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RESEARCH ARTICLE

EFFECT OF HYPOXIC SECRETOME MESENCHYMAL STEM CELLS ON TGF-β EXPRESSION, ESTROGEN HORMONE LEVELS, AND NUMBER OF FOLLICLES (IN-VIVO EXPERIMENTAL STUDY ON FEMALE WISTAR RATS POLYCYSTIC OVARY SYNDROME-LIKE MODELS)

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Abstract

PCOS develops when the ovaries are stimulated to produce more androgen hormones so that the development of follicle count is disrupted. Elevated insulin levels contribute directly to abnormalities in the pituitary hypothalamus of the ovaries which have a direct impact on the occurrence of PCOS by hyperinsulinemia mechanism that increases the frequency of GnRH (Gonadotropin Releasing Hormone) secretion, LH (Luteinizing Hormone) production exceeds FSH, increased ovarian androgen production, decreased follicle count maturation, and increased TGF-β (transforming growth factor β) expression. This study aimed to determine the effect of SH-MSCs administration on TGF-β expression, estrogen hormone levels, and follicle count in female Wistar strain rats with PCOS-like. This study is an in vivo experimental study using a post-test-only control group design. The subjects of this study were female Wistar strain rats divided into 4 treatments consisting of 1 group of healthy mice, 2 negative control groups, 3 treatment groups 1, and treatment group 2 with each different dose. Then the data was analyzed using the One Way Annova Test to determine the influence of each group.One Way ANOVA test showed the results of TGF-β expression ($p = 0.000$), then the results of estrogen hormone levels ($p = 0.000$), then the Kruskall Wallis test showed the number of cystic follicles ($p = 0.045$) in all groups there was a significant difference ($p < 0.05$) except cystic follicle.There is an effect of SH-MSCs secretome administration on decreasing TGF-β expression and caustic follicle count. There is an effect of SH-MSC administration on increasing estrogen hormone levels and corpus luteum count.

Introduction:-

Polycystic Ovary Syndrome (PCOS) is an endocrine disorder characterized by irregular menstruation, hyperandrogenism, and polycystic ovaries.[1] PCOS develops when the ovaries are stimulated to produce more androgen hormones so that the development of the number of follicles is disrupted.

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An increase in insulin levels contributes directly to abnormalities in the hypothalamus, pituitary, ovaries, which has a direct impact on the occurrence of PCOS with the mechanism of hyperinsulinemia, which increases the frequency of GnRH secretion (Gonadotropin Releasing Hormone), LH production (Luteinizing Hormone) excess FSH, increased ovarian androgen production, decreased maturation of the number of follicles, and increased TGF-β expression (transforming growth factor β). These factors contribute to the development of PCOS.[2] Mesenchymal stem cells in hypoxic conditions can increase the secretion of various kinds of solublemolecules or the secretome containing anti-inflammatory cytokines such as Interlekin-10 (IL-10) and regenerative growth factors such as TGF-β.[3] However, there is no standard therapy for PCOS that is effective. Currently, there is still little data regarding research on the influence of the secretome of hypoxic mesenchymal stem cells on TGF-β expression, estrogen hormone levels, and the number of follicles.

Based on diagnostic criteriaNational Institutes of Health, the prevalence of PCOS is 6 to 10% of women of reproductive age.[4] Based on the Rotterdam criteria, the prevalence of PCOS is around 4–6% of women of reproductive age, in Indonesia, around 5.2% and 4.7% of women of reproductive age, and 60% of infertility cases are caused by PCOS.[5] In 2015, of 8,612 women aged 28-33 years, 5.8% had PCOS, and 309 women with PCOS experienced infertility. This data proves that almost 72% of women with PCOS experience infertility.[6] Infertility in PCOS is caused by anovulation, where the development of follicles only reaches a size of 10 mm.[7] PCOS often manifests in the perimenar age. The prevalence of PCOS women with obesity is 42% in Southeast Asia, 30% in Spain, and 38% in Greece. PCOS women in the world with insulin resistance have a prevalence between 25-70%.[8]

Previous research conducted by Han et al stated that culturing mesenchymal stem cells under 2% hypoxic conditions could increase bioactivity and tissue regeneration. Apart from that, it also regulates superoxide dismutase and catalase activity thereby preventing oxidative stress.[9] A study shows that mRNA expressionof Vascular Endothelial Growth Factor (VEGF) in endometriosis model mice that received mesenchymal stem cells was lower than the group that did not. So there is no relationship between VEGF mRNA expression and the number of primary follicles in infertility.[10] Other research says that medium mesenchymal stem cells from adipose tissue secrete proteins in higher amounts compared to proteins produced by umbilical cord tissue. The more mature a cell is, the more protein it produces. However, hypoxic conditions with 2% oxygen have not made a significant difference in protein production related to the need for cell survival.[11] IL-10 is one of the ingredients found in the secretome, IL-10 is a product of monocytes and lymphocytes which has been considered one of the most important anti-inflammatory immune regulatory cytokines, because it effectively downregulates pro-inflammatory cytokines such as IL-1, IL- 6, and TNF-α). Pro- and anti-inflammatory cytokines must be balanced so as not to participate in PCOS. In addition, the IL-10 polymorphic gene can be an important biomarker for PCOS predisposition.[12]

Normally, estrogen hormone levels reach their lowest point when a woman is menstruating. At the same time, LH and FSH levels begin to increase and stimulate the formation of ovarian follicles that contain ova. Mature follicles produce androgen hormones such as testosterone and androstenedione which are released into the blood circulation. Some of these androgen hormones will bind to sexhormone-binding globulin (SHBG) in the blood. Increased androgen production disrupts follicle development so that it cannot produce mature follicles. This results in reduced estrogen produced by the ovaries and no LH surge which triggers ovulation. In addition, insulin resistance causes a state of hyperinsulinemia which leads to a hyperandrogen state, because insulin stimulates androgen secretion and inhibits hepatic SHBG secretion so that androgens are free to bind. TGF-β is also associated with PCOS conditions and insulin resistance, this is because TGF-β is a strong regulator of the proliferation and differentiation of many cell expressions of target genes including insulin.[13]

MSC is an active molecule that can be stable at temperatures of 2-8°C for a long time.[14] MSC-based therapy has shown promise as a treatment option for PCOS due to its self-renewal properties, differentiation potential, and immunomodulatory activity, especially in inflammation-related diseases. Several studies have shown that MSCs have the potential to help restore and improve ovarian function, mediated by paracrine signaling pathways.[15] Research conducted by Chug reported thatBM-hMSC-based therapeutic secretome serves as a novel stem cell therapy for patients with PCOS.[16] Other studies also suggest the use of mesenchymal stem cells and their secretions in reducing inflammatory markers in the treatment of PCOS.[15] Therefore, this study aims to reveal the role of the secretome of hypoxic mesenchymal stem cells on the expression of TGF-b, Estrogen Hormone Levels, and the Number of Follicles in PCOS model mice.

MaterialandMethods:-

This research is experimental researchwith a test control Group Design. The research subjects used 24 female Wistar rats 2-3 months old with a weight between 200-250 grams which meets the inclusion and exclusion criteria, adapted for 3-7 days. This study used 4 treatment groups, a control group of healthy mice (K0), a negative control group of PCOS induced by DHEA 6mg/100gBW for 23 days intraperitoneally which received no treatment (K1), PCOS model mice with DHEA 6mg/100gBW intraperitoneally by administering SH -MSCs dose of 200 μL (K2), and treatment group 2 PCOS model mice with DHEA 6mg/100gBW intraperitoneally by administering SH-MSCs dose of 400 μL (K3), On day 33 female Wistar mice were sampled for examination of TGF- expression β, estrogen hormone levels, and number of follicles.

Research Materials:-

This research uses equipment in the form of cell culture equipment consisting of a Biosafety Cabinet (BSC), micropipette, CO incubator$_2$, dissecting kit, and 75T flask. Hypoxic culture conditions are obtained using a hypoxic chamber. Oxygen meters are used to measure oxygen levels inside a hypoxic chamber. In addition, this study used DHEA induction and administration of SH-MSCs, namely a 3 ml syringe, 23-G syringe, rat cage, 1.5 ml microcentrifuge tube, centrifugation, microscope, microplate reader, and RT-PCR, which were used for expression analysis. TGF-β, estrogen hormone levels, and number of follicles.

Research Equipment

The material for this research consisted of culture material consisting of rat umbilical cord, 0.9% NaCl, PBS, DMEM, FBS, fungizone, and penstrep. Meanwhile, the materials used for the treatment process are water-based gel, 70% alcohol, PBS, Ketamine, and Xylazine. Standard rodent food (Harlan), Sodium citrate 50 mM pH 4.5 (enzyme grade, Fisher), DHEA 6mg/100gBW

Mesenchymal Stem Cell Isolation Procedure from Umbilical Cord

The entire process is done in-house biosafety cabinet class 2, using sterile equipment and working with high sterility techniques.[1]Female mice at 19 days of gestation were anesthetized using vitamin and xylazine.[2] Surgery was carried out on the abdomen, and then the rat fetus along with the umbilical cord (fetoplacental unit) was taken and placed in a sterile container containing 0.9% NaCl.[3]Using tweezers, the fetoplacental unit was placed in a petri dish and washed thoroughly using PBS.[4]The umbilical cord separated from the rat fetus and the blood vessels removed.[5] Complete medium consisting of DMEM, fungizone, penstrep, and FBS) is added slowly until it covers the tissue. (6) The explants were incubated in an incubator at 37°C°C and 5% CO_2. (7) Cells will appear after approximately 14 days from the start of the culture process. (8) The medium was replaced every 3 days by removing half of the medium and replacing it with a new complete medium. (9) Cell maintenance was carried out until the cells reached 80% confluency.

Hypoxia Process

MSCs that had reached 80% confluency were added with a complete medium of up to 10 mL.A flask that has been filled with MSC is then put inside the hypoxic chamber. Nitrogen gas is channeled through the inlet valve and an oxygen meter is placed in the sensor hole to measure the oxygen concentration inside the chamber.

Nitrogen is added until the indicator needle shows a concentration of 5% oxygen. Chamber The containing flask was incubated for 24 hours at 37^0C. After 24 hours, the culture medium was taken and filtered using TFF to obtain SH-MSC which was then mixed with gel according to the dose of K2 and K3.

RNA Extraction and cDNA Synthesis

A 100 mg ovarian sample was then cut into small pieces and put into a tube filled with 50 mL of RNA Iso Plus. On the other hand, there are 10 PBMCs[7] Each cell was transferred to a microtube and given 50 mL of RNA Iso Plus. Ovarian pieces were pounded using a micro pestle and another 50 mL of RNA Iso Plus and kept at room temperature for 5 minutes. Added 20 mL chloroform and vortex until the solution becomes milky white. Incubate at room temperature for 2-3 minutes, and centrifuged at 15,000 rpm for 15 minutes at 40°C until the solution in the tube appears to have 3 layers. The top layer is RNA (liquid phase), the second layer is DNA (semisolid phase) and the bottom layer contains cell debris. The top layer was transferred to a new centrifuge tube and the volume was measured, and a volume of isopropanol equal to the RNA taken from the top layer was added. The Eppendorf tube was shaken until white threads appeared, then centrifuged at 15,000 rpm for 10 minutes at 40°C. The supernatant

was discarded until a white pellet was visible at the bottom of the tube. After drying, 100 mL of 70% ethanol in DEPC (Diethyl pyrocarbonate) solution was added, then turned repeatedly and centrifuged again at 15,000 rpm for 5 minutes at 40°C. The supernatant was discarded and 30-50µm DEPC was added. The mixture was incubated at 55°C for 10 minutes. Next, the total RNA solution was obtained and stored at -80°C. RNA was quantified with Nanodrop. The quantification results were calculated to be 3000ng. Synthesis of cDNA by making mixture A by mixing the calculated RNA sample, 1µL OligoDT, and PCR water until it reaches a volume of 10µL, then incubating for 5 minutes at 70°C. Mixture A was added with mixture B consisting of 5X buffer 4 µL, DEPC-Treated H_2O 5 µL, and ReverTraAce 1 µL. The mixture was incubated at 25°C for 5 minutes, 42°C for 50 minutes, and 85°C for 5 minutes.

Expression of Interleukin 6 and TGF-β Using Real Time-Polymerase Chain Reaction (RT-PCR)

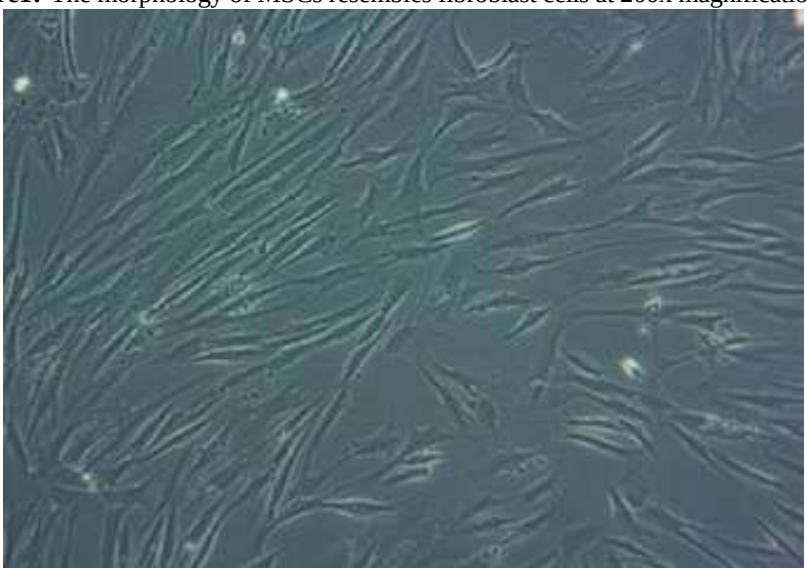
IL 6 and TGF-β expression readings were analyzed using reverse Transcription-Polymerase Chain Reaction (RT-PCR). A mixture of 3 µL cDNA samples, Taq master mix (dNTPs, Taq DNA polymerase, reaction buffer, and $MgCl_2$) as much as 12.5 µL, specific primer for each target gene as much as 0.6 µL for forward and reverse primers and 8.3 µL Nuclease Free Water. PCR products were then analyzed using Illumine qRT-PCR. The GADPH primer sequences used were F: 5'-GCG ACA GTC AAG GCT GAG AATG -3' and R: 5'-TCT CGC TCC TGG AAG ATG GTGA -3'. The IL-6 primer sequences used were F: 5'-TCC TAC CCC AAC TTC CAA TGC TC-3' and R: 5'-TTG GAT GGT CTT GGT CCT TAG CC-3'. The TGF-β primer sequences used were F: 5'-TAC CAT GCC AAC TTC TGT CTG GGA-3' and R: 5'-ATG TTG GAC AAC TGC TCC ACC TTG-3'. The increase in gene expression was analyzed in the ratio of increase to housekeeping genes using the software EcoStudy.

ResultsandDiscussion:-

Validation of MSCs

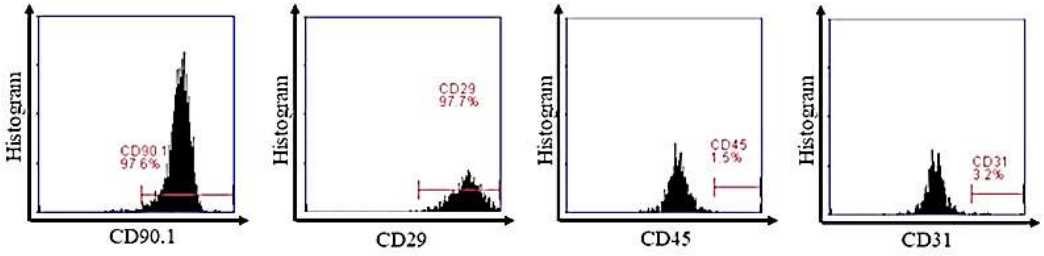
The isolation results were then cultured in T75 flasks with a complete medium. The results of the MSC culture after the 5th passage showed an image of cells attached to the bottom of the flask with morphology spindle-likecells (Figure 1) using microscopic observation.

Figure1:-The morphology of MSCs resembles fibroblast cells at 200x magnification.



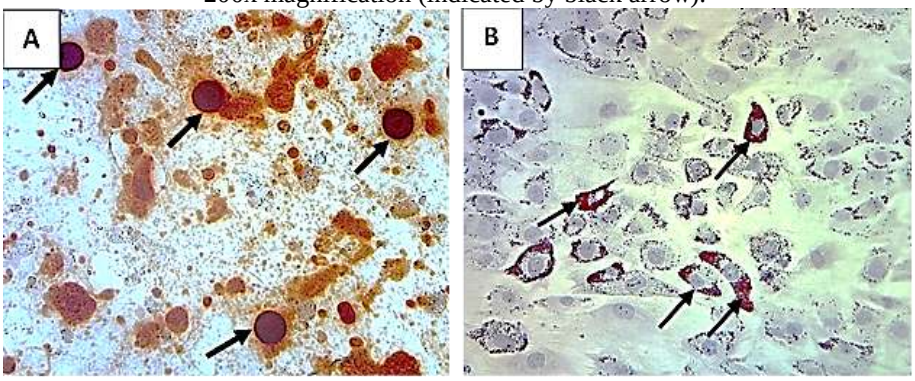
The results of MSC isolation were validated using flowcytometry to show the ability of MSCs to express various surfacemarkers. Quantitative results are the percentage of positive expression of CD 90.1 (97.6%), CD 29 (97.7%), and negative expression of CD 45 (1.5%), and CD 31 (3.2%) (Figure 2).

Figure 2:- Expression of CD90, CD29, CD45, and CD31 on MSCs.



MSC cells were cultured in osteogenic and adipogenic induction medium for 21 days to determine their differentiation ability into osteocytes and adipocytes. The image of osteogenic differentiation of MSCs is shown by arrows on cells containing calcium deposits with stainingAlizarin Red Figure 3. Adipogenic differentiation of MSCs is indicated by arrows on cells containing accumulated lipid droplets with stainingOil Red O (Figure 3). Calcium and fat deposition resulting from the differentiation of MSCs into osteocytes and adipocytes are shown in red in each culture.

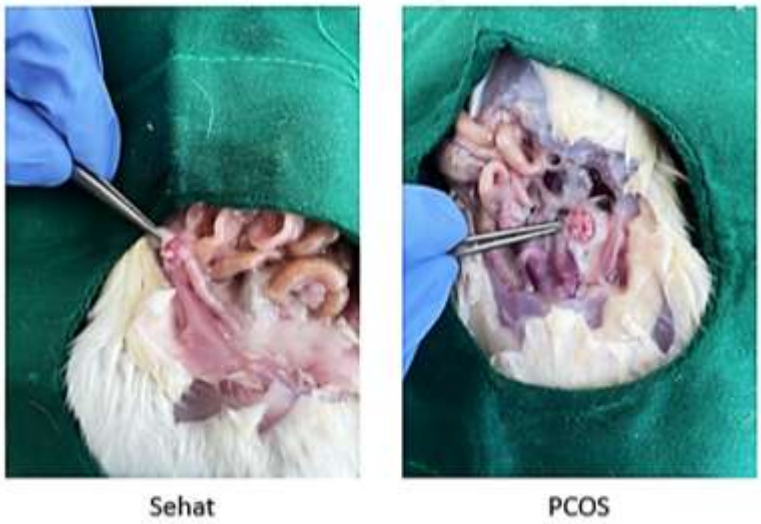
Figure 3:- (A) MSCs can differentiate into osteocytes, (B) adipocytes after staining Alizarin Red and Oil Red O at 200x magnification (indicated by black arrow).



Macroscopic Validation of Animal Tests for PCOS

DHEA induction in mice based on macroscopic observations shows that there are more lumps compared to healthy mice, which are suspected to be cystic follicles, which is a sign of PCOS.

Figure 4:- Macroscopic forms of non-DHEA-induced (Healthy) and DHEA-induced (PCOS) ovaries.



Effect of Administration of SH-Mscs on ExpressionTGF-, Estrogen Hormone Levels, and The Number of Follicles

Study influence of hypoxic mesenchymal stem cell secretome on TGF-b, estrogen hormone levels, and the number of follicles in female Wistar rats, DHEA induction was carried out for 33 days so that the mice became Polycystic Ovary Syndrome-like models. The results of the research are listed in Table1.

Table 1 shows that the lowest mean TGF-β expression was in the K3 treatment group of PCOS model mice given SH-MSCs at a dose of 400 µL, followed by the K2 treatment group, namely PCOS model mice given SH-MSCs at a dose of 200 µL. The highest mean TGF-β expression was in the negative control group, namely PCOS model mice induced by DHEA 6mg/ which received no treatment. All groups of TGF-β expression levels based on the test Shapiro wilk normally distributed with p-value>0.05 and homogeneity test using the Levene test the results are not homogeneous, the p-value is 0.000 (p>0.05), so data analysis uses parametric tests One Way ANOVA. One-way

ANOVA test result showed significant differences between all groups with a p-value of 0.001 (p<0.05), then continued with the Pos Hoc Tukey test to find out which groups are the most influential.

The lowest mean estrogen hormone levels in Table 1 were in the negative control group, namely the PCOS mice group that were not given SH-MSCs, followed by the K2 treatment group, namely PCOS model mice that were given SH-MSCs at a dose of 200 µL. The highest mean estrogen hormone levels were in the K3 treatment, namely PCOS model mice given a dose of 400 µL of SH-MSCs. All groups of estrogen hormone levels based on the Shapiro Wilk test were normally distributed with a p-value>0.05 then homogeneity test using the Lavene test all data was declared homogeneous at 0.136. The data is then analyzed using parametric tests One-Way ANOVA. One Way Anova test result shows significant differences between all groups with a p-value of 0.000 (p<0.05). Then continue using the Pos Hoc LSD test to find out which groups are the most influential.

Table1:- Results of Mean Analysis, Normality Test, Homogeneity Test on TGF-β expression hormone levels and the number of follicles.

Variable	**Group**				
	K1 N=6	**K2 N=6**	**K3 N=6**	**Sig (p)**	
ExpressionTGF-b					
Mean	3.226	0.920	0.784		
Std. deviation	1.598	0.313	0.388		
Shapiro Wilk	0.111*	0.981*	0.059*		
Levene Test				0.000	
One Way Anova				0.000***	
Up to Estrogen					
Mean	77.386	93.300	121.405		
Std. deviation	12.684	27.002	18.008		
Shapiro Wilk	0.968*	0.274*	0.279*		
Low Test				0.136**	
One Way Anova				0.000***	
Cysticfollicle					
Mean	2.333	2.333	1.166		
Std. deviation	1.032	0.816	0.408		
Shapiro Wilk	0.001	0.091*	0.000		
Kruskall Wallis				0.045	
Information: * Normal p>0.05 **Homogeneous p>0.05 ***Significant p<0.05					

Average number of cystic folliclesThe lowest in Table1 is in the K3 treatment group of PCOS model mice with a dose of 400 µL of SH-MSCs, then in the K2 treatment group, namely PCOS model mice with a dose of 200 µL of SH-MSCs and the negative control group, namely PCOS model mice induced by DHEA 6mg/ who did not receive treatment had an average number cystic follicle the tallest. The whole group amount cystic follicles based on the Shapiro Wilk test found two groups did not normally distribute, namely the negative control group with a value of 0.001 and the K3 treatment group with a value of 0.000, p-value>0.05,so data analysis uses non-parametric Kruskal

Wallis test. Kruskal Wallis test results showed significant differences between all groups with a p-value of 0.045 ($p<0.05$). Then proceed using the Mann-Whitney test to find out which group has the most influence.

The differences in TGF-β Expression

The difference in TGF-β expression between the 2 groups was determined by the post-hoc Tukey test as presented in Table 2.

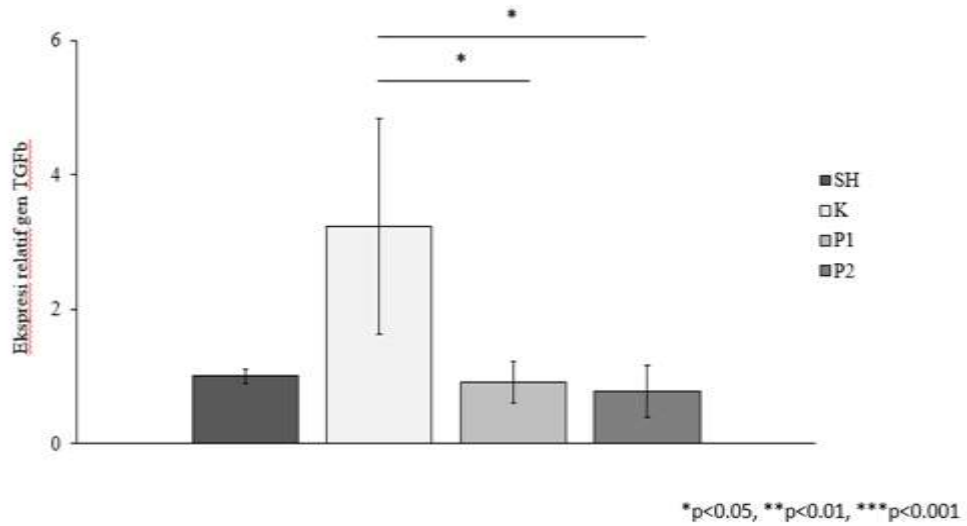
Table 2:- Differences in TGF-β Expression Between the 2 Groups.

Group	p-Value
K1 vs K2	0.001*
K1 vs K3	0.000*
K2 vs K3	0.992

*Tukey's Post Hoc Test with a significant value of $p<0.05$

Tukey test result in Table 2and Figure 5 shows the expression of TGF-β in group (K1) there is a significant difference to group (K2) with a p-value of 0.001 ($p<0.05$) and there is a significant difference in group (K1) to group (K3) with a p-value value 0.000 ($p<0.05$). Meanwhile, there was no significant difference between group (K2) and group (K3) with a p-value of 0.992. Based on the data above, it can be concluded that administration of SH-MSCs at a dose of 200 mL and SH-MSCs at a dose of 400 mL did not significantly influence the reduction in TGF-β expression in female Wistar rats induced by PCOS so the hypothesis statement was accepted.

Figure 5:- Relative Expression of the TGF-β gene.



Differences in Estrogen Hormone Levels

The difference in estrogen hormone levels between the 2 groups was determined by the Pos-Hoc LSD test as presented in Table 3.

Table 3:- Differences in estrogen hormone levels between 2 groups.

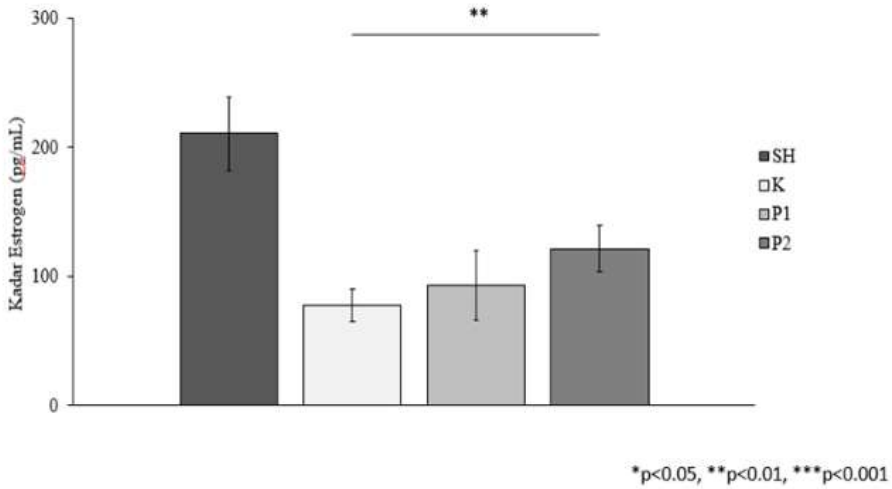
Group	p-Value
K1 vs K2	0.234
K1 vs K3	0.003*
K2 vs K3	0.042*

*LSD Post Hoc Test with a significant value of $p<0.05$

Post Hoc LSD test result in Table 3and Figure 6 shows that there is no significant difference in the levels of the estrogen hormone in group (K1) compared to group (K2) with a p-value of 0.234 ($p<0.05$). However, there is a significant difference in group (K1) to group (K3) with a p-value of 0.003 ($p<0.05$) and group (K2) to (K3) with a p-value of 0.042. However, based on the data above, it can be concluded that administration of SH-MSCs at a dose of 200 mL has no significant effect on increasing the hormone estrogen, and SH-MSCs at a dose of 400 mL

has a significant effect on increasing estrogen levels in female Wistar rats induced by PCOS so that the hypothesis statement is accepted.

Figure 6:- Estrogen hormone levels between groups.



Difference Amount ofCystic Follicles

The amounts of cysticfollicles between the 2 groups is known by the Whitney test as presented in Table 4.

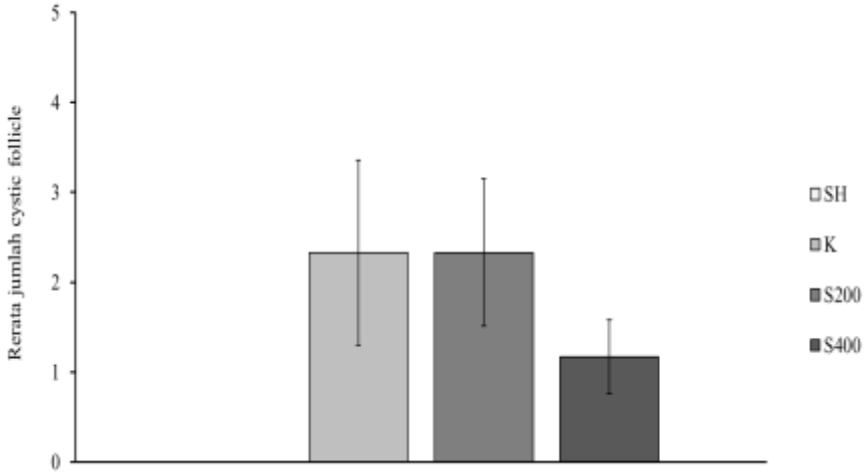
Table 4:- Total differences in cysticfollicles Between 2 Groups.

Group	p-value
K1 vs K2	0.724
K1 vs K3	1.000
K2 vs K3	0.694

*Mann-Whitney test with a significant value of p<0.05

Mann Whitney test result in Table 4and Figure 7 shows that there is no significant difference between the levels of the estrogen hormone in group (K1) compared to group (K2) with a p-value of 0.724 (p<0.05), in group (K1) and group (K3) there is no significant difference in value. p-value 1,000 (p<0.05), then in the group (K2) and group (K3) there was also no significant difference with a p-value of 0.694. Based on the data above, it can be concluded that administering SH-MSCs at a dose of 200 mL and SH-MSCs at a dose of 400 mL does not have a significant effect on reducing the number ofcysticfollicles in Wistar strain female rats induced PCOS so the hypothesis statement was accepted.

Figure 7:- Amount of cystic follicles between group.



Recent advances in stem cell research, especially MSCs have opened new horizons in angiogenesis for the recovery and rescue of ischemic tissue.[17] Angiogenic factors released by MSCs include basic Fibroblast Growth Factor (BFGF), Vascular Endothelial Growth Factor (VEGF), Transforming Growth Factor beta (TGF-β), Platelet-Derived Growth Factor (PDGF), Angiopoietin-1(ANG-1),Placental Growth Factor (PIGF), interleukin 6 (IL-6), and Monocyte Chemoattractant Protein 1 (MCP-1). The MSC secretome positively stimulates angiogenesis in vitro.[18]

MSCs actively participate in angiogenesis through direct differentiation, cell contact interactions with endothelial cell lineages, and the release of pro-angiogenic factors in a paracrine manner. Paracrine factors can increase the blood supply of damaged tissue through the activation and recruitment of stem and progenitor cells. MSCs possess several angiogenesis factors,interleukin-8 (IL-8),Insulin-Like Growth Factor 1 (IGF-1), and Vascular Endothelial Growth Factor (VEGF). These pro-angiogenic factors can form vascular networks and increase the migration of endothelial cell derivatives in vitro. In addition to the secretion of angiogenic factors by MSCs, it has been revealed that various factors present in the secretome can activate angiogenic properties in endothelial cells. For example, peripheral blood angiocrine MSCs were found to be able to stimulate the functional properties of the endothelium by inducing the VEGF-A signaling pathway through several factors such as endothelin-1, IL-8,Platelet-Derived Growth Factor-AA (PDGF-AA), and IGF-2. The useof Mesenchymal Stem Cell Exosomal miRNA content can also control MSC gene expression to improve women's reproductive health.[19]

In this study, there was no significant difference between group (K2) and group (K3) with a p-value of 0.889. Based on the data above, it can be concluded that administration of SH-MSCs at a dose of 200 mL and SH-MSCs at a dose of 400 mL had a significant effect on reducing TGF-β expression in female Wistar rats induced by

PCOS so the hypothesis statement was rejected. This is in line with previous research which stated that administering umbilical stem cells to polycystic ovary model mice could restore TGF-β expression to that of the control group.[20] This shows that there is an effect of SSMPH on reducing TGF-β expression.

The highest levels of the estrogen hormone in Table 2 are in the K3 treatment group of PCOS model mice by giving SH-MSCs at a dose of 400 μL and followed by the K2 treatment group, namely PCOS model mice by giving SH-MSCs at a dose of 200 μL.Polycystic ovary syndrome is a chronic anovulation that causes infertility and is hyperandrogenic, where there is disruption of the feedback relationship between the centers (hypothalamus-pituitary)and ovaries so that estrogen levels are always high which results in an adequate increase in FSH levels never occurring. This research shows that estrogen production (conversion of testosterone to estrogen) is influenced by FSH, aromatase enzyme, and AMH. Meanwhile, in PCOS conditions, there is a surge in LH compared to FSH, resulting in an imbalance and resulting in estrogen production.[21] Androgens are not only obligate intermediates in estradiol biosynthesis. They also have complex effects on follicle growth, including up-regulation of aromatase activity. It is critical for ovarian function that ovarian androgen secretion be coordinated with estrogen formation so that both are optimized for ovulation. Although this process is highly dependent on LH and FSH concentrations, various intrafollicular modulators are essential for the coordinated function of ovarian follicles.[22] This is also because stem cells have provided a therapeutic effect which is thought to be in the form of stopping the rate of apoptosis thereby increasing GDF-9 expression. Stem cells also originate from this extragonadal location and release progenitor germ cells into the circulation and then "homing" to the ovary and engraft as new oocytes in the follicle, but can also be a transformation mechanism to improve the microenvironment in the ovarian follicle.[23] The limitations of this research areLH and FSH hormone levels were not measured.Statisticallycystic follicle There were no significant differences between groups, but clinically there were differences that needed further investigation. Future researchers are expected to measure LH and FSH hormone levels.It is hoped that future researchers can further examine the effect of SH-MSCs on number of cystic follicles.

Conclusion:-

There was an effect of administering SH-MSCs at a dose of 200 μL and a dose of 400 μL on reducing TGF-β expression between treatment groups compared to the control group.There was an effect of giving SH-MSCs at a dose of 200 μL and a dose of 400 μL on increasing estrogen hormone levels between treatment groups compared to the

control group.There was an effect of giving SH-MSCs at a dose of 200 µL and a dose of 400 µL on reducing the number of cystic follicles between treatment groups compared to the control group.

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