Higher endometrial receptivity caused by Letrozole in antagonist protocol-stimulated mouse uterus

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ABSTRACT

Background: The implantation rate of IVF remains low due to the adverse effect of ovarian stimulation on endometrial receptivity. Integrin β3, E-cadherin and Leukemia Inhibitory Factor (LIF) are the best markers known for endometrial receptivity evaluation. In this study, the effect of adding Letrozole to antagonist protocol based on the expression of integrin β3, E-cadherin and LIF level during implantation window was investigated. Letrozole is an inhibitor of aromatase that can prevent the supraphysiological estrogen level. The goal of the study was to prove that adding Letrozole to IVF antagonist protocol can increase the expression of Integrin β3, E-cadherin, and LIF concentration of mice uterine.

Methods: This is an experimental study with a post-test only group design using Balb/c mice mimicked ovarian stimulated IVF. Antagonist protocol was applied to one group as ovarian stimulation, while the other group received the combination of Letrozole and antagonist protocol. Uterus samples were collected 48 hours after ovarian stimulation. Integrin β3 and E-cadherin expression were detected by immunohistochemistry technique and LIF level assay by ELISA. Normality test was carried out using Shapiro–Wilks, and homogeneity test by Levene’s T. Comparison between integrin β3 and E-cadherin expression were tested by Mann-Whitney and Fisher’s exact, while comparison of LIF was tested by Mann-Whitney, with p<0.05 considered as significant. Path analysis was done to measure each variable contribution.

Results: The Letrozole + GnRH antagonist treated mice showed significantly higher integrin β3 (2.71 + 0.61 vs 1.04 + 0.08, p<0.05) and E-cadherin (3.73 + 0.28 vs 1.16 + 0.29, p<0.05) expression in endometrium. It also showed significantly higher level of uterine LIF (1.78 + 0.13 vs 1.66 + 0.17 ng/ml, p<0.05) during implantation window than GnRH antagonist treated mice alone. Expression of integrin β3 (χ2= 22.91, p<0.05) and E-cadherin (χ2=36.00, p<0.05) were significantly higher in Letrozole group compared to control.

Conclusion: Letrozole caused higher expression of Integrin β3, e-cadherin, and LIF concentration in Mice uterus stimulated by antagonist protocol. Letrozole had the highest contribution to the increase of E-cadherin. Integrin β3 together with E-cadherin and Letrozole had 31.4% contribution to the increase of LIF.

Keyword: Letrozole, antagonist protocol, integrin β3, E-cadherin, LIF

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BACKGROUND

During the last decade, the success of In Vitro Fertilization – Intracytoplasmic Sperm Injection (IVF-ICSI) was still low and tends to be constant; especially related to implantation rate (IR). In Indonesia, the average of IVF-ICSI IR in 23 test-tube baby service centers was 13.88%. In Graha Tunjung Clinic, the test-tube baby service center at Sanglah Hospital Denpasar was 9.14% (2005-2010) and 12.66% (2010-2015). In America and Europe, IR was reported 29% and 29.3% where these percentages are relatively constant for the last five years. These numbers were lower than natural cycle IVF program that reaches 50%.

Various efforts had been made to increase IR of IVF-ICSI programs, such as embryo selecting, number and phase of the embryo, and the day of embryo transfer. Those efforts including embryo selecting technique with the best quality, more than one embryo transfer and/or embryo transfer in blastocyst phase as well as the adjustment of embryo transfer (ET) time with implantation window, where ET for eight cells was on the third day since ovum pick up and ET in blastocyst phase was the fifth day since ovum pick up.

Integrin β3, E-cadherin and Leukemia Inhibitory Factor (LIF) are the best known markers for endometrial receptivity evaluation. Integrin subunit β3 (ITGB3) or Integrin β3 is a protein that is expressed by the gene of long arm 17q21.32. This protein has a function in adhesion of endometrium cells, which begin the implantation process. Integrin β3 was located in luminal epithelium and expressed in gland after the nineteenth day of menstruation cycle. The protein that is expressed in the luminous endometrium surface is expected to play a role in the first interaction with embryo trophoblast. Integrin αβ3 is attached to its ligand, osteopontin, together with a hyaluronate-CD44 receptor, forming a “sandwich” pair between an embryo and endometrial epithelium. Integrin is a marker to measure endometrial receptivity regulated by estrogen and progesterone through Homeobox A10 regulating gen (HOXA 10).
Letrozole is the third generation of an aromatase inhibitor. Letrozole selectively inhibits aromatase enzyme that catalyzes androgen (androstenedione and testosterone) to estrogen (estrone and estradiol) in the ovary. One to five mg Letrozole daily can decrease the estrogen levels to 97-99%, which cannot be detected by the most sensitive immunnoassay technique. This drug was fully absorbed by oral route with an average half-life of 45 hours (30-60 hours). The drug was expected to increase the output of IVF-ICSI program by decreasing estradiol level, reducing needed doses of gonadotropin, increasing ovary response to FSH, preventing the fluctuation of premature LH and reducing the risk of ovarian hyperstimulation syndrome.

Since 2001, the use of this drug in ovary stimulation is still off-label. The study about the use of this drug and the Cochrane reviews is still ongoing.

In this study, we investigated the effect of adding Letrozole to antagonist protocol based on the expression of integrin β3, E-cadherin and LIF level during implantation window of human IVF mimicked mice model. Based on the aforementioned evidence, we used Integrin β3, E-cadherin and LIF level as biomarker of endometrial receptivity. We hypothesized that adding Letrozole can cause higher expression of β3-Integrin, E-cadherin, and LIF concentration in mice uterine stimulated by antagonist protocol and reflect different endometrial receptivity.

**MATERIAL AND METHODS**

**Animals:** Animal care and usage procedures were in accordance with the institutional guidelines established by the Animal Care and Use Committee. Virgin 8-12 weeks old female Balb/c mice were housed in a 12hr light and 12hr dark cycle at 25+0.5°C and 50-60% humidity. The mice were fed ad libitum with standard diet and water. To identify the Estrous cycle, vaginal discharge and smear samples were evaluated daily. Mice that exhibited regular 4-days cycles, 18-22 grams body weight, were randomly allocated to two groups with 18 mice in each group. The first group treated with Letrozole + antagonist protocol ovarian stimulation and the second group treated with antagonist protocol alone.

**Ovarian Stimulation:** The ovarian stimulation procedure in two different group were: (1) in the Letrozole + GnRH antagonist group, GnRH antagonist (Cetrotide) was injected (i.p.) at 4.5 μg/100 g body weight dose from the day 3 of estrus to day 9. At 1.00 pm on day 8, Letrozole (Femara) at 1.0 mg/kg body weight dose was given intramuscularly. HMG (Menopur) was injected.
Uterus samples were collected (i.p.) at 40 U/100 g body weight dose at 9:00 am on the day 9, followed by an injection (i.p.) of HCG (Pregnyl) 100 U/100 g body weight dose, 28 h after the injection of HMG; (2) in GnRH antagonist group, GnRH antagonist (Cetrotide) was injected (i.p.) at 4.5 μg/100 g body weight dose from the day 3 of estrus to day 9. HMG (Menopur) was injected (i.p.) at 40 U/100 g body weight dose at 9:00 am on the day 9, followed by injection (i.p.) of HCG (Pregnyl) 100 U/100 g body weight dose, 28 h after the injection of HMG.

**Tissue Collection:** Uterus samples were collected on day 12 of estrus period, at 9.00-10.00 am. Cervical dislocation was used to perform euthanasia, followed by a hysterectomy. The uterus was divided into two parts, for immunohistochemistry analysis and ELISA.

**Immunohistochemistry Analysis and ELISA:**
The uterus was fixated by 10% formalin solution and embedded in paraffin. Paraffinized tissues were sliced into 5 μm thick and deparaffinized in xylene solution and ethanol 90%. Antigen retrieval was conducted by boiling sectioned tissue in TE solution using 700-watt microwave for 15 min. For immunohistochemistry detection of Integrin β3, anti-mouse Integrin beta 3 (Abcam, USA) was used according to the kit standard. E-cadherin antigen retrieval was conducted by boiling sectioned tissue in Tri Sodium Citrate buffer using 700-watt microwave for 10 min. The color was developed using betazoid DAB, counterstain with hematoxylin Gill and dehydrated by alcohol 70-90% and xylene solution. Staining intensity of tissue was evaluated and graded (< 1.1 weak, 1.1-2 moderate, 2.1-3 strong, 3.1-4 very strong), and was assessed using the H-SCORE that calculated using the following equation: H-score = ΣPi(i+1). Where Pi is the proportion of stained epithelial cells in each category of intensity, and i is the staining intensity.

For uterine tissue, BT laboratory mouse LIF ELISA kit E0645Mo was used.

**Statistical Analysis:** Normality data were tested using Shapiro-Wilk Test, homogeneity test by Levene’s T. Comparison of integrin β3 and E-cadherin expression was tested by Mann Whitney and Fisher’s exact test, while comparison of LIF was tested by Mann Whitney, with p < 0.05 considered as significant. Path analysis was done to obtain each variable contribution.

**RESULTS**

There was no significant difference in the average age of mice, pre-intervention body weight and post-intervention body weight in both treatment and control group. Characteristic distribution of age, beginning body weight, and final body weight mean in treatment group and control group shown in [Table 1](#).

Based on mean H-score of Integrin β3 expression, it appeared that Letrozole + antagonist (treatment) group tend to have higher expression of integrin β3 (2.71±0.61) compared to antagonist alone (control) group (1.04±0.08). Statistical analysis for the difference of expression revealed that the result was statistically significant (p=0.001). The detail of the data is presented in [Table 2](#).

Then integrin β3 H-score was classified into these following categories: (a) Weak expression (H-score <1,1), (b) Intermediate expression (H-score = 1.1 – 2), (c) Strong expression (H-score = 2.1 – 3), and (d) Very Strong expression (H-score = 3.1 – 4). To find out the difference between the treatment group and controlled group, Fisher’s exact test was used. The result was presented in [Table 3](#).

Weak category of integrin β3 expression in control group was 17 (94.44%), and the intermediate category was 1 (5.56%). In the treatment group, weak category expression was 4 (22.22%), strong category was 8 (44.45%) and very strong category was 6 (33.33%). By using Fisher’s exact test, χ²=22.91 and p = 0.001 were obtained. This showed that there was a significant difference of integrin β3 expression between treatment group and control group (p<0,05). The addition of Letrozole in antagonist protocol cause an increase in the integrin β3 expression with strong category and very strong category than controlled group.

[Figure 1-4](#) showed the result of integrin β3 immunohistochemical examination in mice’s luminal and glandular epithelium. Color intensity of integrin β3, in endometrium epithelium was showed in brown. The gradation of color was declared as 0 = not colored/negative, 1 = weak intensity, 2 = medium intensity, 3 = strong intensity.

The immunohistochemical examination of mice’s uterine tissue found that E-cadherin mean expression (H-score) in the luminal and glandular epithelium in the treatment group was 3.73 ± 0.28, meanwhile in control group was 1.16 ± 0.29. Mean H-score in the treatment group was significantly higher than in control group. Mann-Whitney test showed p = 0.001 ([Table 2](#)).

E-cadherin H-scores were also classified in the following category: (a) weak expression (H-score < 1.1), (b) intermediate expression (H-score = 1.1 – 2), (c) strong expression (H-score = 2.1 – 3), and (d) very strong expression (H-score = 3.1 – 4). To find out the difference of E-cadherin expression between treatment group and control
### Table 1  Characteristics Distribution of age, beginning body weight, and final body weight mean in treatment group and control group

| Variables                      | Treatment group (n=18) | Control group (n=18) | p   |
|--------------------------------|------------------------|----------------------|-----|
|                                | Mean | SD   | Mean | SD |     |
| Age (days)                     | 62.06 | 6.38 | 63.00 | 6.77 | 0.670 |
| Pre-intervention body weight (grams) | 20.21 | 1.25 | 20.48 | 1.28 | 0.519 |
| Post-intervention body weight (grams) | 22.06 | 1.21 | 22.22 | 1.40 | 0.704 |

### Table 2  Mean of integrin β3, E-cadherin H-score and LIF level in treatment and control group

| Mean                      | Treatment group (n=18) | Control group (n=18) | p   |
|---------------------------|------------------------|----------------------|-----|
| Integrin β3 H-score       | 2.71 | 0.61 | 1.04 | 0.08 | 0.001 |
| E-cadherin H-score        | 3.73 | 0.28 | 1.16 | 0.29 | 0.001 |
| LIF (ng/ml)               | 1.78 | 0.13 | 1.66 | 0.17 | 0.042 |

### Table 3  The difference of integrin β3 expression between treatment and control group

| Group                  | Weak and intermediate | Strong and Very Strong | χ²   | p   |
|------------------------|-----------------------|------------------------|------|-----|
| Treatment              | 4                     | 14                     | 22.91 | 0.001 |
| Controlled             | 18                    | 0                      |      |     |

### Table 4  The difference of E-cadherin expression between treatment group and control group

| Group                  | Weak and Intermediate | Strong and Very Strong | χ²   | p   |
|------------------------|-----------------------|------------------------|------|-----|
| Treatment              | 0                     | 18                     | 36.00 | 0.001 |
| Control                | 18                    | 0                      |      |     |

**Figure 1** Integrin β3 expression in treatment group. H-score = 3.78. Very strong expression. 78% of epithelial cell colored with strong intensity, 22% of epithelial cell colored with moderate intensity. 400 x magnification

**Figure 2** Integrin β3 expression in treatment group. H-score = 2.45. Strong expression. 2.77% of epithelial cell colored with 0 intensity, 67.74 of epithelial cell colored with weak intensity, 11.06% of epithelial cell colored with moderate intensity, and 18.43% of epithelial cell colored with strong intensity. 400 x magnification
group, Fisher’s exact test was used. The analysis result is presented in Table 4.

Table 4 showed that from 18 samples, all of the E-cadherin expression in control group was a weak and intermediate category. Meanwhile, on treatment group, all of the E-cadherin expression was strong and very strong category. Using Fisher’s exact test, $\chi^2 = 36.00$ and $p = 0.001$ were obtained. This showed that there was a significant difference of E-cadherin expression between treatment group and control group ($p<0.05$). The addition of Letrozole on antagonist protocol obtain significantly higher E-cadherin expression with strong category and very strong category than in control group.

Below (Figure 5-8) are several E-cadherin immunostaining results that represent each staining classification.

Our study also found that in the Letrozole + GnRH antagonist-treated mice, the uterine LIF level during a window of implantation was significantly higher (1.78 ± 0.13 vs. 1.66 ± 0.17 ng/ml, $p<0.05$) than in GnRH antagonist-treated mice alone. Results showed in Table 2.

DISCUSSION

Endometrial receptivity is an important factor for embryo implantation. In menstrual cycle, estrogen and progesterone play an important role
in endometrial thickening and vascular development. In the later stage of menstrual cycle, gonad hormones, particularly progesterone, induce development of secretory gland in endometrial tissue which marks the secretory phase of the uterine cycle. Both hormones also modify uterine receptivity in preparation for blastocyst implantation. The three important marker of uterine receptivity is Integrin β3, E-cadherin, and LIF. Several studies revealed that the expression of this molecule appears in a cyclical manner, in accordance with uterine cycle which made it suitable for evaluating uterine receptivity. Because of its expression pattern, it strongly indicates that the expression is influenced by gonad hormones (estrogen and progesterone). The aim of Letrozole + antagonist and antagonist protocol (alone) of ovarian stimulation conducted in our study were to know the expression of integrin β3, E-cadherin in endometrial cell and uterine LIF concentration during window of implantation of mice.

The mean integrin β3 H-SCORE of treatment and control group were 2.71±0.61 and 1.04±0.08 respectively (p=0.001), which showed that integrin β3 was significantly higher in the treatment group. Weak category of integrin β3 expression in control group was 17 (94.44%) and intermediate category was 1 (5.56%). In treatment group, weak category expression was 4 (22.22%), strong category was 8 (44.45%) and very strong category was 6 (33.33%). By using Fisher’s exact test, \( \chi^2 = 22.91 \) and \( p = 0.001 \) were obtained. This showed that there was a significant difference of integrin β3 expression between treatment group and controlled group \( (p<0.05) \). The addition of letrozole to antagonist protocol cause an increase in the integrin β3 expression with strong category and very strong category significantly than controlled group.

Since immunohistochemistry examination was used to measure integrin β3 in the endometrium, it has become the best endometrium receptivity marker. With the development of reproductive technology, controlled ovary stimulation is conducted more often. At the higher levels than physiological, exogenous hormone could affect endometrium morphology changes, mark abnormal expression, and disturb endometrial receptivity.

Integrin is an important cell adhesion molecule that detects extracellular matrix proteins based on integrin arginine-glycine-aspartic acid sequence, mediate cell adhesion with extracellular matrix, stimulate angiogenesis factors production, mediate intra and extracellular signaling transduction, and increase blood supply to endometrium, which affects higher endometrial receptivity.

Integrin beta-3 is secreted in endometrium gland epithelium and luminal epithelium on the 19th day of menstruation cycle, and it plays a role in blastocyst adhesion in implantation. In infertile woman, integrin beta-3 is not expressed or weakly expressed during the implantation window. Miller et al. (2012) found that the number of clinical pregnancy and labor were higher on woman with normal expression of integrin αvβ3, rather than group with negative integrin [ 20/50 (40%) versus 4/29

![Figure 7](image1.png) E-cadherin expression in the control group. H-score = 1.64. Moderate expression. 50% of epithelial cell colored with 0 intensity, 36.49% of epithelial cell colored with weak, 12.16% of epithelial cell colored with moderate intensity, and 1.35% with strong intensity. 400 x magnification

![Figure 8](image2.png) E-cadherin expression in the control group. H-score = 1. Weak expression. 0% of the epithelial cell was colored. 400 x magnification.
(13.8%) respectively; p = 0.02 and 19/50 (38%) versus 2/29 (7%) respectively; p < 0.01. From 18 women who got Letrozole at the beginning of the program, 11 of them was pregnant (61.1%; P < 0.001), compared to negative integrin patient who didn’t get Letrozole. Researchers conclude that the absence of integrin αβ3 expression was related to bad IVF prognosis, which may be improved by the addition of Letrozole.  

On the contrary, research from He et al. (2016) found that integrin αβ3 expression was not disturbed when implantation window at supra-physiological estrogen condition. In this research, estradiol levels in ovary stimulation group were significantly higher than in controlled fertile woman group with natural cycle (6210.81 + 383 vs 190.28 + 18); p <0.05. This research was limited because only used 11 samples in each group.

Estrogen has an important role in integrin expression. The expression of HOXA10 (transcription factor) was regulated by estrogen and progesterone. Then HOXA10 mediate the series of steroid effects by the activation or repression of downstream genes. Integrin β3 is a HOXA10 transcription regulation target. In ovary stimulation condition with antagonist protocol (supraphysiological estrogen), integrin expression was depressed, allegedly caused by the disturbace of estrogen action from down-regulation of Era.  

Immunohistochemical examination of mice’s uterus tissue in this study found that E-cadherin mean expression (H-score) in luminal and glandular epithelium on treatment group was 3.73 + 0.28, meanwhile on the controlled group was 1.16 + 0.29. The mean H-score in the treatment group was significantly higher than the controlled group. Mann-Whitney test showed p = 0.001 (Table 2). The addition of Letrozole on antagonist protocol resulted in E-cadherin expression with strong category and very strong category was significantly higher than controlled group.  

Xiong et al. (2011) found that ovary stimulation, especially by GnRH analog, decrease E-cadherin expression and also Hoxa11, Meis1, mice’s endometrium Ctnnb1 on peri-implantation period. Estrogen stimulation leads to depression of the E-cadherin expression and activity. Meanwhile, transcription factor related to EMT, snail and slug, has a significant upregulation. This upregulation is related to the increasing gene transcription, which is activated by Estradiol through Era. Endogenous snail and slug depletion with small interfering RNA (siRNA) will weaken the decrease of E-cadherin effect because of estradiol.

E-cadherin is a transmembrane cell adhesion molecule that is very important in cell behavior, tissue forming, and cancer suppression. E-cadherin also has an important role in bringing up the cell polarity, glandular differentiation, cellular layering, and morphogenesis, maintaining the normal epithelium architecture. In mice, E-cadherin was expressed either in embryo at peri-implantation and uterus cells, or to contact sites between trophectoderm and uterus epithelium during implantation. E-cadherin expression and glycodelin fix the early stage of implantation, including apposition and adhesion.

The presence of E-cadherin regulation on the cell surface allows the control of cell adhesion. The down-regulation of E-cadherin expression is correlated with metastatic potential of cancer cells. Then the tissue architecture disappears, and lead to cell dissociation and dispersion. Intracellular calcium is important for E-cadherin regulation. Progesterone in mid-secretion phase induces calcitonin expression in endometrium epithelium. The increase of intracellular calcium will down-regulate E-cadherin expression. So, E-cadherin has two functions. In preliminary phase, its expression on cell surface is needed for adhesion. Otherwise, E-cadherin will be downregulated, that lead to epithelium cell dissociation and blastocyst invasion.

E-cadherin expression during implantation window in luminal and glandular epithelium infertile patient because of endometriosis and unexplained infertility higher than the healthy control. E-cadherin expression is very low/none during implantation window in healthy control. On the contrary, Marinakis and Nikolaou found that endometrium E-cadherin expression was depressed on women who were difficult to get pregnant. This controversy showed that E-cadherin function on endometrium is still not clear.

This study found that the mean of uterus LIF level in the treatment group was 1.78 + 0.13 ng/ml. This level was significantly higher than mean LIF level in control group, which was 1.66 + 0.17 ng/ml (Mann-Whitney test, p=0.042) (Table 4.5). This showed that the addition of Letrozole to ovarian stimulation antagonist protocol was significantly affected the LIF levels. LIF level of mice’s uterus on peri-implantation period correlated with metastatic potential of cancer cells. LIF synthesis strongly depends on estrogen hormone. Nidatory estrogen, which is the second bump of estrogen levels on the luteal phase in mice’s estrous cycle, made the uterus receptive to blastocyst. The main function of nidatory estrogen is to induct LIF synthesis because a single dose of LIF injection could induce normal implantation and post-implantation development when there is no nidatory estrogen.
The supraphysiological estrogen that produced by multi follicle development in ovary stimulation disturbs estrogen receptor expression. In the stimulated cycle, estrogen receptor was reportedly disappeared, or significantly downregulated on uterus gland.\(^{40,41}\) This condition resembles a functional hypoestrogenic that disturbs estrogen hormone function, especially in inducting LIF synthesis. The administration of high dose estrogen will lead to the decrease of LIF level in the uterus.\(^{42}\)

Many types of research about the effect of ovarian stimulation to LIF proved that LIF expression decrease in the uterus. LIF gene expression on the endometrium, seven days after hCG administration, was downregulated in the cycle which stimulated by urine gonadotropin and agonist GnRH, compared to control.\(^{43}\) Ruan (2006) found that ovarian stimulation, especially with antagonist protocol, inhibits uterus endometrium LIF expression and disturbs endometrium receptivity in mice.\(^{44}\)

LIF is a glycoprotein secreted by the natural killer cell, included to interleukin-6 cytokine family that regulates human reproduction.\(^{45}\) LIF mRNA is secreted during the menstrual cycle, highly expressed especially on late and mid-luteal phase, also in the early pregnancy.\(^{47}\) Aghajanova et al. (2003) showed that the beginning of human embryo development depends on molecular and morphology change of LIF cell and its receptor, and also pinocytosis. Strong and sudden expression on implantation window, which continued by the disappearing of LIF expression and pinocytosis, showed that LIF has an important role in embryo development, implantation, and pregnancy maintenance phase.\(^{48}\)

The addition of Letrozole in antagonist protocol ovary stimulation will decrease estrogen level especially at the beginning of Gonadotropin administration. By lowering estrogen level, it was expected that the disturbances of estrogen receptor would decrease, so that estrogen can act in inducting LIF synthesis in uterus. This research showed that by Letrozole addition, LIF levels were significantly higher than the standard protocol. Because LIF was an endometrial receptivity marker and very important in implantation process, the addition of Letrozole to ovarian stimulation antagonist protocol will fix the endometrium by causing high levels of LIF uterus, so an increase in implantation rate was expected.

To understand the contribution of Letrozole to integrin \(\beta_3\), \(E\)-cadherin dan LIF, path analysis was used. The goodness of fit model showed the role of letrozole on integrin \(\beta_3\), \(E\)-cadherin expressions and LIF level as presented in figure 9.

Letrozole has a high contribution to the changes of \(E\)-cadherin expression (95.6%), followed by 87.3% contribution on integrin \(\beta_3\) expression. Integrin \(\beta_3\) together with \(E\)-cadherin and Letrozole had 31.4% contribution to the increase of LIF.

**CONCLUSION**

Letrozole caused higher expression of Integrin \(\beta_3\), \(E\)-cadherin, and LIF concentration in Mice uterus stimulated by antagonist protocol. Letrozole had the highest contribution to the increase of \(E\)-cadherin. Integrin \(\beta_3\) together with \(E\)-cadherin and Letrozole had 31.4% contribution to the increase of LIF.

The low implantation rate observed in antagonist ovarian stimulation protocol in IVF clinics might results partly from the impaired endometrial receptivity through impairing integrin \(\beta_3\), \(E\)-cadherin expression, and LIF level and might improve by letrozole administration. Further study in this aspect and to decrease estrogen level in ovarian stimulation are needed.

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