CHAPTER IV

RESULTS AND DISCUSSION

Sperm motility

Percentage of normal sperm motility was significantly decreased in animal treated with METH groups when compared with the control group. Percentage of normal sperm motility in the AB-METH group was significantly decreased (32.79±5.35%) ($p < 0.001$) when compared with the control group (66.50±2.79%). Moreover, percentage of normal sperm motility was significantly decreased in the ED-METH group (45.50±2.83%) and the ED-METH binge group (42.66±4.71%) ($p < 0.01$) when compared with the control group (Figure 21).

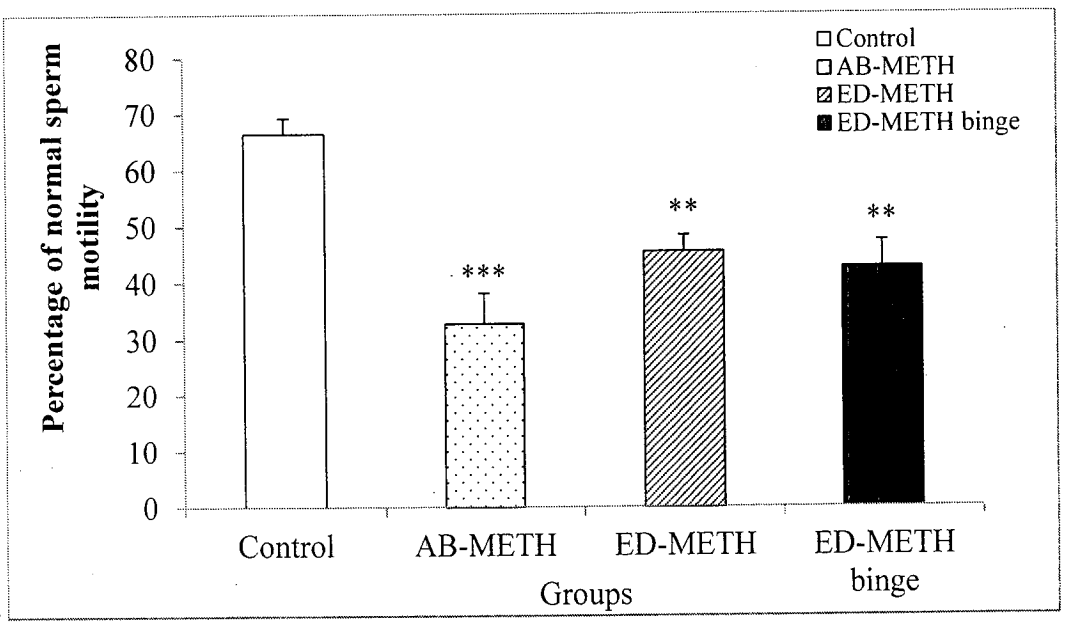

Figure 21 Percentage of normal sperm motility in the control group, acute dose-METH binge (AB-METH) group, escalating dose-METH (ED-METH) group and escalating dose-METH binge (ED-METH binge) group. Data were presented as mean±SEM, n = 6, ** $p < 0.01$ vs control group and *** $p < 0.001$ vs control group (ANOVA post-hoc Dunnett test).

Sperm morphology

Fixed sperm were evaluated sperm morphology by staining sperm with eosin and taken a picture (Figure 22). After that, the sperm were examined morphology. Percentage of normal sperm morphology was significantly decreased in the ED-METH (49.20±1.39%) ($p < 0.05$) and the ED-METH binge groups (44.08±4.53%) ($p < 0.01$) when compared with the control group (68.33±7.41%) (Figure 23).

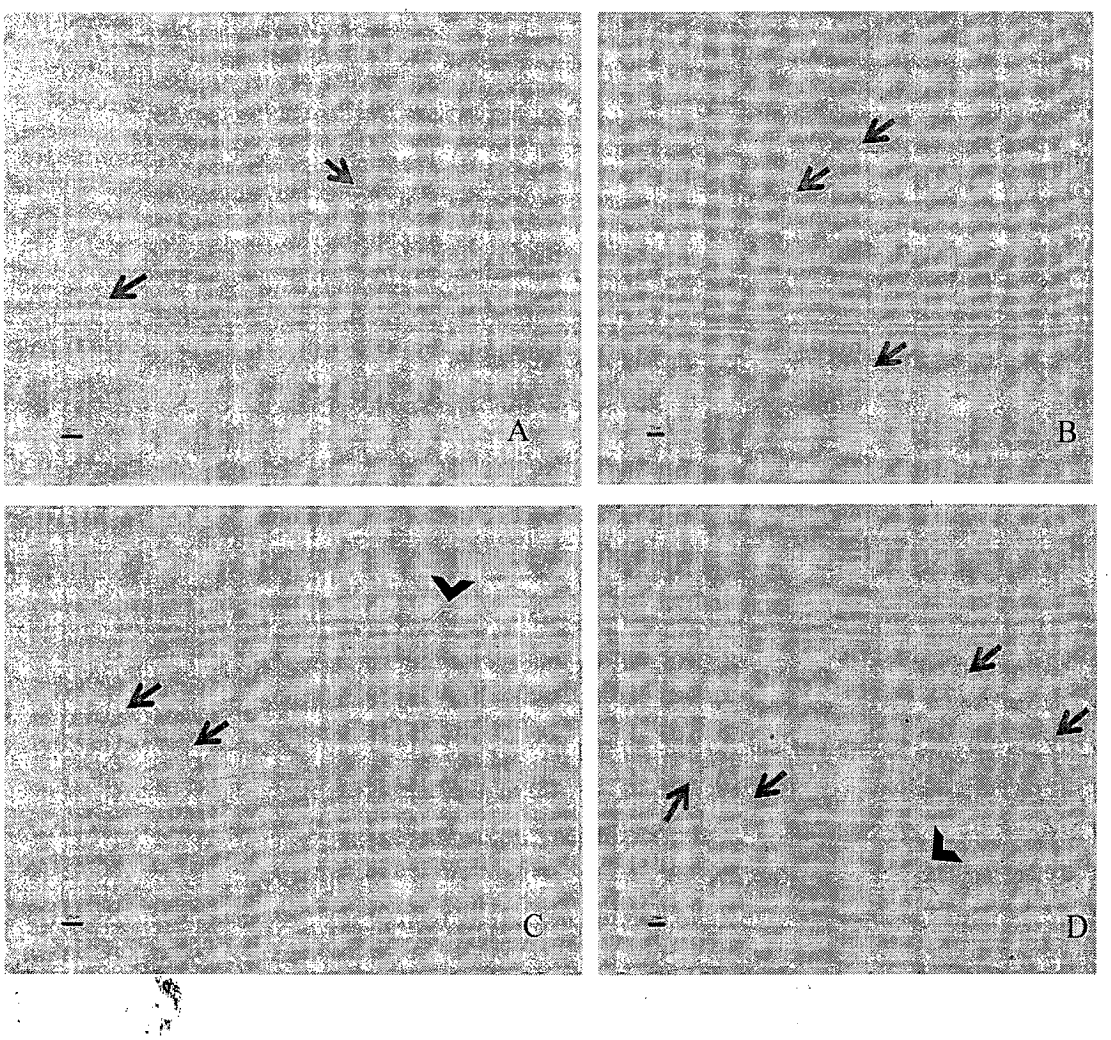

Figure 22 Sperm morphology in male rats in the control group (A), acute dose-METH binge (AB-METH) group (B), escalating dose-METH (ED-METH) group (C) and escalating dose-METH binge (ED-METH binge) group (D). Blue arrow = normal sperm, green arrow = tailless sperm, red arrow = headless sperm, arrow head = bent tail sperm. Scale bar = 25μm. Objective lens = 20x.

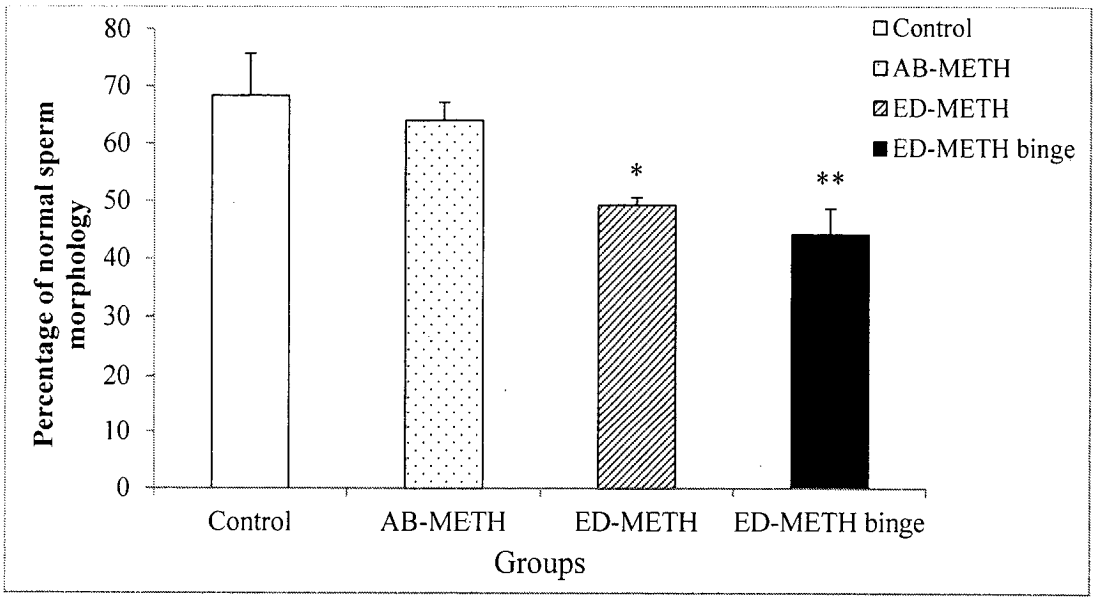

Figure 23 Percentage of normal sperm morphology in the control group, acute dose-METH binge (AB-METH) group, escalating dose-METH (ED-METH) group and escalating dose-METH binge (ED-METH binge) group. Data were presented as mean±SEM, n = 6, * $p < 0.05$ vs control group and ** $p < 0.01$ vs control group (ANOVA post-hoc Dunnett test).

Sperm concentration

The sperm concentration was not changed in the animals treated with METH group when compared with the control group. Although, the sperm concentration in the ED-METH and ED-METH binge groups seemed to decrease when compared with the control group but it just failed to reach significant (Figure 24).

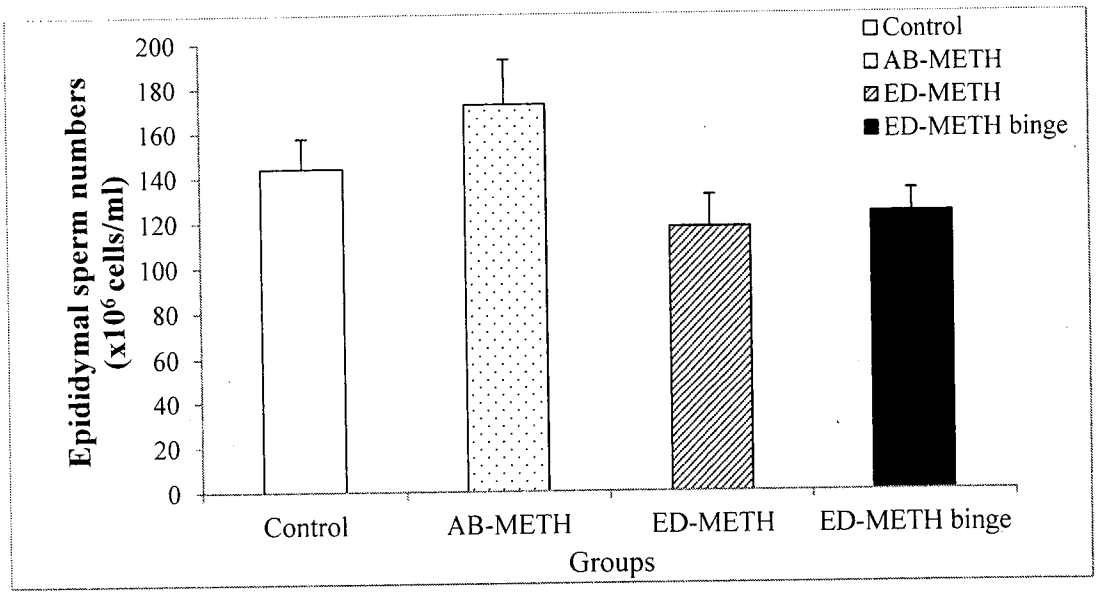

Figure 24 Epididymal sperm number (x 10^6 cells/ml) in the control group, acute dose-METH binge (AB-METH) group, escalating dose-METH (ED-METH) group and escalating dose-METH binge (ED-METH binge) group. Data were presented as mean±SEM, n = 6 (ANOVA post-hoc Dunnett test).

Androgen receptor expression in the testis

The immunohistochemistry study of androgen receptor (AR) expression in the rat testis demonstrated the positive and negative sections. The positive section was stained with anti-androgen receptor primary antibody and the negative section was not stained with anti-androgen receptor primary antibody. The result showed the AR expression in Leydig cells, Sertoli cells, round spermtids and elongated spermatids. Figure 25A showed the section of AR immunostaining with hematoxylin. Figure 25B showed the section of AR immunostaining without hematoxylin. The small block is negative section (Figure 25).

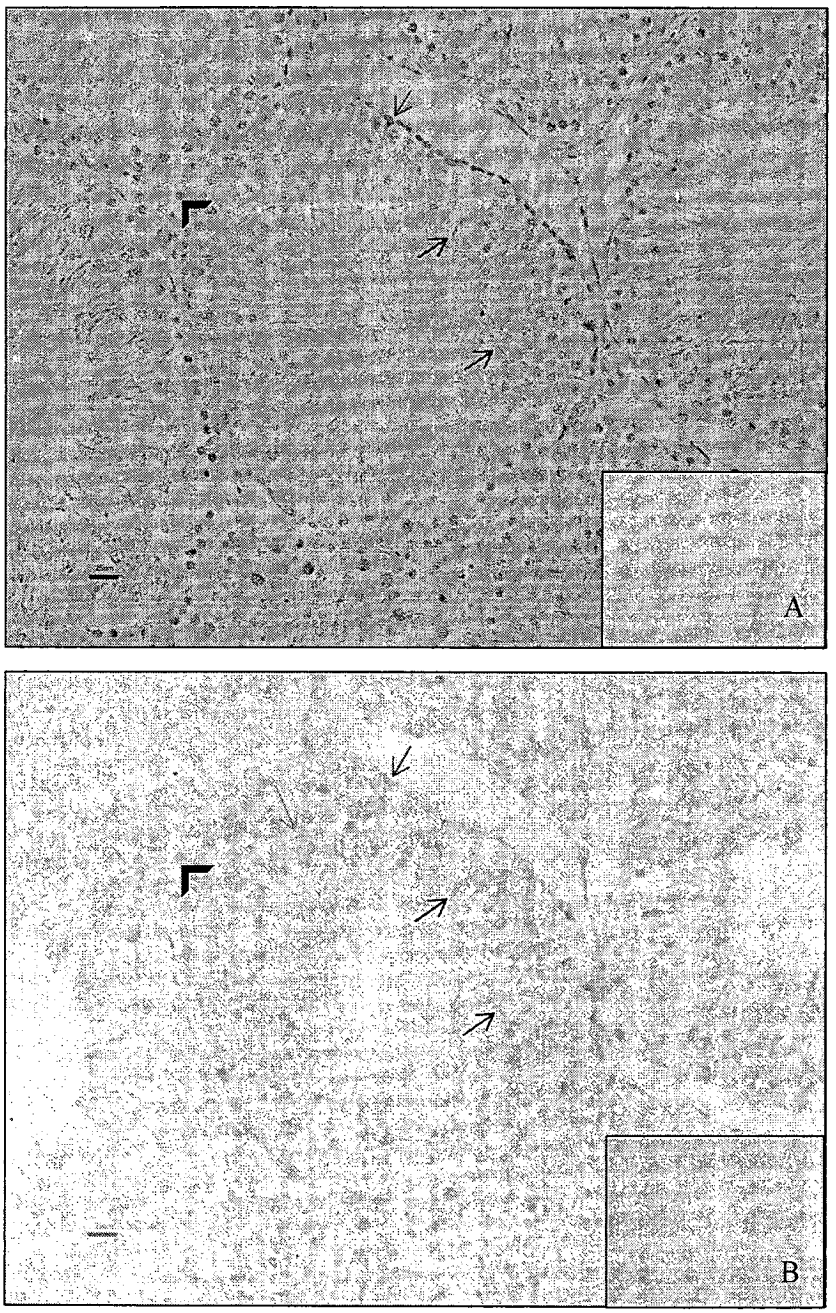

Figure 25 **Sections of rat testis stained with anti-androgen receptor primary antibody. There are the expression of AR in Leydig cell (arrow head), Sertoli cell (red arrow), round spermatid (green arrow) and elongated spermatid (black arrow). Figure A showed the AR immunostaining with hematoxylin. Figure B showed the AR immunostaining without hematoxylin. The small block is negative staining. Scale bar = 25μm. Objective lens = 20x.**

Androgen receptor expression in Leydig cells

Percentage of androgen receptor (AR) expression in Leydig cells was significantly decreased in the ED-METH group (74.54±1.49%) ($p < 0.05$) and the ED-METH binge group (70.72±2.15%) ($p < 0.001$) when compared with the control group (80.48±1.60%) (Figure 26).

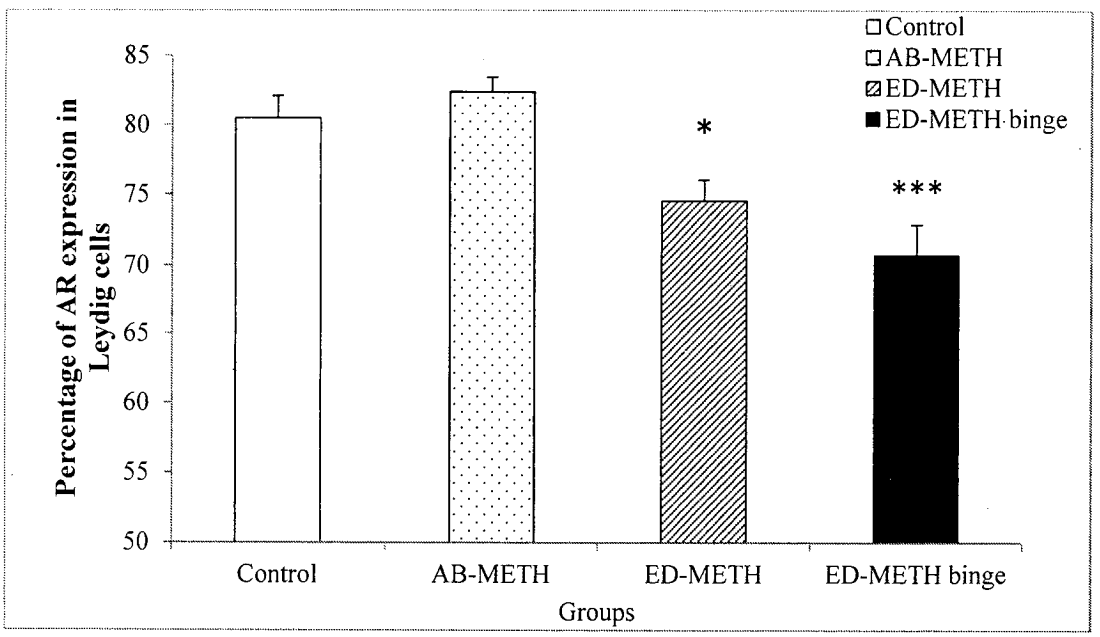

Figure 26 Percentage of AR expression in Leydig cells in the control group, acute dose-METH binge (AB-METH) group, escalating dose-METH (ED-METH) group and escalating dose-METH binge (ED-METH binge) group. Data were presented as mean±SEM, n = 6, * $p < 0.05$ vs control group and *** $p < 0.001$ vs control group (ANOVA post-hoc LSD).

Androgen receptor expression in Sertoli cells

Percentage of AR expression in Sertoli cells in all treated groups was not changed when compared with the control group ($p < 0.05$) (Figure 27).

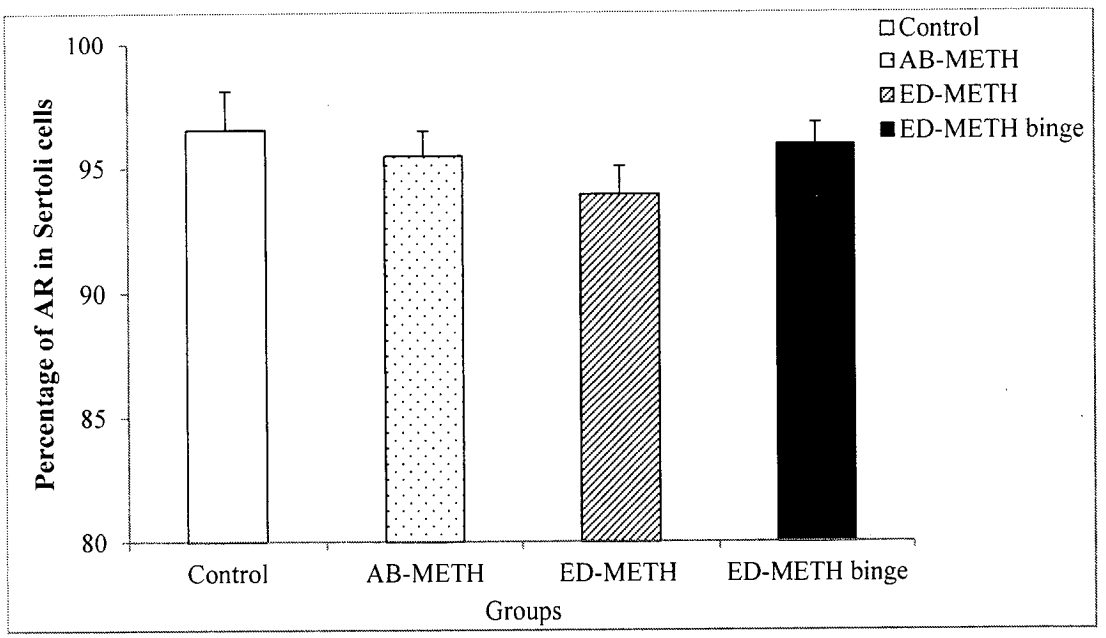

Figure 27 Percentage of AR expression in Sertoli cells in the control group, acute dose- METH binge (AB-METH) group, escalating dose-METH (ED-METH) group and escalating dose-METH binge (ED-METH binge) group. Data were presented as mean±SEM, n = 6, (ANOVA post-hoc LSD).

Androgen receptor expression in round spermatids

The percentage of AR expressions in round spermatids were significantly decreased in the ED-METH group (89.56±3.55%) ($p<0.05$) and ED-METH binge group (88.52±11.19%) ($p<0.05$) when compared with the control group (99.31±0.21%) (Figure 28).

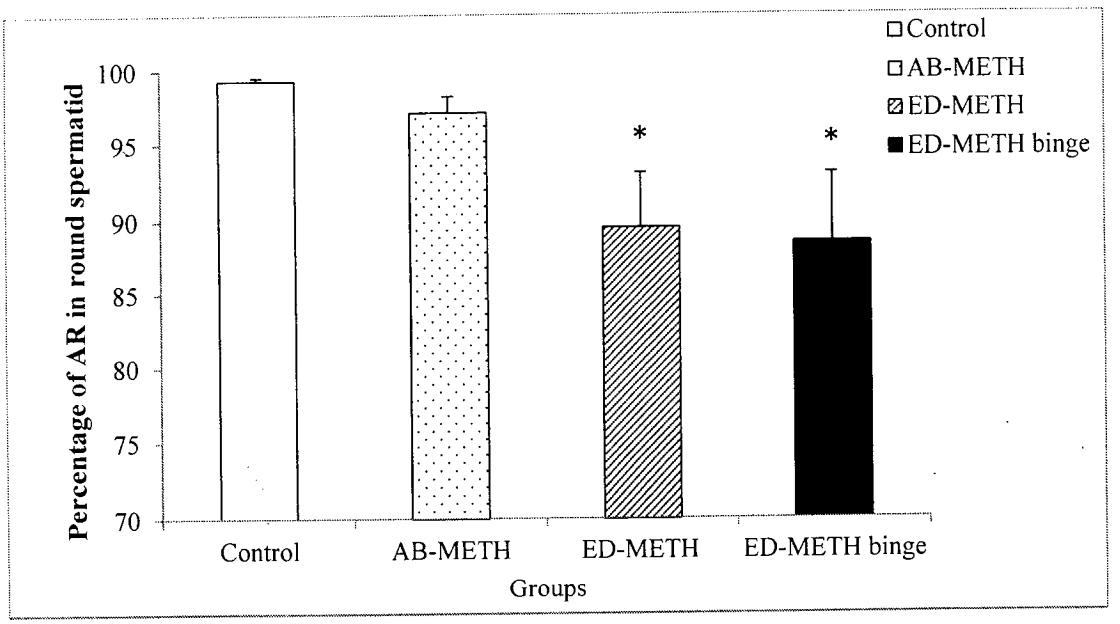

Figure 28 Percentage of AR expression in round spermatids in the control group, acute dose-METH binge (AB-METH) group, escalating dose-METH (ED-METH) group and escalating dose-METH binge (ED-METH binge) group. Data were presented as mean±SEM, n = 6, * $p < 0.05$ vs control group (ANOVA post-hoc LSD).

Androgen receptor expression in elongated spermatids

The percentage of AR expression in elongated spermatids was significantly decreased only in the AB-METH group (60.89±5.10%) ($p < 0.001$) when compared with the control group (84.02±2.53%) (Figure 29).

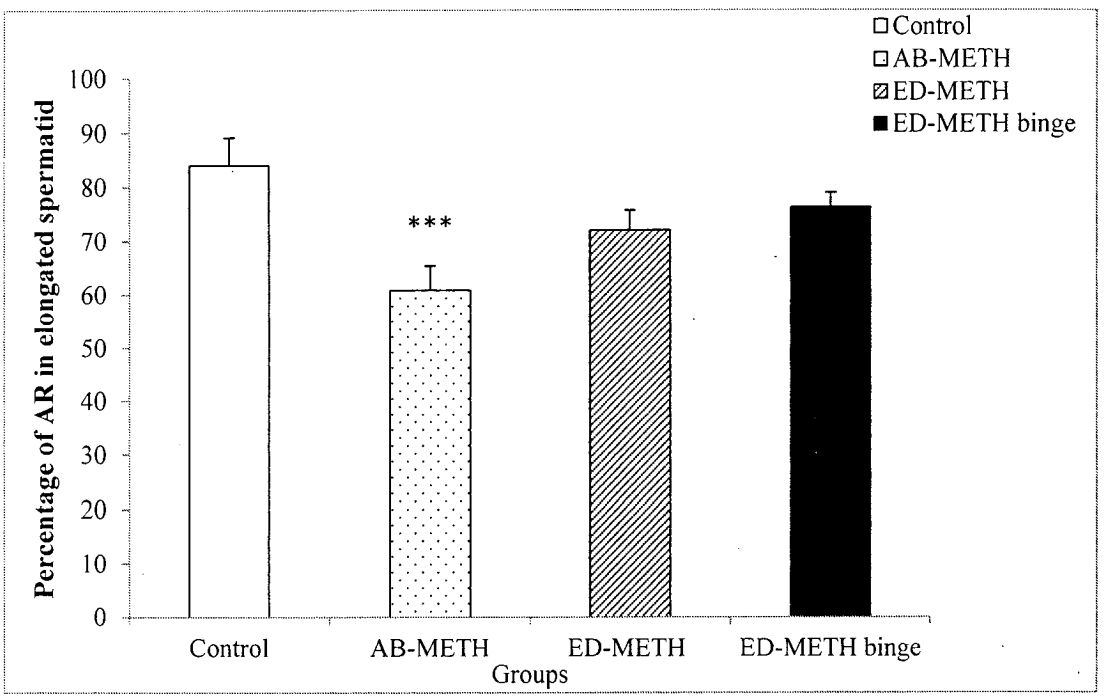

Figure 29 Percentage of AR expression in elongates spermatids in the control group, acute dose-METH binge (AB-METH) group, escalating dose-METH (ED-METH) group and escalating dose-METH binge (ED-METH binge) group. Data were presented as mean±SEM, n = 6, *** $p < 0.001$ vs control group (ANOVA post-hoc LSD)

Plasma testosterone levels in male rats after treated with METH

The plasma testosterone levels were measured by electrochemiluminescence immunoassay (ECLIA). The plasma testosterone levels were not changed in all treated with METH groups when compared with the control group (Figure 30).

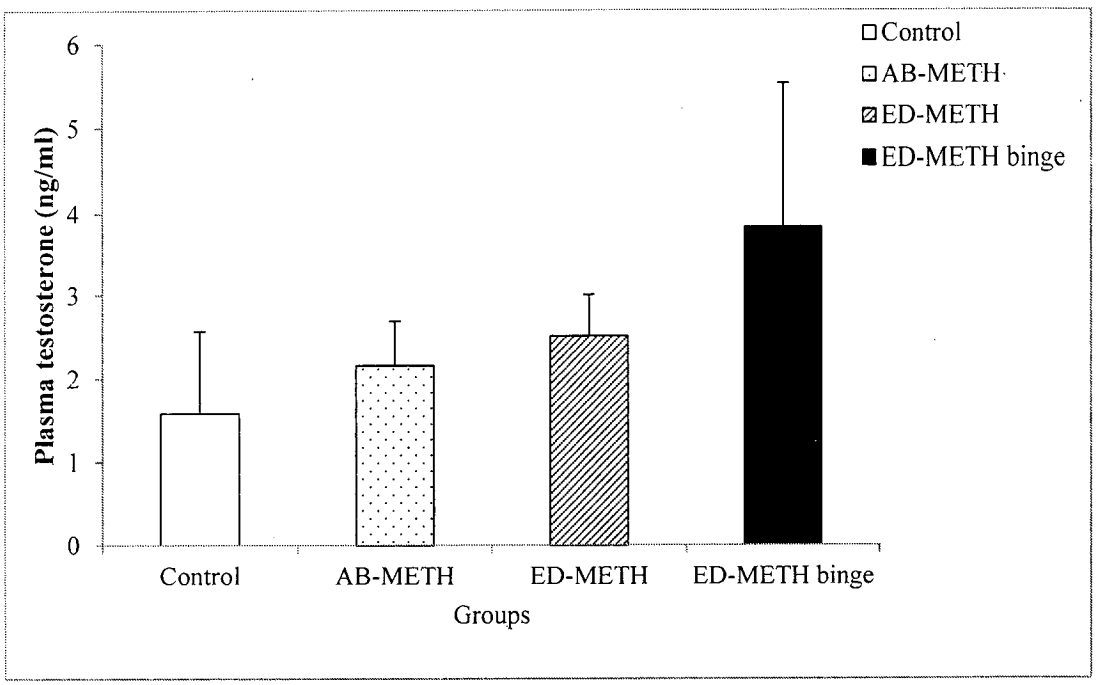

Figure 30 Plasma testosterone levels in the control group, acute dose-METH binge (AB-METH) group, escalating dose-METH (ED-METH) group and escalating dose-METH binge (ED-METH binge) group. Data were presented as mean±SEM, n = 6, (ANOVA post-hoc LSD).

Discussion

The results of present study indicated that METH administration can induce decrease of normal sperm motility, normal sperm morphology and AR expression in Leydig cells, round spermatids and elongated spermatids.

Effect of METH administration on sperm motility in male rat

The normal sperm motility in this study was decreased in all groups that treated with METH when compared with the control group. The result of the present study was consistent with a previous study showed that normal sperm motility was decreased after treated with METH at 15 mg/kg in male mice (Yamamoto, Yamamoto and Hayase, 1999). A decrease of normal sperm motility may due to progesterone receptor (PR) was lower. Although this study was not investigated the PR in male rat but in our team in reproductive biology research group has been studied the expression of PR in seminiferous tubules. PR was decreased in germ cell consist of spermatogonia, spermatocytes, round spermatids and elongated spermatids after treated with METH (Sueudom, 2013). Progesterone has been reported to stimulate hyperactivated sperm motility by induce extracellular calcium (Ca^{2+}) influx in human spermatozoa (Revelli, Massobrio and Tesarik, 1998; Torres-Flores, et al., 2008). Therefore, reduction of PR in germ cell may affect sperm motility by reduced influx of Ca^{2+} cause a decrease of hyperactivated sperm motility after treated with METH.

Effect of METH administration on sperm morphology in male rat

The normal sperm morphology in the present study was decreased in the ED-METH and ED-METH binge groups after treated with METH. A decrease of normal sperm morphology may be related to the effect of METH which can induce an apoptotic cell in the seminiferous tubule and the effect of METH is seemed to be dose dependent manner. The result of the present study is consistent with the previous study of Nudmamud-Thanoi and Thanoi showed that METH can induce abnormal sperm morphology and apoptosis in the seminiferous tubule in male rat (Nudmamud-Thanoi and Thanoi, 2011). Moreover, the previous report of Yamamoto has been showed that apoptotic cell in the seminiferous tubule was increased in dose dependent manner of METH (Yamamoto, et al., 2002). Breaking of DNA strand causes apoptotic cell

(Alavi, Taghavi and Moallem, 2008). Thus, METH exposure may break the DNA strand of germ cell that lead to germ cell apoptosis and the normal spermatogenesis can not process. These may cause a decrease of normal sperm morphology after treated with METH.

Effect of METH administration on sperm concentration in male rat

The sperm concentration in the present study was not changed after treated with METH. But it was numerically decreased in the ED-METH and ED-METH binge groups. Even though, the result of sperm concentration in the present study was not consistent with the previous study. This may due to differential dose and differential time of treated with METH used in the present study that imitates human addiction which may need a longer time to affect. Sperm concentration in the treated groups was not changed but it may reach significant if the animals were treated with METH at the higher dose and/or the longer time.

Effect of METH administration on AR in the testis in male rat

The present study showed that AR express in Leydig cells, Sertoli cells, round spermatids and elongated spermatids in rat testis.

The expression of AR in Leydig cells was decreased in ED-METH and ED-METH binge groups. Function of AR in Leydig cells is regulated autocrine for steroidogenesis that relate to normal spermatogenesis (Tsai, et al., 2006). A decrease of AR expression in Leydig cells is consistent with the previous study of Xu indicated that male mice lacking AR in Leydig cells show that the spermatogenesis was blocked at round spermatids, apoptotic spermatocytes were found in the seminiferous tubules and the serum testosterone level was decreased (Xu, et al., 2007). In the present study, a decrease of AR in Leydig cells may cause a decrease of hormone synthesis especially testosterone, the important hormone for spermatogenesis, leading to suppress spermatogenesis. These may be related to apoptosis in germ cell and cause a decrease of normal sperm morphology.

The expression of AR in Sertoli cells in the present study was not changed in all groups treated with METH. The previous report was showed that the expression of AR in Sertoli cells was highest at stage VII-VIII of rat seminiferous tubule (Shan, Bardin and Hardy, 1997). Moreover, intensity of AR level was highest in these stages (Tirado, et al., 2003). AR expression in the Sertoli cell is required for round spermatid adhesion to the seminiferous epithelium and release of mature sperm (Holdcraft and Braun, 2004). An unchanged of AR expression in the Sertoli cells may lead to round spermatid still attach from seminiferous epithelium and normal of releasing mature sperm. These may be related to an unchanged of sperm concentration.

The expression of AR in round spermatids was decreased in ED-METH and ED-METH binge groups and the expression of AR in elongated spermatids was decreased in AB-METH group. The previous study of Vornberger has been established that AR is expressed in the nuclear at step 11-elongated spermatid of rat (Vornberger, et al., 1994). The expression of AR in elongated spermatids of the present study is similar to the study of Vornberger although it was investigated the step 19-elongated spermatid. Nevertheless, in this study was also investigated step 7 to 8 round spermatids which, the AR has been expressed in these steps of round spermatids. AR is required for the normal spermatogenesis (Holdcraft and Braun, 2004). AR has been reported as a necessity during meiosis I, transition of round spermatids into elongated spermatids and during spermiogenesis at terminal stage (Xu, et al., 2007). Decrease of AR expression in round and elongated spermatids may cause the blockage of the transition of round spermatids into elongated spermatids and abnormality at maturation stage of spermiogenesis. These may relate to decrease of normal sperm motility and normal sperm morphology.

Effect of METH administration on plasma testosterone levels in the testis

The plasma testosterone levels in the present study were not changed in all groups treated with METH but they were numerically increased in a dose dependent manner. However, an increase of plasma testosterone levels just failed to reach significant. The result of plasma testosterone levels was consistent with the previous study of Yamamoto which indicated that METH administration can induce fluctuation of serum testosterone (Yamamoto, Yamamoto and Hayase, 1999; Yamamoto, et al.,

2002). The numerical increase of testosterone in this study may be involved copulatory behavior. Testosterone has not only been report that important in spermatogenesis but also sexual behavior (Holstein, Schulze and Davidoff, 2003). Testosterone has been reported important for copulatory behavior (Hull, et al., 1997). Moreover, the previous report showed that copulatory behavior was increased in male rat after treated with amphetamine (Fiorino and Philips, 1999). Mechanism action of METH and amphetamine are similarity (Kish, 2008). Therefore, METH administration may induce an increase of copulatory behavior in male rat by increasing of plasma testosterone levels. However, the copulatory behavior was not investigated in this study.