



**The Administration of Epididymis Extract Increased the Testosterone Concentration without Affects the Dihydrotestosterone Concentration in Local Male Goat**

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**Abstract**

This study was aimed to determine the effect of epididymis extract (EE) on the testosterone and dihydrotestosterone (DHT) level of local male goat. An experimental study was performed using a completely randomized design (CRD) pattern of one-way analysis of variance (ANOVA). 15 local male goats aged 1.5 years with body weight 14-16 kg were used in this study. The K0 group as a control group, injected with only 1 ml physiological saline, while each KP1, KP2, KP3, and KP4 groups treated with multilevel EE dose, ie 1, 2, 3, and 4 ml / goat for 13 consecutive days. At the end of treatment (day 14<sup>th</sup>), testes, epididymis (caput, corpus, and cauda) and ductus deferens samples were taken through the close-castration method for examining the testosterone and DHT concentration by using enzyme-linked immunosorbent assay (ELISA) technique. Data gathered were later analyzed using ANOVA followed by Tukey's HSD in SPSS 16.0 for Windows. The result showed that the average concentration of testosterone on K0, KP1, KP2, KP3, and KP 4 in testis respectively were 10.00±2.64 ng/ml; 7.66±2.51 ng/ml; 10.00±6.55 ng/ml; 0.66±0.57 ng/ml; 11.66±7.37 ng/ml; caput epididymis; 5.00±1.73 ng/ml; 2.33±1.52 ng/ml; 5.00±2.64 ng/ml; 1.33±0.57 ng/ml; 5.66±1.15 ng/ml; corpus epididymis; 1.33±0.57 ng/ml; 0.66±0.57 ng/ml; 4.00±2.64 ng/ml; 0.66±0.57 ng/ml; 4.33±2.30 ng/ml; cauda epididymis: 1.00±0.00 ng/ml; 0.66±0.57 ng/ml; 1.66±0.57 ng/ml; 1.00 ± 0.00 ng/ml; 2.00±1.73 ng/ml; ductus deferens: 3.66±2.51 ng/ml; 0.66±0.57 ng/ml; 3.00±1.00 ng/ml; 1.00±0.00 ng/ml and 3.66±1.15 ng/ml. While the average concentration of DHT on K0, KP1, KP2, KP3, and KP 4 in testis respectively; 10.00±2.64 ng/ml; 7.66±2.51 ng/ml; 10.00±6.55 ng/ml; 0.66±0.57 ng/ml; 11.66±7.37 ng/ml; caput epididymis; 5.00±1.73 ng/ml; 2.33±1.52 ng/ml; 5.00±2.64 ng/ml; 1.33±0.57 ng/ml; 5.66±1.15 ng/ml; corpus epididymis; 1.33±0.57 ng/ml; 0.66±0.57 ng/ml; 4.00±2.64 ng/ml; 0.66±0.57 ng/ml; 4.33±2.30 ng/ml; cauda epididymis: 1.00±0.00 ng/ml; 0.66±0.57 ng/ml; 1.66±0.57 ng/ml; 1.00 ± 0.00 ng/ml; 2.00±1.73 ng/ml; ductus deferens: 3.66±2.51 ng/ml; 0.66±0.57 ng/ml; 3.00±1.00 ng/ml; 1.00±0.00 ng/ml and 3.66±1.15 ng/ml. Statistical analysis showed that the administration of EE only increased testosterone concentration in testes had significant effect ( $P < 0.05$ ). From this study, it can be concluded that the EE has the potential to improve spermatogenesis and sperm quality through increasing the testosterone concentration in the local male goats.

*Key words : epididymis extract, testosterone, dihydrotestosterone, spermatogenesis, fertility*

**Background**

Spermatogenesis in mammals is a complex process when the spermatogonia undergone differentiation in their step to produce spermatozoa to be released into tubule seminiferous lumen at stage VIII from the cycle of seminiferous epithel. The process is started from a spermatogonium and finalised with the production of 256 spermatozoa within 74 days in human and 58 days in mice (Mruk & Cheng, 2011).

Testosterone (androgen) is a hormon that very vital for spermatogenesis process (Ruwanpura *et al.*, 2010). Androgen is a steroid hormon which important in

determining the expression of male phenotype, the development of secondary sex, initiation, and maintaining spermatogenesis (Wang *et al.*, 2009<sup>a</sup>). Androgen is one of family of main steroids hormone in human, they are progesterone, androgen, oestrogen, glucocorticoids, and mineralocorticoids. The group of androgen is comprising of four main hormon, they are dihydrotestosterone (DHT), testosterone, androstenedione, and dehydroepiandrosterone (Morales *et al.*, 2004). The impairment of testosterone secretion are affected the formation of blood-testis barrier (BTB), meiosis and a

failure for maturation of germinal cells, as well as spermiation, that finally resulted in impairment of spermatogenesis and infertility in male (Walker, 2010).

*Dihydrotestosterone* is a metabolite of testosterone (Meachem *et al.*, 2007) and nature androgen that the most potent in human (Marchetti & Barth, 2013), that plays important role for the development of male reproductive tract (Ketelslegers *et al.*, 1978). Nowadays, DHT is obtaining a high attention for research of male reproductive aspect, especially since the role of DHT for the development of of primary and secondary of sexual characteristic. Then, it is also importance for indicator of the development of initial prostate and breast cancer as well as for Alzheimer disease (Marchetti & Barth, 2013).

Testicular spermatozoa is functionally immature and infertile. During the transit of spermatozoa in epididymis, then resulted in a changes of specific maturity for their fertilization. Among the changes are morphology, motility, chemistry, permeability, density, and metabolism (Cosentino & Cockett, 1986).

The latest research is showed that the epididymis containing proteins or molecules that important for spermatozoa maturity. Sipilä *et al.* (2009) was found *G protein-coupled receptor 64* (GPR64), *Human sperm-associated antigen 11e* (SPAG11e), *Cysteine-rich secretory proteins-1* (CRISP1), *carbonyl reductase P34H*, *cluster of differentiation 52* (CD52), and *Beta-defensin126* (DEFB126) in the epididymis. Then, Tanii *et al.* (2011) reported that the acrosome of spermatozoa is containing of *pituitary adenylate cyclase-activating polypeptide* (PACAP). Therefore, that means the epididymis is rich with PACAP and related with its role as the location for spermatozoa maturation.

Furthermore, Akmal *et al.*, (2014) was found that the administration of Epididymis Extract (EE) could increased the concentration of testosterone, follicle stimulating hormone (FSH), and luteinizing hormone (LH) in the blood serum as well as the expression of follicle-stimulating hormone receptor (FSHR) in the tissue of tubuli seminiferi of testis (Akmal *et al.*,

2015<sup>a</sup>), followed by estrogen concentration (Akmal *et al.*, 2016). Finally, it will affect to the increase of quality of the spermatozoa of male local goat (Akmal *et al.*, 2015<sup>b</sup>). This research was aimed to study the effect of administration of EE to the increase of testosterone and DHT in the testis, caput, corpus, and cauda epididymis, as well as ductus deferens in male local goat.

## Materials and Methods

### Location

This experiment was conducted in Lubok Sukon Village of Subdistrict of Ingin Jaya, District of Aceh Besar. The sample analysis was conducted in Research Laboratory of The Faculty of Veterinary Medicine of Syiah Kuala University, Darussalam, Banda Aceh.

### Animal Model

Goats of age of 1.5 years old were used as animal model in this research. The average body weight of the animals was at 14-16 Kg. During the experimental period, the goats were given fresh forage and water *ad lib*.

### Experiment Materials

The materials used in this study were comprised of *plastic wrap*, knife, centrifuge machine, gloves, mask, *cool box*, *ice pack*, *hot plate*, digital weighing, baker glass, label, *multi tube vortexer*, digital pipette, shaker, automatic pipette, measurement glass, *staining jar*, *microtip*, *microtube*, storage for organs, ELISA *washer*, ELISA *reader*, and computer package. The substances were used comprised of NaCl 0,9%, aquadest, aquabidest, solution of *phosphate buffered saline* (PBS), testosterone ELISA Testosteron(e) DRG EIA1559, DRG instruments Gmbh, Marburg. Then, DHT ELISA DRG. EIA-4132, USA, which comprised of *standart*, *enzyme conjugate*, *control*, *substrate solution*, *stop solution* (H<sub>2</sub>SO<sub>4</sub>), and *wash solution*.

### Research Procedure

#### Preparation of Epididymis Extract (EE).

The preparation of EE was refered to the method by Akmal *et al.* (2014) as

follow: the testis of male local goat collected from the Abattoir of City of Banda Aceh was taken to the Laboratory of Histology of The Faculty of Veterinary Medicine of Syiah Kuala University. Then, this sample was soaked in the water for ease in separation between the testis and epididymis. The obtained epididymis then was sliced to a smaller size and weighed. Then, it was grinded and added with aquabides of 10 ml per gram of epididymis. Next, it was filtered with a filter paper. The obtained solution of EE, then was centrifugated at a speed of 3000 RPM for 20 minutes. Finally, the supernatant was taken and stored into freezer.

### **Animal Treatment**

This experiment were used a number of 15 male local goats of 1.5 years old. The average weight of them were at 14-16 kg. Their were divided into 5 groups, namely K0, KP1, KP2, KP3, and KP4. K0 as a control group was only given of 1 ml NaCl. Then, the groups of KP1, KP2, KP3, and KP4 were administered with different doses of EE at 1 ml, 2 ml, 3 ml, and 4 ml per goat for 13 days, consecutively. After completed the treatment, at day 14 all animals were castrated to collect testis, epididymis epididimis (capute, corpus, and cauda), and ductus defferens.

### **Procedure for Extraction of the Culture of Testis, Epididymis, and Ductus Defferens**

Then, for all collected samples were extracted and measured for the concentration of testosterone and DHT using a method of *enzyme-linked immunosorbent assay* (ELISA). The procedure for extraction of of these samples was a modification of a method by Cusabio (2015). Firstly, the tissue from each sample was collected and taken at 100 mg, and homogenised as well as washed using 1 ml *phosphate buffer saline* (PBS) for one time. Next, the processed samples were stored into freezer at -20°C. Then, those samples were thawed using freezer thawing method for twice and centrifugated for 10 minutes using a speed of 1200 RPM at a temperature of 2-8°C. The obtained supernatants were stored at -

2°C. After that, the concentration of testosterone and DHT were measured.

### **Measurement of concentration of testosterone and DHT**

The available sample were diluted using aquabidestillata at ratio of 1:4. Then, the standard solution of 0.2 ng/ml to 16 ng/ml were prepared and put into microplate ELISA well of 25 µL, followed by adding conjugate enzym, except to blank well. Next, all samples were covered with cling film and homogenized by a slow shaking for 10 seconds as well as incubated for 60 minutes under room temperature. Each microplate was washed using a solution of 300 µL for 3 to 4 times. The substrate of 200 µL was added into each of well and covered with cling film as well as incubated for 15 minutes under room temperature. The enzymatic reaction was stopped by adding a stop solution of 0.5 M H<sub>2</sub>SO<sub>4</sub> at 100 µL to each of well. The reading of absorbance was conducted using ELISA at wavelength of 450 nm

### **Data Analysis**

Data of measurement of estrogen concentration was analysed using analysis of varians (ANOVA). The analysis for significant different of treatment was continued with test of Tukey HSD. Both statistical analyses were conducted using program software SPSS 16.0 for windows.

### **Results and Discussion**

The results of measurement of testosterone concentration in local male goats after treatment with EE is showed in Table 1. Then, the results of measurement for DHT concentration is in Table 2.

Table 1. Average (±SD) of testosterone concentration (ng/ml) in the testis, epididymis, and ductus deferens after treatment with EE in local male goats.

Treatment groups	Testis	Caput Epididymis	Corpus Epididymis	Cauda Epididymis	Ductus Deferens
K0	23.33 ± 15.94 <sup>a</sup>	3.66 ± 3.51 <sup>a</sup>	2.66 ± 2.50 <sup>a</sup>	2.33 ± 2.08 <sup>a</sup>	7.66 ± 2.50 <sup>a</sup>
KP1	20.66 ± 14.84 <sup>ab</sup>	3.00 ± 0.00 <sup>a</sup>	3.66 ± 0.57 <sup>a</sup>	3.33 ± 0.57 <sup>a</sup>	3.00 ± 1.00 <sup>a</sup>
KP2	19.33 ± 8.62 <sup>ab</sup>	5.33 ± 1.52 <sup>a</sup>	6.33 ± 3.21 <sup>a</sup>	4.66 ± 0.57 <sup>a</sup>	7.66 ± 2.88 <sup>a</sup>
KP3	4.33 ± 3.21 <sup>ab</sup>	3.00 ± 1.00 <sup>a</sup>	4.00 ± 1.00 <sup>a</sup>	3.66 ± 2.08 <sup>a</sup>	4.33 ± 1.52 <sup>a</sup>
KP4	65.00 ± 42.79 <sup>c</sup>	5.33 ± 2.51 <sup>a</sup>	5.66 ± 4.04 <sup>a</sup>	4.00 ± 1.00 <sup>a</sup>	8.66 ± 6.42 <sup>a</sup>

Values with different superscript (a-c) in the same column differ significantly (p < 0.05).

Table 2. Average (±SD) of DHT concentration (ng/ml) in the testis, epididymis, and ductus deferens after treatment with EE in local male goats.

Treatment groups	Testis	Caput Epididymis	Corpus Epididymis	Cauda Epididymis	Ductus Deferens
K0	10.00 ± 2.64 <sup>a</sup>	5.00 ± 1.73 <sup>a</sup>	1.33 ± 0.57 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	3.66 ± 2.51 <sup>a</sup>
KP1	7.66 ± 2.51 <sup>a</sup>	2.33 ± 1.52 <sup>a</sup>	0.66 ± 0.57 <sup>a</sup>	0.66 ± 0.57 <sup>a</sup>	0.66 ± 0.57 <sup>a</sup>
KP2	10.00 ± 6.55 <sup>a</sup>	5.00 ± 2.64 <sup>a</sup>	4.00 ± 2.64 <sup>a</sup>	1.66 ± 0.57 <sup>a</sup>	3.00 ± 1.00 <sup>a</sup>
KP3	0.66 ± 0.57 <sup>a</sup>	1.33 ± 0.57 <sup>a</sup>	0.66 ± 0.57 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>
KP4	11.66 ± 7.37 <sup>a</sup>	5.66 ± 1.15 <sup>a</sup>	4.33 ± 2.30 <sup>a</sup>	2.00 ± 1.73 <sup>a</sup>	3.66 ± 1.15 <sup>a</sup>

Values with different superscript (a-c) in the same column differ significantly (p < 0.05).

It can be seen in the Table 1 that the treatment with EE was significantly increased testosterone concentration in the testis (P<0,05), but not in all part of epididymis and ductus deferens. However, the treatment of EE were not affect the concentration of DHT in all locations (P>0,05) (Table 2).

The increased testosterone concentration after administration of EE in the goats in this study is in agreement with the report by Akmal et al. (2014). Perhaps, that effects of EE is related with its content of PACAP, which plays an important role regulation of steroidogenesis in Leydig cells to produce testosterone (El-Gehani *et al.*, 1998; Yanaihara *et al.*, 1998).

The continues production of testosterone is very important for spermatogenesis. Therefore, when the stimulation for testosterone is impaired, then will interrupt spermatogenesis process at stadium of meiosis. In turn, this condition will results in the death of germinal cells. The disturbance during testosterone secretion will cause a problem in spermiation process and spermatogenesis, which could induces infertility in man (Nieschlag *et al.*, 1979; Walker, 2010).

Furthermore, testosterone as an androgen hormone is play a pivotal role in maintaining a continues production of

spermatozoa and the growt of secondary sex organ of prostate gland (Yong *et al.*, 1998). It has been suggested by Verhoeven et al. (2010) that testis, as an organ that produces testosterone, is a target organ for androgen action. This action is importance for initiation of spermatogenesis and the growt of secondary sex organ (Zirkin *et al.*, 1989).

Androgen is a family of steroidal hormones that comprised of DHT, testosterone, androstenedione, and dehydroepiandrosterone (Morales *et al.*, 2004; Marchetti & Barth, 2013). DHT is a natural androgen that very potent (300%) in man (Wang *et al.*, 2004; Marchetti & Barth, 2013). According to Wang *et al.* (2009<sup>b</sup>), the androgen action is mediated by androgen receptor (AR), which is the member of nuclear receptor superfamily. The function of AR is mediated by ligand-dependent transcription factor that regulated expression of androgen-responsive genes.

Normally, when the concentration of testosterone in testis increased, then it will also increased the concentration of DHT. According to Marchetti and Barth (2013), testosterone is irreversibly converted into DHT by NADPH-dependent enzyme 5α-reductase or aromatase. The mechanism of their relationship is initiated by synthesis of testosterone by Leydig cells and then will stimulate sertoli cell to secrete androgen

binding protein (ABP) as well as other proteins to enter lumen of seminiferous tubule. ABP is believed as a paracrine factor that reulated infranuclear, which is the location of enzyme 5 $\alpha$ -reductase. This enzyme is found along of epididymis and controlled by androgen circulation (Robaire et al., 2006). Therefore, a high synthesis of DHT in the caudal epididymis is to support the circulation of androgen-dependent genes (Robaire et al., 2006).

However, in the present study, although the production of DHT was trend to increased, but it was not significant. A further study is needed to clarify this findings.

### Conclusion

In conclusion, the administration of EE was increased the concentration of testosterone in the testis of local male goats.

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