophic factor, BDNF)水平与卵子成熟率及卵裂率正相关[16]。Buyuk等[17]发现卵巢储备下降患者卵丘颗粒细胞中TrkA和p75NTR表达降低。研究NTs在卵泡微环境中的作用对于进一步了解影响卵子发育的机制具有重要意义。

本研究发现人卵泡液中NT-4水平与卵子的受精能力和形成胚胎的质量正相关,这提示NT-4可能影响了卵子的发育潜能。BDNF和NT-4都是TrkB受体的配体,在卵泡募集和生长发育中发挥着同样重要作用,且在卵泡发育过程中能够代偿相互之间的作用[4]。虽然目前仅在小鼠中证实NT-4能促进卵子成熟,但BDNF已在多种哺乳动物及人中发现与卵子成熟和发育潜能相关。BDNF能提高小鼠和猪卵子IVM的成熟率,而且能够促进小鼠体外成熟的卵子形成具有植入潜能的胚胎[18]。在牛IVM体系中添加BDNF有利于卵子成熟和囊胚形成[19]。人卵泡液中BDNF也被认为在卵子成熟和形成胚胎过程中有重要作用[16]。因为和BDNF作用于相同的受体,以及它们之间的相互代偿作用,因此我们认为NT-4同样可能在人卵子成熟发育过程中发挥重要作用。但本研究为观察性研究,NT-4影响人卵子发育潜能的具体作用机制,以及能否将其用于IVM体系中改善胚胎质量,还需要更多的动物及人体实验证实。

表4　不同临床妊娠结局间患者情况比较

Table 4　Comparison of baseline and treatment characteristics of patients between different clinical pregnant outcomes

[$M(P_{25}\sim P_{75})$, $(\bar{x}\pm s)$]

Items	Pregnant (n=24)	Non-pregnant (n=21)	t/Z	P
Age/years	30 ± 3.8	33 ± 3.4	−2.544	0.015[1)]
Duration of infertility /years	3.3 ± 1.8	5.2 ± 3.1	−2.525	0.017[1)]
Body mass index/(kg/m^2)	22 ± 2.8	22 ± 3.6	0.359	0.721
Basal FSH/(U/L)	5.9 ± 1.6	6.6 ± 2.1	−1.286	0.205
AMH/(ng/mL)	2.9 ± 1.4	3.6 ± 2.3	−1.066	0.294
Antral follicle count/n	13± 4.9	14± 5.6	−0.830	0.411
Dose of gonadotropins /U	2 100 ± 710	2 300 ± 770	−0.486	0.630
Gn stimulation duration /days	10 ± 1.9	11 ± 1.3	−1.186	0.242
Retrieved oocytes/n	12 ± 4.1	12 ± 3.5	−0.782	0.438
MⅡ oocytes/n	8.9 ± 3.8	9.8 ± 3.3	−0.830	0.411
Normally fertilized oocytes/n	6.8 ± 3.7	6.4 ± 2.5	0.474	0.638
Available embryos/n	4.8 ± 2.8	4.1 ± 2.8	0.837	0.407
Top-quality embryos/n	4.0 ± 2.7	3.2 ± 2.2	1.018	0.314
Embryo transferred/n	1.6 ± 0.50	1.7 ± 0.48	−0.564	0.575
FF NT-4 level/(pg/mL)	542 (342, 669)	580 (269, 760)	−0.262	0.794
Relative TrkB-fl mRNA	2.10 (1.00, 4.26)	1.60 (0.59, 4.82)	−0.478	0.633
Relative TrkB-t1 mRNA	1.66 (0.69, 3.58)	1.37 (0.86, 2.29)	−0.387	0.699

FSH: follicle-stimulating hormone; AMH: anti-Müllerian hormone; Gn: gonadotrophin; MⅡ: metaphase Ⅱ; FF: follicular fluid; NT-4: Neurotrophin-4; TrkB-fl: full-length tropomyosin-related kinase B receptor. TrkB-t1: truncated tropomyosin-related kinase B receptor. [1)] P< 0.05.

TrkB-fl是典型的酪氨酸激酶受体，与配体结合后磷酸化胞内酪氨酸激酶，进一步激活Ras/MAPK、PLCγ1和PI3K/Akt-mTOR等下游信号通路[20]。TrkB-t1则缺乏胞内酪氨酸激酶结构域，无法激活细胞内信号级联反应。但TrkB-t1能够与TrkB-fl形成异二聚体，抑制TrkB-fl的活性[9]。TrkB-fl和TrkB-t1在神经系统不同的部位以及不同疾病状态下表达不同[21]，同样它们在卵巢中也表现出不同的分布模式。在小鼠早期卵泡发育过程中，TrkB-fl在卵母细胞和颗粒细胞中维持稳定的低水平表达，而TrkB-t1选择性表达于卵母细胞中，排卵前卵母细胞在促性腺激素的作用下TrkB-fl表达迅速升高[8, 22]。Anderson等[23]发现人COCs的卵子和卵丘颗粒细胞中均有TrkB-t1的表达，而TrkB-fl未检测到。本研究在卵丘颗粒细胞中同时检测到TrkB-fl和TrkB-t1的表达，TrkB-t1的表达更为丰富。关于不同TrkB亚型在卵子发育过程中的作用尚无研究报道，本研究结果显示卵丘颗粒细胞中TrkB-t1的表达量与卵子发育成囊胚的能力负相关。在神经细胞中TrkB-t1的高表达会引起TrkB-fl/TrkB-t1之间的失衡，从而加速神经元退化[24]。因此卵丘颗粒细胞中TrkB-t1的高表达可能会抑制TrkB-fl的激活，通过影响卵丘颗粒细胞的功能从而影响卵子的发育。但有研究发现TrkB-t1除了发挥负向调控作用外，还能通过不依赖于NT-4或者BDNF方式激活自身来发挥功能[9]。所以TrkB受体的不同亚型在卵泡发育中作用仍然需要进一步的实验探索。

虽然本研究发现卵泡液中NT-4和卵丘颗粒细胞中TrkB-t1表达与胚胎质量相关，但在不同临床妊娠结局间无差异。这可能是因为纳入本研究的患者基础情况较好，大部分行胚胎移植的患者至少有一个优质胚胎，而我们只比较了新鲜移植周期的妊娠结局。卵泡液中NT-4和卵丘颗粒细胞中*TrkB-t1*基因表达能否预测累计妊娠结局，有待进一步探讨。除此之外，在本研究中卵泡液和卵丘颗

粒细胞并非对应到单个卵子,因此NT-4和不同TrkB受体亚型对不孕症患者卵子发育潜能的预测价值,还需要更多的研究探索。

综上所述,ICSI周期中卵泡液NT-4水平与卵子受精和发育成胚胎的能力正相关,卵丘颗粒细胞中*TrkB-t1*基因表达与囊胚发育能力负相关。NT-4在卵子成熟发育过程中可能发挥着重要作用,而卵丘颗粒细胞中TrkB-t1的高表达与卵子发育潜能受损有关。这为我们未来在IVF/ICSI中寻找预测卵子质量的标志物,和探索提高卵子发育潜能的可能措施提供了线索。

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