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GnRHR-Ⅱ在子宫内膜的分布及表达变化规律研究

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摘要 目的：研究促性腺激素释放激素Ⅱ型受体（GnRHR-Ⅱ）在子宫内膜的分布及表达变化规律。**方法**：选择 2015 年 1 月～2016 年 7 月期间我院收治的 100 例女性不孕患者，经诊断性刮宫技术获取 50 例增生期子宫内膜组织与 50 例分泌期子宫内膜组织，分别作为研究组与对照组；采用免疫组织化学染色法检测两组子宫内膜基质细胞与腺上皮 GnRHR-Ⅱ的表达。**结果**：增生期、分泌期子宫内膜均有 GnRHR-Ⅱ分布；研究组子宫内膜基质细胞的 GnRHR-Ⅱ表达明显高于对照组，而腺上皮则明显低于对照组，差异有统计学意义（P＜0.05）。**结论**：GnRHR-Ⅱ在子宫内膜增生期与分泌期均有表达，且在膜基质细胞与腺上皮均有分布，其分布于表达变化规律在一定程度上与子宫内膜容受性有关，有望成为评估子宫内膜容受性的标记物。

关键词：GnRHR-Ⅱ；子宫内膜；增生期；分泌期

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Research of the Distribution and Expressive Disciplinarian of GnRHR-Ⅱ in Endometrial

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ABSTRACT Objective: To explore the distribution and expressive disciplinarian of GnRHR-Ⅱ in endometrial.Methods:Selected 100 cases of patients with female infertility who treated in our hospital from January 2015 to July 2016, after diagnostic curettage technique to obtain 50 cases of proliferative endometrium and 50 cases of secretory phase endometrium, and as the research group and the control group; Expression of GnRHR- II in two groups of endometrial stromal cells and glandular epithelium were detected by immunohistochemical staining. **Results:** The distribution of GnRHR- in the endometrium of proliferative phase and secretory phase; The expression of GnRHR- II in endometrial stromal cells was significantly higher in the research group than in the control group, the gland epithelium was significantly lower than that of the control group, the difference was statistically significant (P<0.05). **Conclusion:** The expression of GnRHR- II was expressed in the proliferative phase and secretory phase of endometrium, and the distribution of the membrane stromal cells and glandular epithelium, its distribution in expression changes in a certain degree with Endometrial Receptivity about, is expected to become the assessment of endometrial receptivity markers.

Key words: GnRHR-Ⅱ; Endometrial; Proliferative; Secretory

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前言

促性腺激素释放激素 (Gonadotropin releasing hormone, GnRH)是一种与生殖有关的十分重要的激素，参与促性腺激素（Gonadotrophin,Gn）合成、释放的调节，Gn 在生成类固醇激素与形成配子的过程中发挥了重要作用[1]。GnRH-Ⅰ与 GnRH-Ⅱ是迄今在人类基因组中发现的两种 GnRH，后者相对更为常见，在人类的脑干、子宫内膜以及肾脏中均存在表达[2]，二者均对应各自的受体 GnRHR-Ⅰ与 GnRHR-Ⅱ。国外学者早在 20 世纪 90 年代就已证实 [3]，GnRH 及其受体的 mRNA 在育龄女性月经周期各阶段的子宫内膜中均有表达。既往研究提出[4]，GnRHR-Ⅱ可能与子宫内膜对胚胎的容受性表现出正相关的关系，并由此推测 GnRH-Ⅱ的变化是否也与子宫内膜容受性一致。为此，本研究选择增生期、分泌期形态的子宫内膜组织进行临床对照，分析 GnRHR-Ⅱ在子宫内膜的分布及表达变化规律，进而初步明确 GnRHR-Ⅱ与子宫内膜容受性间存在何种关系，现报告如下：

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1 资料和方法

1.1 一般资料

选择 2015 年 1 月～2016 年 7 月期间我院收治的 100 例女性不孕患者，纳入标准：（1）育龄女性，年龄 20~35 岁；（2）不孕原因均为输卵管因素所致；（3）月经周期无异常；（4）无既往宫腔操作史；（5）入组前 3 个月无类固醇激素等药物服用史；（6）无内分泌疾病史；（7）知情同意。通过诊断性刮宫技术获取每位患者的子宫内膜组织，其中 50 例为增生期子宫内膜组织，50 例为分泌期子宫内膜组织，分别作为研究组与对照组，两组患者年龄、体质指数、月经周期、D3 雌二醇、D3 卵泡刺激素、D3 窦卵泡数、D3 内膜厚度对比，差异无统计学意义（均 p>0.

05),详见表 1。

表 1 两组基线资料对比 (n=50)

Table 1 Comparison of baseline data between the two groups (n=50)

Groups	Age (years)	BMI (kg/m²)	Menstrual cycle (day)	D3E2 (pg/mL)	D3FSH (mU/mL)	D3AFC (A)	D3 Intima media thickness(mm)
Research group	28.71± 5.27	21.04± 4.09	31.55± 3.39	50.25± 14.29	6.79± 1.22	12.71± 4.06	5.88± 1.03
Control group	28.44± 6.52	22.33± 1.72	31.73± 4.46	50.74± 15.11	6.49± 1.42	12.66± 5.38	4.71± 1.27
t	0.052	0.066	0.015	0.021	0.066	0.140	0.094
P	0.747	0.758	0.673	0.846	0.326	0.636	0.226

1.2 检测方法

诊刮获取子宫内膜,采用免疫组织化学染色法检测两组子宫内膜基质细胞与腺上皮 GnRHR-Ⅱ的表达,由瑞士 Sigma 公司提供 GnRHR-Ⅱ多克隆抗体,以 1:100 稀释,严格按照试剂盒说明书进行操作,用磷酸盐缓冲液(PBS)代替一抗作阴性对照,用已知子宫内膜癌组织作阳性对照。

1.3 结果判断

在光镜下取 400 个视野,各计数 100 个细胞,以细胞膜、细胞质有浅棕黄色至棕褐色颗粒分布判断为阳性染色,计算每一染色程度细胞所占的比例, 结合组织化学评分法(Histochemistry score,HSCORE)[5]:未着色、无阳性细胞(-)为 0 分,着色不深、浅棕黄色、阳性细胞数 <10%(+)为 1 分,着色较深、棕黄色、阳性细胞数占 10%～50%(++)为 2 分,着色深、棕褐色、阳性细胞数 >50%(+++)为 3 分。每一张玻片均由 2 名检查人员进行判断,以消除主观因素,最后取平均值作为结果。

1.4 统计学方法

采用 SPSS18.0 统计软件进行数据处理,计量资料以($\bar{x}\pm s$)表示采用配对 t 检验, 等级资料采用 Wilcoxon 配对秩和检验。以 P＜0.05 为差异有统计学意义。

2 结果

2.1 两组子宫内膜基质细胞 GnRHR-Ⅱ表达情况对比

两组子宫内膜基质细胞存在 GnRHR-Ⅱ的表达,GnRHR-Ⅱ在研究组基质细胞中的表达明显高于对照组,差异有统计学意义(P＜0.05),详见表 2。

表 2 两组子宫内膜基质细胞 GnRHR-Ⅱ表达情况对比 (n=50)

Table 2 Comparison of the expression of GnRHR- II in two groups of endometrial stromal cells (n=50)

Groups	-	+	++	+++	HSCORE score
Research group	7	21	14	8	1.86± 0.30
Control group	24	17	7	2	0.33± 0.05
Z/t		6.934		5.118	
P		0.000		0.000	

2.2 两组子宫内膜腺上皮 GnRHR-Ⅱ表达情况对比

两组子宫内膜腺上皮存在 GnRHR-Ⅱ的表达,GnRHR-Ⅱ在研究组腺上皮中的表达明显低于对照组,差异有统计学意义(P＜0.05),详见表 3。

表 3 两组子宫内膜腺上皮 GnRHR-Ⅱ表达情况对比 (n=50)

Table 3 Comparison of expression of GnRHR- II in two groups of endometrial glandular epithelium (n=50)

Groups	-	+	++	+++	HSCORE score
Research group	18	17	12	3	0.72± 0.41
Control group	9	19	16	6	1.89± 0.28
Z/t		5.793			5.201
P		0.000			0.000

3 讨论

GnRH 由下丘脑分泌,而目前发现,GnRH 在卵巢、睾丸、子宫内膜、胎盘等组织中也微量存在,而且 GnRH 的生物活性与免疫活性都类似[6]。GnRHR-Ⅰ与 GnRHR-Ⅱ分别是 GnRH-Ⅰ与 GnRH-Ⅱ的受体, 研究发现,GnRH-Ⅱ对其受体的选择敏感度是 GnRH-Ⅰ对其受体的 421 倍[7]。

增生期形态子宫内膜在向分泌期子宫内膜过渡具有如下

的生理变化过程[8-10]:子宫内膜在增生期时,在雌激素的作用下,子宫内膜基质细胞会生成酸性粘多糖,充当内膜支架以为胚胎着床做准备;而到了分泌期时,在孕激素的作用下,子宫内膜腺上皮会有增大的迹象,并且腺体的开口面向宫腔,从而利于为胚胎着床提供糖原等营养物质。早在 1998 年,国外一些学者就首次发现 GnRH 及其受体的 mRNA 在育龄女性月经周期各阶段子宫内膜中均有表达[11];其后又发现 GnRH 前体基因在人类子宫内膜上的表达[12];接着又发现 GnRH 受体在异位子宫内膜中的表达 [13]。而到 21 世纪初期, 国外学者就率先提出, GnRH-Ⅱ在子宫内膜中无论是定量表达还是定位表达均表现出月经周期依赖性的特征[14]。既往研究发现,增生期、分泌晚期形态的子宫内膜上 GnRH-Ⅱ表达的程度要弱于分泌早中期, 从而推测可能是胚胎着床窗的开启与关闭影响到了 GnRH-Ⅱ表达[15,16]。

本研究结果显示,两组子宫内膜基质细胞与腺上皮均存在 GnRHR-Ⅱ的表达,GnRHR-Ⅱ在增生期中主要在内膜基质细胞的细胞膜及细胞质高度表达,但仅在内膜腺上皮的近腔面出现较弱的阳性反应;而在分泌期则主要在内膜腺上皮的细胞膜及细胞质高度表达,但仅在内膜基质细胞轻微表达,这可能是因为子宫内膜为适应胚胎着床而出现了相应的生理、生化及形态的改变并影响 GnRHR-Ⅱ作出相应的分布与表达的变化,进而提示 GnRHR-Ⅱ与子宫内膜对胚胎的容受性呈正相关。

既往有学者推测,GnRHR-Ⅱ与子宫内膜容受性呈正相关,那么与其特异性结合的 GnRH-Ⅱ是否亦和子宫内膜容受性表现出这种关系[17],国外学者研究发现,同一患者进行体外受精 - 胚胎移植(IVT-ET)治疗,与冻融复苏周期相比,新鲜采卵周期的妊娠率有所降低[18]。出现这种情况的原因可能来自两方面:其一为超促排卵的过程中雌 / 孕激素的比例失调,导致子宫内膜对胚胎的容受性降低,引起胚胎着床窗关闭提前,最终使妊娠的几率减小;其二为促性腺激素释放激素激动剂(GnRHa)直接对子宫内膜产生抑制[19]。GnRHa 是天然 GnRH 的类似物,是目前临床在超促排卵长方案中常用的降调药物,具有较强的受体亲和力以及较长的半衰期, 小剂量使用具有兴奋垂体的效果; 而大剂量长时间使用时可通过使垂体 GnRHRG 蛋白偶联受体细胞质内的部分自动磷酸化,使受体亚单位脱偶联,使受体内流来达到“降调”的目的,延缓黄体(LH)峰出现的时间,与此同时对 LH 构成一定的伤害,最终引起子宫内膜容受性的减弱。这说明 GnRHa 对垂体的影响是受剂量决定的,由类似物推及,那么在此是否可初步对 GnRH-Ⅱ与子宫内膜容受性的关系作出推测[20],即二者正相关的关系仅局限在一定的范围内,一旦超出这个范围就会产生反作用。

综上所述,GnRHR-Ⅱ在子宫内膜增生期与分泌期均有表达,且在膜基质细胞与腺上皮均有分布,其分布于表达变化规律在一定程度上与子宫内膜容受性有关,有望成为评估子宫内膜容受性的标记物。

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(下转第 2739 页)

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