Journal Of Health Science Community
ISSN: 2714 7789 (print) – 2745 3561 (online)
DOI : https://doi.org/10.30994/jhsc.v5i2.250
Vol. 5 No. 2 November 2024, Page. 87-96

Decreased TGF-β Expression in PCOS-IR Rat Models after a Low-Carbohydrate High-Protein Diet

Ermawati
Mataram Health Training Center, Ministry of Health of the Republic of Indonesia
***Corresponding author:** erma_nia@gmail.com

ABSTRACT

Transforming growth factor β (TGF-β) is commonly associated with PCOS (Polycystic Ovary Syndrome) conditions and Insulin Resistance (IR). This is because TGF-β is a powerful regulator for the proliferation and differentiation of many cell types by directing the expression of hundreds of target genes including insulin. Hence, this research aims to identify the expression of TGF-β in PCOS-IR Rat Models after a low-carbohydrate, high-protein diet. The research design applied was the true experimental design. The selected population was female white rats (Rattus norvegicus), 3 months old, and weighing 100-200 grams. Modeling of PCOS with insulin resistance was carried out by injecting testosterone propionate hormones at a dose of 100 mg/kg BW for 28 days. On the last day after injection, a vaginal swab was taken to identify diestrus conditions. In the ANOVA test results, it was obtained p-value = 0.172, indicating that there is no significant difference in the mean value of TGF-β expression. It is said to be insignificant since the p-value <0.05. This research concludes that there is no significant difference in the mean value of TGF-β expression. Based on the mean value of TGF-β expression, it shows that the treatment group tends to have a lower mean value compared to the control group.
Keywords: *expression, insulin resistance, low-carbohydrate high-protein, polycystic ovary syndrome, TGF-β*

INTRODUCTION

Ten to fifteen percent of women of productive age suffer from PCOS, caused mostly by endocrine factors which are more widespread in women (Eleni et al., 2018). According to Azizi (2016), this desease is also associated with hormonal problems that disrupt the health of women of productive age and result in infertility. Metabolic disorders, cardiovascular disease, type 2 diabetes, visceral obesity, dyslipidemia, and risk factors for endothelial dysfunction are common in women with PCOS. Thus, PCOS is a serious health problem that can reduce women's life expectancy in addition to being a reproductive problem (Guo et al., 2016).

The underlying etiology of PCOS is still unknown, but there is a possibility of a strong correlation with insulin resistance (Firmansyah et al., 2018). Insulin resistance and hyperinsulinemia occur in 50-75% of PCOS patients and show peripheral insulin resistance similar to type 2 diabetes. Insulin resistance leads to hyperinsulinemia which can increase androgen production in the ovaries and result in hyperandrogenism. This is also related to the increased levels of growth hormone (GH) and insulin-like growth factor (IGF-1) (Hestiantoro et al., 2013; Ibanez et al., 2017).

Transforming growth factor β (TGF-β) is also associated with PCOS and insulin resistance. This is because TGF-β is a powerful regulator for the proliferation and differentiation of many cell types by directing the expression of hundreds of target genes including insulin. Based on the research conducted by Budi et al. (2015), it is known that insulin

increases the signaling of TGF-β by mobilizing intracellular TGF-β receptors. Members of the TGF-ß superfamily are also expressed in the ovaries and have been implicated in the pathogenesis of abnormal follicle development and hyperandrogenism in PCOS, including activin, inhibin, anti-mullerian hormone (AMH), and bone morphogenic protein (BMP). A microarray study of granulosa cells in women with PCOS found increased differential expression of genes involved in TGF-β signaling (Raja-Khan et al., 2014).

Lifestyle modification is recommended as the first-line therapy in PCOS before drug therapy, which includes dietary intervention by regulating nutrient intake and physical activity (Lin et al., 2019). Nutritional regulation includes foods with a lower glycemic index and glycemic load by modifying the amount of carbohydrates, fats, and proteins. A low-carbohydrate, high-protein (LCHP) diet in PCOS with insulin resistance must be proven effective in improving the metabolic and endocrine systems. Therefore, more specific research is needed as a recommendation for appropriate PCOS management in terms of modifying the nutritional intake diet.

METHODS

The research design applied was the true experimental design (pure experiment). It is for the reason that in this design, the researcher can control all external variables that affect the course of the experiment, with the post-test control group design approach (Sugiyono, 2017). This research used female white rats (Rattus norvegicus) as experimental animals, which were made into the PCOS with Insulin Resistance (PCOS-IR) models. The selected population was female white rats (Rattus norvegicus), aged 3 months, and weighing 100-200 grams. Modeling of PCOS with insulin resistance was carried out by injecting testosterone propionate hormones at a dose of 100 mg/kg BW for 28 days. On the last day after injection, a vaginal swab was performed to identify diestrus conditions. After the data was collected and tabulated, normality and homogeneity tests were conducted and then continued with the ANOVA test to see the significance in each group. Furthermore, the data was processed and tested using the Pearson correlation test to determine whether there was a correlation between the research variables or not.

RESULTS

1. TGF-β Expression

Observation of TGF-β expression was carried out on the prepared ovarian tissues with Immunohistochemical staining. Data for each sample was assessed semi-quantitatively according to the modified Remmele method, where the Remmele scale index (Immuno Reactive Score/IRS) is the result of multiplying the percentage score of immunoreactive cells by the color intensity score of immunoreactive cells. Data for each sample is the mean value of IRS observed in ten different Fields of View (FV) at 100x and 400x magnification. From the results of the observations made, the following findings are shown:

Table 1. The Mean Value of TGF-β Expression

Group	n	Mean ± SD (*cells)*
K-	12	3.700±1.974
K+	12	3.233±1.273
KP	12	2.583±0.752

Table 1 shows the differences in each group. The value was derived from the results of histological observations of each rat's ovaries. Then, the total TGF-β expression was averaged and the standard deviation value was calculated.

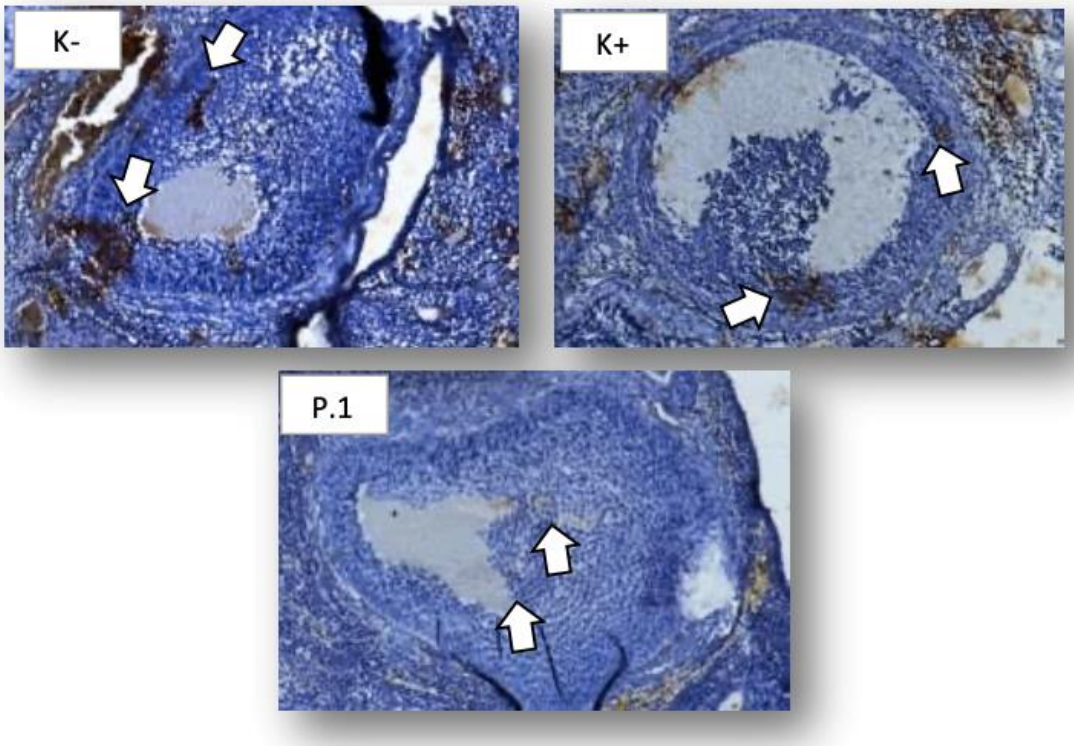

Figure 1 shows the TGF-β expression in each group. It denotes the presence of TGF-β expression in follicle granulosa cells as indicated by the presence of brown chromogen color (arrow), with IHC 400x.

2. Data Analysis

a. TGF-β Expression

The normality test used to examine the mean value of TGF-β expression was the Shapiro-Wilk normality test. The results of the normality test showed that the data distribution of the mean value of TGF-β expression in each group was normal (p>0.05). Therefore, the analysis was continued with a homogeneity test using Levene statistics. The results of the homogeneity test showed significance (p) = 0.133, which means the data is homogeneous. It is said to be homogeneous since the p-value>0.05. Then, it was followed by the ANOVA test. From the results of the ANOVA test, it is known that the p-value = 0.172 indicates that there is no significant difference in the mean value of TGF-β expression. It is said to be insignificant since the p-value <0.05.

b. The Correlation between IGF-1 Levels and TGF-β Expression in PCOS-IR Rat Models

The Pearson correlation test was used to determine the correlation between IGF-1 levels and TGF-β expression as well as the number of corpus luteum. The results of the Pearson correlation test are shown as follows:

Table 2. Pearson Correlation Test Results

Variable	n	Mean±SD	Pearson r	*p-value*
TGF- β Expression (*cells)*	36	3.172±1.459	0,098	0,571

Based on Table 2, the results of the Pearson correlation test show that there is no correlation between IGF-1 levels and TGF-β expression as well as the number of corpus luteum ($p>0.05$).

DISCUSSION

Based on the research results, it is known that there is no significant difference in the mean value of TGF-β expression. Based on the mean value of TGF-β expression, it is indicated that the treatment group tends to have a lower mean value compared to the control group. These results are in line with the results of a research conducted by Markova et al. (2020) which concluded that providing a low-carbohydrate, high-protein diet with a composition of 40% carbohydrates, 30% protein, and 30% fat decreased TGF-β expression.

The decrease in TGF-β expression is likely due to the influence of low-carbohydrate, high-protein diet (LCHP). Low-carbohydrate and high-protein intake can activate calcium-activated potassium channels (BKCa), voltage and calcium in endothelial cells. BKCa activation promotes signaling through proline-rich tyrosine kinase-2 (Pyk2), c-Src, Akt and mitogen-activated protein kinase (MAPK) to suppress TGF-β production (Hovater and Sanders, 2012).

The PI3K-Akt signaling pathway plays a central role in mobilizing TGF-β receptors to the cell surface to enhance cell responsiveness to TGF-β. Akt signaling can be activated through growth factors to induce hyperactive TGF-β responses. Additionally, IGF-1 can also activate Akt to induce the enhancement of autocrine TGF-β responsiveness (Budi et al., 2015). Insulin also enhances TGF-β responses by mobilizing intracellular TGF-β receptors. FSH also promotes preantral follicle development along with a large number of paracrine factors derived from oocytes and granulosa cells, namely GDF9 and BMP15 (TGF-β superfamily), which are able to stimulate primary stage follicle development (Hsueh et al., 2015).

When FSH levels increase, androgen hormones decrease (Rosenfield and Ehrmann. 2016) and signal ovarian follicles for folliculogenesis. Decreased androgen hormones signal ovarian follicles for folliculogenesis as well as AMH as an intrafollicular modulator for follicle growth and maturation. The process of folliculogenesis will directly increase the secretion of several local cytokines in the ovaries, such as TGF-β, causing cumulus cell expansion which will initiate the mechanism of egg maturation (Santoso and Sulistiyono, 2014).

CONCLUSION

There is no increase in TGF-β expression in PCOS-IR rat models after following a low-carbohydrate, high-protein diet. Based on the mean value of TGF-β expression, it indicates that the treatment group tends to have a lower mean value compared to the control group.

REFERENCES

Aguirre, G.A., De Ita, J.R., and de la Garza, R.G. 2016.Insulin-like growth factor-1 deficiency and metabolic syndrome. J Transl Med 14:3. doi:10.1186/s12967-015-0762-z

Azhar, Minda. 2016. Biomolekul sel: Karbohid

, protein, dan enzim. Padang: UNP Press. ISBN: 978-602-1178-12-6

Azziz, R., Carmina, E., Chen, Z.J., Dunaif, A., Laven, J.S.E., and Legro, R.S. 2016. Polycystic ovary syndrome. J Nature Rev 2:1–18.

Baerwald, A. R., Adams, G. P., and Pierson, R. A. 2005. Form and function of the corpus luteum during the human menstrual cycle. Ultrasound in obstetrics & gynecology : the official journal of the international society of ultrasound in obstetrics and gynecology, 25(5), 498–507. doi:10.1002/uog.1891

Beale, E.G. 2013. Insulin signaling and insulin resistance. Journal of investigative medicine : the official publication of the American federation for clinical research, 61(1), 11–14. doi:10.2310/JIM.0b013e3182746f95

Boucher, J., Kleinridders, A., and Kahn, C. R. 2014. Insulin receptor signaling in normal and insulin-resistant states.Cold spring harbor perspectives in biology, 6(1), a009191. doi:10.1101/cshperspect.a009191

Budi, E. H., Muthusamy, B. P., and Derynck, R. 2015. The insulin response integrates increased TGF-β signaling through akt-induced enhancement of cell surface delivery of TGF-β receptors. Science signaling, 8(396), ra96. doi:10.1126/scis i gna l. aaa9432

Clemmons, D.R. 2012. Metabolic actions of insulin-like growth factor-I in normal

Corre, I., Paris, F., and Huot, J. 2017. The p38 pathway, a major pleiotropic cascade that transduces stress and metastatic signals in endothelial cells. Oncotarget, 8(33), 55684–55714. doi:10.18632/oncotarget.18264

Clark, J. J., Benson, M. L., Smith, R. D., and Carlson, H. A. 2019. Inherent versus induced protein flexibility: Comparisons within and between apo and holo structures. PLoS computational biology, 15(1), e1006705. doi:10.1371/jour na l.pcb i.1006705 physiology and diabetes. Endocrinology and metabolism clinics of North America, 41(2), 425–viii. doi:10.1016/j.ecl.2012.04.017

Copp, T., Hersch, J., Muscat, D. M., McCaffery, K. J., Doust, J., Dokras, A., Mol, B.W., and Jansen, J. 2019. The benefits and harms of receiving a polycystic ovary syndrome diagnosis: a qualitative study of women's experiences. Human reproduction open, (4), hoz026. doi:10.1093/hrope n/ ho z026

Coyle, C., Rebecca, E., and Campbell. 2019. Pathological pulses in PCOS. Molecular and cellular endocrinology. 498: 110561.

DeFronzo R., A. 2004. Pathogenesis of type 2 diabetes mellitus. Med Clin North Am;88(4):787–835.

Das, D., and Arur, S. 2017. Conserved insulin signaling in the regulation of doi:10.1186/s12958-016-0173-x

Dennet, C., and Simon, J. 2015. The role of polycystic ovary syndrome in reproductive and metabolic health: overview and approaches for treatment. Diabetes spectrum 28(2): 116-120. https://do i.or g/10.2337/d iaspec t.28.2.116

Desai, N. A., and Patel, S.S. 2015. Increased insulin-like growth factor-1 in relation to cardiovascular function in polycystic ovary syndrome: friend or foe?, Gynecological endocrinology. ISSN: 1473-0766 doi.org/10.3109/09513590.2015.1075497

Diamanti-Kandarakis, E., dan Dunaif, A. 2012. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endocr Rev 33:981–1030. doi:10.1210/er.2011-1034.

Du, T., Yuan, G., Zhang, M., Zhou, X., Sun, X. and Yu, X. 2014. Clinical usefulness of lipid ratios, visceral adiposity indicators, and the triglycerides and glucose index as risk markers of insulin resistance. Cardiovascular diabetology, 13, 1-10.

Eleni, A.G., Lauri, A.P., Marcelle, I., Richard, S. L., Esther, E., and Heather, G. 2018. American society for reproductive medicine: Insulin resistance is associated with depression risk in polycystic ovary syndrome. Fertility and sterility Vol. -, No. -, - 2018 0015-0282.

Firmansyah, A., Chalid, M. T., Farid, R. B., and Nusratuddin, N. 2018. The correlation between insulin-like growth factor binding protein 1 (IGFBP-1) and homeostasis model assessment of insulin resistance (HOMA-IR) in polycystic ovarian syndrome with insulin resistance. International journal of reproductive biomedicine (Yazd, Iran), 16(11), 679–682.oocyte growth, development, and maturation. Molecular reproduction and development, 84(6), 444–459. doi:10.1002/mrd.22806

De Leo, V., Musacchio, M. C., Cappelli, V., Massaro, M. G., Morgante, G., and Petraglia, F. 2016. Genetic, hormonal and metabolic aspects of PCOS: an update. Reproductive biology and endocrinology : RB&E, 14(1), 38.

Dumesic, D. A., Oberfield, S. E., Stener-Victorin, E., Marshall, J. C., Laven, J. S., Legro, R. S. 2015. Scientific statement on the diagnostic criteria, epidemiology, pathophysiology, and molecular genetics of polycystic ovary syndrome. Endocrine reviews, 36(5), 487–525. doi:10.1210/er.2015- 83

Friedrich, N., Thuesen, B., Jørgensen, T., Juul, A., Spielhagen, C., Wallaschofksi, and Linneberg, A. 2012. The association between IGF-I and insulin resistance. American diabetes association: Diabetes care 2012 Apr; 35(4): 768-773. Doi.org/10.2337/dc11-1833.

Faghfoori, X., Fazelian, S., Shadnoush, M., and Goodarzi, R. 2017. Nutritional management in women with polycystic ovary syndrome: a review study. Diabetes & rome: clinical research & reviews, 11: S429 S432. doi.org/10.1016/j.dsx.2017.03.030.

Gill R, Verma C, Wallach B, Urso B, Pitts J, Awollmer, De Meyts, P., and Wood, M. 1999. Modeling of the disulphide-swapped isomer of human insulin-like growth factor-I: Implication for receptor binding. Protein engineering 12(4):297-303

Govers E, Slof E, Verkoelen H, Ten Hoor-Aukema NM, KDOO. 2015. Guideline for the management of insulin resistance. International journal endocrinol metabolic disorder 1(4). doi.org/10.16966/2380- 548X.115 .

Guo Yanjie,Qi Yane,Yang Xuefei,Zhao Lihui,Wen Shu,Liu Yinhui,and Tang Li. (2016). Association between polycystic ovary syndrome and gut microbiota. .

Guzick, D.S. 2004. Polycystic ovary syndrome. Obstet gynecol, 103(1): 181-193. doi.org/10.1097/01.AOG.0000104485.44999.C6.

Hall JE. 2016. Guyton and hall textbook of medical physiology. 13th ed. Philadelphia (PA): Elsevier, Inc. and Harvard Health Publishing. Juli 2015. Low-carb, high-protein diets. https://www.health.harvard.edu/healthy-eating/low-carb-high-protein- diets. (Diakses pada 13 Februari 2020)

Gower, B. A., and Goss, A.M. 2015. A lower-carbohydrate, higher-fat diet reduces abdominal and intermuscular fat and increases insulin sensitivity in adults at risk of type 2 diabetes. The journal of nutrition, 145(1), 177S–83S. doi:10.3945/jn.114.195065

Graham, A., and Hamoda, H. 2016. Treatment of polycystic ovarian syndrome in primary care. Prescriber volume 27(11): 36-45. https://do i.or g/10.1002/psb.1515 doi: 10.1371/journal.pone.0153196 PLos one. 11(4): e0153196.

Hardy, O. T., Czech, M. P., Corvera, S. 2012. What causes the insulin resistance underlying obesity?. Current opinion in endocrinology, diabetes, and obesity, 19(2), 81–87. doi:10.1097/MED.0b013e3283514e13

Herr, D., Bekes, I., and Wulff, C. 2013. Regulation of endothelial permeability in the corpus luteum: a review of the literature. Geburtshilfe und frauenheilkunde, 73(11), 1107–1111. doi:10.1055/s-0033-1351032

Hestiantoro, A., and Natadisasatra, M. 2013. Current Updates on: Polycystic ovary sindrome, endometriosis, adenomyosis. Sagung Seto, Jakarta. P 1- 52

Himpunan endokrinologi reproduksi dan fertilitas indonesia (HIFERI) Perkumpulan obstetri dan ginekologi indonesia (POGI). 2016. Konsensus tata laksana sindrom ovarium polikistik. Jakarta:5-69.

Hirschberg, A.L. 2009. Polycystic ovary syndrome, obesity and reproductive implications. Women's health, 5(5): 529-542. doi.org/10.2217/whe.09.39. Holesh, J.E., and Martin, A. 2019. (www.ncbi.nlm.nih.gov/books/NBK459280/#_NBK459280_pubdet). (Diakses pada 28 Desember 2019)

Hovater, M.B. and Sanders, P.W. 2012. Effect of dietary salt on regulation of TGF-β in the kidney. Semin nephrol, 32(3): 269–276. Hermendy B. E., (TGF- Hinck, A. P., Mueller, T. D.,Springer, T. A. 2016. Structural biology and evolution of the TGF-β family. Cold spring harbor perspectives in biology, 8(12), a022103. doi.org/10.1101/cshperspect.a022103 Physiology, carbohydrates. in: statpearls [Internet]. Treasure island (FL): StatPearls publishing. Holesh, J.E., Hazhirkarzar B., and Lord M. Physiology, ovulation. 2019. In:statpearls. Treasure island (FL): StatPearls publishing; 2020 Jan- . Available from: https://www.ncbi.nlm.nih.gov/books/NBK441996/ (Diakses pada 31 Januari 2020).

Homburg, R. 2014. Physiology of ovulation. Ovulation induction and controlled ovarian stimulation, 7–23. doi:10.1007/978-3-319-05612-8_2

Hsueh, A. J., Kawamura, K., Cheng, Y., and Fauser, B.C. 2015. Intraovarian control of early folliculogenesis. Endocrine reviews, 36(1), 1–24. doi:10.1210/er.2014-1020

Ibáñez, L., Oberfield, S.E., Witchel, S., Auchus, R.J., Chang, R.J., Codner, E., et al. 2017. An international consortium update: pathophysiology, diagnosis, and treatment of polycystic ovarian syndrome in adolescence. Horm res pediatr 371-395. doi: 10.1159/000479371.

Jones, R.E., and Lopez, K.H. 2014a. The female reproductive system. Human reproductive biology: 23-50. doi.org/10.1016/B978-0-12-382184-3.00002- 7

Kelana, E., Nasrul, E., Yaswir, R., and Desywar. 2015. Korelasi indeks 20/(C-peptide puasa×glukosa darah puasa) dengan HOMA-IR untuk menilai resistensi insulin diabetes melitus tipe 2. MKA, 3 (38).

Jones, R. E., and Lopez, K. H. 2014b. The Menstrual cycle. Human reproductive biology, 51–66. doi:10.1016/b978-0-12-382184-3.00003-9 and Treasure island (FL): StatPearls publishing; 2020 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK545270/. (Diakses pada 13 Februari 2020)

Lagiou Pagona, Sandin Sven, Lof Marie, Trichopoulos Dimitrios, Adami Hans- Olov, and Weiderpass Elisabete. 2012. Low carbohydrate-high protein diet and incidence of cardiovascular diseases in Swedish women: prospective cohort study. BMJ 344 :e4026. doi.org/10.1136/bmj.e4026

Laron Z. 2001. Insulin-like growth factor 1 (IGF-1): a growth Lebinger, T.G. 2007. Metformin and polycystic ovary syndrome. Current opinion endocrinology diabetes obesity, 1(14): 132–40. And Kirkendoll, SD.,

Bacha, D. 2019. Histology, corpus albicans in: statpearls. Lansdown, A.,

Rees DA. 2012. The sympathetic nervous system in polycystic ovary syndrome: a novel therapeutic target? Clinical endocrinology (Oxf). 77(6):791–801. hormone. Molecular pathology : MP, 54(5), 311–316. doi.org/10.1136/mp.54.5.311

Li, J., Bai, L., Wei, F., Zhao, J., Wang, D., Xiao, Y., Yan, W., Wei, J. 2019. Therapeutic mechanisms of herbal medicines against insulin resistance: a review. Frontiers in pharmacology, 10, 661. doi.org/10.3389/fp har.2 019.00661

Spandorfer, S. D., and Lujan, M.E. 2019. Dietary and physical activity behaviors in women with polycystic ovary syndrome per the new international evidence-based guideline. Nutrients, 11(11), 2711. doi:10.3390/nu11112711

Łucja C.M., Teresa, G., Marta, K., Krystyna, C., and Henryk, W. 2014. The use of low-carbohydrate diet in type 2diabetes – benefits and risks.

Ludwig, D. S., Hu, F. B., Tappy, L., and Brand-Miller, J. 2018. Dietary

Mani, A.M., Fenwick, M.A., Cheng, Z., Sharma, M.K., Singh, D., and Wathes, D.C. 2010. IGF1 induces up-regulation of steroidogenic and apoptotic regulatory genes via activation of phosphatidylinositoldependent kinase/AKT in bovine granulosa cells. Reproduction(Cambridge, England) 139(1):139-11. DOI: 10.1530/rep-09-0050 PMID: 19819918

Marissa, A.V., and Heba, I. M. 2019. PCOS and type II diabetes. Pediatric type II diabetes. Elsevier Inc. All rights reserved. Doi.org/10.1016/B978-0-323- 55138-0.00010-3.

Mareta, R., Amran, R., and Larasati, V. 2018. Hubungan polycystic ovary syndrome (PCOS) dengan infertilitas di praktik swasta dokter obstetri Ann agric environ med. 21(2):320–326. doi.org/10.5604/1232-1966.1108597 carbohydrates: role of quality and quantity in chronic disease. BMJ (Clinical research ed.), 361, k2340. doi:10.1136/bmj.k2340 ginekologi Palembang. Majalah kedokteran sriwijaya, 50(2): 85-91. doi.org/10.36706/mks. v50 i2.8552.

Markova, M., Koelman, L., Hornemann, S., Pivovarova, O., Sucher, S., Machann, J., Rudovich, N., Thomann, R., Schneeweiss, R., Rohn, S., Pfeiffer, A.F.H. and Aleksandrova, K. 2020. Effects of plant and animal high protein diets on immune-inflammatory biomarkers: A 6-week intervention trial. Clinical nutrition, 39(3):862-869. doi.org/10.1016/j.clnu.2019.03.019.

Meier, R.K., 2018. Polycystic ovary syndrome. Nursing clinics of North America. doi.org/10.1016/j.cnur.2018.04.008. and Pasquali R., L. Zanotti., F. Fanelli., M. Mezzullo., A. Fazzini., A.M.M. Labate., A. Repaci., D. Ribichini., and A. Gambineri. 2016. Defining hyperandrogenism in women with polycystic ovary syndrome: a challenging perspective. Journal clinical endocrinology metabolism. 101 (5): 2013-2022.

Peigné, M., and Dewailly, D. 2014. Long term complications of polycystic ovary syndrome (PCOS). Ann endocrinol paris;75(4):194-9 doi: 10.1016/j.ando.2014.07.111

PERKENI. 2015. Pengelolaan dan pencegahan diabetes melitus tipe 2 di Indonesia. www.perkeni.org. (Diakses pada 31 Desember 2019).

Plant, T. M., and Zeleznik, A. J. 2015. Knobil and Neill's physiology of reproduction. 4$^{t\ h}$ edition. Academic press. P 2684. //doi.org/10.1016/C2011-1-07288-0.

Poniatowski, Ł.A., Wojdasiewicz, P., Gasik, R., and Szukiewicz, D. 2015.

Transforming growth factor Beta family: insight into the role of growth factors in regulation of fracture healing biology and potential clinical applications. Mediators of inflammation, 2015, 137823. doi:10.1155/2015/137823.

Puttabyatappa, M., and Padmanabhan, V. 2018. Ovarian and extra-ovarian

Murphy, B. D. (2012). Adiponectin and the control of female reproductive functions. Vitamins and hormones, 239–287. doi:10.1016/b978-0-12-398313-8.00010-5

Petersen, M.C., and Shulman, G.I. 2018. Mechanisms of insulin action and insulin resistance. Physiological reviews, 98(4), 2133–2223. doi:10.1152/physrev.00063.2017.

Puche, J.E., and Castilla-Cortázar, I. 2012. Human conditions of insulin-like growth factor-I (IGF-I) deficiency. J Transl Med 10, 224 doi:10.1186/1479-5876-10-224. mediators in the development of polycystic ovary syndrome. Journal of molecular endocrinology, 61(4), R161–R184. doi:10.1530/JME-18-0079.

Raja-Khan, N., Urbanek, M., Rodgers, R. J., and Legro, R. S. 2014. The role of TGF-β in polycystic ovary syndrome. Reproductive sciences (Thousand oaks, Calif.), 21(1), 20–31. doi:10.1177/1933719113485294.

Robert, J.S., Perets, R.A., and Sarfert, S. 2017. High-fat high sugar diet induces polycystic ovary syndrome in a rodent model. Biology of reproduction, 96(3): 551–562. doi.org/10.1095/biolreprod.116.142786.

Rosenfield dan Ehrmann. 2016. The pathogenesis of polycystic ovary syndrome (PCOS): The hypothesis of PCOS as functional ovarian hyperandrogenism Endocr Rev. 2016 ct;37(5):467-520. revisited. DOI:10.1210/er.2015- 1104.

Samuel, V. T., and Shulman, G. I. (2016). The pathogenesis of insulin resistance: Santoso, B., dan Sulistiyono, A. 2014. Ekspresi GDF9 dan kadar estrogen pada rattus norvegicus model sindroma ovarium polikistik pasca injeksi stem cell red bone marrow. Majalah obstetri & ginekologi, 22(1): 9-12.

Sherwood, LZ., 2014. Fisiologi manusia dari sel ke sistem. Edisi 8. Jakarta: EGC, 595-677.

Sibarani NMH, Berata IK, Ajana AAG. 2013. Studi histopatologi hepar tikus putih yang diinduksi aspirin pasca pemberian madu per oral. Indonesia medicus veterinus 2(5): 488-95.

Silva, J.R.V., Figueiredo, J.R., and Hurk, R.V.D. 2009. Involvement of growth hormone (GH) and insulin-like growth factor (IGF) system in ovarian folliculogenesis. Theriogenology 71:1193-1208.

Spanos, S., Becker, D.L., Winston, R.M.L., and Hardy, K. 2000. Anti-apoptotic action of insulin-like growth factorI during human preimplantation embryo development. Biology reproduction 63:1413-1420.

Speroff, L., and Fritz, M. 2005. Clinical gynecologic endocrinology and infertility. Seventh edition. Philadelphia: Lippincott Williams & Wilkins.

Speroff, L. and Marca, A.F. 2011. Clinical gynecologic endocrinology and Infertility. Edisi ke-8. Baltimore. USA: William Lippincot William & Wilkins. integrating signaling pathways and substrate flux. The Journal of clinical investigation, 126(1), 12–22. oi:10.1172/JCI77812.

Scherer, T., Lindtner, C., O'Hare, J., Hackl, M., Zielinski, E., Freudenthaler, A., Baumgartner-Parzer, S., Tödter, K., Heeren, J., Krššák, M., Scheja, L., Fürnsinn, C., and Buettner, C. 2016. Insulin regulates hepatic triglyceride secretion and lipid content via signaling in the brain. Diabetes, 65(6), 1511–1520. https://doi.org/10.2337/db15-1552

Slavin, J., and Carlson, J. 2014. Carbohydrates. Advances in nutrition (Bethesda, Md.), 5(6), 760–761. doi:10.3945/an.114.006163 Sriwastva, M.K., and Ganie, M.A. 2016. Polycystic ovarian syndrome: a multi- specialty, multi system galloping epidemic. Diabetes Obes Int J.;1(3):1–4.

Steward K, and Raja A. Physiology, ovulation, basal body temperature. 2019. Treasure Island (FL): StatPearls publishing; 2020 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK546686/ (Diakses pada 03 Februari 2020)

Sugiyono. 2017. Metode penelitian kualitatif. Bandung:Alfabeta. ISBN:978-602- 289-325-7.

Sutadarma, I.W.G and Wiryanthini, I.A.D. 2015. Terapi diet pada obesitas. Denpasar: Fakultas Kedokteran Universitas Udayana. 25 hal.

Swarjana, I.K. 2016. Statistik kesehatan. Yogyakarta:Penerbit andi. ISBN:978- 979-29-5562-0.

Taylor, H.S., Pal, L., and Seli, E. 2019. Speroff's clinical gynecologic and endocrinology and infertility. 9th edition. Philadelphia: Lippincott Williams and Wilkins.

Tomac, J., Cekinovic, D. and Arapovic, J. 2011. Biology of the corpus luteum. Periodicum biologorum, 113(1): 43–49.

Tremellen, K., and Pearce, K. 2012. Dysbiosis of gut microbiota (DOGMA) a novel theory for the development of polycystic ovarian syndrome. Medical hypotheses 79(1):104-12.

Vasudevan, D., and Vaidyanathan, K. 2017. Protein: structure and function. Textbook of biochemistry chapter 04. DOI: 10.5005/jp/books/13014_5

Wibawa, Putu Putra. 2017. Bahan ajar biokimia: karbohidrat. Fakultas Peternakan Universitas Udayana Bali.

Williams C.J., Erickson G.F. Morphology and physiology of the ovary. 2012. In:

Wongsari, Melisa Heidy. 2018. Kesesuaian triglyceride-glucose index (TyG INDEX) dan homeostasis model assessmentinsulin resistance (HOMA-IR) pada pria dewasa muda dengan obesitas sentral. Skripsi: Program pendidikan dokter spesialis-1 (SP.1) Program studi ilmu patologi klinik fakultas kedokteran Universitas Hasanuddin Makassar.

Yakar, S., and Adamo, M.L. 2012. Insulin-like growth factor 1 physiology:

Feingold KR, Anawalt B, Boyce A, et al., editors. MDText.com, Inc.; 2000-.Available from: https://www.ncbi.nlm.nih.gov/books/NBK278951/. Xu, N., Kwon, S., Abbott, D.H., Geller, D.H., Dumesic, D.A., Azziz, R., and

Goodarzi, M.O. 2011. Epigenetic mechanism underlying the development of polycystic ovary syndrome (PCOS)-like phenotypes in prenatally androgenized rhesus monkeys. PloS one, 6 (11), e27286. doi:10.1371/jour na l.po ne.0027286 lessons from mouse odels.Endocrinology and metabolism clinics of North America, 41(2), 231–v. doi:10.1016/j.ecl.2012.04.008

Yen and Jaffe. 2018. Reproductive endocrinology physiology, pathophysiology, and clinical management. 8th edition. Philadelphia: Wolters Kluwer.

Youngjae Ryu , Sung Woo Kim , Yoon Young Kim and Seung-Yup Ku. 2019. Animal models for human polycystic ovary syndrome (PCOS) focused on the use of indirect hormonal perturbations: a review of the literature. Int. J. Mol. Sci. 20, 2720; doi:10.3390/ijms20112720

Youzhu Li, Qiaobin Liu, Qionghua Chen, Xiaohong Yan, and Na Li. 2016. Insulin like growth factor 1 promotes cumulus cell expansion and nuclear maturation of oocytes via Pi3K/Akt pathway. Int J Clin Exp Pathol 9(11):11436-11443. ISSN:1936-2625/IJCEP0040089.

Zetira, Z., and Rodiani, Fakhruddin, H. 2019. pengaruh metformin terhadap wanita infertilitas dengan sindrom polikistik ovarium. Majority, 8(1): 172- 177.

Zhang, Y. E. 2018. Mechanistic insight into contextual TGF-β signaling. Current opinion in cell biology, 51, 1–7. doi:10.1016/j.ceb.2017.10.001