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Effect of Tryptophan Administration on Luteinizing Hormone Levels and Number of Leydig Cells

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ABSTRACT
This study observed the effect of giving tryptophan on the levels of Luteinizing Hormone (LH) and the number of Leydig cells. The study used the design of the post test only control group design, where the sample was dividedinto 4 groups consisting of 1 control group without treatment and 3 treatment groups with the provision of tryptophan doses of 40mg, 50mg and 60mg respectively. The treatments are given for 14 days and measured hormone levels using RIA. Counting the number of Leydig cells contained in the interstitial network between seminiferous tubules using an electric microscope. The results showed a statistically significant effect, there was a decline in the average levels of the hormone LH from 5.60 ± 0,30 nmol/L and a decreased in the number of leydig cells by 20.20 ± 2.06%. The levels of Luteinizing Hormone and the number of Leydig cells decreased in accordance with increasing the dose of tryptophan given on the white male rats Rattus Novergicus when compared with the control group. It could be concluded that the tryptophan that works as a neurotransmitter/ serotonin is able to affect the hypothalamus which then to the anterior pituitary which works as a regulator of the secretion of reproductive hormones.

Keywords: Tryptophan Administration, Luteinizing Hormone Levels, Number Of Leydig Cells

I. INTODUCTIONS

Infertility is still a health problem experienced by men and women of childbearing age. Infertility is a couple who undergo regular sexual intercourse without protection for 1 year and do not occur pregnancy, cases of infertility since several years increase[1.2]. According to statistics, 60-80 million couples experience cases of infertility each year[3].Based on Household Health Survey (SKRT) there are approximately 3.5 million couples are infertile, the number of infertility has increased by 15%-20% from about 50 million couples in Indonesia. According to data from the Central Bureau of Statistics in Indonesia in 2008, infertility events have increased every year. Masoumi and his team's 2015 study found infertility factors caused by women (88.6%) has the highest score due to menstrual disorders, diseases (obesity, thyroid disease, and diabetes), ovulation dysfunction, uterine factors, fallopian tubes and cervical factors have the highest prevalence. Causes of male infertility based on frequency include abnormalities in semen fluid, genetic factors, vascular abnormalities, and antispermatogenesis factors[4].

The research that has been done to reveal the problem of infertility, the recent studies explain that possible malfunctions in Leydig cells, has include as one of the causes of infertility. Testosterone production occurs inside the testicular Leydig cells. When production fails at this level, then spermatogenesis will be disrupted. There have been many testosterone formulations that have been developed, none of which are fully capable of replicating the physiological pattern of testosterone secretion[5]. Leydig cells stimulated by hormone

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luteinizing (LH) plays a role to produce testosterone hormone in the testes, in addition follicle stimulating hormone (FSH) also serves to stimulate Sertoli cells in the formation of proteins namely androgen binding protein (ABP), where proteins plays a role in the transport of testosterone[6.7].

Tryptophan is one of the essential amino acids that the body cannot produce, but only obtained from food has a role in the reproductive cycle[8]. Tryptophan plays a role in the synthesis of neurotransmitter/ serotonin in the brain associated with reproductive hormone secretion[9–11]. Research conducted by Shibata found that, no effects were obtained from testosterone administration on serotonin levels in the brain. However, other researchers obtained with the administration of tryptophan tested in humans, found a decrease in LH levels, but did not have a significant influence on the concentration of spermatozoa[12].

Putri and the team have found that there has been a decrease in the average level of hormone testosterone with tryptophan administration, although the decrease is not significant. Therefore, the researchers wanted to see more of the effect of tryptophan administration on Leydig and LH cells where this is a continuous series of processes[13]. Based on the background above, the authors wanted to conduct research on tryptophan administration on Luteinizing Hormone levels and the number of Leydig cells in male Rattus Novergicus".

II. Materials and Methodologies

The research was experimental with the design of the post test only control group design which is a design used to measure the influence of treatment on the experimental group by comparing that group with the control group[14].

1.1 Animals

Samples in this study are white male rats (Rattus norvegicus) has a weight of 200-250 grams, age 12 weeks contained in the Experimental Animal Maintenance Unit (UPHP). These laboratory animals were used as models for research before being treated in humans. The samples were divided into 4 groups consisting of 1 control group and 3 treatment groups using simple random sampling techniques (4-1) (n-1) ≥ 15, n ≥ 6. So the number of samples according to the above formula is 6 and to serve in case of drop out, then the sample is added 1 to become 7 heads per group. So, the total number of samples is 7 x 4 = 28 rats.

1.2 Tryptophan

Tryptophan in the form of powder dissolved with aqua pro injection with a solubility level of 10 g/l was administered to rats in intraperitoneal injection (ip). Tryptophan was injected with 3 doses of 40mg, 50 mg and 60 mg/kg of body weight for 2 weeks to the treat group. While the control group was not given the tryptophan.

1.3 LH hormone

The levels of the hormone LH in the serum of the blood which is taken intra-venous in the morning after treatment for 14 days using of capillary microhematocrit. The collected blood is then put in a measuring cup and placed on the tube test rack, then let it stand for approximately 10 minutes. After the centrifuges 3000 RPM for 15 minutes, blood serum is now obtained. The blood serum is then separated into a new measuring cup and then measured it hormone levels by radioimmunoassay (RIA) method.

1.4 Leydig cells

Counting the number of Leydig cells contained in the interstitial network between seminiferous tubules using an electric microscope with 400x magnification and

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observed in every interstitial region and then calculated per 100 cells and in each preparation of the left and right testicles, then summed and averaged. Leydig cells obtained through paraffin method. Paraffin method is a way of making permanent preparations that use paraffin as an embedding medium with slices thickness approximately 6 microns-8 micron[15]

III. Results

Research has been conducted on the effect of tryptophan administration on Luteinizing Hormone levels and the number of Leydig cells in male white rat Rattus Novergicus. Initial normality test conducted with Kolmogorov-Smirnov method. Then followed by ANOVA statistical test that conducted statistical test Multiple Comparison (Post Hoc Test Type Bonferroni) which can be seen in the following tables.**Table 1** : Normality Test Results of Kolmogorov Smirnov Luteinizing Hormone (nmol/L) Of Male White Rat (Ratuss Novergicus) and Number of Leydig Cells per 100 cells (%) In The Control Group and Treatment Group

	Average ± SD	**p**
LH Level	3.91 ± 1.079	0.243
Number of Leydig cells	20.20 ± 2.06	0.729

Based on normality test it turns out value (p>0.05), so it is assumed data is normally distributed (table 1). thus, the next test can be performed by parametric ANOVA as seen in table 2

Table 2 : Effect of Tryptophan Administration on LH Levels of Male White Rats (Rattus Novergicus) and Number of Leydig Cells in Research Group

	LH	**Leydig Cell**
Control	5.60 ± 0.30	22.50 ± 1.22
P1	3.78 ± 0.29	21.00 ± 1.09
P2	3.32 ± 0.35	19.66 ± 1.03
P3	2.96 ± 0.28	17.66 ± 0.81
p	0.000	0.000

Table 2 showed a decrease in average levels of Luteinizing Hormone and the number of Leydig cells in the group given tryptophan (treatment) compared to the group which is not given tryptophan (control) in male white rattus Novergicus. From the ANOVA test table obtained p value of <0.05 which means there is a significant difference between control group and treatment group in male white rats (Rattus novergicus). Thus, it can be continued with the Post Hoc Test Bonferroni Test, to see the difference in average Luteinizing Hormone levels and the number of Leydig cells between groups contained in the table below:

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Table 3 : Concentration Levels of LH and the Number of Leydig Cells of Male Rats (Rattus Novergicus) In the Control Group and the Treatment Group After the Administration of Tryptophan

		LH	Leydig Cell
Control	**P1**	0.000*	0.136
	P2	0.000*	0.001*
	P3	0.000*	0.000*
P1	**P2**	0.107	0.241
	P3	0.001*	0.000*
P2	**P3**	0.345	0.022*

(* There are significant differences
From Table 3 it is known that the LH levels of each control group with treatments 1, 2 and 3 with doses of 40mg, 50mg and 60mg respectively, there are significant differences ($p>0.05$). As for the number of cells Leydig, only in the control group and treatment 1 group that does not have a significant difference.

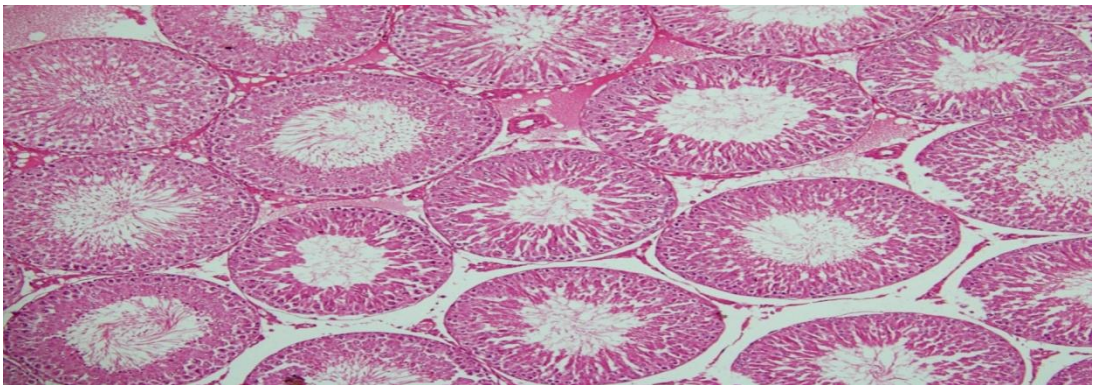

Figure.1. Histology of the Seminiferous Tubules of the Testes of Male Rats (Rattus Novergicus) in the control group (HE : 100x)

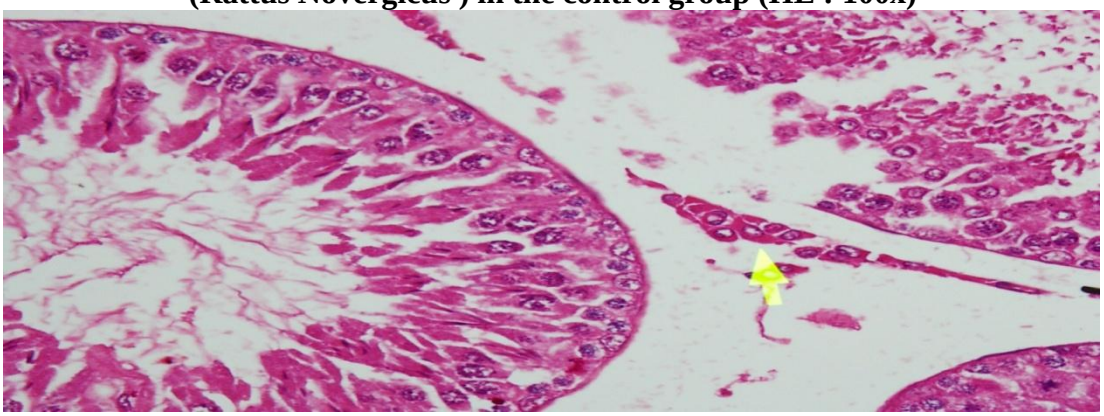

Figure.2. Histology of the Seminiferous Tubules of the Testes of Male Rats (Rattus Novergicus) in the Control group (HE : 400x) arrow indicates leydig cells

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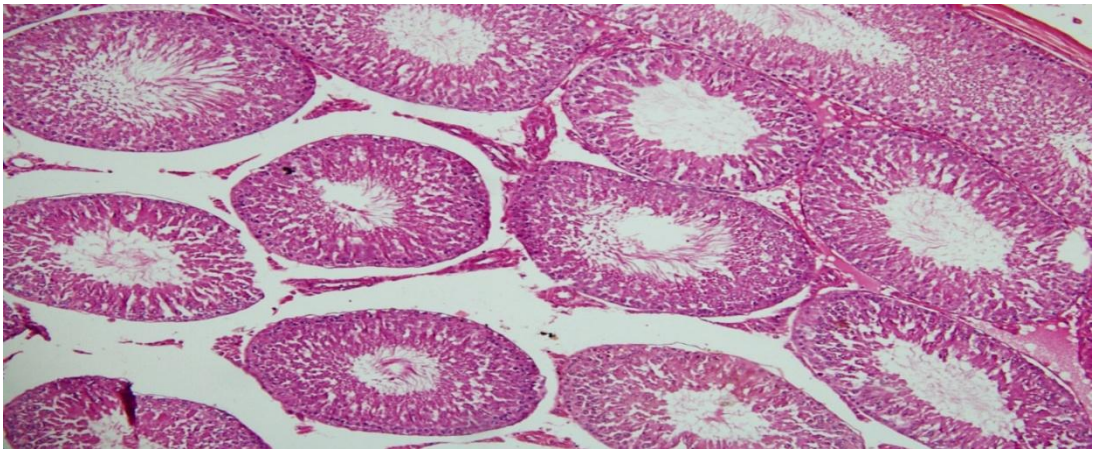

Figure.3. Histology of the Seminiferous Tubules of the Testes of Male Rats (Rattus Novergicus) in the Treatment group 1 (HE : 100x)

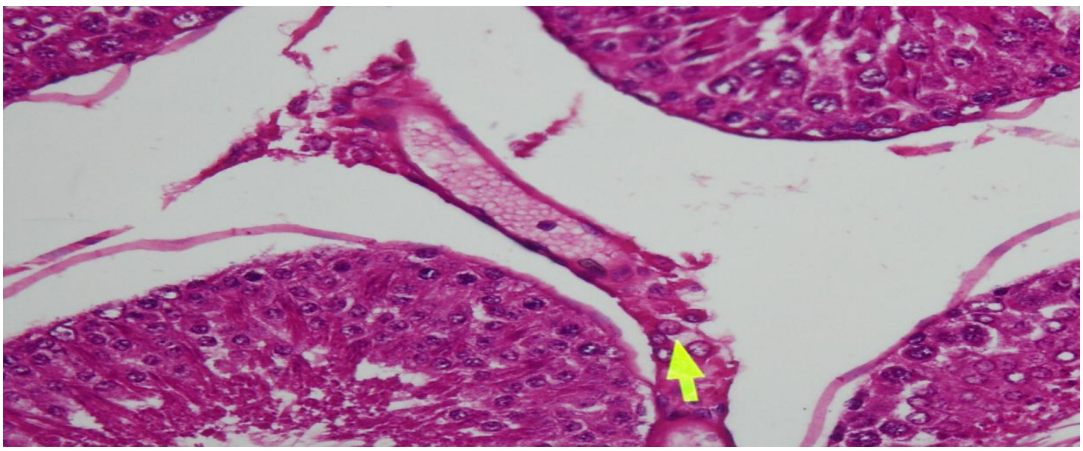

Figure.4. Histology of the Seminiferous Tubules of the Testes of Male Rats (Rattus Novergicus) in the treatment group 1 (HE : 400x) arrow indicates leydig cells

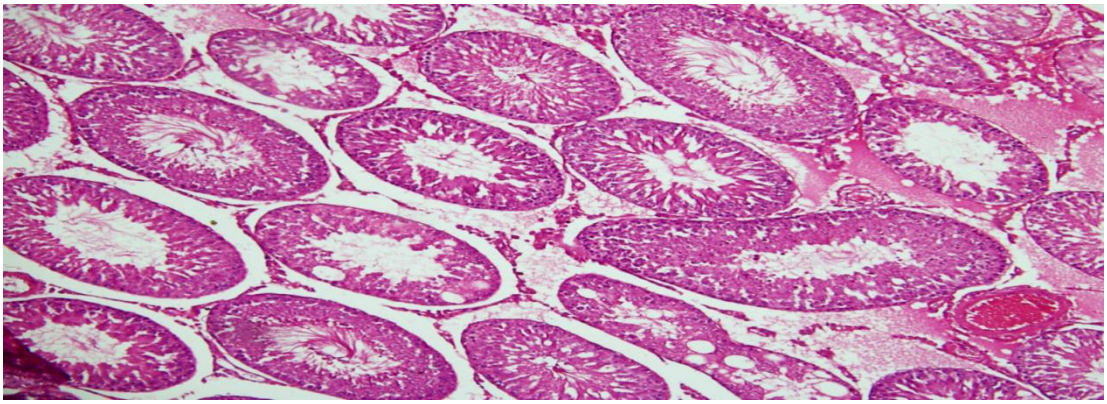

Figure.5. Histology of the Seminiferous Tubules of the Testes of Male Rats (Rattus Novergicus) in the Treatment group 2 (HE : 100x)

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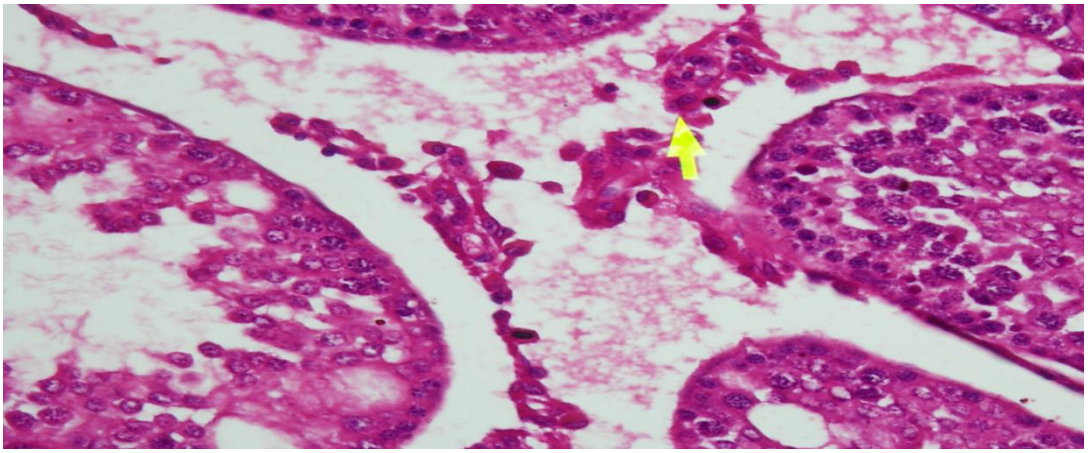

Figure.6. Histology of the Seminiferous Tubules of the Testes of Male Rats (Rattus Novergicus) in the Treatment group 2 (HE : 400x) arrow indicates leydig cells

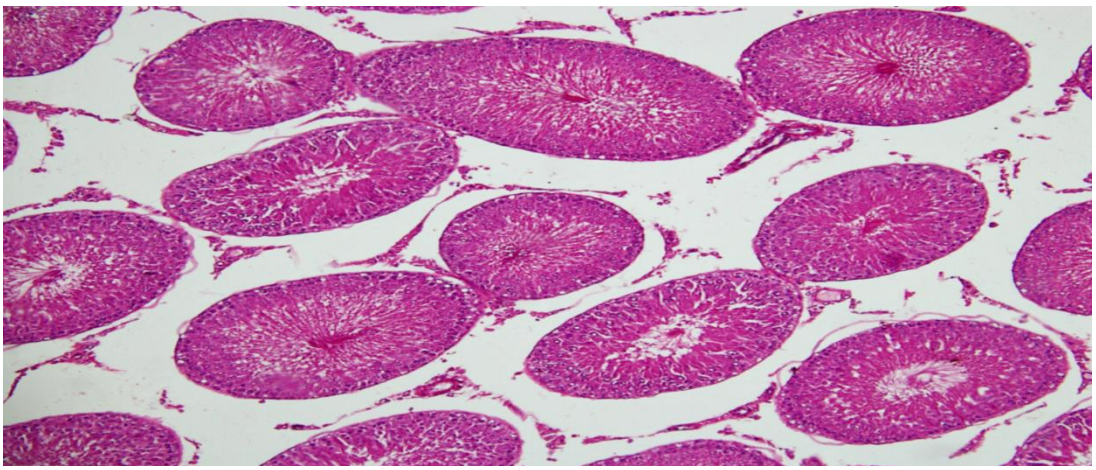

Figure.7. Histology of the Seminiferous Tubules of the Testes of Male Rats (Rattus Novergicus) in the Treatment group 3 (HE : 100x)

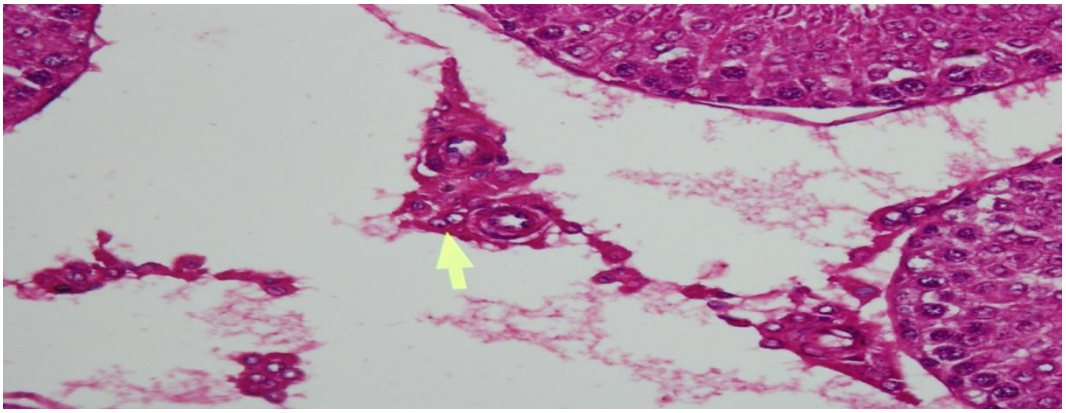

Figure.8. Histology of the Seminiferous Tubules of the Testes of Male Rats (Rattus Novergicus) in the treatment group 3 (HE : 400x) arrow indicates leydig cells

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IV. DISCUSSION

On the results of the study found that there was an average decrease in LH hormone levels of 5.60 ± 0.30 nmol/L and the number of Leydig cells 20.20 ± 2.06% after the administration of tryptophan. The findings are in line with previous research which has also found that by the time the fetus the number of Leydig cells will be increased during pregnancy, but after give birth the number will be decreased. However, this number of Leydig cells will increase again during puberty, where these cells reappear with stimulation of the hormone LH[15.16]. Serotonin acts as a secretion regulator of testosterone hormone produced by tryptopan[17]. While testosterone itself in its production through stages involving the role of Leydig cells as producers of cAMP towards the translocation of cholesterol in the mitochondria which will then be converted into pregnenolone and subsequently into testosterone[18]. So, with this continuously event, starting from the decrease in LH secretion followed by a decrease in the number of Leydig cells, which then impacted the decrease in levels of hormone testosterone[19]. Putri's research also explained a similar thing, whereby giving tryptophan it can actually lower the average levels of hormone testosterone[13]. These results have supported other studies stating that there is a link between decreased testosterone levels followed by inhibition of LH secretion and negative feedback mechanism through the role of estradiol in the hypothalamus that suppresses GnRH frequency, resulting in LH secretion decreasing [20.21]

Conflict of intersts statement

We declare that we have no conflict of interest.

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