



# The Number of Leydig Cells, Sertoli Cells, and Spermatogonia are Lower towards a Little Rats that Their Parent Given Genistein during Periconception Period



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## Abstract

The testis was composed by the cells of Leydig, Sertoli, and Spermatogenic. The cells formation itself was able to be disrupted by exposure to an *endocrine disrupting chemical* (EDC) since the prenatal period. The research was intended to prove that Genistein could obstruct the formation of Leydig cells, Sertoli cells, Spermatogenic of rats. The randomized post-test only control group design method was conducted towards white female Wistar rats, 12-13 weeks old, has been had one child, could normally eat and drink. The analysis unit was the child of mother rat treatment group that given Genistein of 10 mg/kg/day and the control group that received distilled water, each of them were 15. The research was done in the Laboratory of Faculty of Veterinary Medical, Udayana University, from January to July 2016. The computer was used for analyzing the data using, with  $\alpha$  of 0.05. The result was the Genistein child had an average of 5.464 Sertoli cell, 11.120 Leydig cell, and 48.427 spermatogonia, whereas, the control group had an average of 8.173 Leydig cells, 12.987 Sertoli cells, and 69.547 spermatogonia. There were significant differences between the two groups ( $p$  0.000). The conclusion was that an average of Leydig cells, Sertoli cells, and Spermatogonia lower in children whose their parent rats given Genistein during periconception period.

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## 1. Introduction

The testis has a very important role in the reproduction process. It is composed based on the seminiferous tubules in which there are Sertoli cells and spermatogenic cells, and interstitial tissue, unlike the Leydig cells. The interaction between these cells may develop effect and the other function cells of the testis. A disorder that occurs in one cell will interfere other function cells. Basic Health Research (BHR) in 2013, was found 15.5% of Reproductive Age Couples (RAC) never use contraception due to they want to have a child (WHO, 2013). An infertility is caused by several factors, including the spermatozoa abnormalities of 25%, and ovulation disorders of 27%, Abnormalities in spermatozoa, including deformities (morphology), movement disorders (motility), and sperm concentration were less (Barbieri, 2014).

The testis forerunner is determined soon after conception. The *gonad indifferent* forming in humans occurs when primordial germ cells at getting the gonad, i.e. the gestation is 4-6 weeks, which is influenced by *Steroidogenic factor 1 (SF1)*. In term of this, the embryonic gonad is identical towards the men or women, thus, it is difficult to distinguish. Furthermore, the sex subsequent differentiation that involves many genes, unlike SRY, Sox 8, 9. The Sertoli cells are first differentiated somatic cells. It is followed by Leydig cells subsequent differentiation (Fritz & Speroff, 2011).

The Sertoli cells are formed at 6 to 7 weeks of pregnancy. These cells combine to form the testis cords. In fetal life, Sertoli cell is functioned maintaining and playing a role in the germ cells differentiation. These cells function is supported by a Testosterone hormone (Sadler, 2014). The fetal Leydig cells synthesize testosterone that is required for masculinization, including primordial germ cell differentiation and development of male sex organs (Erb, 2006; Weinbauer *et al.*, 2010). The Leydig cells of fetal rat begin to emerge and developed when the gestational age of 14,5 days. It begins to secrete testosterone and other sex hormones on 15 days (Delbe *et al.*, 2006).

An Estrogen Receptor (ER) presents towards testis tissue since the fetus period. ER  $\beta$  is in the seminiferous cords for controlling gametogenesis, whereas ER  $\alpha$  found in fetal Leydig cells that regulate steroidogenesis, thus, Estrogen hormones are involved and able to control the testis development and their functions during fetal life and neonates (Delbe *et al.*, 2006). An estrogen as well as regulates the several genes expression that involved in the sex hormones synthesis, unlike StAR protein gene and *CYP11A1* (Craig *et al.*, 2011).

An estrogenic is had by Isoflavones due to it has a chemical formula is very similar to estrogen. Thus, isoflavones are able to bind to estrogen receptors and action to the estrogen target cells. Isoflavones affinity and ER  $\beta$  are stronger than ER  $\alpha$ . Isoflavones can be agonist when estrogen level is low, and antagonistic when high estrogen level (Kim & Park, 2012).

An isoflavones derivatives include Genistein, daidzein, and *glisitein*. The most powerful isoflavone is an *equol* (a metabolite of daidzein) (Patisaul & Whitten, 2005; Bucar, 2013). An ability to animate the estrogen properties causes isoflavones to be one of the compounds endocrine disruptors or *endocrine disrupting chemical (EDC)*. The earlier exposure to EDC, its impact is detrimental. EDC exposure during preimplantation can change three main epigenetic mechanisms, that directs gene expression changes (Uzumcu *et al.*, 2012).

Based on the above exploration, it is necessary to do research on the Genistein effects towards the formation and development of the child testis. This study aims at proving the Genistein effect on the formation of the Leydig cell, Sertoli cells, and spermatogonia at the Wistar rat pups.

## 2. Materials and Methods

The present paper is an experimental study, it is applied the randomized post-test only control group design. The research is conducted at the Pharmacology Laboratory, Laboratory of Veterinary Medical, in January and July 2016. The sample is a female rat Wistar aged 12-13 weeks, weight from 140 to 145 grams, have been childless one time, eating and drinking normally. The sample quantity is 5 animals per group. The analysis unit is the child of the mother who treated, every 3 rats per parent. The Genistein or 5,7,4'-Trihydroxyisoflavone, CAS: 446-72-0, C<sub>15</sub>H<sub>10</sub>O<sub>5</sub> formula, 99% purity is produced by Indofine Chemical Company, Inc., Hillsborough, New Jersey, USA.

The rats are given the standard feed pellets 594 about 15 gram/rat/day and given water to drink from the government water. They were placed in individual cages on the size of 40 cm x 15 cm x 10 cm. The preparations anatomical pathology of testis organ using Hematoxylin-Eosin (HE) staining and observed with a microscope in a 10 view field.

The treatment is given from one week before it is mated, during a pregnant, giving birth, to finish breastfeeding/weaning. After weaning, three male pups per litter chose at random to be sacrificed. The mother and calf were left alive and returned to the initial population after conducted a washing out for 2 weeks.

The euthanasia is conducted before surgery by cervical dislocation. One of the testicles is taken, put in a formalin buffer solution and then made preparations for the Anatomic Pathology (AP) examination. The tissues rest and organs, as well as other surgical garbage, is collected, put into ground holes and burned. The rest subsequent combustion is in the grave.

### 3. Results and Discussions

The treatment is given towards five breeding, then 3 male rats from each parent are sacrificed and the testis was taken for examination (AP). A little rat is dissected at 21 days old. Thus, the analysis unit in this study is 30 testes.

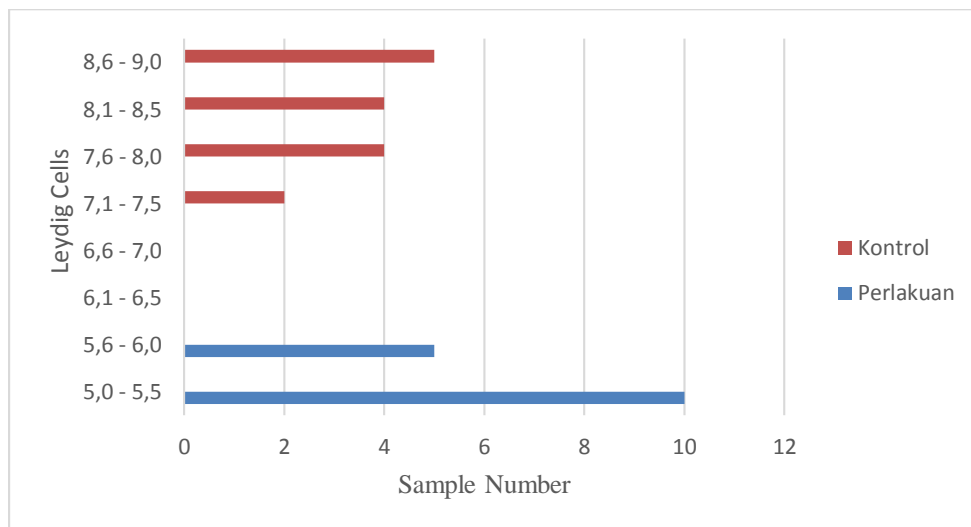


Figure 1. The number of Leydig cells of a little rat

Figure 1. Illustrates of the most of the treatment groups had Leydig cell number between 5.0-5.5. No testis of little rats of their parent in the control group who has Leydig cell count less than 7 cells.

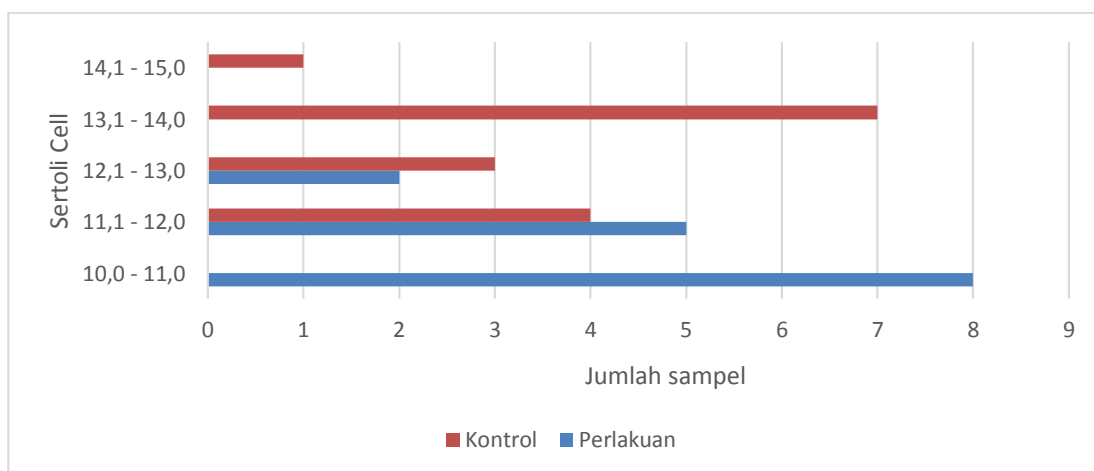


Figure 2. The number of Sertoli cells of a little rat

Figure 2. illustrates that over 50% of the treatment group has a Sertoli cell count less than 11 cells. The most Sertoli cell counts (more than 14 cells) found in the testis of little rats of the control group parent.

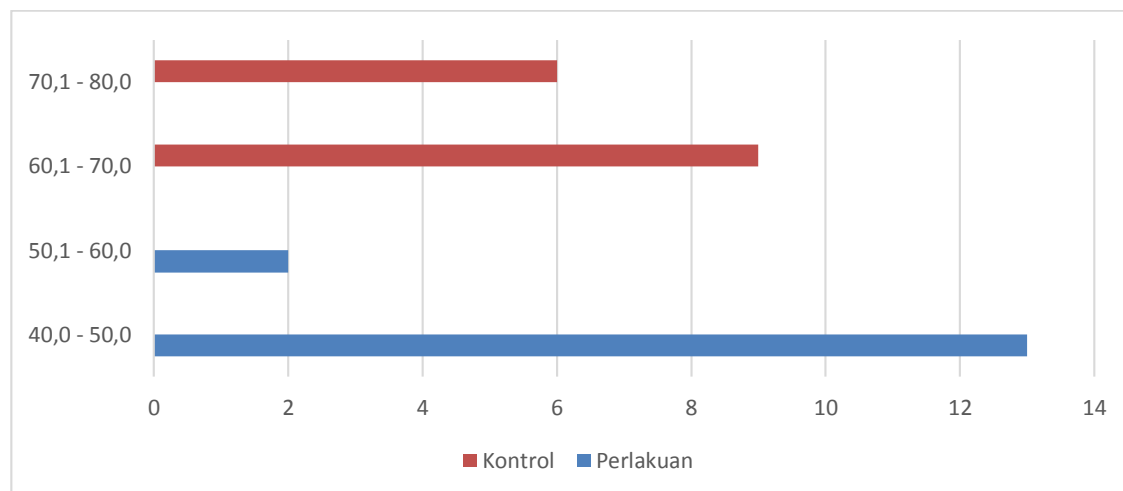


Figure 3. The number of spermatogonia of little rats

The whole a little rats testis that is born by their parent of a control group that appears in Figure 3, it has more than 60 cells spermatogonia. It as well as seems that the majority of little rats to the parent in the treatment group has 50 cells spermatogonia or less.

Table 1

The differences of the average Leydig cell, Sertoli cell, and spermatogonia among the control group with treatment

Group	N	Leydig cell		Sertoli cell		Spermatogonia	
		Average	p	Average	p	Average	p
Control	15	8,173	0,000	12,987	0,000	69,547	0,000
Treatment	15	5,467		11,120		48,427	

Table 1. shows that the average number of Leydig cells, Sertoli cells and spermatogonia was higher in the control group compared with the treatment group. There are significant differences meaning of Leydig cells, Sertoli cells, and spermatogonia between control group with treatment group (p 0.000). This difference shows that the effect is given by Genistein towards the child testicular development, in term of this is the development of Leydig cells, Sertoli cells, and spermatogonia.

### Discussion

Leydig cells, Sertoli cells, and germ cells is a part of testicle constituent, interconnected and influence each other. The Sertoli cell has an ability as a "mother" for the germ cells. Sertoli cells in fetal life serve to provide nutrients and protect germ cells, play a role in the germ cells differentiation, preventing the germ cells enter meiosis. Sertoli cell function is influenced by androgen hormones that are produced by Leydig cells fetus (Huff, 2011). Thus, if it occurs that leads to the germ cells differentiation is as well as disrupted.

Genistein is one of the isoflavone compounds. Genistein has the ability to bind estrogen receptors  $\alpha$  and  $\beta$  is the strongest compared with other isoflavone compounds, except *equol* (Bucar, 2013; Casanova *et al.*, 1999). Genistein as well as has a greater molecular weight and low water solubility, therefore, it is needed longer metabolic processes. This causes alive time in the body becomes longer than before (Larkin, *et al.*, 2008).

Therefore, Genistein is able to animate the endogenous estrogen activities. The estrogen hormone is able to influence the growth and function of male fetal gonads. Endogenous estrogens inhibit testicular development and function during fetal life and neonatus (Delbe *et al.*, 2006). Isoflavones can as well as adjust an endogenous estrogen

concentration by binding or deactivate some enzymes such as P450 aromatase, 5 $\alpha$ -reductase. Isoflavones are able to influence the sex hormones bioavailability to bind or stimulate the synthesis of *sex hormone binding globulin* (SHBG) (Retana-Marquez *et al.*, 2012). Genistein is able to penetrate the placenta (Todaka, *et al.*, 2005). It is as well as found in breast milk. It is to show that the fetus gets exposed isoflavones mother through the placenta and the child by breast milk when future in the lactation period (Franke *et al.*, 2006).

The female white Wistar rats (parent) in this study are given a treatment (Genistein ) since *preconception* period, i.e. one week before they are mated (preconception), during conception (*inter conception*), and for pregnant and breastfeeding period (*pascaconception*). The finding is that the average number of Leydig cells, Sertoli cells, and spermatogonia a little rat that was born in the control group were higher than the treatment group. An average of three variables (Leydig cells, Sertoli cells, and spermatogonia), was found to differ significantly (p 0.000) between control and treatment groups. The number of Leydig cells in the treatment group were a little possibility of causing testosterone hormone synthesis are as well as less.

This condition is able to inhibit the Sertoli cells differentiation so that the number of Sertoli cells is slight. The testosterone hormone that is a bit unable to support the functions of the Sertoli cell as a mother for their germ cells. As a result, the gnosis differentiation becomes spermatogonia to be blocked, therefore, the number of spermatogonia is lower in the treatment group compared with the control group. The results showed that the treatment that is given (Genistein) is able to influence or give an effect of inhibiting the Leydig cells development, Sertoli cells, as well as spermogonia.

The results are consistent with the research on isoflavones supplementation of high doses towards female rats (150 mg/kg a weight/day) during *preconception*, giving birth, a male rats that have a tubular seminiferous is broken / incomplete and spermatogenic cells appear not developed with the image that is not clear, the head of the epididymis atrophy. The medium-dose (100 mg/kg) and low dose (50 mg/kg) showed a condition that is similar to the control group, however, the majority of the seminiferous tubules looked shrink (Lofamia *et al.*, 2014).

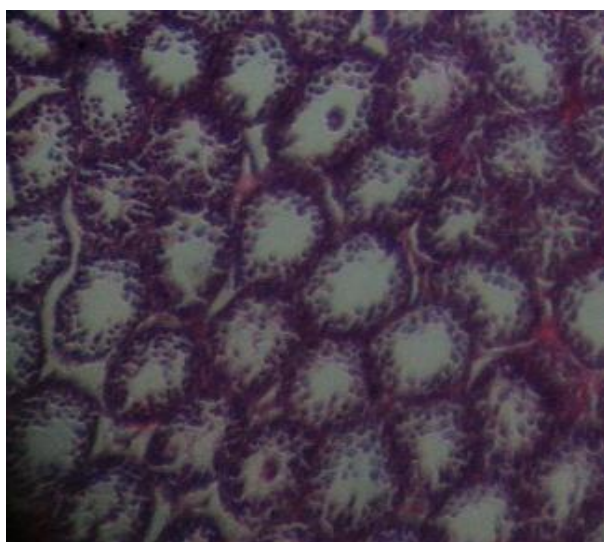


Figure 1.

An overview testis of the little rat of treatment group towards 100 times magnification

It is to show that rarely spreads from Leydig cells (white arrow), Sertoli cells (yellow arrows), and spermatogonia (green arrow)

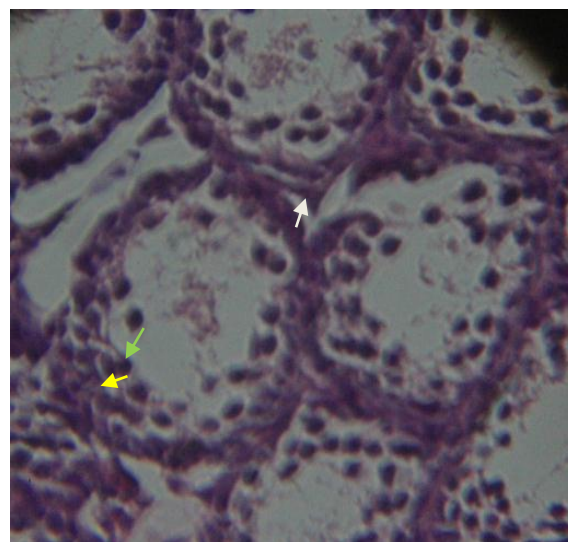


Figure 2.

An overview testis of the little rat of treatment group towards 400 times magnification

It is to show that rarely spreads from Leydig cells (white arrow), Sertoli cells (yellow arrows), and spermatogonia (green arrow)



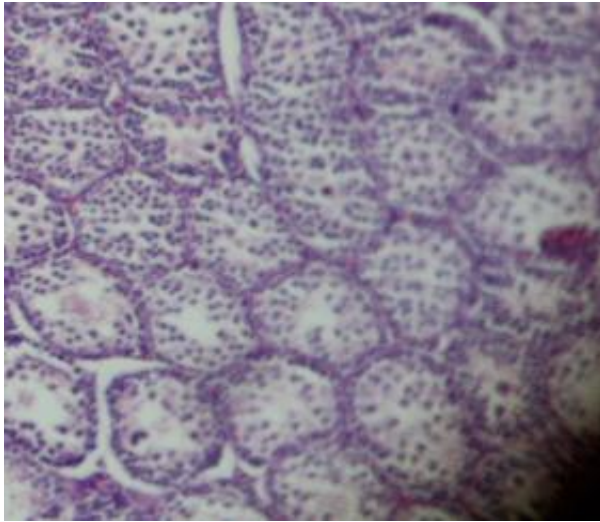


Figure 3.  
An overview testis of the little rat of control group towards 100 times magnification

It is to show that dense distribution of Leydig cells (white arrow), Sertoli cells (yellow arrows), and spermatogonia (green arrow)

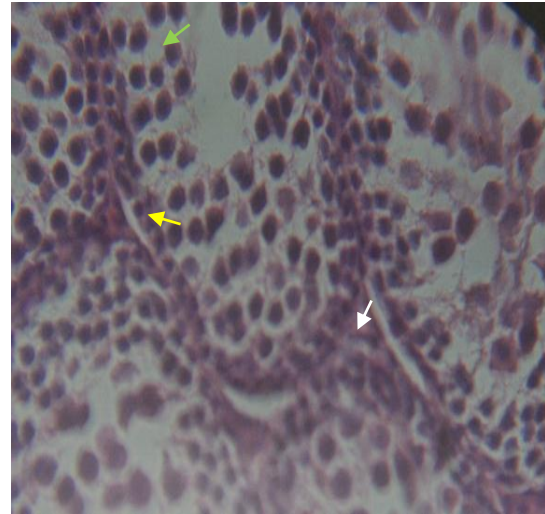


Figure 4.  
An overview testis of the little rat of control group towards 400 times magnification

It is to show that dense distribution of Leydig cells (white arrow), Sertoli cells (yellow arrows), and spermatogonia (green arrow)

#### 4. Conclusion

Provide a statement that what is expected, as stated in the "Introduction" chapter can ultimately result in "Results and Discussion" chapter, so there is compatibility. Moreover, it can also be added to the prospect of the development of research results and application prospects of further studies into the next (based on result and discussion).

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The author(s) declared that (s)he/they have no competing interest. The study was financed by independent funding.

##### *Statement of authorship*

The author(s) have a responsibility for the conception and design of the study. The author(s) have approved the final article.

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The average number of Leydig cells, Sertoli cells, and Spermatogonia towards a little rats testis of the control group were higher than the group which given a Genistein. Thereby, the genistein could inhibit the Leydig cells developing, Sertoli cells and Spermatogonia of little rats.

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