International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2020): 7.803

The Effect of Autologous Platelet Rich Plasma (PRP) Administration on Leukemia Inhibitory Factor (LIF) Level, Integrin β3, ER α, and HOXA10 Expression on Endometrial Damage in Wistar Rats

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Abstract: ***Objectives: To determine the effect of PRP on rats' endometrial damage to endometrial receptivity through β3 integrin, LIF, ER α expression and HOXA10 expression and LIF levels in the laboratory. Biomedical unit Animal Lab, Udayana University, Denpasar for the period December 2019 - February 2020. Materials and Methods: An experimental study, the randomized posttest only controlled group design, used female rats of the Wistar/C strain. The data were processed with the SPSS for Windows 17-Version program to test the normality, homogeneity, comparison, and significance of the sample. Results: A total of 40 rats were divided into two groups, 20 as a treatment group with PRP and 20 as control after previously receiving Intra Uterine ethanol. There was no significant difference between the bodyweight of rats from the treatment group and the control group at the start of the study (p = 0.519) and the end of the study (p = 0.704). The mean expression (H-score) of Integrin β3 in the endometrial epithelium of the treatment group (3.68 + 0.49) was higher than the control group (1.54 + 0.52); ER α expression (H-score) in the endometrial epithelium in the treatment group (2.42 + 0.63) was higher than that in the control group (1.50 + 0.04) with p-value <0.001. The mean expression (H-score) of HOXA 10 in the endometrial epithelium in the treatment group (3.07 + 0.66) was higher than that in the control group (1.52 + 0.05) with p <0.001. The mean LIF level in the treatment group (356.79 + 25.43 ng / ml) was higher than the control group (151.12 + 11.59 ng / ml). There was a significant difference in Integrin β3 integrin, LIF, ER αexpression and HOXA10 expression between the treatment and control groups (p <0.05). Conclusion: The addition of APRP to endometrial damage with ethanol causes a significant increase in integrin β3 integrin, LIF, ER αexpression and HOXA10 levels.***

Keywords: β3-integrin, ERα, HOXA10, LIF, APRP

1. Introduction

Endometrial receptivity is one of the essential factors in implantation, both in apposition, adhesion, and invasion, which is directly proportional to the implantation rate (IR), in addition to the quality of the embryo itself. Decreased endometrial receptivity is often associated with endometrial damage due to trauma, infection, or other factors. Endometrial receptivity correlates directly with the implantation rate (IR) in addition to embryo quality. In Indonesia, the average IR of the In Vitro Fertilisation (IVF) program at 36 centers of IVF service is 19.3%.[1,2] About 3% of IVF cycles are postponed due to suboptimal endometrial conditions; it is feared that it can interfere with embryo implantation.Low IR is clinical evidence of impaired endometrial receptivity associated with failure at the apposition, adhesion, and invasion stages in the initial implantation and placentation processes.[3]

Estrogen is the primary trophic hormone for the uterus, which mediates endometrial growth via estrogen receptor alpha (ER α), where its concentration is highest during the proliferative phase of the menstrual cycle and decreases after ovulation in response to increased progesterone.[4,5] Estrogen has a vital role in the development and differentiation of the reproductive system, estrogen will enter the body's cells, but only cells that contain estrogen receptors will respond to it, which will activate the transcription process, which results in control of gene expression. At the molecular level, endometrial damage is thought to be related to the role of adhesion and inflammation molecules, especially ß-3 integrin and leukemia inhibitory factor (LIF). The two molecules above are thought to work optimally in administering autologous platelet-rich plasma (APRP) to the damaged endometrium.

Leukemia inhibitory factor (LIF) is a family of cytokines IL-6, whose expression is regulated by estrogen and progesterone levels. This protein has been shown to play a role in apposition, adhesion, and invasion of the blastocyst implantation process.[6] Expression of LIF in the endometrium increases rapidly at the implantation window, and levels are regulated by estrogen and progesterone levels. While ß3 integrin is a heterodimer transmembrane glycoprotein composed of α and β subunits.[4] Several integrins are found in the epithelial lumen and glandular endometrial tissue.[7] Integrin β3 and its ligands (osteopontin) were detected by immunohistochemical examination on the epithelial surface of the endometrium when it first interacted

with trophoblasts. Based on the location and pattern of expression, β3 integrin is thought to be a receptor for embryo implantation[7] and a good marker of endometrial receptivity.[8,9] Integrin ß3 is found in the luminal epithelium and gland, expressed after day 19 of the menstrual cycle. This protein expressed on the surface of the endometrial luminal epithelium is thought to play a role in the first interaction with the embryonic trophoblast. Integrin is a marker for measuring endometrial receptivity[10] whose expression is regulated by estrogen and progesterone through HOXA10.[11]

One of the best-recognized sequences for signaling the occurrence of implantation in the endometrium. Homeobox A10 (HOXA 10), which is the. HOXA 10 is the GATA family of transcription factors and is usually up-regulated in the endometrium during the implantation window period, and its levels increase dramatically during the mid-secretion phase of the menstrual cycle.[12] Integrin β3, which is an adhesion molecule in the endometrium that is locally responsible for the presence of pinopod, and β3 integrin is directly regulated by HOXA 10.[5] Animal studies have shown that decreased expression of HOXA 10 as a result of anatomical defects in the reproductive system can lead to infertility.[13]

This study aims to determine the effect of PRP on endometrial receptivity through the role of integrin β3 integrin, LIF, ER α expression and HOXA10 levels in endometrial damage. The Declaration of Helsinki (2015) states that this experimental study cannot be applied to humans who have endometrial disorders. Therefore rat endometrium is used as a model in this study, where the rat endometrium is damaged with ethanol. If proven, the results of this study can be used as a reference or basis for researching in humans so that, in the end, it can increase endometrial receptivity in humans.

2. Materials and Methods

This research is an experimental, randomized posttest only controlled group design, using female rats of Wistar / C strain. Observation or maintenance measures, physical examination, vaginal swab examination, and rat endometrial tissue retrieval were carried out at the Biomedical Laboratory of the Animal Lab unit, Udayana University, Denpasar, from December 2019 to February 2020. The inclusion criteria of this study were healthy female Wistar rats, never married, 10-12 weeks old, weighing 230 to 500 grams. While the exclusion criteria were mice with irregular estrous cycles and the drop out criteria, mice were dead or missing during the study.

Experimental Animal Treatment

Wistar / c female rats were treated following the Animal Care and Use Committee (ACUC). Rats were kept in an adaptation of 12 hours light-12 hours of darkness, temperature 25 ± 0.5 ° C, and humidity 50-60%. Was fed ad libitum with standard feed and water. The estrous period was observed daily by looking at vaginal secretions and then doing a swab. Rats with four days of regular estrus, 8-12 weeks of age, 200-230 grams of body weight were used as the study target population. A simple random sampling technique was conducted using a random number table in the Microsoft Excel for Windows program. Furthermore, rats number 1-18 were included in the treatment group by administering PRP therapy after receiving intra-uterine ethanol, and rats number 19-36 were included in the group without PRP therapy after previously receiving Intra Uterine ethanol.

Platelet Rich Plasma Administration

In the treatment group, PRP was given at a dose of 0.25 ml in the uterine 30-G cavity on day three after previously being given ethanol at a dose of 0.5 ml of 95% ethanol on day 0 using a 1ml syringe using a 30-G needle. The PRP was made using the rat's blood which previous anesthetic procedures had carried out. 1.5 ml of blood was taken through retro-orbital plexus puncture, then 160 g centrifuge was performed for 20 minutes so that the blood was divided into three layers: plasma, buffy coat (Platelets and leucocytes), and erythrocytes. The layer above the line dividing the buffy coat and red blood cells is removed and transferred to a new sterile tube. This layer is then subjected to a 400 g centrifuge for 15 minutes to obtain the lowest layer, namely the platelets. To activate PRP has added 0.15 ml of 3% calcium chloride. In the control group, they were giving ethanol at a dose of 0.5 ml of 95% ethanol on day 0 using a 1 ml syringe using a 30-G needle without administering PRP.

Immunohistochemical Examination and ELISA Procedures

Immunohistochemical staining using the Ultravision kit (Thermo Scientific, USA) and anti-mouse E integrin monoclonal primary antibody [M168], ERα [M168], and HOXA and anti-mouse Integrin beta 3 (Abcam, USA). Other materials use materials from Sigma-Aldrich (USA).

The manufacture of histological preparations is divided into three stages, namely the fixation, dehydration, clearing, and embedding stages. Endometrial tissue resulting from hysterectomy of rats, each with a diameter of 8 mm and depth until the myometrium is treated following these steps. The fixation stage means that the endometrial tissue is immersed in 10% phosphate-buffered formalin for 24 hours then trims the part of the tissue to be taken. Furthermore, the tissue was immersed with graded alcohol (dehydration stage) soaked 50%, 70%, 90%, 96%, and 100%, respectively, for 2 hours. Then enter the clearing stage by inserting the tissue into the clearing agent (xylene) for 24 hours until it is transparent. The embedding stage begins with the infiltration process two times for 1 hour each with pure liquid paraffin (histoplast) (temperature 60o C), then the tissue is implanted into liquid paraffin and allowed to form a block takes one day so that it is easily sliced with a microtome. The cutting was done using a rotary microtome (Jung Histocut Leica 820), five micrometers thick and then attached to the object-glass, then incubated at 60o C for 2 hours. Especially for slides painted with immunohistochemistry, use a glass object coated with adhesion such as Poly-Lysine or the like.

Before painting, the slide went through deparaffinization and rehydration processes, including immersion in xylene solution 2 x 5 minutes, 100% ethanol for 2 minutes, 96%

ethanol 2 x 2 minutes, 70% ethanol for 2 minutes, and PBS for 2 minutes.

Furthermore, the retrieval antigen was carried out, in which the slide was immersed in TE solution then heated in the microwave for 15 minutes using 700 Watt power, cooled then washed with PBS 2 x 5 minutes. Furthermore, endogenous peroxidase blocking was performed in a plastic box with 3% H2O2 for 30 minutes. Then washed with PBS 1X for 5 minutes each two times. Dropped 5% FBS 100 µL for two hours at room temperature, and the box is closed. Followed by washing PBS 1X for 5 minutes each two times, then dropping the primary antibody Integrin beta 3 100 µL (1: 250 using FBS 1% as solvent) for one night in a closed box. After one night, it was washed with PBS 1X for 5 minutes in a glass jar twice while shaking it. Followed by a biotinylated link which is dripped on the entire surface of the tissue, then incubated for 30 minutes in a closed box, then washed in PBS 1X for 5 minutes in a glass jar twice each while shaking. Next, put streptavidin peroxidase, then let stand for 30 minutes in a closed box, washed again in a glass jar using PBS 1X four times each for 3 minutes while shaking. Dropped DAB until brown, then washed with PBS 1X until clean and dried. Dropped with Hematoxylin Gill, let stand for five minutes, then washed with running water. It was soaked in absolute ethanol twice for five minutes each, followed by soaking in xylene twice for five minutes each. After drying, the slides were mounted with xylene-based medium (DPX) and covered with a cover glass.

Observation of the number of expressions of Integrin beta three was carried out using digital analysis methods. A 400-fold magnification using an Olympus CX41 (Japan) microscope, photographed with an Optilab Pro camera (Miconos, Indonesia). Each preparation was photographed three times using the JPEG format using Optilab Viewer Plus 2.2 and Image Raster 3.0 software (Miconos, Indonesia).

Data Analyze

Descriptive statistical tests were carried out to describe the essential characteristics of each group and the frequency distribution of various variables in the form of weight data and PRP administration, data on the results of an immunohistochemical examination of rat endometrial preparations. Data on the results of the Integrin β3 integrin, LIF, ER α expression and HOXA10 concentration of rats endometrium.

Also shown is the proportion of the Integrin β3 integrin, LIF, ER α expression and HOXA10 H-score in the PRP therapy group and without PRP and comparing the mean LIF concentration in the PRP therapy group and the group without PRP.

The data obtained will be processed with the SPSS for Windows 17-Version program, with the following steps:

1) The data normality test of the rats' age, initial and final body weight, the Integrin β3 integrin, LIF, ER α expression and HOXA10 using the Shapiro Wilk test.
2) Data homogeneity test of rat age, initial and final body weight using the Levene's test.
3) Comparison test:
   a) To test the comparability of data on the characteristics of research subjects (age, body weight at the beginning and the end of the study), an independent t-test was used.
   b) To test the data comparison, the H score of the Integrin β3 integrin, ER α expression and HOXA10: the data were not normally distributed, the Mann Whitney test was used.
   c) To test the data comparison of The Integrin β3 integrin, LIF, ER α expression and HOXA10, the Chi-Square test was used.
4) The significance test used a p-value <0.05.
5) Path analysis was used to determine the simultaneous contribution of PRP to the integrin β3 and LIF variables.

3. Results and Discussion

This study is an experimental study with the randomized posttest only controlled group design. The research was conducted on 40 rats divided into two groups, namely 20 as the treatment group and 20 as the control. This research was conducted at the integrated Biomedical Laboratory of the Faculty of Medicine, University of Udayana Denpasar.

Mice with a regular 4-day estrous cycle, aged 8-12 weeks, with a 200-230 grams body weight were used as the study target population. The affordable population found in the Pharmacology Laboratory during the study period amounted to 47 mice. The samples were determined through a simple random sampling technique using random number tables in Microsoft Excel for Windows. Furthermore, rats in column A numbered 1-20 were entered into the treatment group with PRP, and rats in column B were numbered 21-40 were entered into the control group without PRP administration.

In this study, the mean age of rats when the study started in the treatment group was 62.06 + 6.77 days, while in the control group, it was 63 + 6.38 days (Table 1). There was no significant difference in the mean age between the rats in the treatment group and the control group at the start of the study. The t-test obtained p = 0.570.

Table 1: Demographic characteristics of the treatment and control groups

Characteristics	Treatment Group (N=20)		Control Group (N=20)		p
	Mean	SD	Mean	SD	
Age (days)	62,06	6,77	63	6,38	0,570*
Initial BW (gram)	210,00	11,50	212,30	12,28	0,519
Final BW (gram)	225,10	10,50	232,20	10,40	0,704

*Tes Mann-Whitney

The mean body weight of rats from the treatment group when the study started was 210.00 + 11.50 grams, while the mean bodyweight of the control group was 212.30 + 12.28 grams. There was no significant difference between the body weight of rats from the treatment and control groups at the start of the study (p = 0.519). At the end of the study, the mean weight of the treatment group was 225.10 + 10.50 grams, while the mean bodyweight of the control group was 232.20 + 10.40 grams. Similar to the beginning of the study, there was also no significant difference between the mean

body weight of rats from the treatment group and the control group at the end of the study. The t-test got p = 0.704.

In this study, an examination of the LIF levels in the endometrium of rats from both study groups was examined using the ELISA technique. Based on Table 2, the mean LIF level in the treatment group was 356.79 + 25.43 ng / ml endometrial tissue. This level was significantly higher than the mean LIF level in the control group, 151.12 + 11.59 ng/ml (p = 0.000). This indicated that the addition of Autologous PRP to endometrial damage by ethanol significantly caused higher LIF levels in rats' endometrium than those without Autologous PRP (p <0.05).

Table 2: Distribution of average LIF levels in the treatment and control groups

Mean	Treatment Group (n = 20)		Control Group (n = 20)		U	*P*
	Mean	SD	Mean	SD		
LIF level (ng/ml)	356,79	25,43	151,12	11,59	95	0,000*

Based on immunohistochemical examination of rat endometrial tissue, the mean expression (H-score) of Integrin β3 in the endometrial epithelium in the treatment group was 3.68 + 0.49 control group the mean H-score of Integrin β3 was 1.54 + 0.52. The mean ER α expression (H-score) in the endometrial epithelium in the treatment group was 2.42 + 0.63, while in the control group, the mean ER α H-score was 1.50 + 0.04; H-score of HOXA 10 in the endometrial epithelium in the treatment group was 3.07 + 0.66, while in the control group the mean H-score for HOXA 10 was 1.52 + 0.05. The mean H-score in the treatment group was significantly higher than in the control group. The Mann-Whitney test obtained p <0.001 (Table 3). The H-score was divided as follows: (a) weak expression (Hscore<1,1), (b) medium expression (Hscore = 1,1 - 2), (c) strong expression (Hscore = 2, 1- 3), and (d) the expression is very strong (Hscore = 3.1 - 4).

Table 3: Distribution of mean LIF levels and H-score of integrin β3, ERα, HOXA10respectively treatment and control groups

Mean	Treatment group (n=20)		Control group (n=20)		*P*
	Mean	SD	Mean	SD	
LIF (ng/mL)	1,77	0,05	1,66	0,17	0,032*
H-score integrin β3	3,68	0,49	1,54	0,52	<0,001*
H-score ERα	2,42	0,63	1,50	0,04	<0,001*
H-score HOXA 10	3,07	0,06	1,52	0,05	<0,001*

*Tes Mann-Whitney

In the treatment group, all 20 mice (100%) had strong-very strong β3 integrin, ER α expression and HOXA10 expression and no mice had weak and moderate β3 integrin expression. In contrast, in the control group, 18 mice (90%) had weak and moderate β3 integrin, ER α expression and HOXA10 expression, and only two mice (10%) had very strong-strong expression. The Chi-Square test found that the value of $\chi 2$ = 28.8 and the value of p <0.001. This indicates a significant difference in β3 integrin expression between the treatment and control groups (p <0.05), where the addition of autologous PRP to endometrial damage due to ethanol can significantly increase the strong expression of β3 integrin, ER α expression and HOXA10 in rat endometrial epithelium.

Table 4: Differences in the Integrin β3, ER α expression and HOXA10expression between the treatment group and the control group

Group		Weak Moderate	Strong Very Strong	$\chi 2$	*p*
Integrin β3 expression					
Treatment		0	20	28,8	<0,001
Control		18	2		
ER α expression					
Treatment		0	20	28,8	<0,001
Control		18	2		
HOXA 10 expression					
Treatment		0	20	28,8	<0,001
Control		18	2		

Figures 1 and 2 show the results of the β3 integrin immunohistochemical examination in the rat endometrial epithelium. The intensity of the β3 integrin stain is shown in brown on the endometrial epithelium. The gradations of the coloring intensity are expressed as 0 = unstained / negative, 1 = weak intensity, 2 = moderate intensity and 3 = strong intensity. H-score is calculated by formula H-score = $\Sigma P_i(i + 1)$ where Pi is the proportion of cells that are colored in each intensity category, i is the intensity of the staining

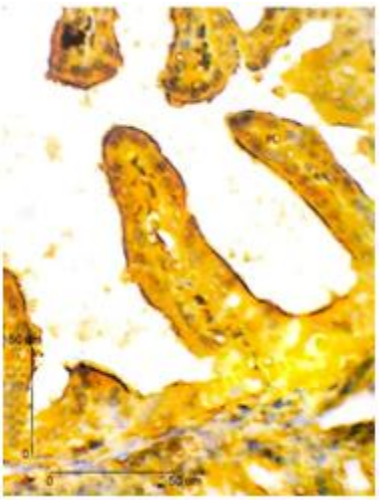

Figure 1: Integrin expression β3 of the treatment group (sample no 9). H-score = 3.89. Very strong expression. Of the 161 epithelial cells, 144 epithelial cells were stained with strong intensity, 17 epithelial cells were stained with moderate intensity. Magnification of 400 X. Note: intensity 0 = absent, 1 = weak, 2 = moderate, 3 = strong

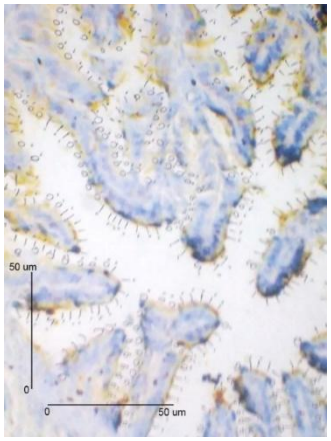

Figure 2: Expression of β3 integrin control group (sample no. 14). H-score = 1.21. Weak expression. From 392 epithelial cells, 0 epithelial cells stained with strong intensity, 1 epithelial cell stained with moderate intensity, 93 epithelial cells stained with weak intensity, 298 epithelial cells were not stained. Magnification 400 X. Note: intensity 0 = absent, 1 = weak, 2 = moderate, 3 = strong

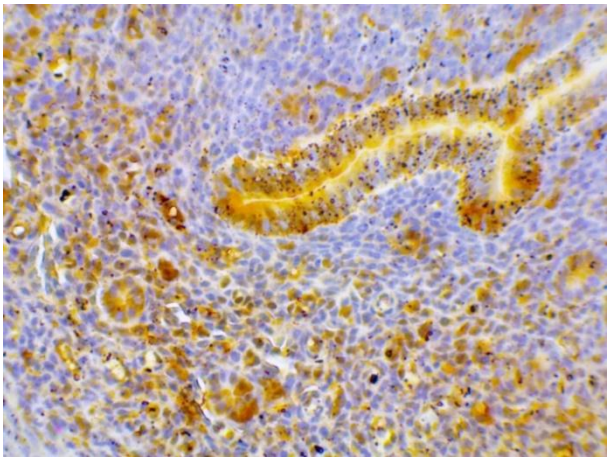

Figure 3: HOXA expression of 10 treatment groups (sample no 7). H-score = 3.41. Strong expression. Of the 314 epithelial cells, 176 epithelial cells were stained with strong intensity, 103 epithelial cells were stained with moderate intensity. Magnification of 400 X. Note: intensity 0 = absent, 1 = weak, 2 = moderate, 3 = strong

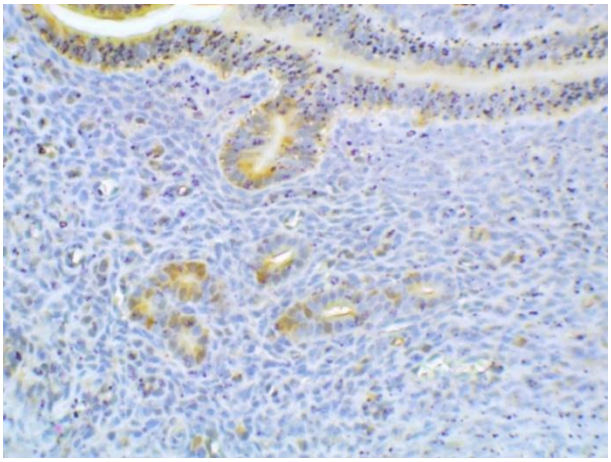

Figure 4: HOXA expression of 10 control groups (sample no 5). H-score = 1.32. Weak expression. Of the 288 epithelial cells, one epithelial cell was stained with strong intensity, 23 epithelial cells were stained with moderate intensity, 43 epithelial cells were stained with weak intensity, 221 epithelial cells were not stained. Magnification of 400 X. Note: intensity 0 = absent, 1 = weak, 2 = moderate, 3 = strong

Figure five and six shows the results of the immunohistochemical ER α on the endometrial epithelium of Wistar rats. The intensity of the ER α stain is indicated by brown coloration of the endometrial epithelium. The gradations of the intensity of the coloring are expressed as 0 = unstained / negative, 1 = weak intensity, 2 = moderate intensity and 3 = strong intensity. H-score is calculated by the formula H-score = ΣPi (i + 1) where Pi is the proportion of cells that are colored in each intensity category, i is the intensity of the staining (Cassals, 2012).

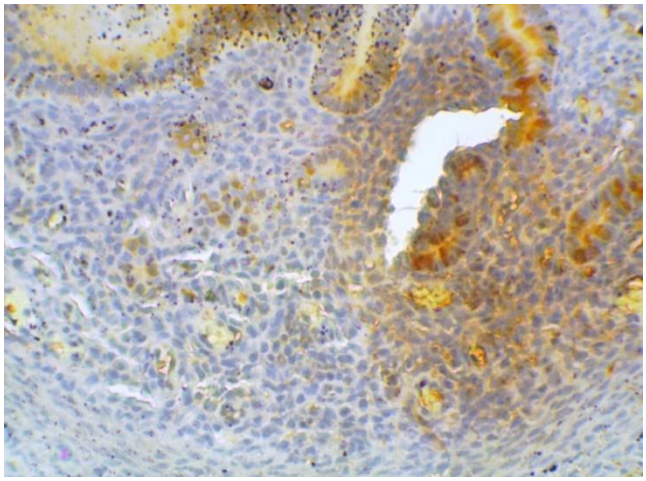

Figure 5: Expression of ER α treatment group (sample no.13). H-score = 2.20. Strong expression. Of the 412 endometrial cells, 58 epithelial cells were stained with strong intensity, 95 epithelial cells were stained with moderate intensity and 132 epithelial cells were stained with weak intensity. Magnification of 400 X. Note: intensity 0 = absent, 1 = weak, 2 = moderate, 3 = strong

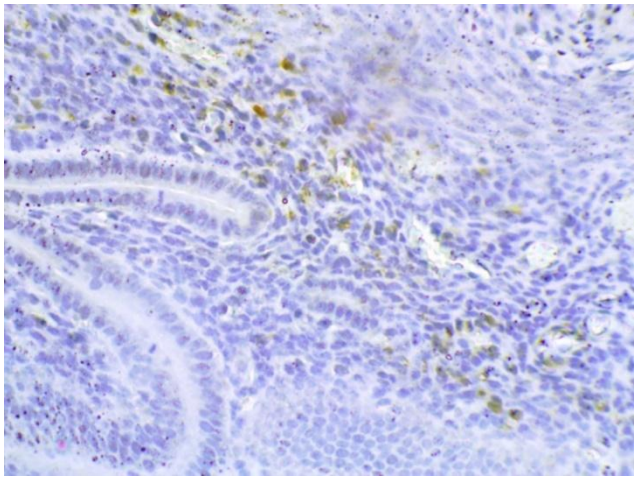

Figure 6: Expression of ER α control group (sample no.15). H-score = 1.25. Medium weak expression. Of the 302 epithelial cells, 2 epithelial cells were stained with strong intensity, 13 epithelial cells were stained with moderate intensity, 43 epithelial cells were stained with weak intensity, 298 epithelium was not stained. Magnification of 400 X. Note: intensity 0 = absent, 1 = weak, 2 = moderate, 3 = strong

4. Discussion

The mean body weight of rats from the treatment group and the control group increased from the start of the study to the end of the study, although there was no significant difference. Rat body weight is mainly influenced by age, sex, and diet.[14] Growth rate can reflect the health of rats in general, where the growth rate itself is influenced by several factors such as cage density, food, and water acidity.[13] The increase in mean body weight in both groups in this study from the time the study started to the end of the study reflected the good health condition of the study subjects.

Integrin β3 is a marker of endometrial receptivity. Integrin is a cell adhesion molecule that plays a role in detecting extracellular matrix proteins based on the arginine-glycine-aspartic acid integrin sequence, mediating cell adhesion to the extracellular matrix, stimulating the production of several angiogenesis factors through various signals, mediating intra and extracellular transduction, and increasing blood supply to the endometrium. increases endometrial receptivity.[15,16]In this study, the expression of β3 integrin was examined in the endometrium of rats from both study groups using immunohistochemical techniques. Based on immunohistochemical examination of rat endometrial tissue, the mean expression (H-score) of Integrin β3 in the endometrial epithelium in the treatment group was significantly higher than in the control group. The Mann-Whitney test found p <0.001. This shows that the addition of autologous PRP to endometrial damage because ethanol can significantly increase the strong-strong expression of β3 integrin in rat endometrial epithelium. The intensity of β3 integrin stain is shown in brown on the endometrial epithelium. The integrin expression β3 in the treatment group (sample no 9) obtained H-score = 3.89, and the expression was very strong. The control group's β3 integrin expression (sample no. 14) obtained H-score = 1.21, and the expression was weak.

PRP has been applied as a cell culture supplement for stem cell expansion and progenitor cells for tissue engineering and cell therapy applications. Cell cultivation with PRP significantly altered the protein expression profile in cells compared to FCS, suggesting that the effect of PRP on cell behavior should be thoroughly investigated.[17]Therefore, the measurement of β3 integrin expression has been implemented to predict success in the IVF program.[18,19] Research by Miller et al. (2012) found significantly higher clinical pregnancy and delivery rates in women with normal αvβ3 integrin expression compared to the group without αvβ3 integrin expression (pregnancy 20/50 (40%) versus 4/29 (13.8%); p = 0.02 ; deliveries 19/50 (38%) versus 2/29 (7%); p <0.01).[20]

In this study, an examination of the LIF levels in the endometrium of rats from both study groups was examined using the ELISA technique. Based on Table 4, the mean LIF level in the treatment group was 356.79 + 25.43 ng / ml endometrial tissue. This level was significantly higher than the mean LIF level in the control group, 151.12 + 11.59 ng/ml (p = 0.000). LIF is a glycoprotein secreted by natural killer cells. LIF belongs to the cytokine family interleukin 6, which regulates human reproduction.[10] LIF mRNA is expressed throughout the menstrual cycle, especially in the late and mid-phase of secretion and early pregnancy. Based on research by Aghajanova et al. (2003), the molecular, morphological changes of LIF and its receptors also play a role in implantation, human embryo development, and the phase of maintaining pregnancy. The LIF level obtained in uterine rinses is proven to predict the success of implantation.[18,21]Expression of uterine biopsy inflammatory biomarkers obtained from a mare with endometritis induced by intrauterine PRP addition showed that mRNA expression of the interleukins IL-1β, IL-6, and IL-8 was significantly downregulated (P <0.05) when compared. A mare who received PRP.[22] IL1-β is a pro-inflammatory cytokine known to increase in the mid-phase of human endometrial secretion, essential for embryo implantation (Laird et al., 2006). PRP can effectively decrease the expression of pro-inflammatory factor genes IL-1β, I-L8, COX2, and iNOS after stimulation by LPS, possibly its use in regenerative therapy in endometritis in vivo.[23]

HOXA 10 plays an essential role in decidualization in humans by regulating the expression of cell cycle inhibitors P57, IL-11, and IL-15 during steroid hormone-mediated decidualization of human endometrial stromal cells in vitro.[24]HOXA 10 affects several genes required for normal decidua development. In humans, HOXA 10 is highly expressed in endometrial cells during the mid-secretory phase of the menstrual cycle, during which the stroma initiates decidual differentiation. This shows the vital role of HOXA 10 during decidualization.[25] HOXA 10 also regulates the expression of a decidualization marker, namely insulin-like growth factor-binding protein (IGFBP) -1.

In this study, HOXA 10 expression was classified into weak, moderate, strong, and very strong for each treatment and control group. From this study, the statistical test results showed that there was a significant difference in the expression of HOXA 10 between the control and treatment groups with a P-value <0.001. From these results, it appears that a higher expression of HOXA 10 was found in the treatment group. With the Mann-Whitney test, it was found that the administration of PRP therapy was associated with improvements in HOXA 10 expression. The study by Hang et al. examined how PRP administration's effect on the endometrium of damaged mice showed results similar to this study. Based on the ELISA examination, it was found that there was a significant difference in HOXA 10 expression between the group of mice receiving PRP therapy compared to the control group with a p-value <0.001. This shows that the expression of HOXA 10 was higher in the group that received PRP therapy.[26]

From this study, statistical tests showed significant differences in the expression of ERα between the treatment and control groups with a p-value <0.001. From these results, it appears that a higher ERα expression was obtained in the treated group. With the Mann-Whitney test, it was found that PRP was associated with improvements in ERα expression.One of the genes whose expression is increased is ERα. ERα expression increased nine times in the group that received PRP therapy with a p-value <0.001. Based on research conducted by Fuentes et al., that ER α is associated with endometrial receptivity. ERα increases during the

proliferative phase in response to estrogen and is downregulated during the implantation period in response to progesterone.[4] Loss of ERα at implantation has been reported in most mammal species. The decrease in ERα coincides with endometrial gene expression in the mid-luteal phase and is a critical event in the formation of endometrial receptivity. The loss of ERα at implantation can disrupt the expression pattern of proteins that regulate endometrial receptivity.[5,23]

The first time PRP was used in infertility, the main idea of using PRP in IVF was based on its ability to increase endometrial growth and tissue healing ability in women with thin endometrium.[37] A study to evaluate the effectiveness of PRP in the treatment of infertile women with a thin (≤ 7 mm) endometrium found successful endometrial expansion, and pregnancy was observed in all patients after PRP infusion. Intrauterine infusion of PRP is a new method for thin endometrium with inadequate response.[27] Intrauterine administration of PRP increases pregnancy and birth rates, especially in patients with poor endometrial growth.[28]

Another study using an in vitro bovine endometritis model showed that PRP-treated cells had an increased proliferation rate and higher expression of some of the genes involved in implantation.[23] The exact molecular basis of PRP action in the implantation process has not been determined, but several possible mechanisms have been proposed as follows: (1) activated PRP promotes migration of human primary endometrial epithelial cells, endometrial stromal fibroblasts, endometrial mesenchymal stem cells (MSC), and bone marrow-derived MSCs, (2) through its regulatory action on proliferation, apoptosis, inflammation, cell adhesion, chemotaxis, and immune response during blastocyst implantation (3) enhances cell regeneration, proliferation, and vascularization by several growth factors including VEGF, TGF -b, PDGF, IGF1, EGF, HGF, (4) cell migration via chemo, differentiation of trans mesenchyme to the epithelium and perhaps most importantly inflammation and (5) stimulatory effects on the expression of several pro-inflammatory cytokines (IL1A, IL1B, IL1R2), chemokines (CCL5, CCL7, CXCL13) and matrix metalloproteins (MMP3, MMP7, MMP26).[27]

Integrin β3 integrin, LIF,ER α expression and HOXA10 are significant markers of receptivity in the process of trophoblast apposition, adhesion, and invasion, so the expression of these three parameters during the implantation window is essential to study because the ovarian stimulation protocol harms its expression.[29]

5. Conclusion

Based on the results of the study and discussion, it can be concluded that there is a difference in the effect of autologous PRP on rats' endometrial damage where the expression of the Integrin β3 integrin, LIF, ER α expression and HOXA10 of the rat endometrial epithelium is higher than that without autologous PRP administration. The integrin β3 integrin, LIF, ER α expression and HOXA10 levels in this study proved that giving autologous PRP to endometrial damage with ethanol improved endometrial receptivity.

6. Acknowledgment

We would like to thank the director and the head of the Obstetrics and Gynecologic Department Faculty of Medicine, Udayana University and Sanglah Hospital, Bali, Indonesia, for supporting this study.

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