

Depot Medroxyprogesterone acetate reduces spermatogonia cells and spermatid cells in the seminiferous tubules of male mice



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ABSTRACT

Background: Until now the existing male contraceptive methods are periodic abstinence, interrupted intercourse (coitus interruptus), the use of condoms, and vasectomy. Another alternative method of contraception for men is the use of the hormone depot medroxyprogesterone acetate (DMPA) as has been done in female contraceptives. Research with DMPA at a dose of 1.25 mg was effective in reducing the concentration and viability of spermatozoa and testosterone secretion in male rats.

Aim: to discover if DMPA as a gonadotropin hormone could effectively inhibit the process of spermatogenesis in male mice.

Methods: This research is an experimental research with post-test control group design. A total of 36 samples were grouped by simple random sampling into two groups. Control group (P0): consisted of 18 male mice given 0.3 ml of intramuscular aquabides. Treatment group (P1): 18 male mice were given DMPA intramuscularly (IM) with a dose of 3 mg as much as 0.3 ml. Data obtained from examination of the mean of 60 tubules (30 tubules from the right testis and 30 tubules from the left testis) for each treatment with HE staining at 400x magnification.

Results: The results showed a significant difference in the decrease in the number of spermatogonia cells in the treatment group compared to the control group (9.83 ± 2.33 vs 12.78 ± 1.67 cells/tubule), with p value = 0.000 ($p < 0.01$) and Spermatid cells (76.50 ± 7.66 vs 86.44 ± 6.06 cells/tubule), with p value = 0.000 ($p < 0.01$). The administration of DMPA could significantly reduce the number of spermatogonia and spermatid cells in the seminal tubules of male mice.

Conclusion: The results of the study have implications for further research on the use of DMPA as a male contraceptive in higher experimental animals such as rabbits, then clinical trials in humans.

Keywords: DMPA, spermatogonia, spermatid, male contraception.

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INTRODUCTION

One of the main problems faced in Indonesia is the uncontrollable increase of population. This condition has made it difficult to improve the welfare of the people, because the higher the population growth, the greater the efforts needed by the government to improve the welfare of the people.¹ To avoid population explosion and achieve the formation of a happy and prosperous small family norm (NKKBS), the family planning program (KB) has been made a national program in Indonesia. To achieve the goals and objectives of the policies in the population sector, various policies have been formulated, including increasing population quality and controlling growth in order to suppress and control the population growth.²

Data from the National Population and Family Planning Agency (BKKBN) shows that female contraceptive methods used (93.66%) are much higher than male contraceptive methods (6.34%). This shows that the participation of men in using contraceptives is still very small. The use of contraceptives is still dominated by women.³

One of the efforts made to suppress population growth is through the Family Planning program. Without an increasingly intensive family planning movement, the Indonesian population will be trapped in poverty, destitution, ignorance which is the most terrible and gripping human calamity. In order for the family planning program to be successful, the family planning program must be carried out by all parties, both men and

women. In fact, family planning programs are still dominated by women while men have not participated much; only about 5% compared to women.⁴ In addition, it can be seen that the target of family planning clinic services is mostly aimed at women, even though the role of men in family planning programs is very important because men are usually more dominant as policy makers in the family. Even though contraception works for women, it doesn't mean that men absolutely can't take part in the family planning program with one of the contraceptives that is effective for men.^{5,6}

Until now the existing male contraceptive methods are periodic abstinence, interrupted intercourse (coitus interruptus), the use of condoms, and vasectomy. Periodic abstinence is very

simple contraception, in which men do not have sexual intercourse during the fertile period. This method is generally carried out by men with strong religious beliefs and consider it taboo to share this method of family planning with others, so the number is not recorded. Interrupted intercourse (coitus interruptus) is an old natural method of contraception. This method is done by shedding sperm outside the vagina during the fertile period. This method is still used in some countries, but the results are less effective.⁶ Condoms will be successful as a male contraceptive if the rubber covering the male genitalia does not leak. Although the percentage of condom use is high and tends to increase over time, some users often complain of being uncomfortable and less practical because they are used every time they have coitus.^{5,6} A vasectomy is a reliable form of contraception in which the sperm duct (vas deferens) is severed so that sperm from the testicles do not come out during coitus. This contraceptive is a contraceptive that can be trusted for its stability because it is permanent.^{6,7}

Another alternative method of contraception for men is the use of the hormone depo medroxyprogesterone acetate (DMPA) as has been done in female contraceptives. Research found that DMPA at a dose of 1.25 mg was effective in reducing the concentration and viability of spermatozoa and testosterone secretion in male rats. Progesterone is expected to further suppress gonadotropin hormones thereby suppressing spermatogenesis.^{8,9} Based on the above background, the researchers wanted to prove the effect of DMPA on the inhibition of the spermatogenesis process in male mice, by looking at the decrease in spermatogonia cells and spermatids of Balb/c male mice. From this study it is expected that DMPA may be used as a new contraceptive for men.

METHODS

Research Design

This study is an experimental research with post-test control group design. This research was conducted at the Pharmacology Department, Faculty of Medicine, Udayana University and the joint laboratory of the Faculty of Medicine,

Udayana University from July 2020 until June 2021.

Sampling

The selected samples were male mice of the Balb/C strain, 12 weeks old, weighing 20-22 grams. Samples were divided into 2 groups, the control group was given aquabides (P0), and the treatment group was given depo medroxyprogesterone (P1). To find out the number of replications in each group, Freederer's formula was used: $(t-1)(n-1) \geq 15$.¹⁰ Because there were two types of treatments, we found there had to be sixteen times replication. Therefore, 32 mice were needed in this study. To anticipate drop out, the sample was added by 10%, so there was 36 samples involved, which were then grouped by simple random sampling as followed: controlled group (P0) consisted of 18 male mice given 0.3 ml of intramuscular aquabides, treatment group (P1) consisted of 18 male mice given DMPA intramuscularly (IM) with a dose of 3 mg (0.3 ml). The inclusion criteria were adult male mice of Balb C strain, age of mice is about 12 weeks with weight of 20-22 grams. Exclusion criteria were sick mice which characterized by skinny mice that didn't want to eat, with slow movement, dull fur and pale mucosa of the eyes, nose and mouth. Drop out criteria was mice that died.

Identified Variables

Independent variable in this study was DMPA of 0.3 ml and dependent variables were spermatogonia and spermatid cells. Controlled variables were age and weight of the mice, as well as gender of the mice.

Sample Preparation

Thirty-six adult male mice of the Balb-C strain, aged 12 weeks and weighing 20-22 grams (as measured by electrofit scale 1140 by Tanita Corporation, Japan) were adapted to the same environment and food for 1 week. The cage was made of a plastic container measuring 23 cm x 17 cm x 9.5 cm with a base of rice husks and a lid made of strong, bite-resistant woven wire that was not easily damaged so the animals could not easily escape. The cage was lighted, placed in a room with good ventilation, sufficient light, quiet, not noisy and the temperature was set at room

temperature around 25°C with humidity around 50%. The cage was cleaned every 3 days. Mice were adapted for 7 days and given a standard diet in the form of standard diet fodder with HI-GRO 552 and vegetables by using disposable sput of 1 ml. The composition of the diet of mice consisted of 17-20% protein, 3-4% fat, 35-40% carbohydrates. For drinking, boiled water was used ad libitum. Drinking water was put in bottles that were hung on the walls of the cage.^{citation smith 1988} Then the 36 male mice were grouped randomly into 2 groups of 18 each.

Data Collection

After completion of the treatment, the control group (which was given 0.3 ml of aquabides given intramuscularly 1x) and the treatment group (which was given 3 mg of DMPA with 0.3 ml given intramuscularly 1x) were taken randomly and then cleaned at the testicular area with 70% alcohol swab. Then euthanasia was performed using a combination of ketamine (50 mg/kg BW) and xylazine (10 mg/kg BW) intramuscularly, then neck dislocation was performed, then the testes were taken and fixed in 10% formalin PBS buffer overnight. The testes were taken for examination of spermatogonia cells and spermatids using HE staining. After all the mice were euthanized and buried properly (buried according to local customs as well as burying humans where at least the mice's bodies were given offerings of canang and accessories) because they could no longer be used for other research.

Data obtained from examination of 60 tubules (30 tubules from the right testicle and 30 tubules from the left testis) for each treatment. Evaluation was carried out at stage VII of the mice spermatogenesis cycle. This examination was carried out in a joint laboratory of the Faculty of Medicine, Udayana University. We observed all sixty tubules for the spermatogonia and spermatid cells.

Data Analysis

We conducted normality (Shapiro-Wilk test) and homogeneity (Leven's test) for all the obtained data to decide which type of analysis to use and find out the variance of the data consecutively. Bivariate analysis was then conducted to find the difference

of the result between controlled and treatment groups, in which significant if $p \leq 0,05$.

RESULTS

The number of spermatogonia and spermatid cells in each group was tested for normality with the results showed that the data were normally distributed ($p > 0,05$, as shown in Table 1). Homogeneity test also showed that the number of spermatogonia cells and spermatid cells in this research were homogeneous ($p > 0,05$, as shown in Table 2).

parability test as a part of bivariate analysis was conducted to differentiate the means of both control and DMPA group. As the data were normally distributed and homogeneous, we used the means of each group's spermatogonia and spermatid cells to compare by T independent test as shown in Table 3 and 4. There was a significant difference between the DMPA group and the control group in terms of the number of spermatogonia cells ($t(34) = -4.359$, $p = 0.000$) and spermatid cells ($t(34) = -4.318$, $p = 0.000$). Based on those results, DMPA administration was

proved to significantly reduce the number of spermatogonia and spermatid cells in the seminal tubules of male mice. The difference in the means of the number of Spermatogonia and Spermatid cells could also be seen in Figure 1 and 2, whereas visualization of the cells under the microscope were also shown in Figure 3.

DISCUSSION

From this experiment, we found that DMPA administration could significantly reduce the number of spermatogonia cells. DMPA is an esterification of progesterone which can suppress the secretion of the pituitary gland so that the production of FSH and LH decreases, by decreasing FSH and LH, the secretion of the hormone testosterone will also decrease. The decrease in the hormone testosterone will disrupt the process of spermatogenesis. Progesterone contained in DMPA will cause the secretion of the FSH hormone to decrease, with the decrease in the FSH hormone causing disruption of the process of mitosis and the proliferation of spermatogonia cells so that the number of spermatogonia cells will also decrease.¹¹

Our results also proved that administration of DMPA could significantly decrease the number of spermatocyte cells. Progesterone contained in DMPA will suppress the pituitary gland so that the FSH and LH hormones decrease. Reducing FSH secretion will cause disruption of Sertoli cells causing disruption of lactate and pyruvate supply. Lactic acid and pyruvate are energy sources for spermatid cells.¹² The decrease in the hormone FSH will disrupt the spermiogenesis process, causing the spermatids to decrease. The decrease in FSH will also cause disruption of the meiotic process so that it causes disruption of spermatids.¹³

The limitation of this research is that we only explored two types of the germ cells, where leydig cell could also be explored. Future research conducted may identify all the three androgen receptors of the spermatid cell, spermatogonia cells and Leydig cells in the seminiferous tubule of male mice.

Table 1. Descriptive analysis of spermatogonia cells in each treatment group.

Treatment group	Mean	SD	Normality (p value)	Homogeneity (p value)
DMPA	9.83	2.333	0.630	0.116
Control	12.78	1.665	0.126	

Table 2. Descriptive analysis of spermatid cells in each treatment group.

Treatment group	Mean	SD	Normality (p value)	Homogeneity (p value)
DMPA	76.50	7.664	0.933	0.494
Control	86.44	6.061	0.667	

Table 3. T independent test of spermatogonia cells.

Treatment group	Mean	SD	t-test for equality of means		p value
			t	df	
DMPA	9.83	2.333	-4.359	34	0.000
Control	12.78	1.665			

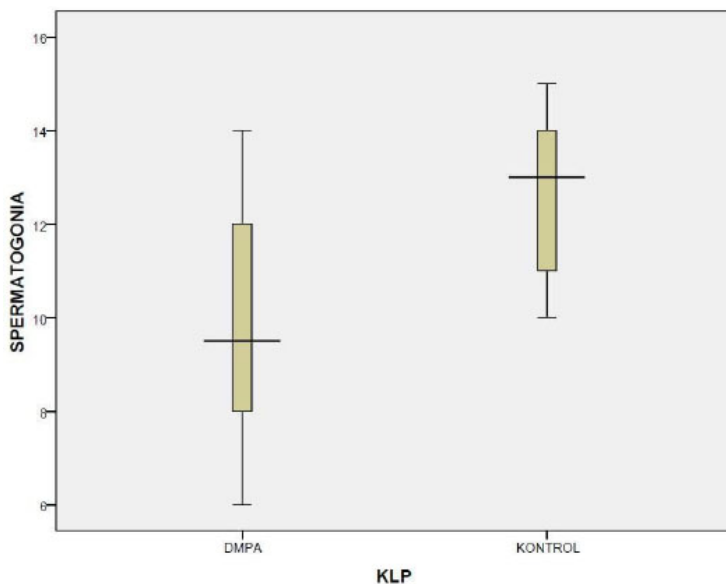
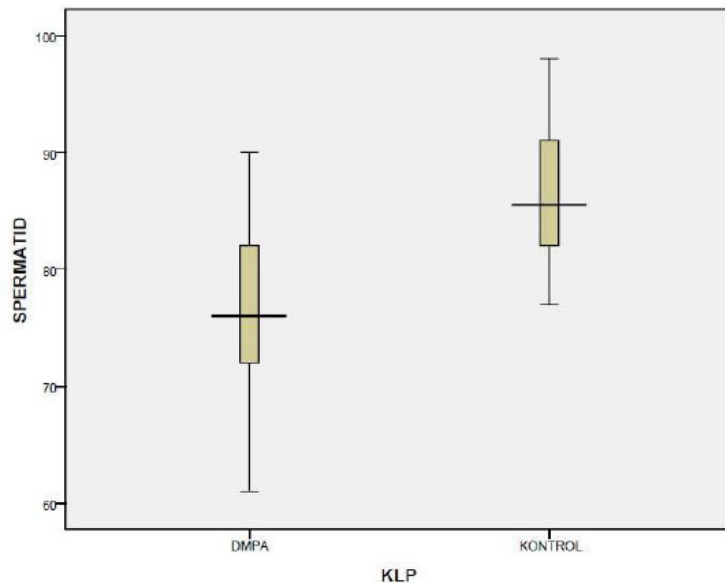
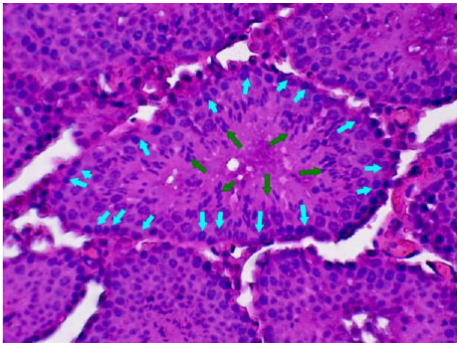
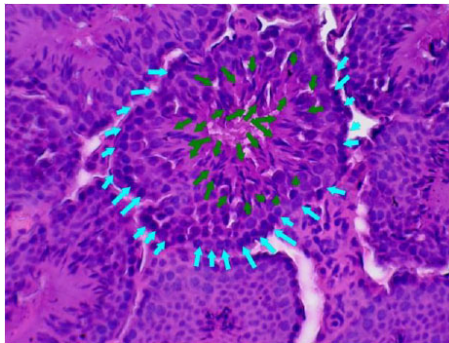


Figure 1. Difference of means of spermatogonia cells shown by box plots of each group.

Table 4. T independent test of spermatid cells.

Treatment group	Mean	SD	t-test for equality of means		p value
			t	df	
DMPA	76.50	7.664	-4.318	34	0.000
Control	86.44	6.061			

**Figure 2.** Difference of means of spermatid cells shown by box plots of each group.**A. DMPA group****B. Control group****Figure 3.** Overview of Spermatogonia Cells and Spermatid Cells Between Groups (400x Magnification). Spermatogonia cells (green arrow) were seen less in the DMPA group than in the control group. Spermatid cells (blue arrow) were seen less in the DMPA group than in the control group. Spermatid cells in the DMPA group also appeared to be less mature.**CONCLUSION**

Administration of DMPA can significantly reduce the number of spermatogonia cells and spermatid cells in the seminiferous tubules of male mice. It is necessary to test

on higher animals such as rabbits before clinical trials on humans are carried out.

AUTHOR CONTRIBUTION

All authors contributed equally in the writing of this manuscript.

ETHICAL STATEMENT

This research protocol was approved by the Ethics Committee of the Faculty of Medicine, Udayana University.

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DISCLOSURE

No conflict of interest was found in this research.

REFERENCES

- Yurnadi, Suryandari, D.A., Moeloek, N. Pengaruh penyuntikan dosis minimal depo medroksiprogesteron asetat (DMPA) terhadap berat badan dan kimia darah tikus jantan galur Sprague-dawley. 2009. 13(2):189-194.
- Moeloek, N. Kontrasepsi Hormonal Pada Pria. Pertemuan Ilmiah Tahunan Persandi V-Pandi XIX, dengan judul Current Hopes in Sexual Dysfunction and Infertility Solution. 27-29 April. Grand Sahid Jaya Hotel Jakarta. 2011.
- Proporsi metode kontrasepsi menurut jenis kelamin, BKKBN. 2014. Dapat diakses di <http://www.depkes.go.id/download.php?file=download/pusdatin/infodatin/infodatin-harganas.pdf>
- Biro Pusat Statistik, Badan Koordinasi Keluarga Berencana Nasional, Depkes, Macro International Inc, Survei Demografi dan Kesehatan Indonesia, 2011: XXIV+228
- Sumaryati, A. "Tahun Ini KB Pria Mulai Dibalakkan". Badan Koordinator Keluarga Berencana Nasional. 2004. Available from: http://www.bkkbn.go.id/article_detail.php. Accessed, 26, Oct. 2011.
- Hartono, H. "Keluarga Berencana dan Kontrasepsi." Pustaka Sinar Harapan. Jakarta. 1996.
- Tjokronegoro, A. "Penyebab dan Patofisiologi Infertilitas Pria." Pertemuan Ilmiah. 2002.
- Johnson, M. and Eviritt. "Essensial Reproduction." 3 nd Edition. Blackwell Sci. Pub. 1990.
- Matsumoto, A.M. "Spermatogenesis" In: Adashi, E.Y., Rock, J.A. Zev. 1996
- Charan J, Kantharia ND. How to calculate sample size in animal studies?. *J Pharmacol Pharmacother.* 2013;4(4):303-306.
- Turek, P.J. "Hypothalamic-Pituitary-Gonadal (HPG) Axis and Control of Spermatogenesis". In: Endocrine Evaluation. Male Reproductive Laboratory Departement of Urology Universitas of California at San Francisco. San Francisco, California. 2005.

12. Ilyas S. Effect of Methanolic *Momordica charantia* Seed Extract and Depot Medroxyprogesterone Acetate (DMPA) to Quantity and Quality of Rat Sperm. *International Journal of Pharm Tech Research*, 2014;6(6):1818.
13. Kansil SE, Kundre R, Bataha Y. Hubungan Penggunaan Kontrasepsi Suntik Depo Medroksiprogesteron Asetat (DMPA) dengan Perubahan Fisiologis pada Wanita Usia Subur (WUS) di Puskesmas Ranomuut Kota Manado. *EJournal Keperawatan (Ekp)*. 2015;3(3):4-6.



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