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Increasing Mature Spermatids Formation Using Pasak Bumi

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Abstract

Pasak bumi as a rare plant with eroded status is benefit as aphrodisiac for Borneo men. This study investigate the effect of pasak bumi (*Eurycoma longifolia*) on spermatozoon cell stages development. The research was conducted by giving pasak bumi steeping doses of 18 mg/200 g (=90 mg/kg) of bodyweight (bw) for three days to 15 white male rats as the experiment group, and 15 white male rats also given with distilled water as the control group. Micromorphological analysis was carried out using histological staining of Hematoxylin Eosin (HE) in the seminiferous tubule tissue. The result showed that pasak bumi treatment until day-3 is: 1) clearly the amount of mature spermatids was increased, 2) maintaining the amount of spermatogonia, 3) meanwhile the amount of primary spermatocyte was decreased, in term of related to primary spermatocyte formation. It is concluded that pasak bumi increased mature spermatids formation.

Keywords: Eurycoma longifolia; Pasak bumi; endemic of Borneo; Hematoxylin Eosin; mature spermatids.

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1. Introduction

Borneo traditional people in Indonesia believe that drinking root steeping of pasak bumi (*Eurycoma longifolia*) can increase man's vitality ability [1, 2]. Pasak bumi is known by Indonesian people especially Borneo district having a benefit as anti-fever, anti-malaria, aside from increasing man's libido and stamina, the name of pasak bumi in Malaysia and Vietnam is tongkat ali [3, 4]. So far, Pasak bumi has been taken from the forest without any cultivation, even though its presence in the forest is dwindling [5]. The research of pasak bumi benefit in white male rat libido explains that pasak bumi treatment with the steeping doses of 18 mg/200 g bodyweight (bw) in 1 ml of distilled water increases the highest libido compared to the steeping doses of 100 mg/200 g bw, steeping doses of 200mg/200 g bw, and distilled water control of 1 ml [6]. The steeping treatment doses of 18 mg/200 g bw (=90 mg/kg bw) is the safe doses because it is relatively far below Lethal dose (LD)50 which is 34.65 /kg bw [7]. A preparation having LD50 value greater than 15 g/kg bw is stated as non-toxic practical [8]. Further, pasak bumi treatment to etawa breeding male goat is relatively safe because it does not affect the sugar level, cholesterol, and uric acid in its blood [9].

The use of pasak bumi as aphrodisiac and treatment for man having sexual disorder is strongly caused by the work of chemical substance of pasak bumi activating an inhibition of Rho-kinase (ROCK II). Chemical substances contained in pasak bumi among others are Eurycomanone, Longilactone, and its derivation [10, 11, 12]. ROCK II inhibition causes the blood vessel in corpus cavernosum is clogged so it tightens and enlarge penis muscles [13].

Pasak bumi's work in pituitary anterior cells has been observed through staining method of immunohistochemical antigen anti Luteinizing hormone (LH). The finding through the method is obtained that basophil cells strongly reacted to pasak bumi treatment are the LH producer cells, [10], while Folicle Stimulating Hormone (FSH) producer cells are relatively stable and not increase its production after pasak bumi steeping treatment [14]. Cell activity increase producing intracellular LH hormone in pituitary anterior of white mature male rat significantly occurs (Duncan test $\alpha = 0.05$) after pasak bumi treatment until day-3. Therefore, it is concluded that pasak bumi is the strong trigger in producing LH [10].

LH hormone identified inside activated cells in pituitary anterior has been distributed to the blood on day-3. LH increase occurs will increase the Leydig cells activity to produce testosterone inside blood serum on day-3 of pasak bumi treatment (from average 4.00 ng/ml increases to average 9.73 ng/ml) [15]. The total testosterone level in mature male white rat in normal condition, is varied from the average of 0.5 ng/ml to 5.4 ng/ml [16]

LH has been reported has a role in stages development of sperm cell formation or spermatozoon in spermatogenesis process. In males, the hormone LH secreted by the pituitary gland is responsible for stimulating androgen production in the Leydig cells of the testes. Furthermore, secreted testosterone stimulates sperm production until, when the level of this hormone increases to a certain concentration, a signal is sent to the brain to stop producing more LH. Through this mechanism, the processes of steroidogenesis and spermatogenesis are perfectly regulated. [17,18]. However, only a few researches explaining which spermatozoon cell form stages influenced by LH. Based on the need, it needs to be explained further how pasak bumi performance increasing

LH in spermatozoon form stages in spermatogenesis process. The research was conducted by analyzing pasak bumi performance in spermatogenesis stage development inside seminiferous tubules of male white rat's testis. Spermatozoon cell form stages in spermatogenesis observed and calculated is display; spermatogonia (SPG), primary spermatocyte (PSPT), and mature spermatids (MSPD).

2. Method

2.1 Field research

The research was conducted in Histology Laboratory of Anatomy Department, Physiology, and Pharmacology, Veterinary Medicine Faculty of IPB Dramaga Bogor. White rat breeding was carried out in experimental animal cage in Animal Hospital of IPB Dramaga Bogor for 2 weeks.

Experimental animal using mature male white rat of Rattus norvegicus strain Sprague Dawley in age of 3 ½ months with the weight of 121-194 grams.

The rats were obtained from Experimental Animal Laboratory of Indonesia drug and food control center Institution in amount of 30 rats, for treatment with the following details: fifteen mature male white rats with pasak bumi treatment to be sacrificed respectively 5 rats (n=repetition=5) on day-1 and 3 using ketamil anesthesia. Fifteen male white rats were used as the control group by giving distilled water which also sacrificed 10 rats respectively 5 each on day 1^{st} and day 3^{rd} . One female mouse in estrus condition from 5 female rats supply were chosen to be used as seducer. Before given by pasak bumi treatment, the white rats were adapted in experimental animal cage in anatomy and histology laboratory of Animal Medical Faculty of Bogor Agricultural Institute. Adaptation time for each one is 2 weeks. During the research, the rats were given feed and drink of ad libitum.

Pasak bumi (*Eurycomae longifolia* Jack) simplicial making is the root part was skinned like any other herb powder making [19, 20].

The wood part was cut into the size of 7 cm length, 2 cm wide, and 2 cm thickness. The pieces were dried in oven with 50^{o}C temperature for 5 days, then cut into small pieces, and ground until smooth using special grinding mill, and filtered with 50 Mesh sieves (1 inch contains 50 holes, with pore size = 300 µm). The bext simplicia is stored in jars inside dry cupbard.

2.2 Data sampling

2.2.1 Determining Pasak Bumi in Spermatogenesis Stage Development

Spermatogenesis stage development inside seminiferous tubules was learned by an approach using HE staining in transverse piece preparat of rat testis. Testicular tissue sample taken on day 1^{st} and day 3^{rd} was performed with the following design, table 1:

Table 1: Activity design before testicular tissue samples were taken.

Time	At 9.00 WIT	5 hours interval	At 14.00 Western Indonesian Time (WIT)	At 14.15 (WIT)
Day-1, 2, 3	1.Experiment group: pasak bumi treatment 2.Control group: distilled water treatment	♂ Rat taking a break	Libido seduction for 10 minutes, by estrous female rat, inside cage with wire mesh screen.	sampling testis day 1st day 3rd

The treatment with female estrous rat seducer was performed at 14.00, after 10 minutes it was continued with testis tissue sampling. Before testis tissue sampling, the white rat were intraperitoneal anesthetized with Ketamine of 100 mg/ml with 0.2 cc doses for 100-200 g of body weight (bw). The use of rat sacrificed for tissue sampling is as follows:

Day 1st → 15 rats were given pasak bumi, 5 of them were sacrificed for testis sampling.

Day 2nd → 10 rats were given pasak bumi.

Day 3rd → 10 rats were given pasak bumi, 5 of them were sacrificed for testis sampling.

As control, 1 ml of distilled water was given per oral per one white rat, with repetition of 5 rat (n=5). Testicular tissue sampling of control group rat was performed like the one in treatment group, which is on day 1st and day 3rd . All testicular tissue samples obtained were fixated in 4% formaldehyde solution in 0.9% physiological NaCl stored for 1 week then the next stage is to analyze histologically micromorphology using Hematixilin Eosin (HE) staining.

2.2.2 Micromorphology Analysis of Seminiferous Tubules

Testicular tissue sample obtained, was sliced with a blade, the middle part piece was taken transversely in 1-2 mm thickness, and fixated for 1 week. Next, it is dehydrated with a set of multilevel alcohol for 4 days. Then it was cleared with a set of xylol and blocked with paraffin histo. The tissue blocked was stored in refrigerator for several days, then it was sliced with microtome device carefully in 4-5 μm size.

Micromorphology analysis of seminiferous tubules content inside testis especially to determine spermatozoon cells form stages in spermatogenesis development using HE histochemical staining following Kiernan's [21] method. Spermatozoon cells observed and calculated includes: spermatogonia quantity (SPG), primary spermatocyte (PSPT) quantity, and mature spermatids (MSPD) quantity, the display shape of spermatozoon cell stage is like in the figure 1.

2.3 Data Analysis

Calculation of spermatozoon stage cell quantity was performed to 10 fields of view from a number of slides

chosen from each treatment group, so that n=10 for each treatment group.

Then statistical analysis by SPPSS 20 program was performed by comparing the calculation result of spermatozoon cell stage form between control group and pasak bumi treatment group, using Duncan test (α=5%).

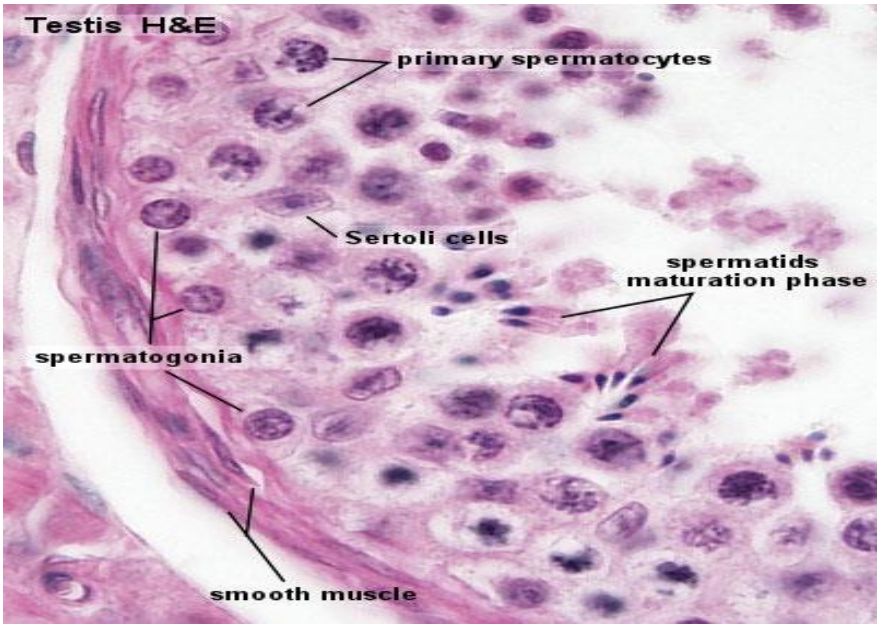

Figure 1: Spermatozoon cells form stage in spermatogenesis process inside seminiferous tubules [22].

3. Result And Discussion

Another approach to find out the pasak bumi mechanism that overcomes erectile dysfunction is the binding of the ligand interaction molecule with the receptor.

Ligands derived from pasakbumi are 5-methoxycanthin-6-one, 9-methoxycanthin-6-one, eurycomalactone, eurycomalide A, eurycomanone, eurycomaoside, laurycolactone A, longilactone, niloticin.

The best result from the mooring of the pasak bumi molecule is niloticin, its interaction value on the PDE5 (human Phosphodiesterase 5) receptor is 93.71% from Sildenafil, Sildenafil is a chemical compound from Viagra [23]. After explaining how the alleged mechanism of the pasak bumi is in overcoming erectile dysfunction, it is further necessary to explain the effect of the pasak bumi on the process of spermatogenesis stages.

Spermatogenesis stage inside seminiferous tubules observed and calculated after pasak bumi steeping treatment of 18 mg/200g bw in 1 ml distilled water and control on day 1st and day 3rd is: spermatogonia (SPG), primary spermatocyte (PSPT), and mature spermatids (MSPD). Spermatozoa cells stage profile inside seminiferous tubules after treatment is shown in Table 1.

Table 1: Quantity of spermatogenesis stage cells after treatment on day 1st to day 3rd.

No	Treatment	Spermatozoon stage cell quantity (Average±SD)		
		SPG	PSPT	MSPD
1	Distilled water day 1st	25,2±3,9 a	61,4±5,4 a	51,1± 8,7 a
2	Distilled water day 3rd	28,4±4,2 a b	56,8±5,4 a	29,3± 2,8 b
3	Pasak bumi day 1st	33,8±3,6 a b	34,1±3,5 b	52,1±7,7 a
4	Pasak bumi day 3rd	39,7±5,0 b	31,4±4,6 b	91,1± 7,6 c

Description: different superscript small letter in the same column states significant difference on 5% level, Duncan Test $\alpha = 0.05$. Spermatogonia (SPG), Primary spermatocyte (PSPT), and mature Spermatids phase (MSPD)

Based on the data in Table 1, spermatogenesis development on the stage of mature spermatids form, it can be seen that mature spermatids quantity is increased significantly in spermatogenesis development after pasak bumi treatment from day 1st to day 3rd , which is 52.0 cell becoming 91.0 cell compared to control (Duncan test $\alpha = 0.05$). Mature spermatids form quantity in control group decreased significantly from day 1st to day 3rd (51.0 cell becomes 29.2 cell).

Mature spermatids quantity in control group from day 1st to day 3rd is decreased, this might be because a number of mature spermatids move from seminiferous tubules to epididymis. Meanwhile, there is an increase of mature spermatids quantity on pasak bumi treatment from day 1st to day 3rd significantly might be caused by the formation process of secondary spermatocyte to spermatids. In relation to an increase of LH cell activity affected by pasak bumi treatment up to day 3rd from previous finding [10], then it is expected that mature spermatids formation is affected by LH performance. Pasak bumi roots contain several active compounds, such as canthin, β-carboline alkaloids, derivatives of squalene tirucallane triterpenes, biphenylneolignans, and quassinoids [24, 25].

The possibility was suggested the compound of the root of pasak bumi such as: eurycomanone and longilactone or eurycomalactone in figure 2 serves as Gonadotropin releasing hormone-LH (GnRH-LH).

The chemical structure of eurycomanone and longilactone is somewhat similar to that of testosterone. While testosterone tied by Androgen Binding Protein (ABP) is carried out to lumen of seminiferous tubules stimulating meiosis process generally and spermiogenesis [26].

The process of spermatogenesis in the testes is especially safe because there is BTB.

The blood-testis barrier (BTB) in mammals, such as the male white rat, consists of a tight junction (TJ), basal ectoplasmic specialization (basal ES), basal tubulobulbar complex (basal tuberculosis) (both of which are testis-specific actin). by type of adherent junction [AJ]), and desmosome-like junctions that are present side by side in the seminiferous epithelium. BTB physically divides the seminiferous epithelium into basal and apical (or adluminal) compartments, and is critical for spermatogenesis [26].

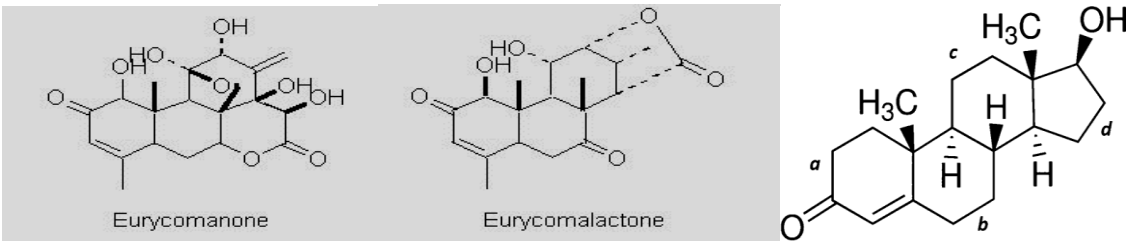

Figure 2: Chemical structure of Eurycomanone, Eurycomalactone, and Testosteron [27, 28, 29].

Based on the data in Table 1, spermatogenesis development in primary spermatocyte stage, it can be seen that primary spermatocyte cell quantity in pasak bumi treatment group decreased significantly. Spermatocyte cell quantity decrease from day 1st to day 3rd of treatment (34.2 cell becoming 31.3 cell) is significantly different compared to control group (61.5 becoming 56.9 cell). This phenomenon explains that pasak bumi encourages the formation/escalation process of mature spermatids because a part of it has become secondary spermatocyte. Mature spermatids stage comes from initial young spermatids stimulated to develop more actively after pasak bumi treatment up to day 3rd. Data on Table 1 explaining spermatogonia stage, it can be seen that there is an increase if spermatogonia quantity significantly occurs on day 3rd (39.6 cell) compared to control on day 1st (25.1 cell) but not significantly different from the control treatment on day 3rd (28.5 cell) and pasak bumi on day 1st (33.7 cell). However, relatively there is a little increase of spermatogonia in pasak bumi treatment up to day 3rd compared to control but not significantly different statistically. Pasak bumi treatment in spermatogonia development stage, does not have an effect in increasing spermatogonia quantity. However, pasak bumi maintains the quantity or stable existence of spermatogonia compared to control.

Spermatogenesis process cycle in white rat occurs approximately every 12 days inside seminiferous tubules, including the stage of: spermatogonia, primary spermatocyte, secondary spermatocyte, early spermatids, mature spermatids. This period is ¼ of the actual time needed in complete spermatogenesis process of male rat generally (48 days) to produce becoming mature spermatozoon, to be released for fertilization function after copulation occurs [30]. Complete spermatogenesis process in human and male animal occurs in testis (seminiferous tubules) and epididymis requiring the as shown in Table 2.

Table 2: Period of spermatogenesis process in some species [30].

Species	Time for complete spermatogenesis (days)	Time for spermatogenesis cycle in Seminiferous tubules (days)
Male	64	16
Male cow	54	13,5
Male goat	49	12,25
Male pig	34	8,5
Male rat	48	12

In relation with research in the previous stage, it was found that pasak bumi treatment up to day 3rd with the steeping doses of 18 mg/20 g bw, significantly increases pituitary cells activity producing LH hormone compared to control. Experiments on anterior pituitary cells of male white rats that produce LH were observed

through histological staining techniques, increased activity after administration of pasak bumi infusion [10]. Besides, it was also found that pasak bumi treatment up to day 3rd significantly increases the testosterone hormone level in blood serum [15]. This research explains that pasak bumi activate mature spermatids formation increase in seminiferous tubules influenced by the increase of testosterone serum by Leydig cells. Testosterone hormone directly affects an actively running spermatogenesis process [31]. Whereas, pituitary cells producing FSH is maintained so that it affects in seminiferous tubules by not increasing spermatogonia quantity and relatively the same as control [14].

In line with the finding, it is known that FSH and LH control spermatogenesis process separately. FSH shows significant effect on the increasing of secondary spermatocyte quantity and spermatogonia maturity. While the significant increase of changes becoming mature spermatids is influenced by LH [32], the mechanism of the changes is unclear. FSH level is associated to spermatogenesis stage development based on male rat age, Lee and his colleagues [33] found that in male Sprague Dawley rat of age 0-5 days have high/increased FSH level (600 ng/ml) function to increase spermatogonia formation from spermatogonia candidate cell. FSH level is decreased when male rat in age of 11-15 days (282ng/ml), at that time the spermatogenesis histology display in seminiferous tubules does not change compared to control. FSH level becomes high/increase again (421 ng/ml) in 26-30 days old rat and function to increase secondary spermatocyte formation to be early spermatids. While mature spermatids formation and spermiogenesis (spermatozoon release process to lumen) is stimulated by LH [32]. Then for 51-60 days old rat show a decreased FSH level (193 ng/ml) and it is related to the decrease of spermatozoa quantity because most of them have been released from seminiferous tubules [33, 34].

Spermatogenesis stage development in testicular seminiferous tubules in control group on day 3rd using HE (figure 3) staining method, and in pasak bumi treatment group on day 3rd (figure 4), the display is as follows:

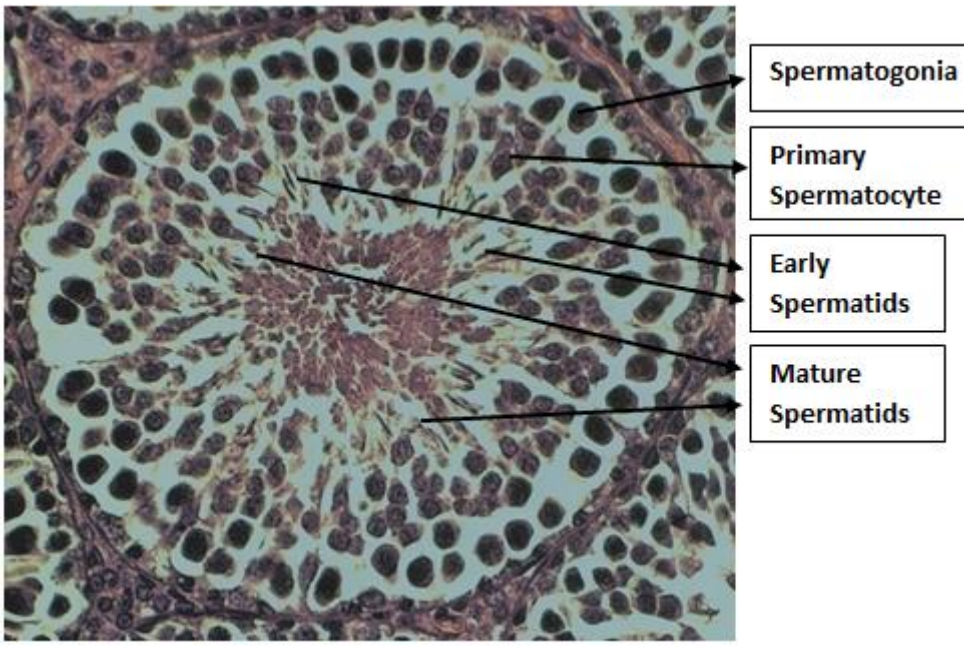

Figure 3: Spermatogenesis stage development in testicular seminiferous tubules in control on day 3rd (HE staining method).

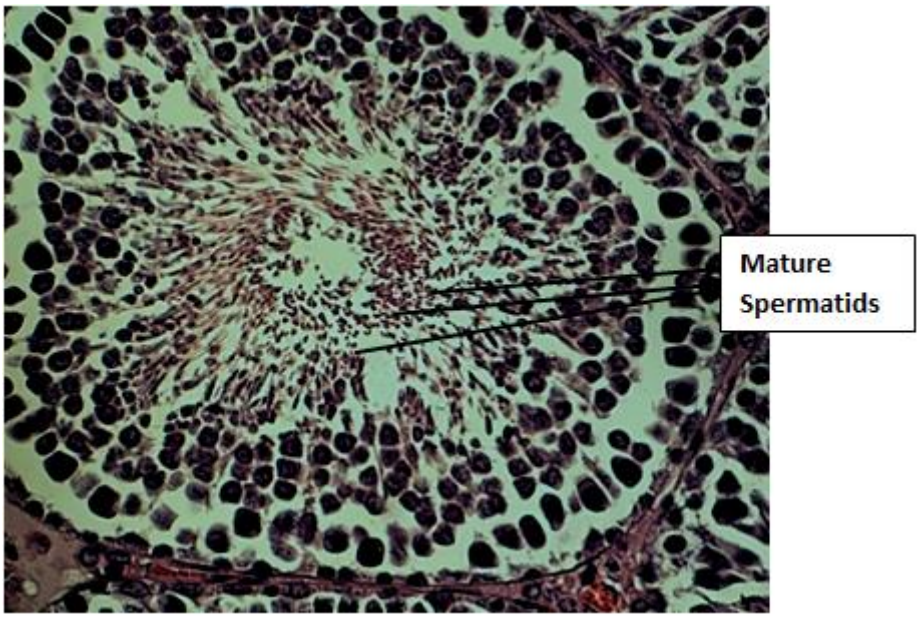

Figure 4: Spermatogenesis stage development in testicular seminiferous tubules in pasak bumi treatment on day-3. There is a significant increase of mature spermatids quantity compared to control on day 3rd . (HE staining method).

An increase in mature spermatids in this study, supports the results of research on pasak bumi treatment on Etawa crossbreed male goats (EC) [34]. A study aims to determine the performance of pasak bumi (*Eurycoma longifolia* Jack) administration on testosterone levels and quality spermatozoa of Etawa crossbreed goats (EC) for six days. The control group (k1) was given distilled water, and group 2 (k2) was given a dose infusion of pasak bumi 90 mg/kg body weight in 20 ml of distilled water orally every morning at 9:00. Testosterone concentration measured on days 1, 3, and 6 using the enzyme-linked immunosorbent assay (ELISA) method. Researcher also observed the quality of spermatozoa includes concentration and the percentage of live spermatozoa. The results showed that testosterone levels in k2 increased from day 1 to day 6th. The concentration of spermatozoa did not increase significantly on k2 day 1 to day 6 compared with k1. The percentage of live spermatozoa increased on k2 compared to k1 on day 3 until the 6th day. The research was concluded the administration of 90 mg/kg body weight in 20 ml of distilled water for 6 days can increase testosterone hormone levels and the percentage of live spermatozoa, although it did not increase the spermatozoa concentration of EC male goats [34].

4.Conclusion

Pasak bumi treatment once a day for 3 days encourages spermatogenesis process, especially in mature spermatids formation significantly (Duncan test $\alpha = 0.05$). In spermatogonia formation stage inside seminiferous tubules, pasak bumi maintains the spermatogonia quantity up to day 3rd equal compared to control.

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