

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

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

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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 H₂O₂ 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%,

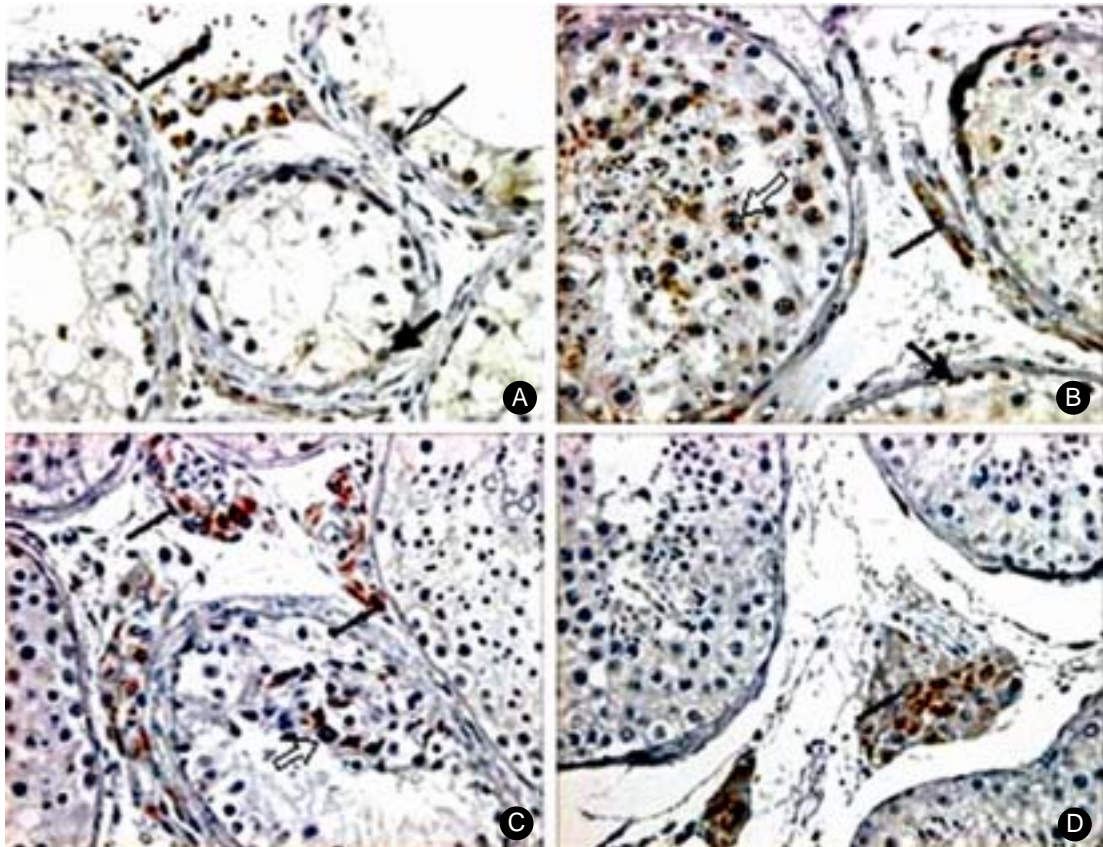


Figure 1. Immunohistochemical stains for VEGF ($\times 400$). **A;** Sertoli cell only syndrome **B;** maturation arrest **C;** hypospermatogenesis **D;** control group. Sertoli cell; thick black arrow, Leydig cell; thin black arrow, Spermatogonia; thin white arrow, Spermatocyte; thick white arrow.

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

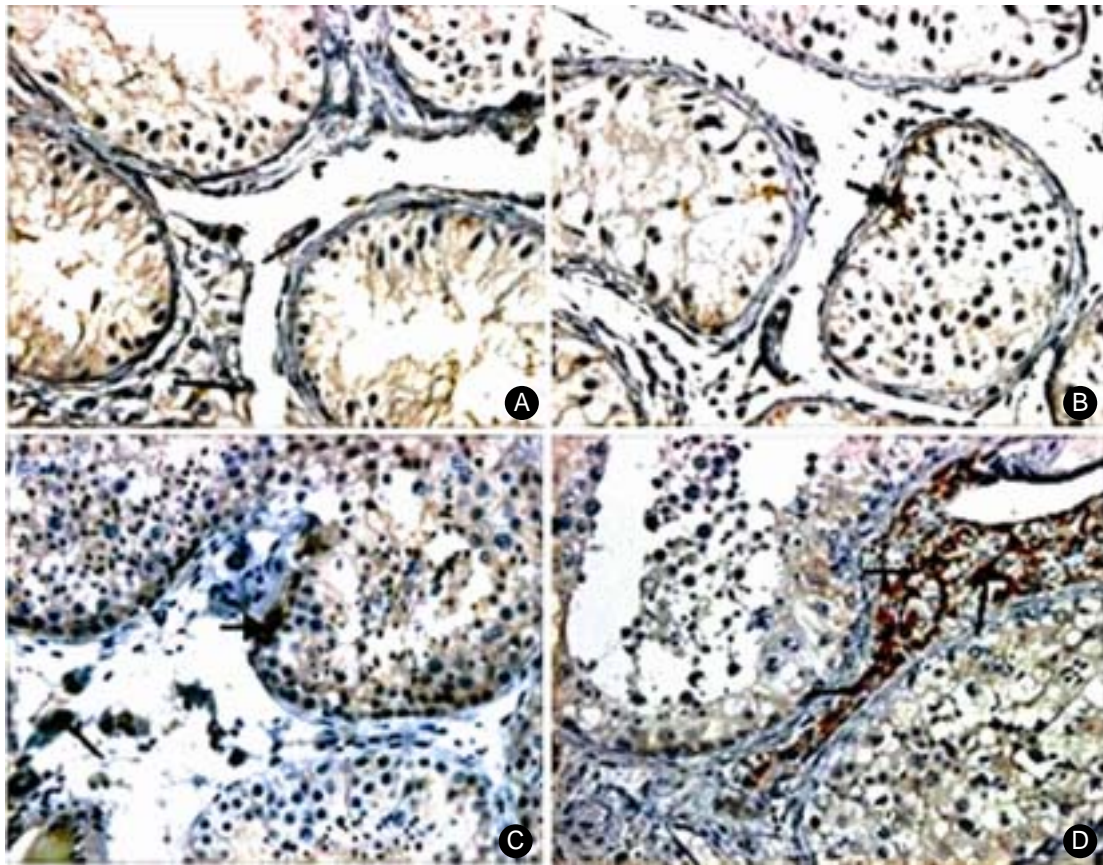


Figure 2. Immunohistochemical stains for VEGF ($\times 400$). **A**; Sertoli cell only syndrome **B**; maturation arrest **C**; hypospERMATogenesis **D**; control group Sertoli cell; thick black arrow Leydig cell; thin black arrow.

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

(Table 3) (Figure 2).
ET-1 (p<0.05).

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

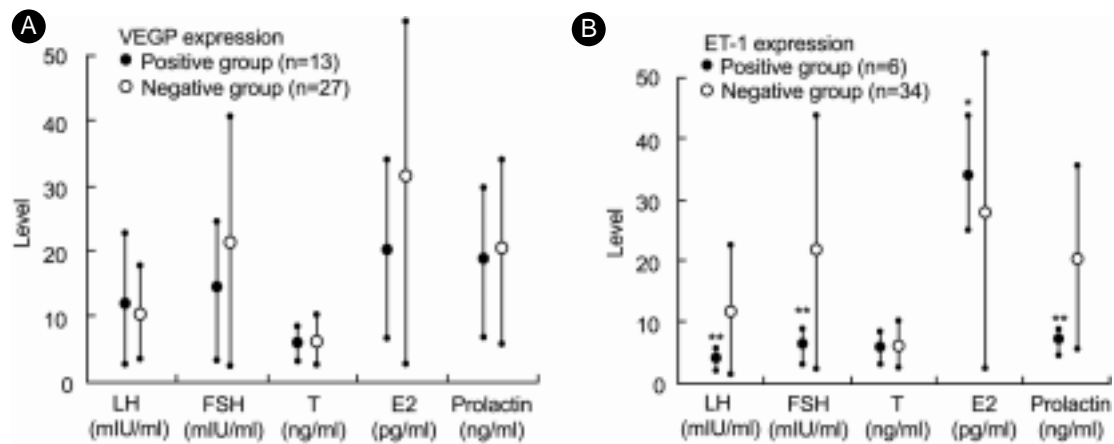


Figure 3. The relationship between serum hormonal level and VEGF, ET-1 expression on germ cell in infertility group. *; p<0.05, **; p<0.001.

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
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 ⁶ VEGF가 VEGF가

Korpelainen ⁷ VEGF가

VEGF

가 가 가 (fi-brinogen) (fibrin)가

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

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

VEGF VEGF

17

B, C D

Ergün ⁵ 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
^{20,21} ET-1 Sertoli Leydig
 100 Sertoli 가
 가 ^{21,22} ET-1 Leydig , Sertoli
^{8,9} ET-1
 ET_A ET_B가 ET_A ET-1 Se-
 ET_B rtoli 가
 가 ^{9,23} ET_A Leydig ET-1
 ET_B
^{24,25} ET_A ET_B Fantoni ³⁰
 Leydig , Sertoli ET_A ET-1
 ET_B (lamina propria) Romanelli ³¹ (leu-
 tenizing hormone releasing hormone, LHRH) ET-1
⁹ ET-1 , Ergul ³² ET-1
^{8,9,26,27} Leydig
 Collin ²⁷ Sprague-Dawley rat
 ET-1 VEGF
 ET-1 ET_A 가
 ET-1 VEGF
 1 ET-3 ET-1 VEGF
 nitric oxide (NO) prostacyclin ET-1
²⁰ (myoid cell)
 Tripiciano ²⁸ 가
 Sertoli ECE-1 (endothelin converting en-
 zyme-1) VEGF ET-1
 arginine
 vasopressin, TGF-β, PDGF, oxytocin, prostaglandin
 adreno-
 medullin ²⁹ Sertoli
 ET-1 ET-1

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