

GnRH-agonist에 의한 인간 과립-황체화 세포의 세포사멸과 PBR 단백질의 발현

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Apoptosis and Peripheral Benzodiazepin Receptor (PBR) Expression in Human Granulosa-Luteal Cells by GnRH-agonist

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Objective: To investigate whether GnRH-agonist (GnRH-Ag) using in IVF-ET affects apoptosis of human granulosa-luteal cells and expression of peripheral benzodiazepine receptor (PBR) protein involved in the apoptosis of the cells.

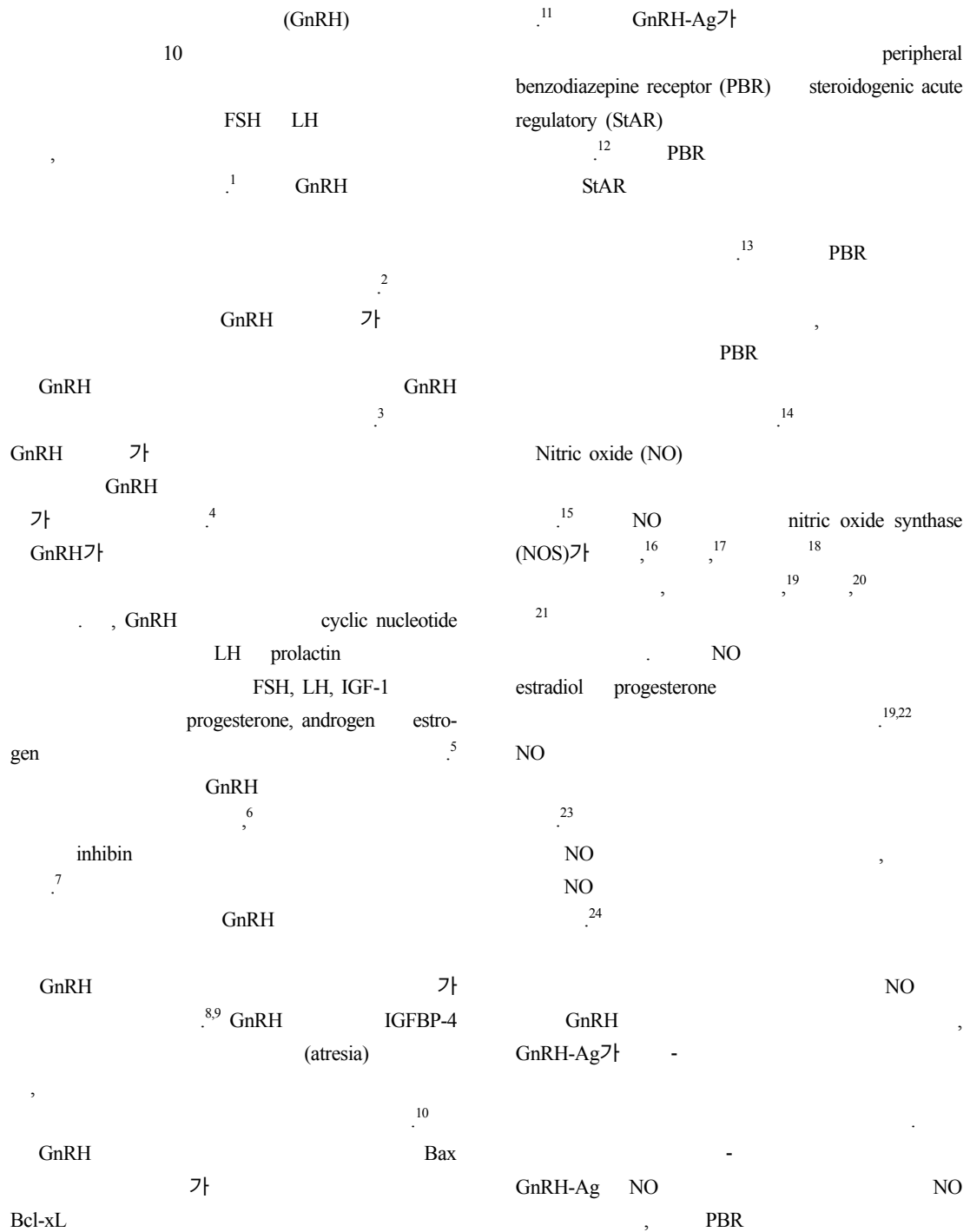
Methods: Granulosa-luteal cells obtained during oocyte retrieval were cultured and treated with 10^{-5} M GnRH-Ag. Apoptosis of the cells by the treatment was confirmed using DNA fragmentation analysis 24 h after culture. The presence of PBR protein within the cells was examined by immunofluorescence staining and the expression of the protein was analyzed by Western blotting. In addition, it was measured for progesterone and nitric oxide (NO) produced by granulosa-luteal cells after GnRH-Ag treatment. To evaluate the relationship between NO production and PBR expression, sodium nitroprusside (SNP) as a NO donor was added in media and investigated the expression of PBR protein by Western blotting.

Results: Apoptosis increased in the granulosa-luteal cells 24 h after GnRH-Ag treatment, whereas the expression of PBR protein significantly decreased. Furthermore, the production of progesterone and nitric oxide (NO) by the cells significantly fell from 12 h after the treatment. In the results of Western blotting after SNP treatment, the expression of PBR protein increased in the treatment with SNP alone to the granulosa-luteal cells, but was suppressed in the treatment with GnRH-Ag and SNP. Additionally, the staining result of PBR protein in the cells showed the even distribution of it through the cell.

Conclusion: These results demonstrate that GnRH-Ag treatment induces apoptosis, decreasing expression of PBR protein and NO production in human granulosa-luteal cells. The present study suggests that one of the apoptosis mechanism of human granulosa-luteal cells by GnRH-Ag might be a

signal transduction pathway via NO and PBR.

Key Words: Apoptosis, GnRH-agonist, Granulosa-luteal cells, Nitric Oxide (NO), Peripheral benzodiazepine receptor (PBR)



, 0 2.5
 100% ethanol 가, -70
 1 4
 14,000 xg 30 DNA
 50 μ l 1X TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) 1 μ l DNase-free RNase (500 μ g/ml; Boehringer-Mannheim, IN) 가 60 37
 DNA phenol : chloroform : isoamyl alcohol chloroform: isoamyl alcohol 0.1 3 M sodium acetate 0 2.5 100% ethanol DNA -70 60 4 14,000 xg 30 , 0 0.2 ml 80% ethanol 25 μ l , 260 nm DNA -20 DNA lane 5 μ g 1.5% agarose gel loading , running buffer TBE , 50 V 3 ethidium bromide trans-illuminator
 5. NO NO nitrate/nitrite colorimetric assay kit (Alexis Biochemicals, San Diego, CA) 10 kDa molecular mass cut-off filter (Amicon, Millipore Co., Bedford, MA) nitrate reductase enzyme cofactor가 3 sulfanilamide N-[1-naphthyl] ethylenediamine 가 10 microplate reader (Spectra Max 250, Molecular Devices, Sunnyvale, CA) 540 nm
 6. PBR Western blot 50 mM Tris-base (pH 7.4), 150 mM NaCl, 10 mM EDTA, 0.1% Tween-

20, protease inhibitors (0.1 mM phenyl methyl-sulfonyl-fluoride, 5 g/ml aprotinin, and 5 g/ml leupeptin)가 homogenization buffer 12,000 xg 30 DC protein assay kit (Bio-Rad Laboratories, Inc., Hercules, CA, U.S.A.) 10% SDS-PAGE blotting nitro-cellulose membrane , 5% non-fat dry milk가 Tris-buffered saline (TTBS; 10 mM Tris (pH 7.6), 150 mM NaCl, 0.1% Tween-20) 1 가 blocking . Blocking membrane rabbit polyclonal anti-human PBR (Trevigen, Gaithersburg, MD) 1:1,000 TTBS 1 TTBS 1 3 anti-rabbit horseradish peroxidase-conjugated antibody (Santa Cruz Biotechnology, Santa Cruz, CA) TTBS 1:1,000 40 membrane chemiluminescence (ECL kit; Amersham Life Science, Buckinghamshire, U.K.) X-ray film (Hyperfilm, Amersham Life Science, Buckinghamshire, U.K.)
 7. PBR PBR 1% paraformaldehyde PBS goat normal serum 가 30 rabbit polyclonal anti-human PBR , 1:100 PBS fluorescein isothiocyanate (FITC)가 anti-rabbit IgG (Jackson Immuno Research laboratories, West Grove, PA) 가 30 Propidium Iodide 가 (Nikon, Tokyo, Japan)

8.

one-way ANOVA stu-

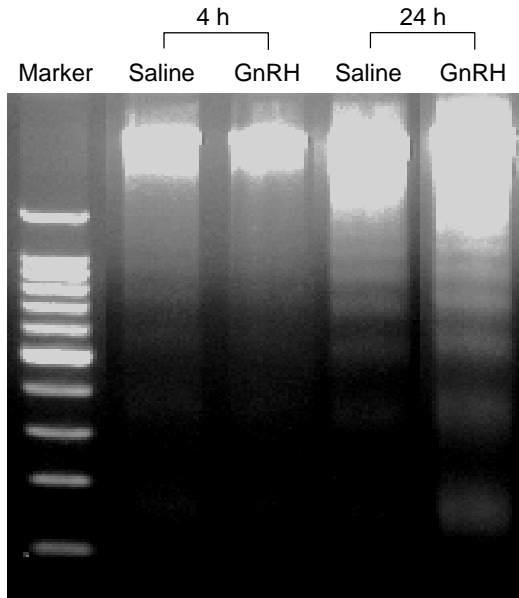


Figure 1. Effects of GnRH-Ag treatment on DNA fragmentation in the cultured human granulosa-luteal cells. The cells cultured for 24 h showed an increase in DNA fragmentation in GnRH-Ag treatment compared with those in the saline.

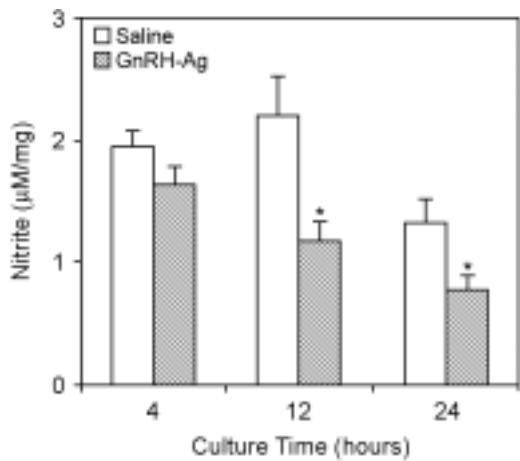


Figure 2. Nitric oxide levels in the medium (mean \pm EM; n = 5) at points timed after initiation of the treatment. *p<0.05 compared with corresponding saline controls.

dent's t-test p 0.05

1. GnRH - Ag

GnRH-Ag - DNA 4
 DNA 24 GnRH-Ag 가 (Figure 1).

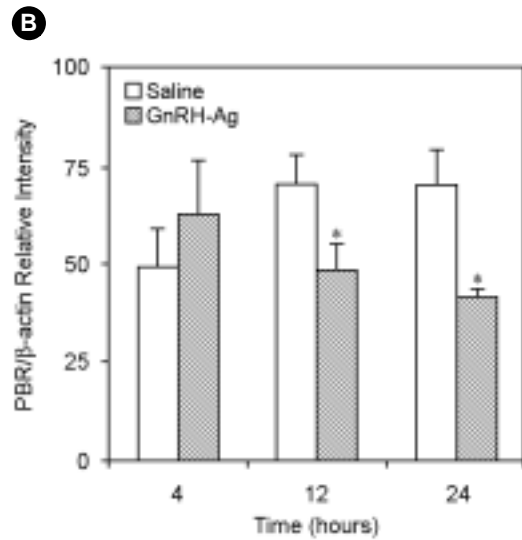
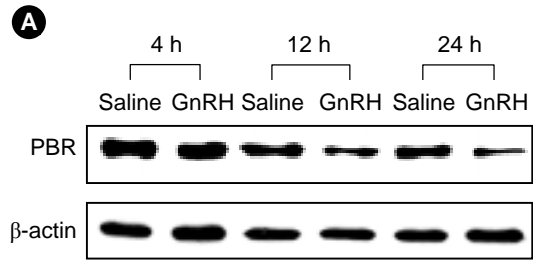


Figure 3. Western blot analysis (mean \pm EM; n = 5) of PBR proteins in the granulosa-luteal cells at points timed after the commencement of treatment. *p<0.05 compared with corresponding saline controls.

2. GnRH - Ag

NO

Figure 2 GnRH-Ag

NO

NO

	12	GnRH-Ag
	$3.33 \pm 0.66 \mu\text{M/ml}$	$6.21 \pm 1.52 \mu\text{M/ml}$
		($p < 0.05$), 24
	$2.35 \pm 0.91 \mu\text{g/ml}$	$4.04 \pm 1.23 \mu\text{g/ml}$

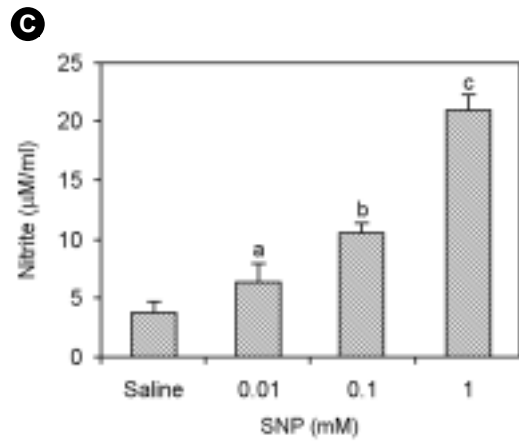
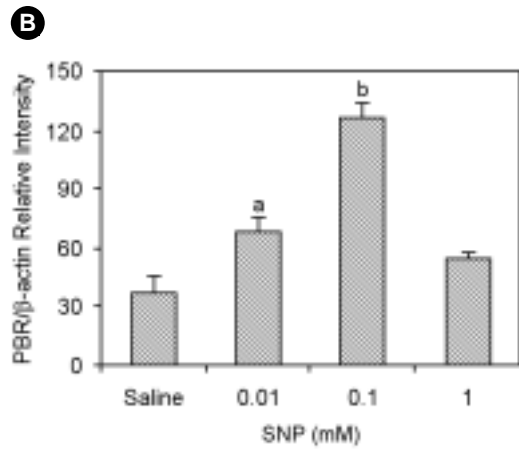
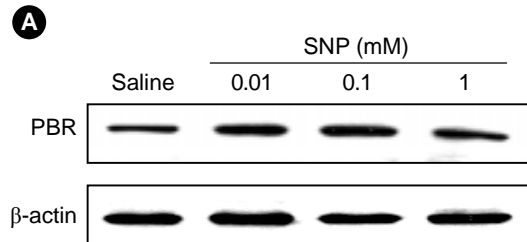


Figure 4. Western blot analysis (mean \pm EM; $n = 5$) of PBR protein in the granulosa-luteal cells (**A & B**) and nitric oxide levels (mean \pm EM; $n = 5$) in the medium 24 h after sodium nitroprusside (SNP) treatment in a dose-dependent manner. * $p < 0.05$ compared with corresponding saline controls.

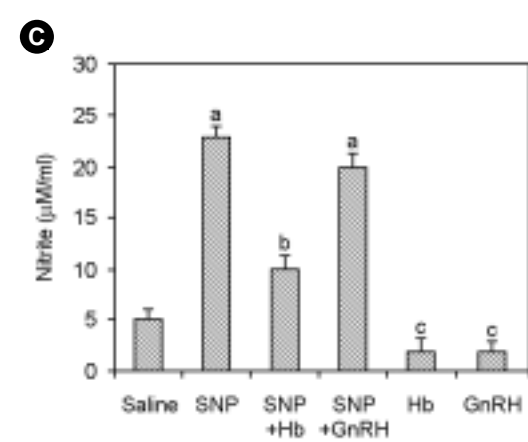
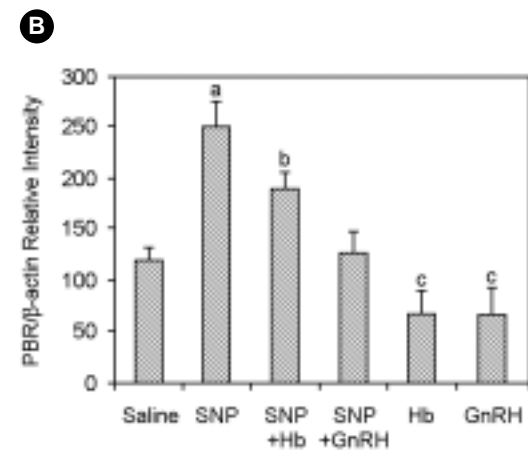
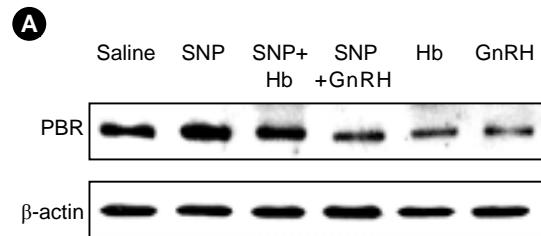


Figure 5. Western blot analysis (mean \pm EM; $n = 5$) of PBR protein in the granulosa-luteal cells (**A & B**) and nitric oxide levels (mean \pm EM; $n = 5$) in the medium 24 h after treatment with GnRH-Ag, sodium nitroprusside (SNP), or hemoglobin (Hb). * $p < 0.05$ compared with corresponding saline controls.

(p<0.05).

3. GnRH - Ag PBR

- , 4 PBR

가, 12 GnRH-Ag (71.34±

10.21) (48.67±6.98)

(p<0.05). 24 GnRH-

Ag (72.78±12.32) (43.34±

5.22) (p<0.05) (Fi-

gure 3).

4. SNP PBR

NO SNP -

PBR ,

PBR 0.01 mM SNP (62.48±

9.20) 0.1 mM SNP (121.09±11.11) 가

가 1 mM SNP (48.76±4.23)

(Figure 4B). NO

SNP 가

(Figure 4C). Figure 5 0.1 mM SNP, Hb GnRH-

Ag PBR

NO

PBR

SNP

가 Hb

(239.11±23.12) (178.56±13.32)

(123.98±21.87) SNP PBR

GnRH-Ag Hb (72.13±

17.09) GnRH-Ag (81.98±19.09)

PBR

(113.60±13.54) (Figure

5B). NO SNP

(23.21±2.12 µg/ml) 가 ,

Hb (8.95±1.93 µg/ml)

GnRH-Ag

NO 가 SNP

19.78±2.67 µg/ml

Hb (2.10±1.09 µg/ml) GnRH-Ag (1.97±0.91 µg/ml)

PBR

(5.66± 1.24 µg/ml)

(Figure 5C).

5. PBR

- PBR

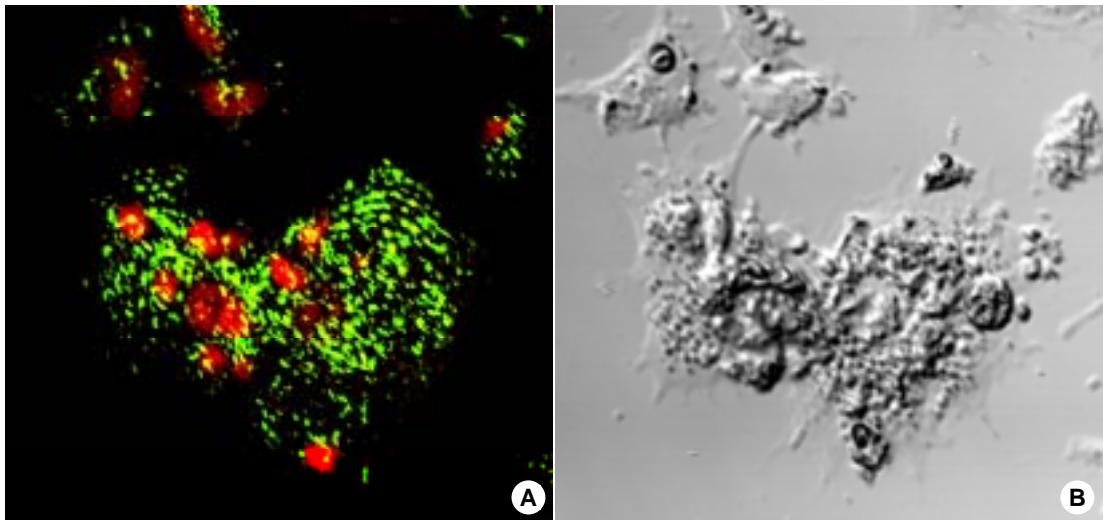
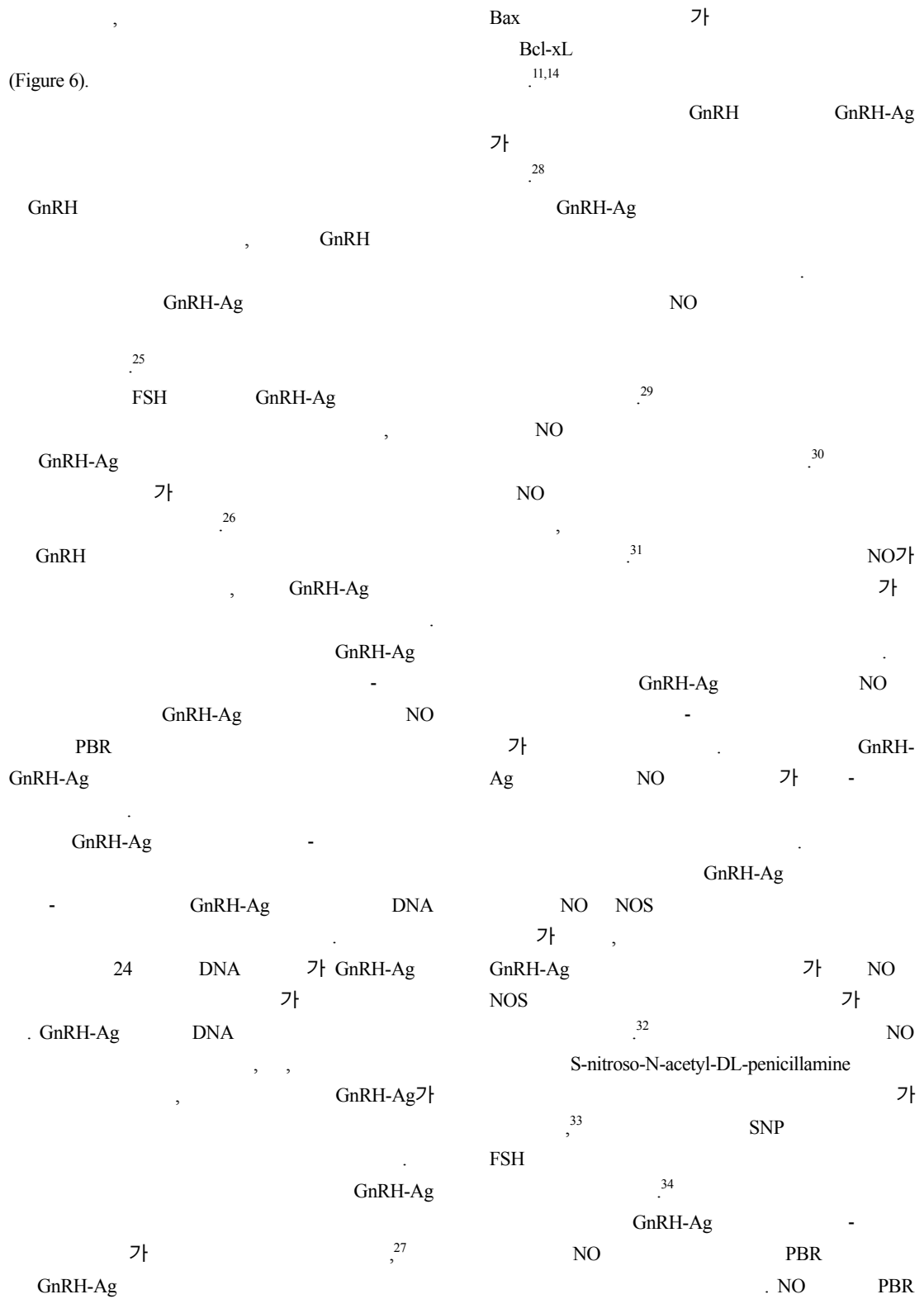


Figure 6. Localization of PBR protein in the cultured human granulosa-luteal cells. Green spots displaying PBR expression localize on mitochondria in even throughout the cell. **A;** Fluorescent and **B;** Light microphotographs. Original magnification X400.



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