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성균관대학교 의과대학 삼성제일병원 생식생물학 및 불임연구실¹, 산부인과²,
서울대학교 수의과대학 공중보건학교실³

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Alteration of Gene Expressions in Human Endometrial Stromal Cells by Exogenous FSH Treatments

Hye Won Choi¹, Jin Hyun Jun^{1*}, Hyoung Song Lee¹, In-Sun Hong³,
Kyung-Sun Kang³, Mi Kyoung Koong²

¹Laboratory of Reproductive Biology & Infertility, ²Department of Ob/Gyn, Samsung
Cheil Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea

³Department of Veterinary Public Health, College of Veterinary Medicine
Seoul National University, Seoul 151-742, Korea

Objective: To evaluate the effects of recombinant FSH (rFSH) and urinary FSH (uFSH) on the gene expressions of human endometrial stromal cells *in vitro*.

Methods: Endometrial tissue was obtained from a pre-menopausal women undergoing hysterectomy. Primary endometrial stromal cells were isolated and *in vitro* cultured with FBS-free DMEM/F-12 containing 0, 10, 100, and 1,000 mIU/ml of rFSH and uFSH for 48 hours, respectively. Total RNA was extracted from the cultured cells and subjected to real time RT-PCR for the quantitative analysis of *progesterone receptor* (PR), *estrogen receptor α/β* (ER- α/β), *cyclooxygenase 2* (Cox-2), *leukemia inhibitory factor* (LIF), *homeobox A10-1 and -2* (HoxA10-1/-2).

Results: Both hormone treatments slightly increased (< 3 folds) the expressions of PR, ER- β and HoxA10-1/-2 gene. However, ER- α expression was increased up to five folds by treatments of both FSH for 48 hours. The LIF expression by the 10 mIU/ml of uFSH for 12 hours was significantly higher than that of rFSH (p<0.01). After 24 hours treatment of two kinds of hormones, the expression patterns of LIF were similar. The 100 and 1,000 mIU/ml of rFSH induced significantly higher amount of Cox-2 expression than those of uFSH, respectively (p<0.05).

Conclusion: This study represents no adversely effect of exogeneous gonadotropins, rFSH and uFSH, on the expression of implantation related genes. We suggest that rFSH is applicable for the assisted reproductive technology without any concern on the endometrial receptivity.

Key Words: Recombinant FSH, Urinary FSH, Endometrial stromal cell, Implantation related genes, Endometrial receptivity

(follicle stimulating hormone, FSH)
(assisted reproductive technology, ART)

urinary FSH
1.

(uFSH)가
recombinant FSH (rFSH)가
¹⁻³ 가 FSH Julia ^{16,17}

uFSH
(luteinizing hormone, LH) (2×3
FSH cm) (M199/F-12 medium;
(consistency) Gibco Life Technologies, MD, USA)

FSH (FSH receptor, 1~2 mm²
FSH-R) LH (LH receptor, LH-R) collagenase (2 mg/ml;
Sigma, MO, USA) 가 2~3

^{4,5}
wire sieve (140 μm, 37 μm; Newark Wire
Co., NJ, USA)

LH-R FSH-R
FSH LH sieve

가
⁶⁻⁹ 10% fetal
LH-R bovine serum (FBS), insulin, transferrin, selenium, bo-
vine serum albumin, linoleic acid 가

(chorionic gonadotropin, CG) 2~3
(decidualization)

prolactin cyclooxygenase-2 (Cox-2)
가 ^{10,11}

2.
cytokeratin

가 rFSH vimentin (immu-
가 가 ¹⁻³ nocytochemical staining)

LH uFSH 3.7% formaldehyde-PBS 10
, 100% methanol 100% acetone

가 rFSH 10 5 , 0.4% Triton X-100-PBS 10

가 ¹²⁻¹⁴ 2% goat serum 4% bovine
serum albumin 가 PBS 1

FSH cytokeratin 8/18 (Santa Cruz Bio-
uFSH rFSH tech., CA, USA) vimentin (chemicon, CA, USA)
가 ¹⁵ 1:100 1 , PBS

가 rFSH uFSH FITC conjugated anti-
mouse IgG (Zymed Lab. Inc., CA, USA) 1:100

1
anti-fade mounting medium (Fisher Scientific, PA, USA)

3.
(FSH)
가
95%

가
가
sterone 가 4~5
FSH 가
rFSH (Puregon;
Organon, Netherlands) uFSH (Metrodin HP; Serono,
Switzerland) 10, 100, 1,000 mIU/ml
가 12, 24, 48
FSH 24

4.
RT - PCR
(Qiagen, WA, USA)
-70
M-MLV kit (Promega Co., WI, USA)

real time
Qiagen RNeasy kit
RNA
RNA
Dynamo kit (Finnzyme,
Finland) DNA engine Opticon 2 fluorescence detec-
tion system (MJ Research, MA, USA)
real time RT-PCR
primer
Table 1
primer real time PCR
, internal control β -actin
 $2^{-\Delta\Delta Ct}$

Table 1. Sequences of oligonucleotide primers and PCR conditions for implantation related genes

Genes	Forword primer Reverse Primer	Annealing Tm ()	Product size (bp)
PR	5'-GATTCAGAAGCCAGCCAGAG-3' 5'-AGTAGTTGTGCTGCCCTTCC-3'	62	163
ER- α	5'-CCACCAACCAGTGCACCATT-3' 5'-GGTCTTTTCGTATCCCACCTTTC-3'	65	108
ER- β	5'-AGAGTCCCTGGTGTGAAGCAAG-3' 5'-GACAGCGCAGAAGTGAGCATC-3'	65	143
Cox-2	5'-CTGGCTGAGGGAACACAACA-3' 5'-GCAATTTGCCTGGTGAATGA-3'	62	381
LIF	5'-GCTGTTGTTCTGCACTGGA-3' 5'-TCCCCCTGGGCTGTGTAATA-3'	59	183
HoxA10-1	5'-GAGAAGGATTCCTGGGCAA-3' 5'-TTCATCCTGCGGTTCTGAAA-3'	59	227
HoxA10-2	5'-GTGTCAAGGCAATCCAAAG-3' 5'-CCGGTTTCTCGATTCAATT-3'	59	247
β -actin	5'-TGGCACCACACCTTCTACAATGAGC-3' 5'-GCACAGCTTCTCCTTAATGTCACGC-3'	62	396

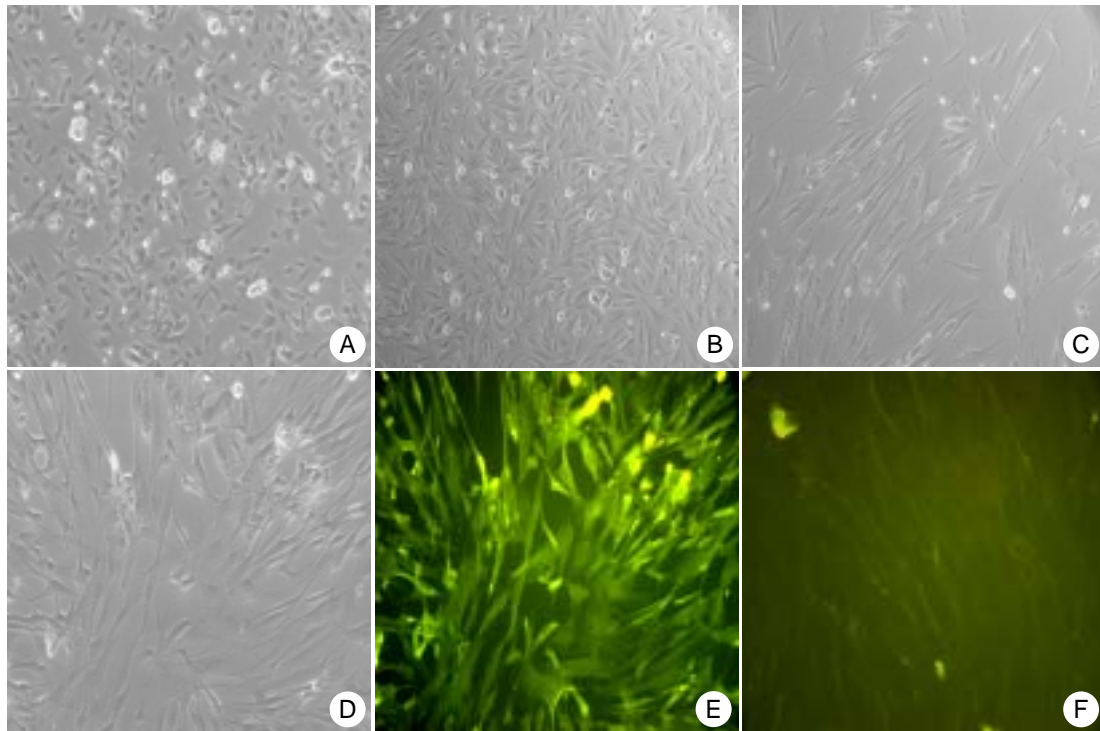


Figure 1. Morphological changes and immunocytochemical staining of endometrial stromal cells during *in vitro* culture. Early passage cells (A) were polygonal. It became more spindle-shaped in passage 3 (B) and more than passage 5 (C) of subculture. Stromal cells (D) was localized with the primary antibody to vimentin (E) and without the primary antibody (F). Bar indicates 10 μ m.

18
 Student's *t*-test
 p 0.05
 2.
 Real time RT-PCR
 FSH
 Figure 2
 1,000 mIU/ml FSH
 1.
 가 , rFSH
 uFSH 가가 3
 PR, ER- β HoxA10
 가 Cox-
 가 (Figure 1). 2가 . LIF 10 mIU/ml uFSH 12
 vimentin rFSH
 95%
 (Figure 1). , (p<0.01), 24 100 1,000 mIU/ml
 FSH-R LH-R RT- 가 . Cox-2
 PCR (data not shown). 100 1,000 mIU/ml rFSH 48
 , uFSH

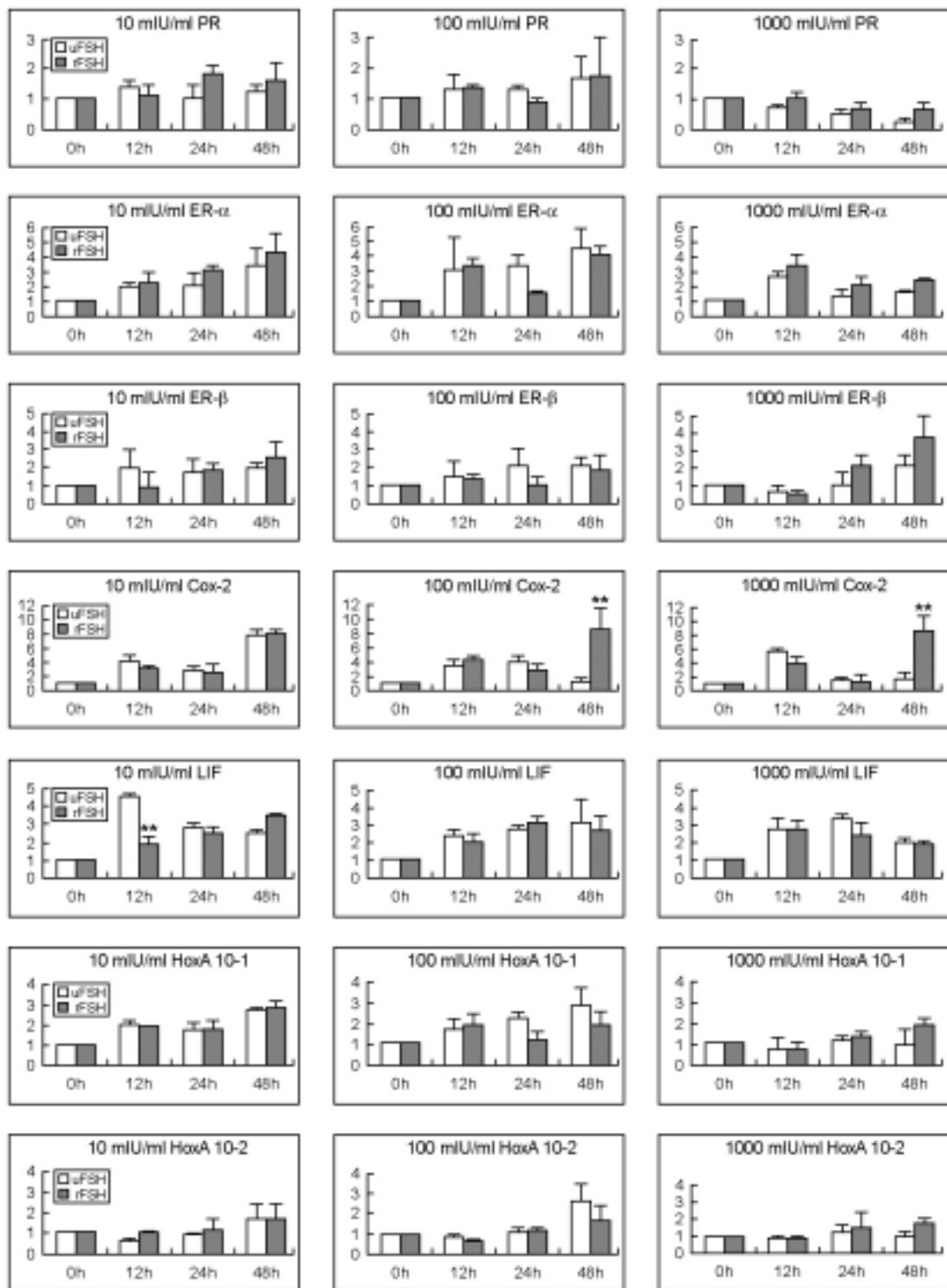


Figure 2. Effects of recombinant FSH and urinary FSH on the expression of implantation related genes by quantitative analysis using real time RT-PCR in human endometrial stromal cells. ** indicate the significant difference ($p < 0.01$).

가 ($p < 0.01$).

FSH FSH가

¹⁸⁻²¹

rFSH uFSH 가

PR, ER- α/β , Cox-2, LIF, HoxA10-1/-2

FSH 가

rFSH uFSH

(Figure 2).

PR

가 , FSH

estrogen ER 가

LIF

10 mIU/ml uFSH 12 가 , uFSH

LH 가

LH-R LH-R

Cox-2 가 rFSH CG

progesterone

Cox-2 가

^{10,11}

rFSH HoxA10

Ishikawa cell line

GnRH

agonist, GnRH antagonist rFSH

¹⁵

HoxA10

FSH

가 FBS 가

FSH

rFSH uFSH

in vivo FSH

가 , 가

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