

## 정자형성 과정에서 Vascular Endothelial Growth Factor 및 Endothelin-1 발현의 면역조직화학적 연구

부산대학교 의과대학 비뇨기과학교실

### The Influences of Vascular Endothelial Growth Factor and Endothelin-1 on Spermatogenesis in Testis

Sung Woo Park, Hyun Jun Park, Nam Cheol Park

Department of Urology, Pusan National University Hospital, Pusan, Korea

**Objective:** The effects on spermatogenesis by expression of vascular endothelial growth factor (VEGF) and endothelin-1 (ET-1) were investigated.

**Materials and Methods:** Testicular specimens were obtained from 40 infertile males due to primary testicular failure and from 10 fertile males with other urologic problems. The specimens of infertile males were divided into 4 groups according to histologic findings; Sertoli cell only syndrome (A), maturation arrest (B), hypospermatogenesis (C) and sloughing and disorganization (D). VEGF and ET-1 expression were detected with immunohistochemical stain.

**Results:** VEGF expression on Leydig cell was detected in all cases. But, VEGF expression rates on germ cell were significantly higher in infertile group B, C, D compared to that of the control group ( $p < 0.05$ ). ET-1 expression rates on Leydig cell was significantly lower in all infertile group compared to that of the control group ( $p < 0.05$ ). But, ET-1 expression rates on Sertoli cell was significantly higher in all infertile group compared to that of the control group ( $p > 0.05$ ). In germ cell of infertile group, LH, FSH and prolactin were significantly decreased, and estradiol is increased in positive stain group on ET-1 immunohistochemical stain ( $p < 0.05$ ). VEGF and ET-1 expression were not correlated mean seminiferous tubule diameter ( $p > 0.05$ ).

**Conclusions:** Abnormal spermatogenesis would be reflected in VEGF expression in germ cell.

**Key Words:** Vascular endothelial growth factor, Endothelin-1, Spermatogenesis, Testis

cytoplasmic sperm injection, ICSI)

(spermatogenesis)

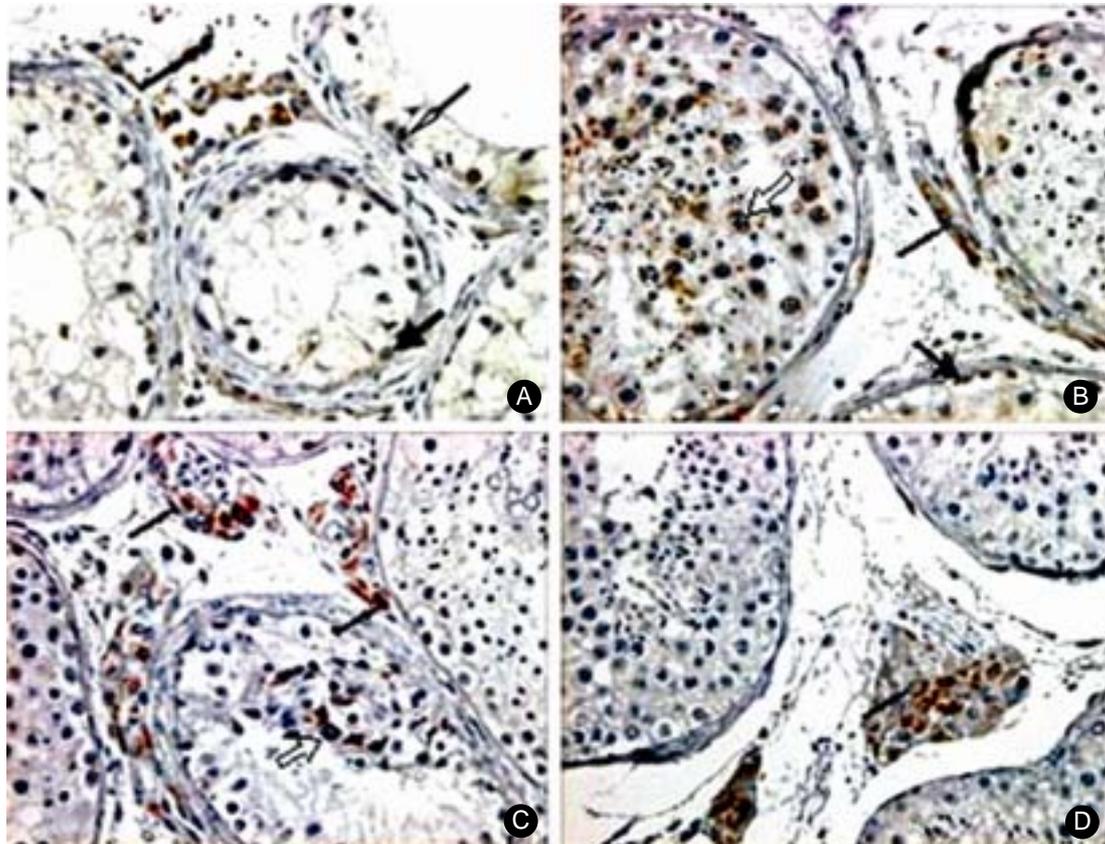
50~75%

(intra-

(germ cell) ,  
 가 2~4  
 vascular endothelial growth factor (VEGF) endothelin-1 (ET-1)  
 Sertoli , Leydig  
 5-9  
 가  
 VEGF ET-1  
 1.  
 ( ) ,  
 10 ( )  
 Sertoli  
 cell only syndrome, maturation arrest, hypospermatogenesis sloughing and disorganization  
 A, B, C D A, B, C,  
 D 10 50  
 2.  
 1)  
 (luteinizing hormone, LH),  
 hormone, FSH),  
 (estradiol),  
 (follicular stimulating hormone, FSH),  
 (testosterone),  
 (prolactin)  
 2)  
 window  
 hematoxylin-eosin (H-E)

3)  
 (1) VEGF  
 4~5 μm  
 1 가 가 xylene 5 4 ,  
 alcohol 2 3  
 , citrate buffer au-  
 toclave 15 H<sub>2</sub>O<sub>2</sub> 10  
 . PBS buffer 2 3  
 1:250 VEGF 1  
 (mouse monoclonal antibody, Santa Cruz Biotechnol, USA) 4  
 biotin streptavidin 30  
 PBS 5 3 , AEC  
 . Hematoxylene  
 mounting solution  
 (2) ET - 1  
 1:250  
 ET-1 1 (mouse monoclonal antibody, Abcam Biotechnol, USA)  
 4)  
 VEGF ET-1  
 400  
 (-), 25%  
 (1+), 25~75% (2+)  
 75% (3+) 10,11  
 3.  
 Fisher's exact test Mann Whitney U test , VEGF ET-1  
 Chi-square test  
 . p 0.05  
 1.  
 1) VEGF  
 (1)  
 VEGF A

D (spermatogonia) +1 2 B, C (20%), 2 (20%) 1 (10%) , +2 C 1 (10%) 20%,



**Figure 1.** Immunohistochemical stains for VEGF ( $\times 400$ ). **A;** Sertoli cell only syndrome **B;** maturation arrest **C;** hypospermatogenesis **D;** control group. Sertoli cell; thick black arrow, Leydig cell; thin black arrow, Spermatogonia; thin white arrow, Spermatocyte; thick white arrow.

**Table 1.** VEGF expression rate on germ cell according to the grade of maturation

	No. cases	SG		SC		ST	Overall (%)
		+1 (%)	+2 (%)	+1 (%)	+2 (%)	+1 (%)	
Infertility group	40						
A	10	-	-	-	-	-	-
B	10	2 (20)	-	3* (30)	-	-	3* (30)
C	10	2 (20)	1 (10)	5* (50)	2 (20)	2 (20)	7* (70)
D	10	1 (10)	-	3* (30)	-	-	3* (30)
Control group	10	-	-	-	-	-	-

A; Sertoli cell only syndrome, B; maturation arrest, C; hypospermatogenesis, D; sloughing and disorganization, SG; spermatogonia, SC; spermatocyte, ST; spermatid \*,  $p < 0.05$  vs control group. calculated by Mann-Whitney U test

30% 10% (spermatocyte) +1 20% (0%~30%)  
 3 (30%), 5 (50%) 3 (30%) , +2 10% 가 ,  
 C 2 (20%) 30%, (Table 2) (Figure 1).  
 70% 30% (spermatid) (3) Leydig  
 C +1 2 (20%) Leydig VEGF  
 VEGF A, B, C, D  
 A , B, C D +1 5 (50%), 6 (60%), 6  
 42.5% (30%~70%) (60%), 7 (70%) 5 (50%), +2 5 (50%),  
 4 (40%), 2 (20%), 2 (20%) 4 (40%), +3  
 C, D 2 (20%), 1  
 (Table 1) (Figure 1).  
 (2) Sertoli (10%) 1 (10%) (p>0.05). Leydig  
 Sertoli VEGF A VEGF 가 ,  
 B, C, D 2 (20%), 3 100% 가 ,  
 (30%), 3 (30%) 1 (10%) Sertoli  
 +1 Sertoli VEGF (Table 2) (Figure 1).

**Table 2.** VEGF expression rate on Sertoli cell and Leydig cell according to the grade of maturation

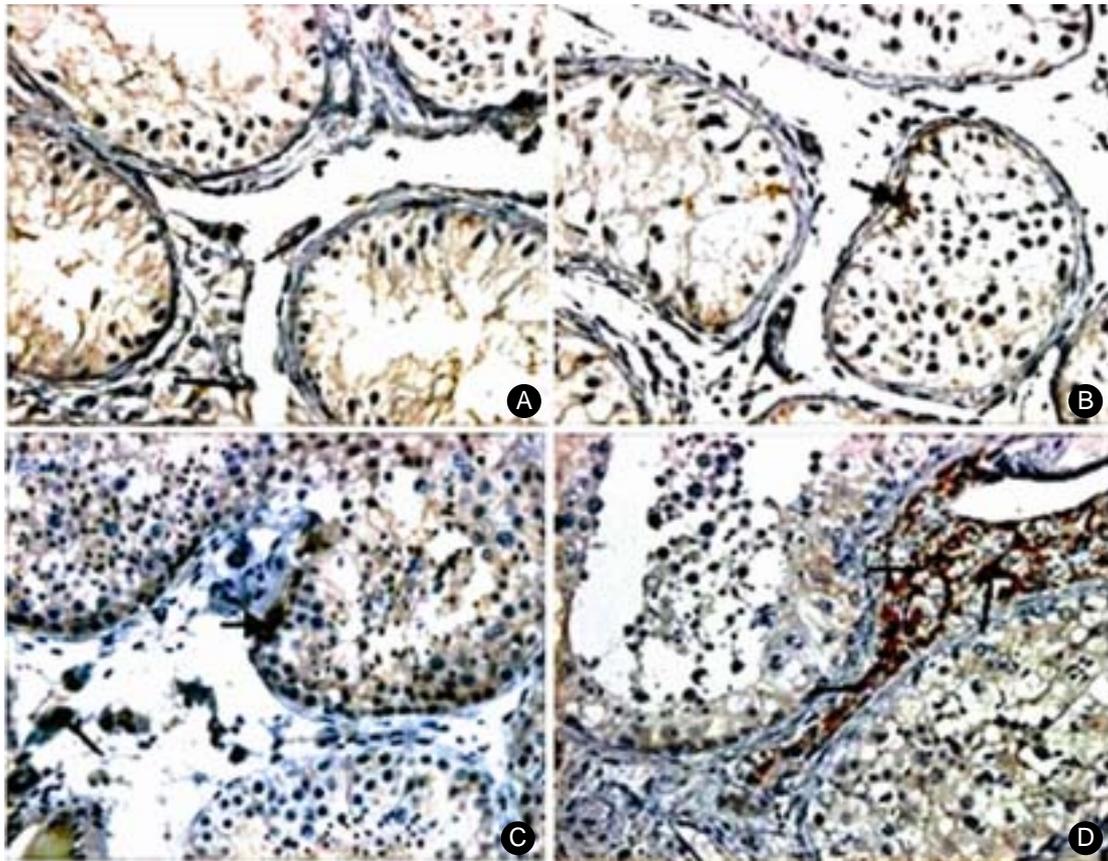
	No. cases	Ser (%)		Ley			Overall (%)
		+1 (%)	+1 (%)	+2 (%)	+3 (%)		
Infertility group	40						
A	10	-	5 (50)	5 (50)	-	10 (100)	
B	10	2 (20)	6 (60)	4 (40)	-	10 (100)	
C	10	3 (30)	6 (60)	2 (20)	2 (20)	10 (100)	
D	10	3 (30)	7 (70)	2 (20)	1 (10)	10 (100)	
Control group	10	1 (10)	5 (50)	4 (40)	1 (10)	10 (100)	

A; Sertoli cell only syndrome, B; maturation arrest, C; hypospermatogenesis, D; sloughing and disorganization, Ser; Sertoli cell, Ley; Leydig cell, In all, p>0.05 vs control group. calculated by Mann-Whitney U test

**Table 3.** ET-1 expression rate on germ cell according to the grade of maturation

	No. cases	SG (%)	SC (%)	ST (%)	Overall (%)
		+1	+1	+1	+1
Infertility group	40				
A	10	-	-	-	-
B	10	1 (10)	1 (10)	-	2 (20)
C	10	-	1 (10)	-	1 (10)
D	10	1 (10)	2 (20)	1 (10)	3* (30)
Control group	10	-	-	-	-

A; Sertoli cell only syndrome, B; maturation arrest, C; hypospermatogenesis, D; sloughing and disorganization, SG; spermatogonia, SC; spermatocyte, ST; spermatid\*; p<0.05 vs control group. calculated by Mann-Whitney U test



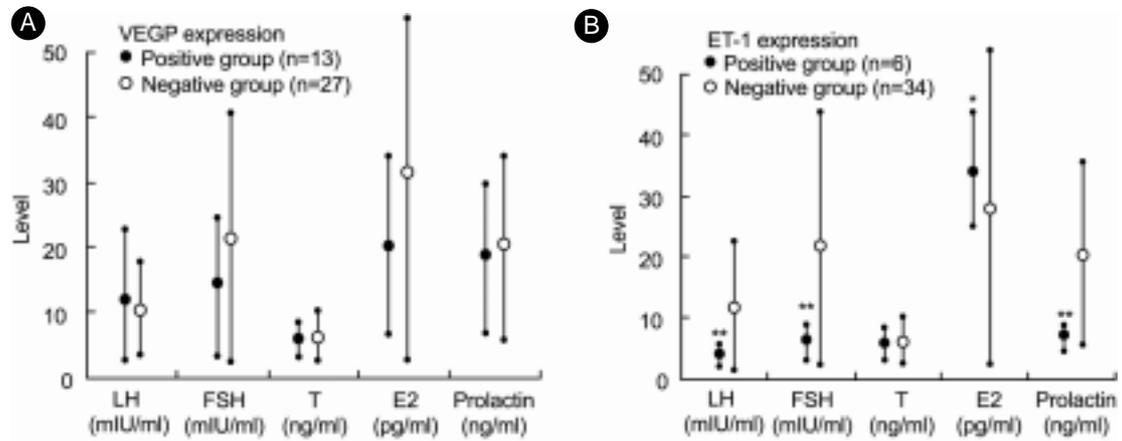
**Figure 2.** Immunohistochemical stains for VEGF ( $\times 400$ ). **A**; Sertoli cell only syndrome **B**; maturation arrest **C**; hypospERMATogenesis **D**; control group Sertoli cell; thick black arrow Leydig cell; thin black arrow.

2) ET - 1				(2) Sertoli			
(1)				Sertoli ET-1			
	ET-1		B D	A, B, C	D	+1	3
	+1	1 (10%)	,	(30%), 6	(60%), 3	(30%) 3	(30%) .
B, C	D		+1 1	Sertoli	ET-1		
(10%), 1	(10%) 2	(20%) .		37.5% (30%~60%)			
	D 1	(10%) +1		(Table 4) (Figure 2).			
		ET-1		(3) Leydig			
A		B, C D		Leydig	ET-1	A, B, C, D	
	20.0% (10%~30%)			+1	7 (70%), 6	(60%), 7	
			(Table 3) (Fi-	(70%), 4	(40%) 7	(70%) , +2	
gure 2).	D		(p<0.05).	3	(30%)		
ET-1				Leydig			
					60.0% (40%~70%)	100%	

**Table 4.** ET-1 expression rate on Sertoli cell, Leydig cell and germ cell according to the grade of maturation

	No. cases	Ser (%)		Ley (%)		Overall
		+1	+1	+2		
Infertility group	40					
A	10	3* (30)	7 (70)	- *	7* (70)	
B	10	6* (60)	6 (60)	- *	6* (60)	
C	10	3* (30)	7 (70)	- *	7* (70)	
D	10	3* (30)	4 (40)	- *	4* (40)	
Control group	10	-	7 (70)	3 (30)	10 (100)	

A; Sertoli cell only syndrome, B; maturation arrest, C; hypospermatogenesis, D; sloughing and disorganization, Ser; Sertoli cell, Ley; Leydig cell, Germ; germ cell, \*; p<0.05 vs control group. calculated by Mann-Whitney U test



**Figure 3.** The relationship between serum hormonal level and VEGF, ET-1 expression on germ cell in infertility group. \*; p<0.05, \*\*; p<0.001.

**Table 5.** Correlation with expression of VEGF and ET-1 on germ cell in infertility group

	ET-1	
	Positive rate (%)	Negative rate (%)
VEGF		
Positive rate (%)	2 (5.0)	11 (27.5)
Negative rate (%)	4 (10.0)	23 (57.5)

Calculated by Chi-square test

3) VEGF ET - 1  
VEGF ET-1  
32.5% 15.0%  
VEGF ET-1  
(p>0.05) (Table 5).

3. VEGF ET - 1  
LH,  
FSH, testosterone, estradiol prolactin  
6.40±5.85 mIU/ml 5.58±4.20 mIU/ml, 11.16±7.13  
mIU/ml 13.72±11.62 mIU/ml, 4.69±1.39 ng/ml  
4.51±1.30 ng/ml, 22.85±12.04 pg/ml 31.99±16.71 pg/

(Table 4) (Figure 2).

ml, 15.10±9.44 ng/ml 11.51±7.39 ng/ml (p>0.05, crine effect) (paracrine effect)

). ET-1 VEGF

LH, FSH, testosterone, estradiol prolactin

2.92±1.36 mIU/ml 6.36±4.94 mIU/ml, 4.38±1.74 mIU/ml 14.38±10.52 mIU/ml, 4.51±1.65 ng/ml 4.58±1.28 ng/ml, 37.23±6.09 pg/ml 27.99±15.43 pg/ml, 5.36±0.92 ng/ml 13.41±7.85 ng/ml testosterone

(p=0.001, <0.001, >0.05, 0.01, <0.001, ) (Figure 3).

Huminiiecki <sup>6</sup> VEGF가

Korpelainen <sup>7</sup> VEGF가

VEGF

가 가 가 (fi-brinogen) (fibrin)가

c-myc, p53, Bax Fas, Bcl-2, Obermair <sup>18</sup> (in vitro fer-tilization, IVF) VEGF가

c-kit <sup>12-16</sup> 가 가 가 (vasculogenesis), (angiogenesis), (wound healing), (myocardial ischemia), (ocular neovascular disease) (embryogenesis), (tumor growth),

VEGF VEGF

17

VEGF VEGF

B, C D

Ergün <sup>5</sup> VEGF Leydig C 가 VEGF

Sertoli VEGF Leydig, Sertoli 가 Endothelin ET-1 1988 Yanagisawa

ephrine 가 , norepin-  
 100 , angiotensin II 10 Leydig ET-1  
<sup>20,21</sup> ET-1 Sertoli Leydig  
 가 <sup>21,22</sup> ET-1 Leydig , Sertoli  
 ET<sub>A</sub> ET<sub>B</sub>가 ET<sub>A</sub> ET-1 <sup>8,9</sup>  
 ET<sub>B</sub> Sertoli 가 ET-1  
 가 <sup>9,23</sup> ET<sub>A</sub> Leydig ET-1  
 ET<sub>B</sub> Fantoni <sup>30</sup> ET-1  
 Leydig , Sertoli ET<sub>A</sub> Romanelli <sup>31</sup> (leu-  
 ET<sub>B</sub> (lamina propria) tenizing hormone releasing hormone, LHRH) ET-1  
 ET-1 <sup>9</sup>  
 , , , Ergul <sup>32</sup> ET-1  
 , <sup>8,9,26,27</sup> Leydig  
 Collin <sup>27</sup> Sprague-Dawley rat  
 , ET-1  
 , ET-1 ET<sub>A</sub>  
 ET-1  
 ET<sub>B</sub> ET- 가 VEGF  
 1 ET-3 , VEGF  
 nitric oxide (NO) prostacyclin ET-1  
<sup>20</sup>  
 (myoid cell)  
 Tripiciano <sup>28</sup> 가  
 Sertoli ECE-1 (endothelin converting en-  
 zyme-1) VEGF ET-1  
 arginine  
 vasopressin, TGF-β, PDGF, oxytocin, prostaglandin  
 adreno-  
 medullin <sup>29</sup> Sertoli  
 ET-1 ,  
 ET-1

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