CHAPTER IV

RESULTS

Experiment 1; Effects of *Mucuna collettii* on hormone-related ovarian functions and reproductive organs in cyclic female rats.

1.1 Effects of *Mucuna collettii* on hormone-related ovarian functions

1.1.1 Serum estradiol levels

When compared to the pre-treatment levels (D_1), there were no significant differences of serum E_2 levels in rats treated with DW, Mc-1 and Mc-10 throughout the study periods (Figure 7). In Mc-100 and TP groups, serum E_2 levels were significantly increased only at D_{31} or 15 days after the administration of Mc-100 and TP.

Comparison to the DW group, serum E_2 levels of Mc-1 group were significantly higher than that of the DW group at D_1, D_{46} and D_{61}. There were no significant differences from the DW group throughout the study periods in Mc-10 group. Serum E_2 levels of Mc-100 group at D_{16} and D_{31}, and TP group at D_{31} were significantly higher than those of DW group.

Comparison to the TP group, serum E_2 levels of Mc-1 group showed the similarity of significant difference as it was to the DW group, the serum E_2 levels were significantly higher than that of TP group at D_1, D_{46} and D_{61}. Serum E_2 levels of Mc-

10 and Mc-100 groups did not significant differences from the TP group throughout the study periods, except for D_{46} of Mc-100 group, the level was significantly higher.

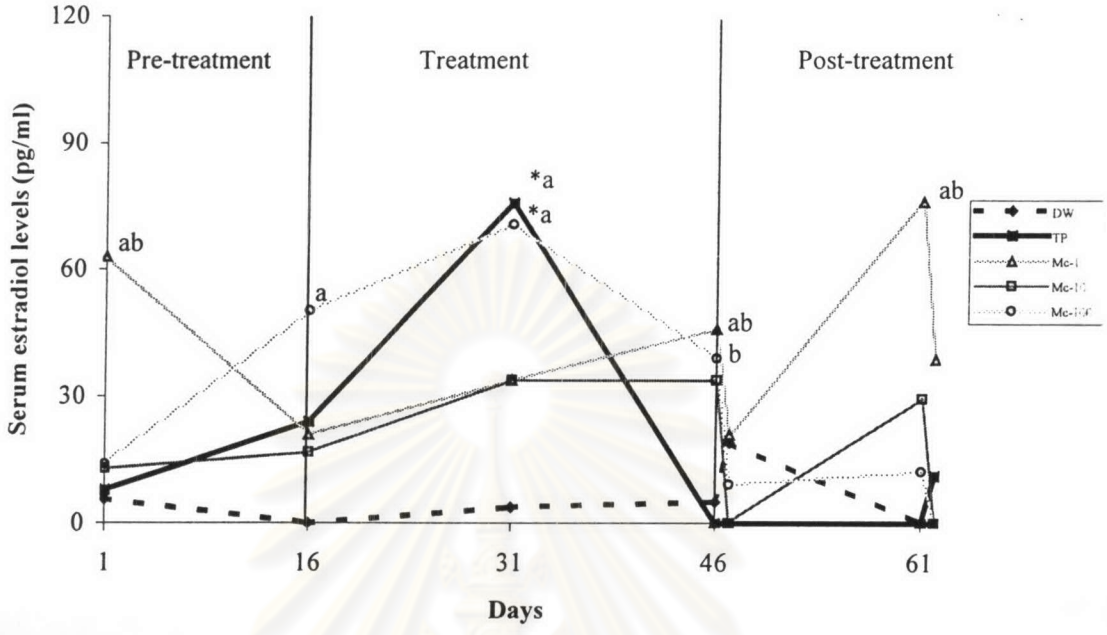
1.1.2 Serum LH levels

When compared to the pre-treatment levels (D_1), serum LH levels of DW and Mc-100 groups did not significant differences throughout the study periods (Figure 8). Serum LH levels of TP group were significantly decreased during D_{31}-D_{62}. Serum LH levels of Mc-1 and Mc-10 groups were significantly increased at D_{62} and D_{61}, respectively.

Comparison to the DW group, serum LH levels of Mc-1 and Mc-10 groups were no significant differences throughout the study periods. Serum LH levels of Mc-100 group were significantly higher than the DW group at D_1, D_{16}, D_{46} and D_{47}. Serum LH levels of TP group were significantly lower than the DW group at only D_{47}.

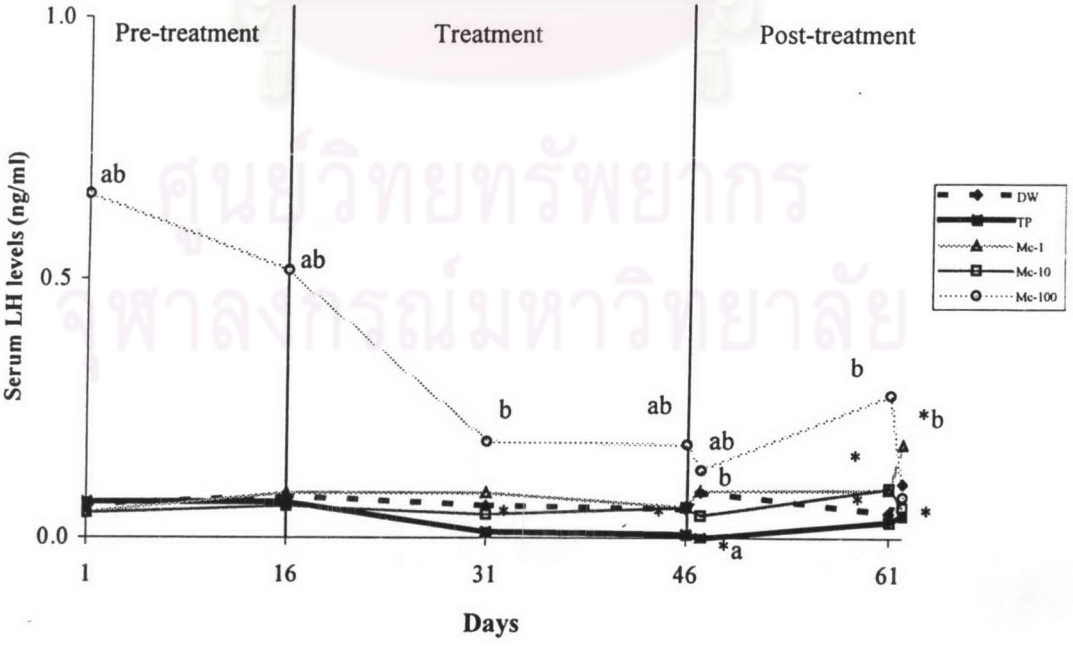
Comparison to the TP group, serum LH levels of Mc-1 group at D_{47} and D_{62} were significantly higher than the TP group. Serum LH levels in Mc-100 group were also higher than that of TP group at D_1, D_{16} and D_{46}-D_{61}. Serum LH levels of Mc-10 group did not significantly different throughout the study periods.

Figure 7 Changes of serum estradiol levels in cyclic female rats treated with distilled water, testosterone propionate and *M. colettii*.



* Significantly different compared to D_1 levels.
[a] Significantly different of TP and Mc groups compared to DW group.
[b] Significantly different of Mc groups compared to TP group.

Figure 8 Changes of serum LH levels in cyclic female rats treated with distilled water, testosterone propionate and *M. colettii*.



* Significantly different compared to D_1 levels.
[a] Significantly different of TP and Mc groups compared to DW group.
[b] Significantly different of Mc groups compared to TP group.

1.1.3 Serum FSH levels

When compared to the pre-treatment levels (D_1), serum FSH levels of Mc-1 group at D_{47} and Mc-10 group at D_{16} and D_{62} were significantly increased (Figure 9). In Mc-100 and TP groups, serum FSH levels did not changes throughout the study periods.

Comparison to the DW group, serum FSH levels of Mc treated groups were not significant differences throughout the study periods, except serum FSH levels of Mc-1 group were significantly higher at D_{47}. In contrast, serum FSH levels of TP group were significantly lower at D_{62}.

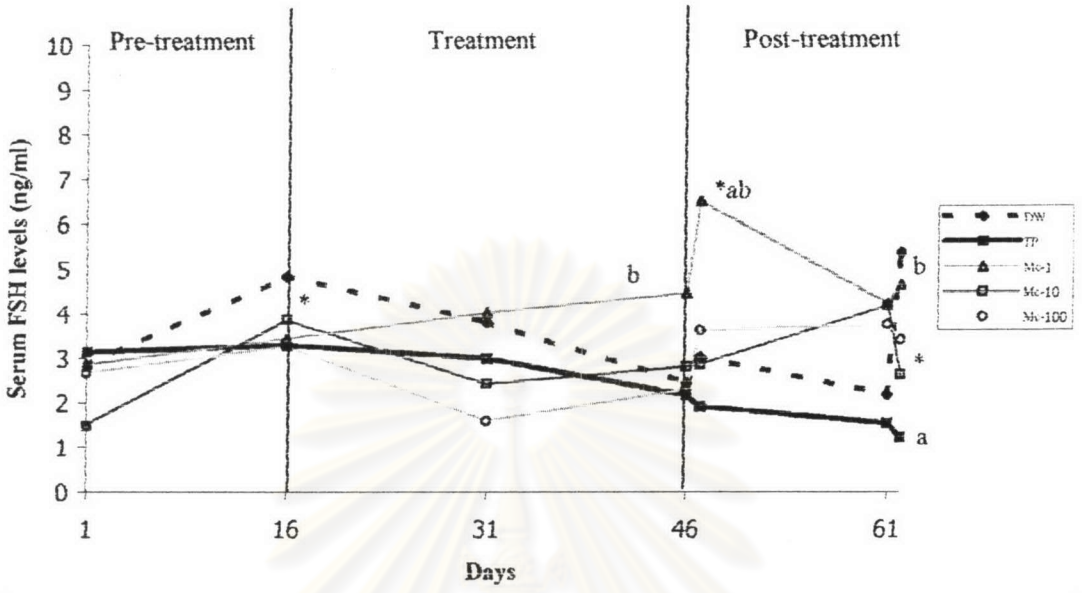
Comparison to the TP group, serum FSH levels of Mc treated groups were no significant differences throughout the study periods, except serum FSH levels of Mc-1 group were significantly higher at D_{46}, D_{47} and D_{62}.

1.2 Effects of *Mucuna collettii* on ovaries and uteri.

Comparison the weight and absolute weight of ovaries and uteri between the end of treatment to the end of post-treatment period, there were no significant differences in DW and all Mc groups (Table 1). The TP group showed the increase of weights and absolute weights of ovaries and uteri at the end of post-treatment period.

Comparison to the DW group, the uterine weight of Mc-10 group at the end of treatment period was significantly higher, while the absolute ovarian weight of Mc-

Figure 9 Changes of serum FSH levels in cyclic female rats treated with distilled water, testosterone propionate and *Mucuna collettii*.



* Significant difference compared to D_1 levels.
[a] Significant difference of TP and Mc groups compared to DW group.
[b] Significant difference of Mc groups compared to TP group.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 1 Changes of the weights and absolute weights of ovaries and uteri at the end of treatment and at the end of post-treatment periods of cyclic female rats treated with distilled water, testosterone propionate and *Mucuna collettii*.

Group	Organs weight (grams)				Absolute weight (x 10^{-3} grams)			
	Ovaries		Uteri		Ovaries		Uteri	
	Treatment	Post-treatment	Treatment	Post-treatment	Treatment	Post-treatment	Treatment	Post-treatment
DW	0.16±0.01	0.15±0.01	0.42±0.05	0.41±0.03	0.55±0.02	0.57±0.03	1.49±1.50	1.54±0.15
TP	0.11±0.01[a]	0.09±0.01*[a]	0.68±0.02[a]	0.32±0.03*	0.41±0.02[a]	0.30±0.02*[a]	2.55±0.09[a]	1.06±0.09*[a]
Mc-1	0.14±0.01[b]	0.15±0.01[b]	0.34±0.02[b]	0.45±0.05[b]	0.54±0.03[b]	0.55±0.02[b]	1.33±0.08[b]	1.64±0.19[b]
Mc-10	0.16±0.01[b]	0.14±0.01[b]	0.31±0.04[ab]	0.36±0.04	0.64±0.03[ab]	0.58±0.03[b]	1.21±0.14[b]	1.46±0.19
Mc-100	0.14±0.01[b]	0.13±0.01[b]	0.33±0.02[b]	0.37±0.04	0.53±0.02[b]	0.47±0.02[ab]	1.24±0.07[b]	1.32±0.15

* Significant difference compared to at the end of treatment period.
[a] Significant difference of TP and Mc groups compared to DW group.
[b] Significant difference of Mc groups compared to TP group.

100 group was significantly lower at the end of post-treatment period. In TP group, the weight and absolute weight of ovaries were significantly lower than the DW group in both periods. The uterine weight of TP group were significantly higher at the end of treatment period and recovered during the end of post-treatment period, but the absolute uterine weight was lower than that of DW group at the end of post-treatment period.

Comparison to the TP group, the weight and absolute weight of ovaries in Mc treated groups were significantly higher in both periods. Uterine weight and its absolute weight in all Mc treated groups were significantly lower at the end of treatment period and only in Mc-1 group were significantly higher at the end of post-treatment period.

1.3 Effects of *Mucuna collettii* on histological changes of ovaries and uteri

1.3.1 Histological changes of ovaries

The ovarian tissue of DW and all Mc treated groups at the end of treatment period presented normal ongoing folliculogenesis and corpus luteum formation (Figure 10A-D). There were many corpus lutea in all Mc treated groups than DW group. In contrast, the ovaries treated with TP at the end of treatment period showed some large follicles atresia. In addition, some large corpora lutea were occurred in this group (Figure 10E-F).

As regards to ovary of DW and all Mc treated groups at the end of post-treatment period, it demonstrated normal ongoing folliculogenesis and corpus luteum formation. There were some atretic follicles with some large corpora lutea in TP treated group (Figure 10G).

When the ovarian histology at the end of treatment period was compared to ovary at the end of post-treatment period, it was found that there were no differences in all groups.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

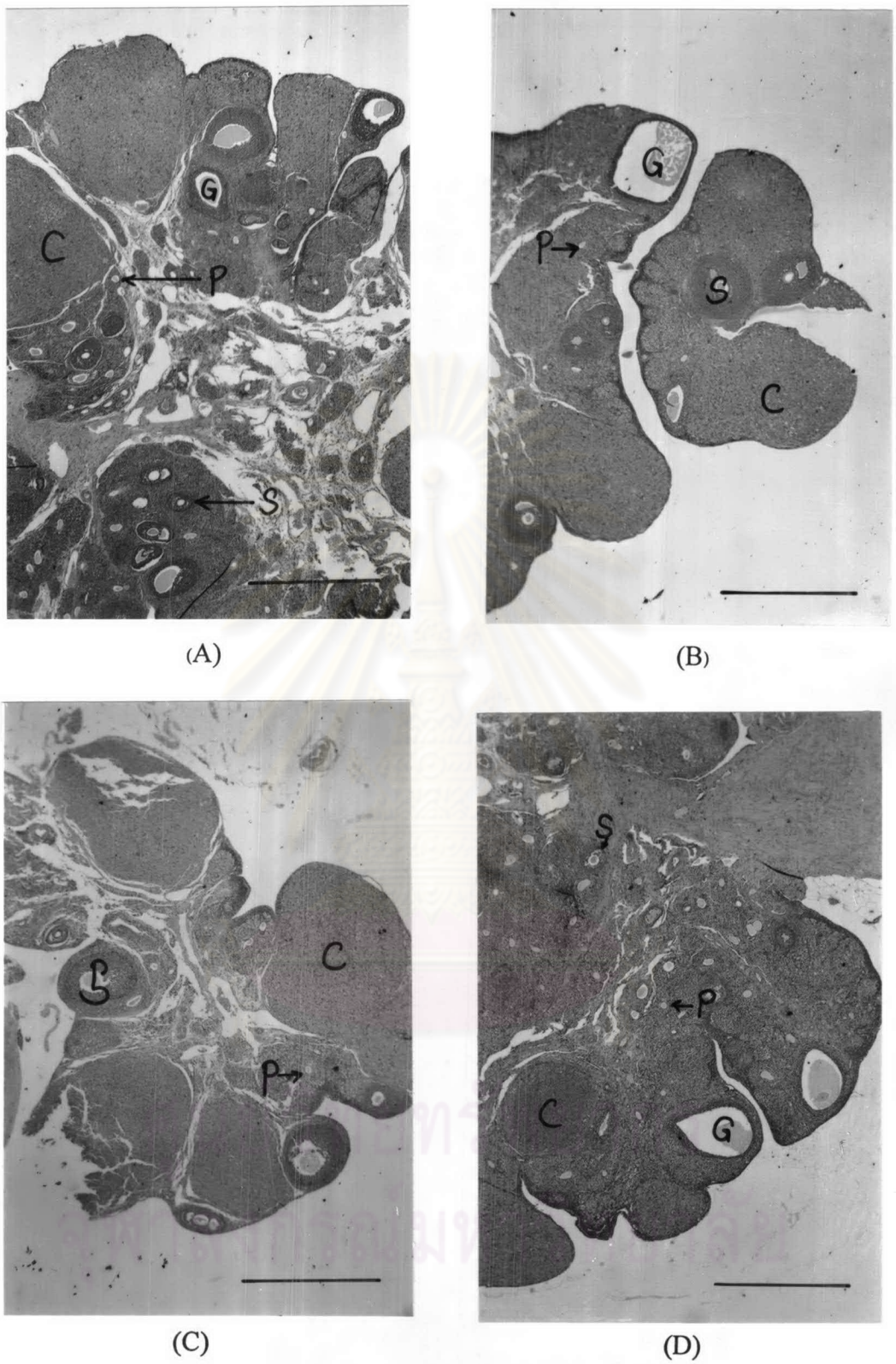


Figure 10 Ovarian tissue of cyclic female rats treated with distilled water (A), Mc-1 (B), Mc-10 (C), Mc-100 (D) and TP at the end of treatment (E,F) and TP at the end of post-treatment periods (G) with H & E staining for primary follicles (P), secondary follicles (S), Graafian follicles (G) and corpus lutea (C). Bar scale = 200 μm

(E)

(F)

Figure 10 (continued) Ovarian tissue of cyclic female rats treated with distilled water (A), Mc-1 (B), Mc-10 (C), Mc-100 (D) and TP at the end of treatment (E,F) and TP at the end of post-treatment periods (G) with H & E staining for primary follicles (P), secondary follicles (S), Graafian follicles (G) and corpus lutea (C). Bar scale = 200 μm

1.3.2 Histological changes of uteri

The uterine histological study of DW and all Mc treated groups at the end of treatment period showed the normal proliferation and no differences between all 4 groups. The mucosal epithelial cell lining was a layer of cuboidal cells (Figure 11A-D). In contrast, the uteri treated with TP revealed the marked increased in endometrial thickness, number and diameter of endometrial glands when compared to DW group. The mucosal epithelial cell lining was a layer of columnar cells (Figure 11E-F).

According to the uterine morphology of DW and all Mc treated groups at the end of post-treatment period, it was found that these groups revealed normal proliferation and no differences between all 4 groups. The mucosal epithelial cell lining was a layer of cuboidal cells. In case of TP group, the endometrial layer, number and diameter of endometrial glands were increased when compared to DW group. In addition, the epithelial cell lining was a layer of columnar cells (Figure 11G-H).

The histology of uteri at the end of treatment period was compared to the end of post-treatment period, there were no changes of uterine histology in all groups, except for TP group, endometrial thickness, number and diameter of endometrial glands at the end of treatment period were higher than at the end of post-treatment period.

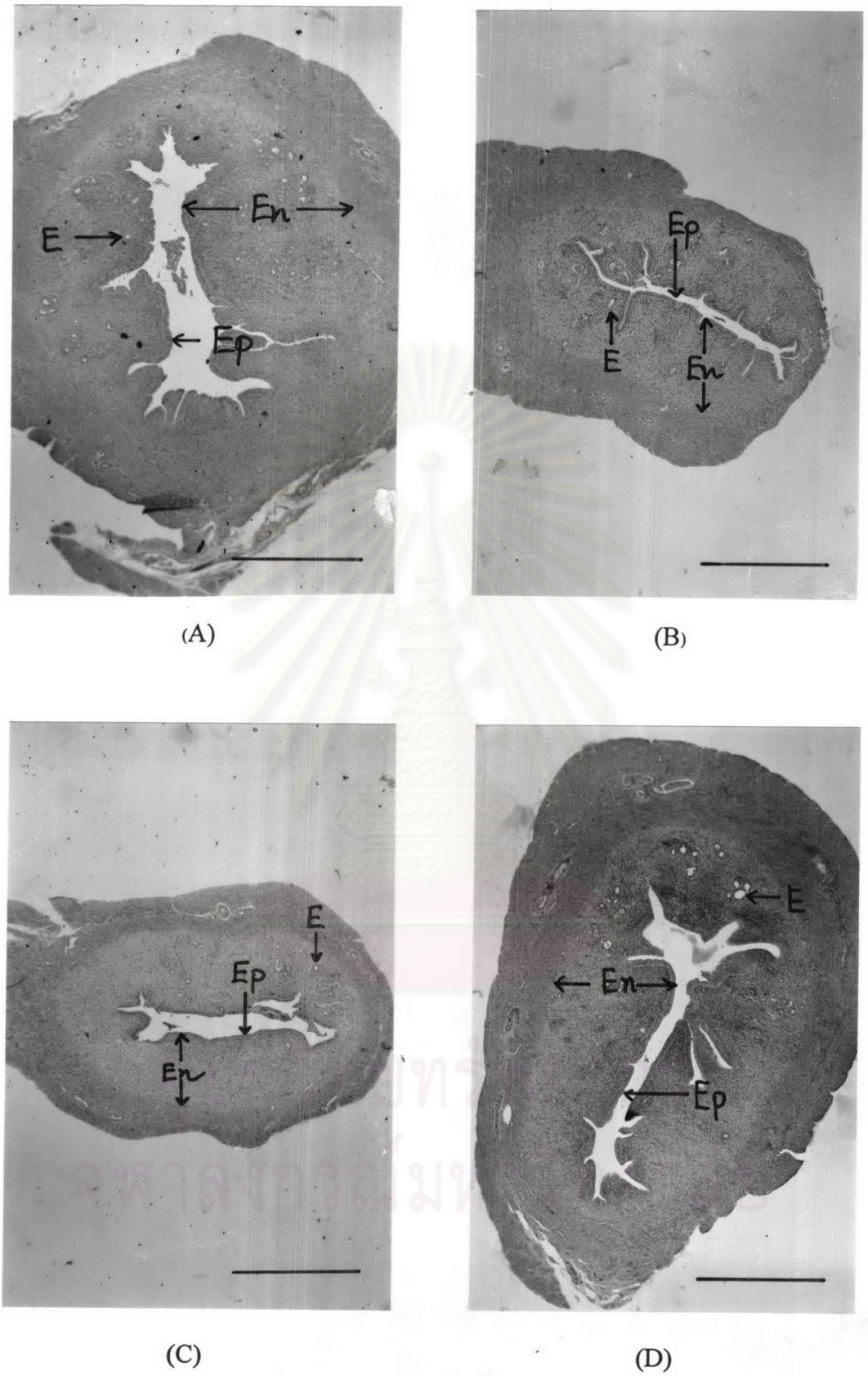
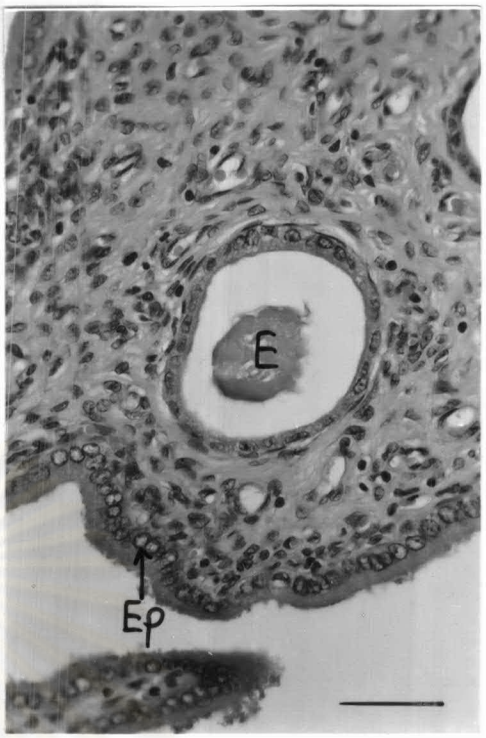
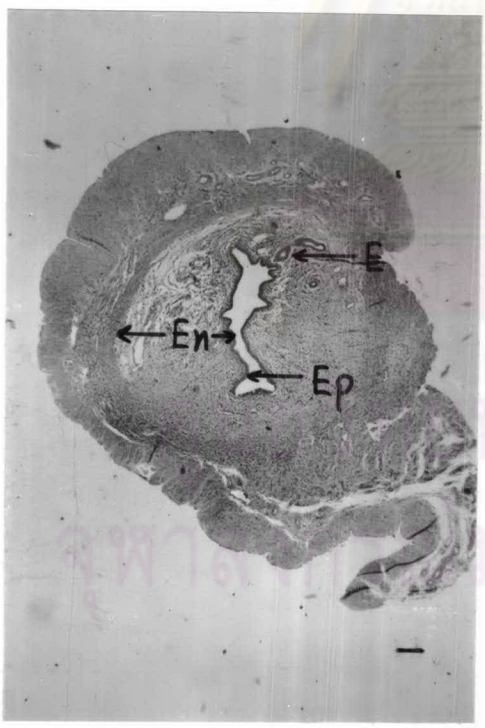


Figure 11 Uterine histology of cyclic female rats treated with distilled water (A), Mc-1 (B), Mc-10 (C), Mc-100 (D) and TP at the end of treatment period (E,F) with H & E staining for endometrium (En), epithelial cells (Ep) and endometrial gland (E). Bar scale = 200 μm

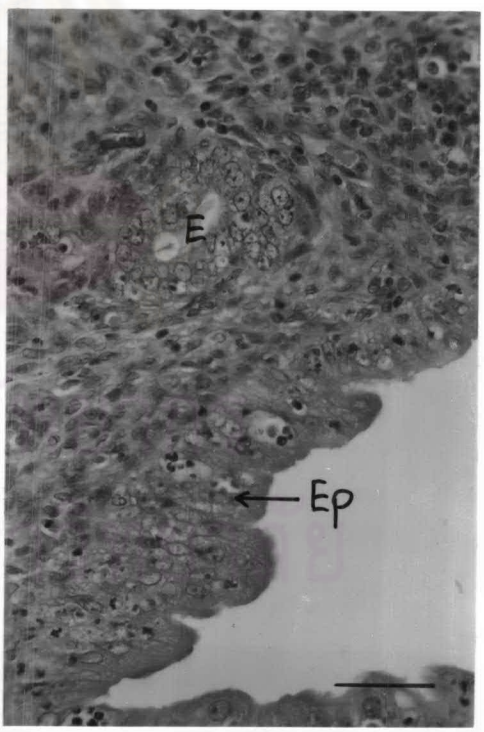
(E)



(F)



(G)



(H)

Figure 11 (continued) Uterine histology of cyclic female rats treated with distilled water (A), Mc-1 (B), Mc-10 (C), Mc-100 (D) and TP at the end of treatment (E,F) and TP at the end of post-treatment periods (G,H) with H & E staining for endometrium (En), epithelial cells (Ep) and endometrial gland (E). Bar scale = 50 μm

1.4 Effects of *Mucuna collettii* on estrous cycles

The estrous cycles in DW and all Mc treated groups were regular approximately 4-5 days throughout the study periods (Table 2,4-6), except for the Mc-100 group, the estrous cycles tended to be prolonged during treatment period (estrous cycle length = 5.40±0.11 days, p=0.058). In TP group, all rats showed leukocytes after TP treatment for approximately 1 estrous cycle until the last day of study periods (Table 3).

Estrous cycle length were no significant differences between each period between all Mc treated groups (Table 7), except for Mc-100 group, estrous cycle length in treatment period tended to be longer (p=0.058) when compared to DW group (Table 7).

Experiment 2; Efects of *Mucuna collettii* on hormone-related ovarian functions and reproductive organs in ovariectomized female rats.

2.1 Effects of *Mucuna collettii* on hormone-related ovarian functions

2.1.1 Serum estradiol levels

Basal serum E_2 levels (D_1) in femal rats after ovariectomy were significantly lower than the cyclic female rats (0±0 and 19.56±7.04 pg/ml in ovariectomized rats and cyclic rats, respectively). Serum E_2 levels were kept in the low levels throughout the study periods (Figure 12). No significant differences were found between that 3

periods in any treated groups, and also between that 4 groups in any treatment periods.

2.1.2 Serum LH levels

Basal serum LH levels (D_1) in female rats after ovariectomy were significantly higher than the normal cyclic female rats in Experiment 1 (12.67±1.08 and 0.18±0.12 ng/ml in OVX rats and normal cyclic female rats, respectively) (Figure 13). In DW and all Mc treated groups, serum LH levels were significantly increased since D_1 until the end of study period. Serum LH levels of TP group were significantly increased at D_1-D_{16}, and then decreased to the D_{14} levels after 2 weeks of TP injection until 2 weeks of TP withdrawal.

When compared to the pre-treatment levels (D_1), serum LH levels of DW and Mc-1 groups during D_{31}-D_{62} were significantly increased. However, the LH levels of Mc-10 and Mc-100 groups were not significantly different from the D_1 levels at D_{31} and D_{16}-D_{31}, respectively. Serum LH levels of TP group were significantly higher than the D_1 only at D_{16}.

Comparison to the DW group, serum LH levels of Mc-1 group were significantly higher than the DW group at D_{61}. There were not significant differences in serum LH levels of Mc-10 and Mc-100 groups throughout the study periods except at D_{31} of Mc-10 LH levels was lower than the DW group. In TP group, serum LH levels were significantly lower than the DW group since 2 weeks of TP injection until 2 weeks of TP withdrawal.

Table 2 Changes of vaginal cytology in cyclic female rats treated with distilled water. ▮ and ▪indicate fully and partial cornification, respectively.

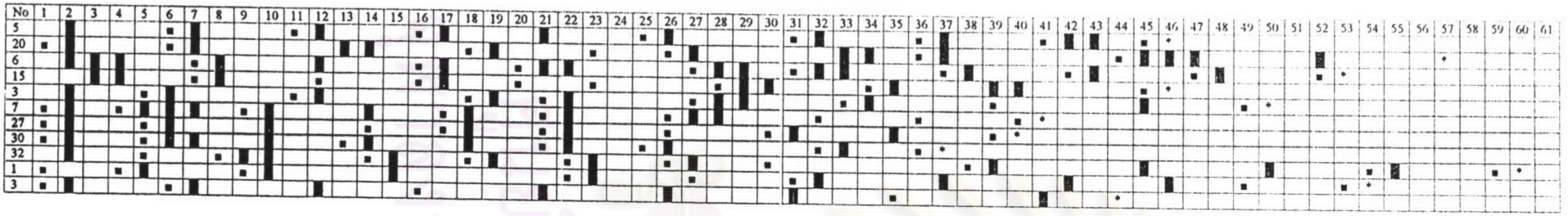
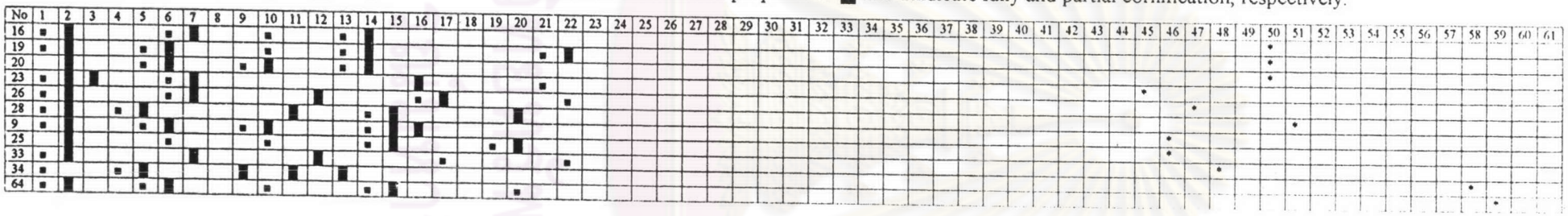
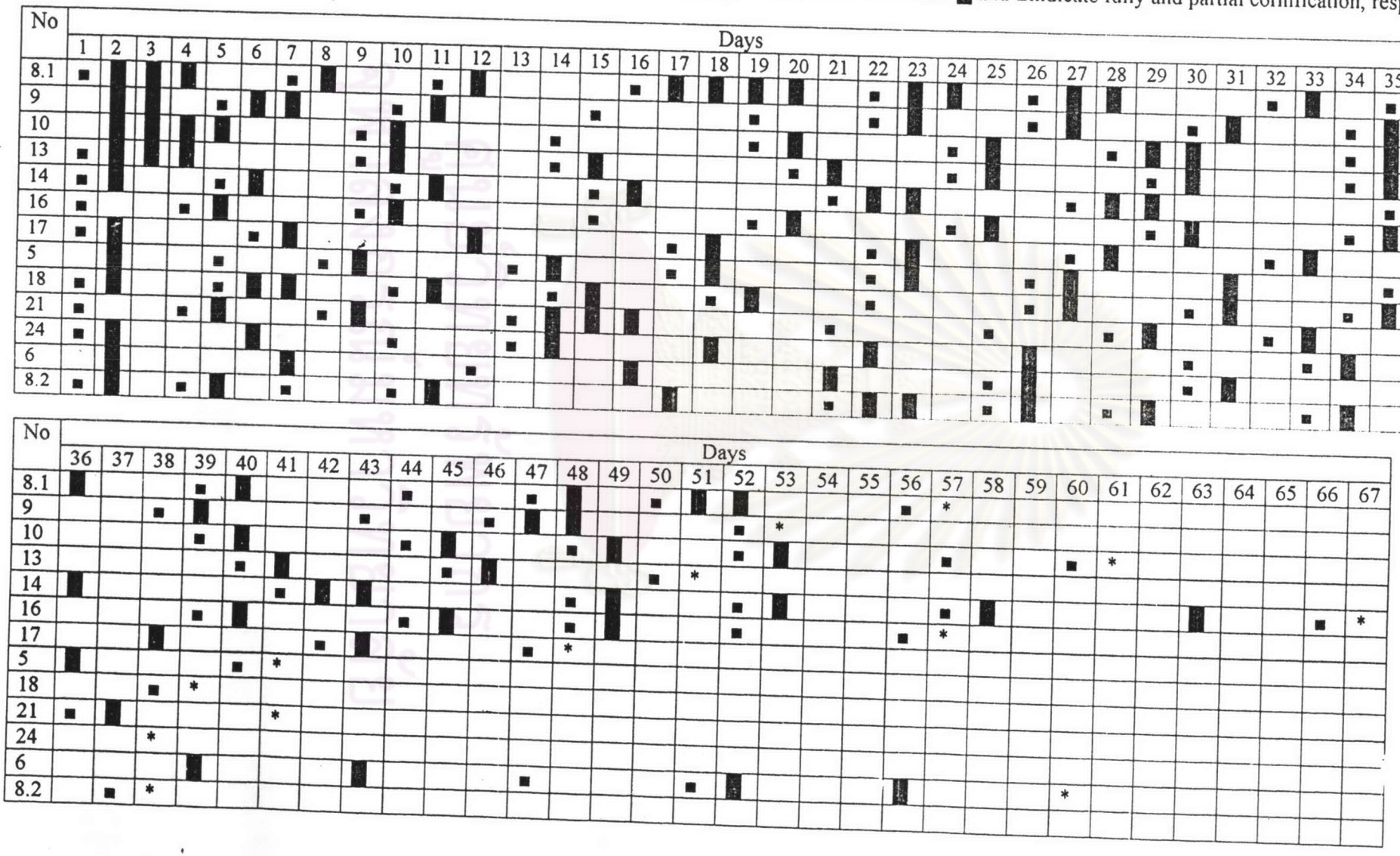


Table 3 Changes of vaginal cytology in cyclic female rats treated with testosterone propionate. ▮ and ▪indicate fully and partial cornification, respectively.



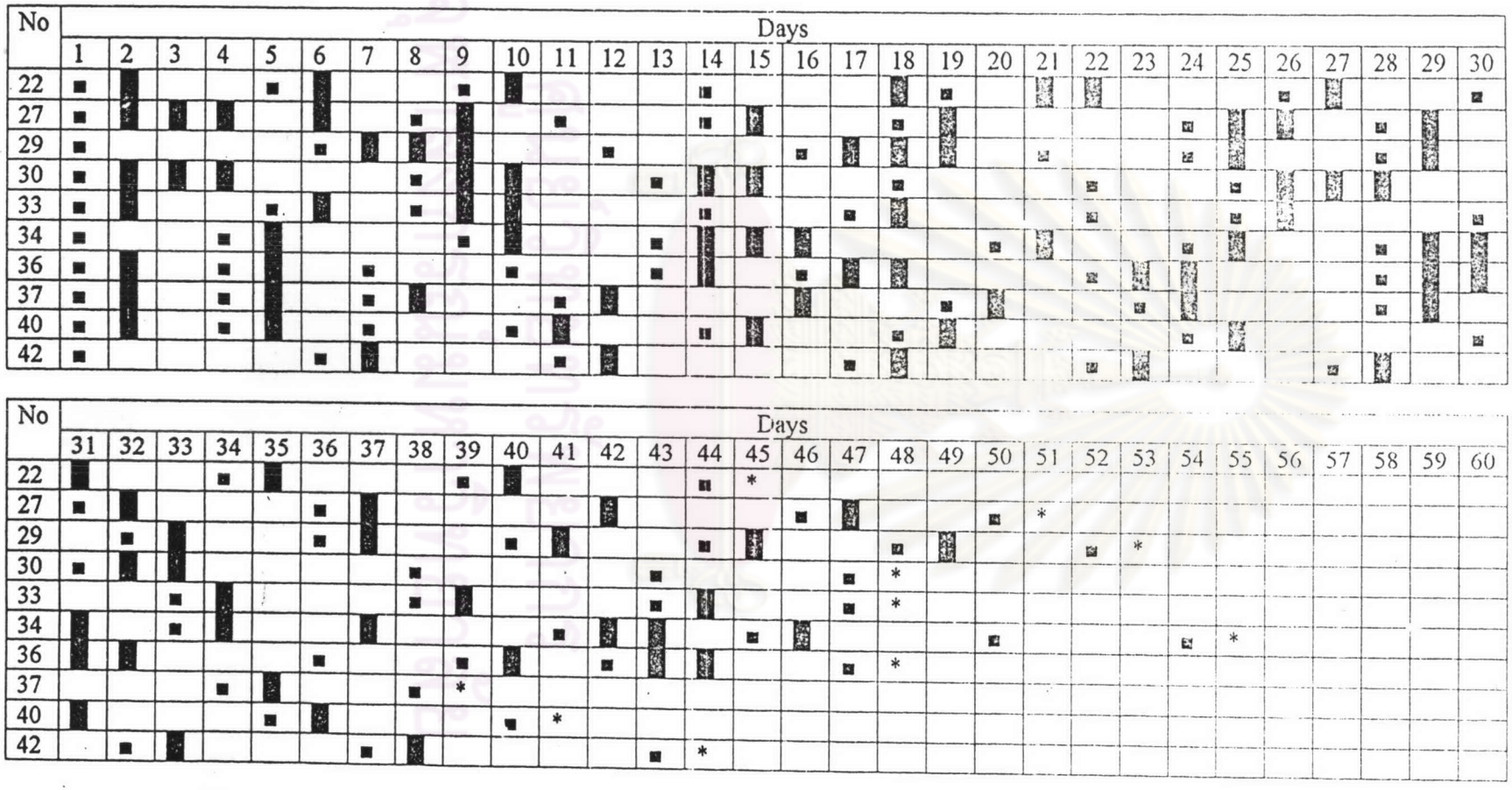
* indicated the sacrifice day of the rats

Table 4 Changes of vaginal cytology in cyclic female rats treated with 1 mg/kgBW of *Mucuna collettii*. ■ and ▪ indicate fully and partial cornification, respectively



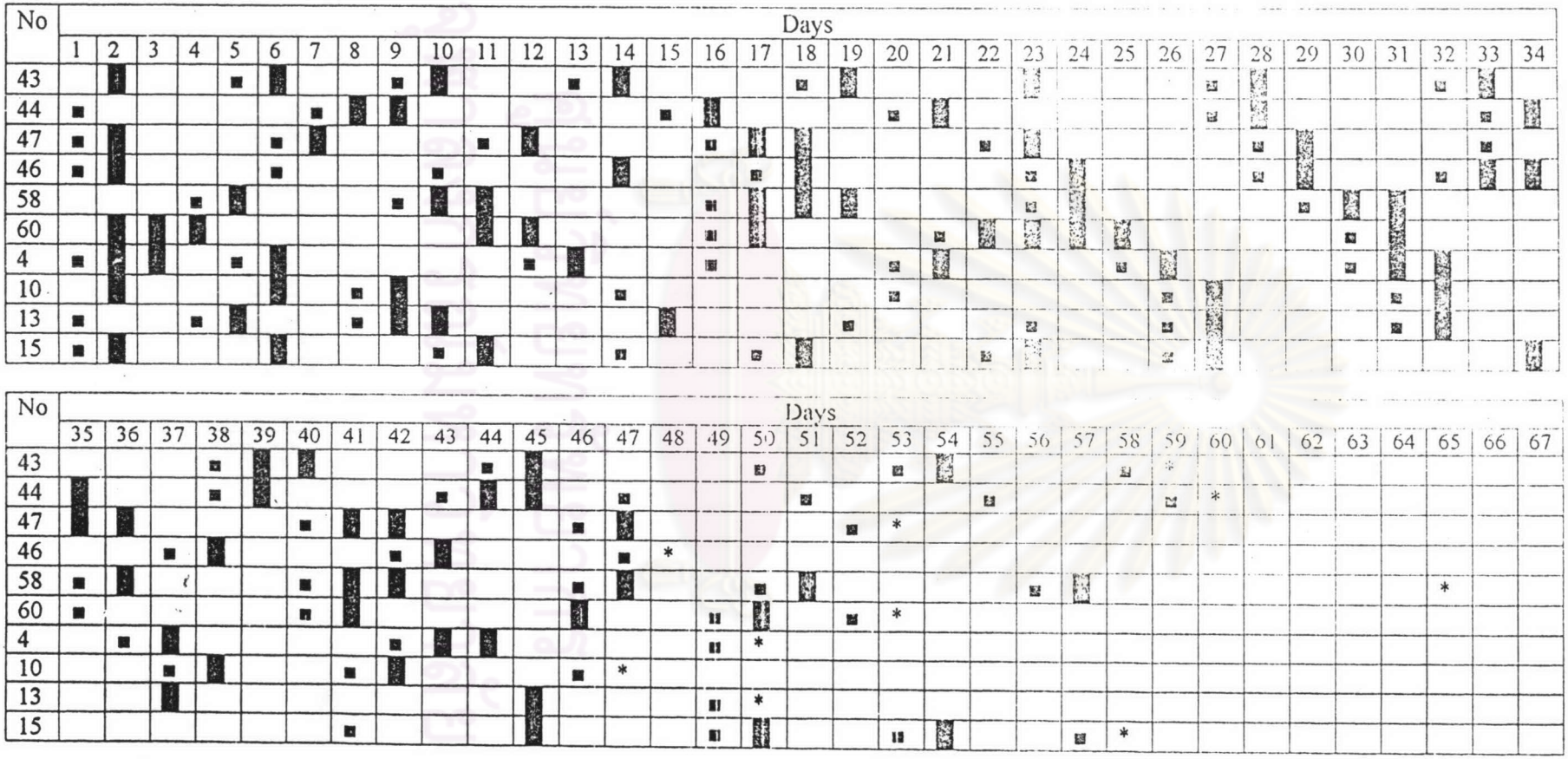
* indicates the sacrifice day of the rats

Table 5 Changes of vaginal cytology in cyclic female rats treated with 10 mg/kgBW of *Mucuna collettii*. ▩ and ▪ indicate fully and partial cornification, respectively.



* indicates the sacrifice day of the rats

Table 6 Changes of vaginal cytology in cyclic female rats treated with 100 mg/kgBW of *Mucuna collettii*. ▪ and ▫ indicate fully and partial cornification, respectively.



* indicates the sacrifice day of the rats

Table 7 Changes of estrous cycle length in each study period in cyclic female rats treated with distilled water, testosterone propionate and *Mucuna collettii*.

Group	Estrous cycle length (days)		
	Pre-treatment	Treatment	Post-treatment
DW	4.76±0.18	4.44±0.16	4.75±0.25
TP	4.36±0.15	-	-
Mc-1	4.54±0.19	4.71±0.17	4.22±0.14
Mc-10	4.03±0.32	4.48±0.15	4.27±0.19
Mc-100	4.87±0.24	5.40±0.11	4.42±0.16

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Figure 12 Changes of serum estradiol levels in ovariectomized female rats treated with distilled water, testosterone propionate and *Mucuna collettii*.

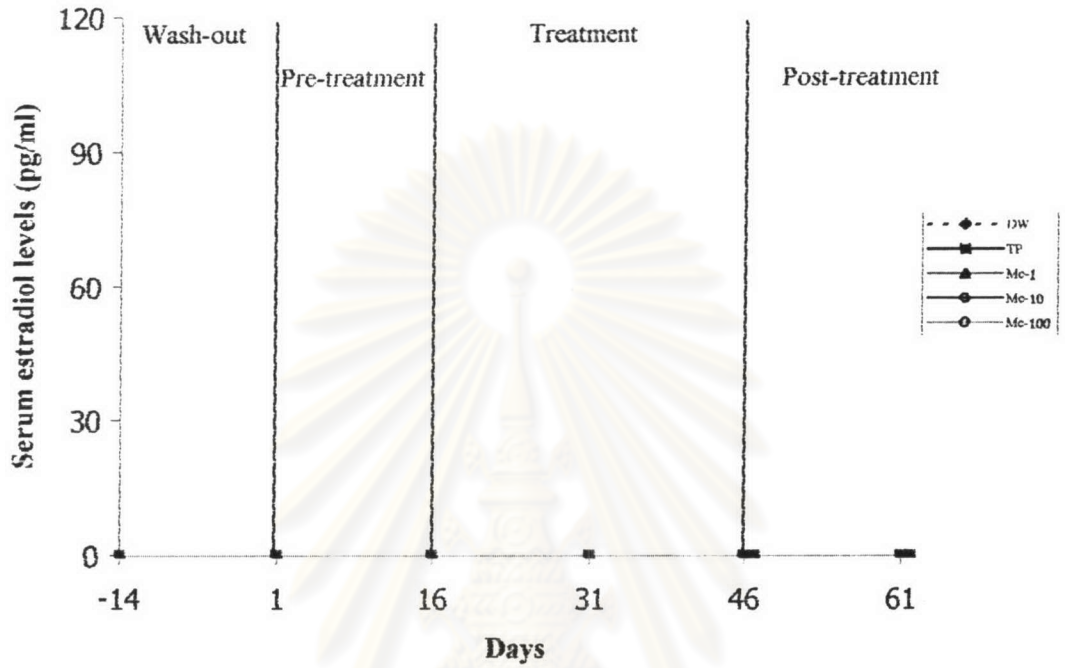
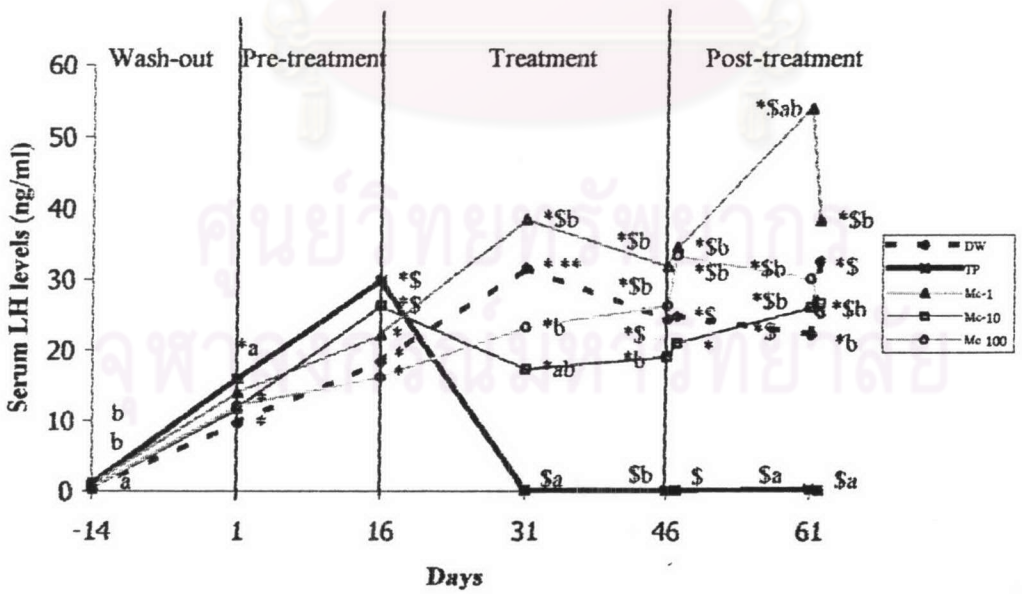


Figure 13 Changes of serum LH levels in ovariectomized female rats treated with distilled water, testosterone propionate and *Mucuna collettii*.



* Significant difference compared to D_{-14} levels.
$ Significant difference compared to D_1 levels.
[a] Significant difference of TP and Mc groups compared to DW group.
[b] Significant difference of Mc groups compared to TP group.

2.1.3 Serum FSH levels

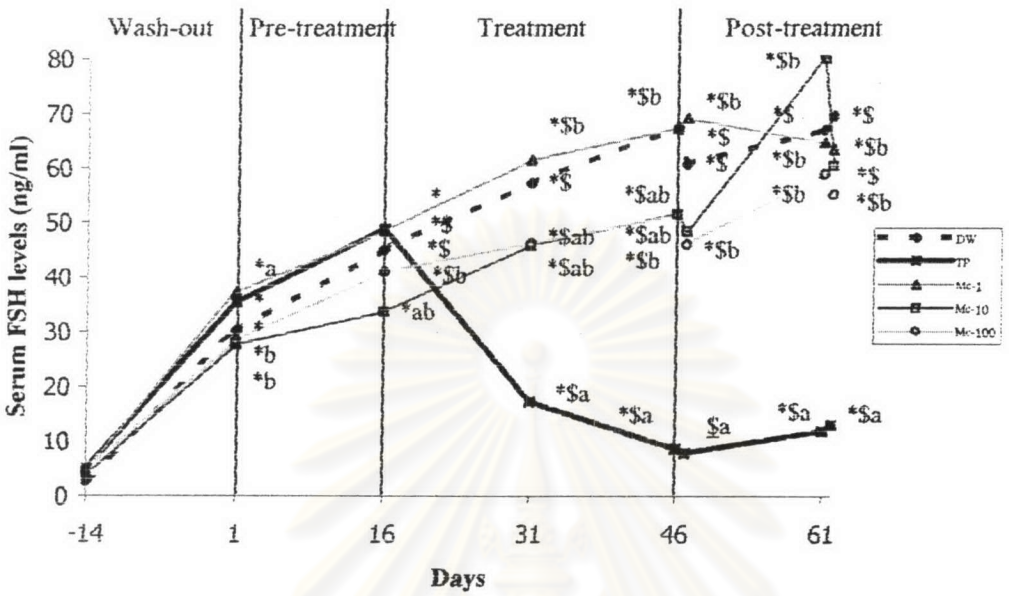
Basal serum FSH levels (D_1) in female rats after ovariectomy were significantly higher than the normal cyclic female rats in Experiment 1 (31.75±1.93 and 2.60±0.29 ng/ml in OVX rats and normal female rats, respectively) and the levels at D_{-14} as shown in Figure 14. Serum FSH levels were kept in the high levels throughout the study periods in DW and all Mc treated groups. Except for the TP group, serum FSH levels at D_{47} were not significantly differences from D_{-14}.

When compared to the pre-treatment levels (D_1), serum FSH levels of DW and all Mc treated groups were significantly increased during D_{16}-D_{62}, except for D_{16} of the Mc-1 and Mc-10 groups. In TP group, serum FSH levels were significantly increased higher than D_1 at only D_{16} while the levels were significantly decreased at D_{31}-D_{62}.

Comparison to the DW group, there were no different of serum FSH levels between Mc-1 and DW group, except at D_1. The serum FSH levels of Mc-10 group during D_{16}-D_{46} and Mc-100 group during D_{31}-D_{46} were significantly lower and recovered to the DW levels thereafter. On the other hand, serum FSH levels in Mc-10, Mc-100 and TP groups were significantly lower than that of DW group at D_{16}-D_{46}, D_{31}-D_{46} and D_{31}-D_{62}, respectively.

Comparison to the TP group, serum FSH levels of Mc-10 and Mc-100 groups were significantly lower at D_1-D_{16} while the FSH levels of all Mc treated groups were significantly higher during D_{31}-D_{62}.

Figure 14 Changes of serum FSH levels in ovariectomized female rats treated with distilled water, testosterone propionate and *Mucuna collettii*.



* Significant difference compared to D_{-14} levels.
$ Significant difference compared to D_1 levels.
[a] Significant difference of TP and Mc groups compared to DW group.
[b] Significant difference of Mc groups compared to TP group.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

2.2 Effects of *Mucuna collettii* on uteri

There were no significant differences of uterine weight and its absolute weight in DW and all Mc treated groups at the end of post-treatment period when compared to that of the end of treatment period (Table 8). In TP group, uterine weight and its absolute weight at the end of post-treatment period were significantly decreased when compared to the treatment period.

Comparison to the DW group, uterine weight and its absolute weight of DW and all Mc treated groups were no significant differences between groups. In TP group, uterine weight and its absolute weight were significantly higher in both treatment and post-treatment periods.

Comparison to the TP group, uterine weight and its absolute weight of all Mc treated groups were significantly lower in both treatment and post-treatment periods.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 8 Changes of the weight and absolute weight of uteri at the day of treatment and at the day of post-treatment periods in ovariectomized female rats treated with distilled water, testosterone propionate and *Mucuna collettii*.

Group	Uterine weight (grams)		Absolute uterus weight (x 10^{-3} grams)	
	Treatment	Post-treatment	Treatment	Post-treatment
DW	0.13±0.01	0.12±0.00	0.38±0.02	0.33±0.03
TP 600 µg/100gBW/day	0.50±0.01[b]	0.29±0.03[ab]	1.62±0.03[b]	0.84±0.06[ab]
M. collettii 1 mg/kg/day	0.12±0.01[c]	0.13±0.02[c]	0.39±0.03[c]	0.39±0.06[c]
M. collettii 10 mg/kg/day	0.11±0.01[c]	0.12±0.01[c]	0.32±0.02[c]	0.34±0.02[c]
M. collettii 100 mg/kg/day	0.12±0.01[c]	0.12±0.01[c]	0.37±0.03[c]	0.37±0.03[c]

[a] Significant difference compared to the end of treatment period.
[b] Significant difference of TP and Mc groups compared to DW group.
[c] Significantl difference of Mc groups compared to TP group.

2.3 Effects of *Mucuna collettii* on histological changes in uteri

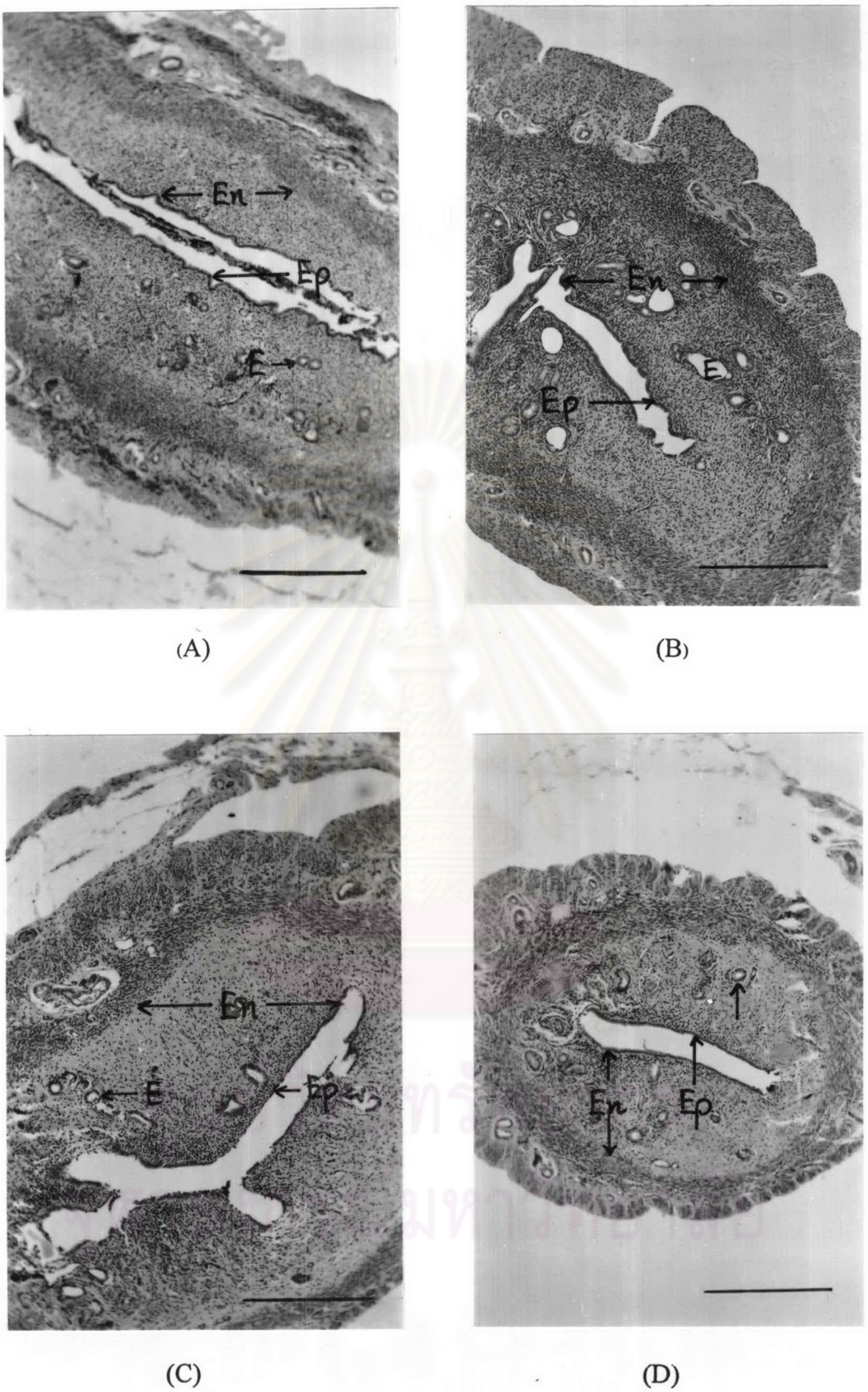
Histological changes in uteri

The uterine morphology of DW and all Mc treated groups at the end of treatment period appeared to be the thin endometrial mucosa layers. The mucosa epithelial cell lining was a layer of low cuboidal cells. Few number and narrow lumen of endometrial glands that dispersed in some area of endometrium (Figure 15A-D). In controversy, the endometrium of rats in TP group was thick. The epithelial cell lining was a layer of columnar cells. Number and diameter of endometrial glands were increased when compared to DW group (Figure 15E).

The histology of uteri of DW and Mc treated groups at the end of post-treatment period demonstrated the thin endometrium. The epithelial cell lining was a layer of low cuboidal cells. Few dispersal endometrial glands with narrow lumen were noticed. In contrast, the endometrial thickness, number and diameter of endometrial glands of TP group increased when compared to DW group. In addition, the epithelial cell lining was a layer of columnar cells (Figure 15F).

There were no different of histological structure of uteri at the end of treatment period and at the end of post-treatment period in all treatment groups, except endometrial layer thickness, number and diameter of endometrial glands of rats in TP group at the end of treatment period were higher than that of post-treatment period.

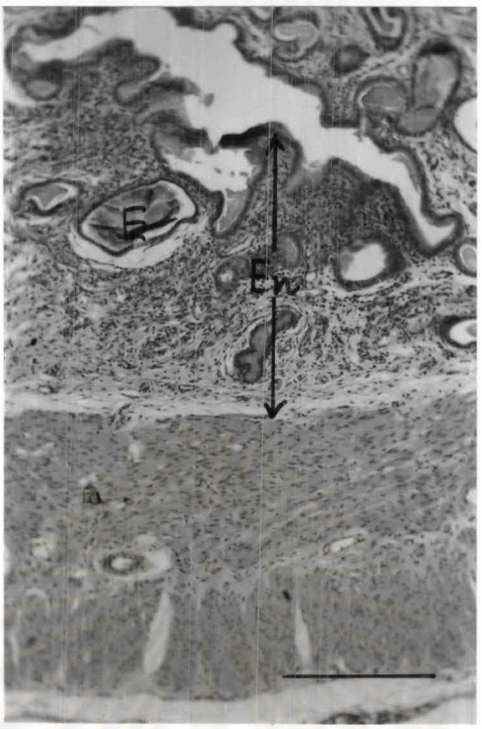
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



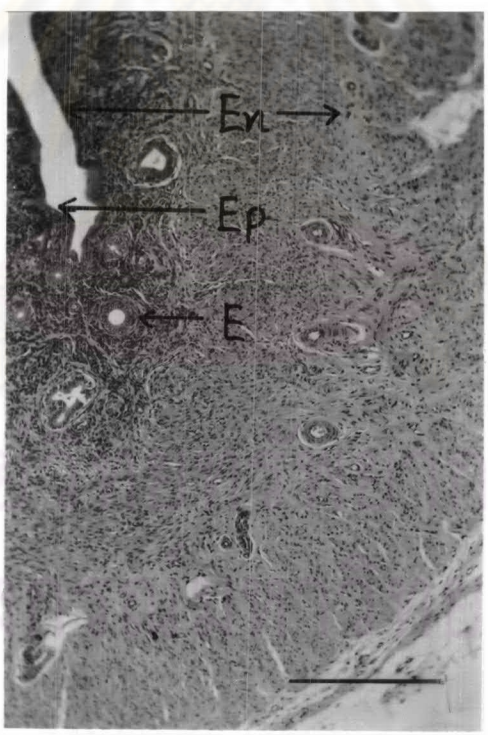
(A) (B)

(C) (D)

Figure 15 Uterine histology of ovariectomized female rats treated with distilled water (A), Mc-1 (B), Mc-10 (C), Mc-100 (D) and TP at the end of treatment (E) and TP at the end of post-treatment periods (F) with H & E staining for endometrium (En), epithelial cells (Ep) and endometrial gland (E). Bar scale = 200 μm



(E)



(F)

Figure 15 (continued) Uterus of ovariectomized female rats treated with distilled water (A), Mc-1 (B), Mc-10 (C), Mc-100 (D) and TP at the end of treatment (E) and TP at the end of post-treatment periods (F) with H & E staining for endometrium (En), epithelial cells (Ep) and endometrial gland (E). Bar scale = 200 μm

2.4 Effects of *Mucuna collettii* on estrous cycles

There were no estrous cycles in all of the experimental groups and vaginal smear was completely found leukocyte cells throughout the experiment (Table 9-13).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 9 Changes of vaginal cytology in ovariectomized female rats treated with distilled water. © indicates vaginal leukocytes.

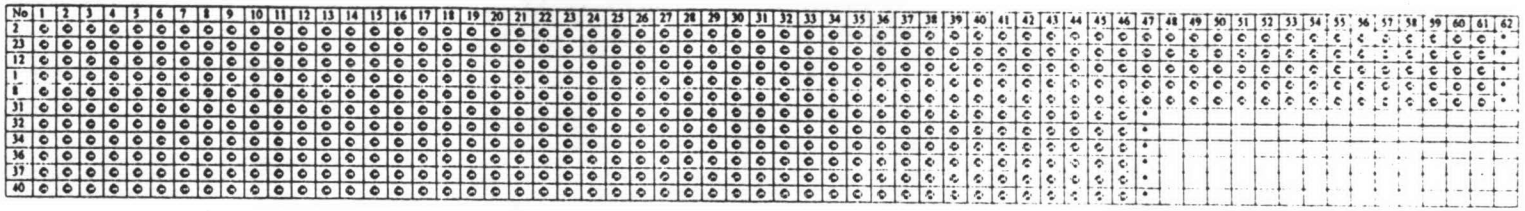


Table 10 Changes of vaginal cytology in ovariectomized female rats treated with testosterone propionate. © indicates vaginal leukocytes.

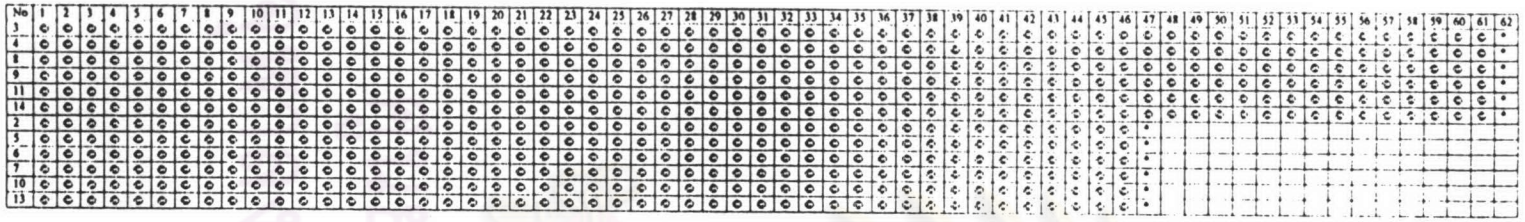


Table 11 Changes of vaginal cytology in ovariectomized female rats treated with 1 mg/kgBW of *Mucuna collettii*. © indicates vaginal leukocytes.

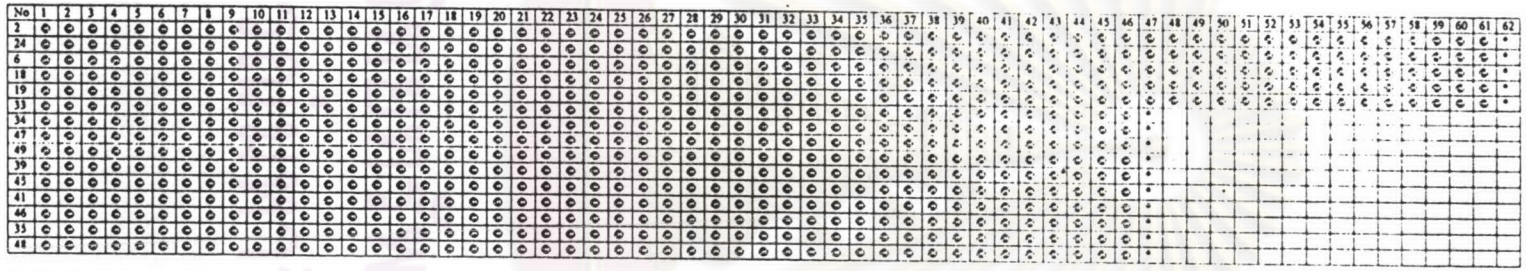


Table 12 Changes of vaginal cytology in ovariectomized female rats treated with 10 mg/kgBW of *Mucuna collettii*. © indicates vaginal leukocytes.

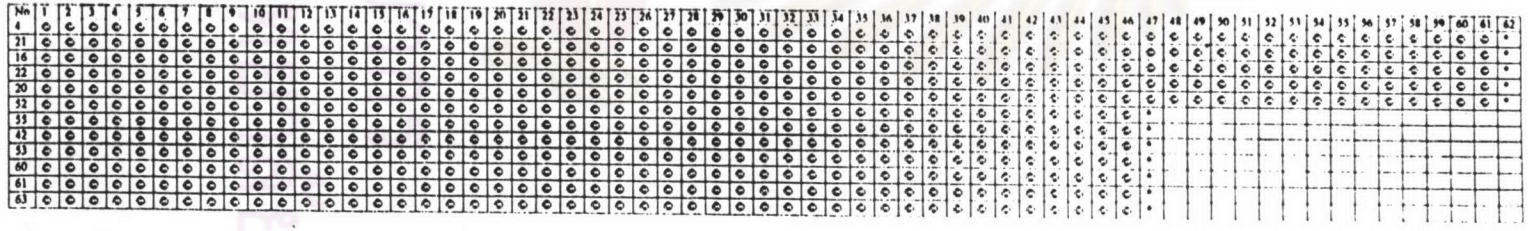
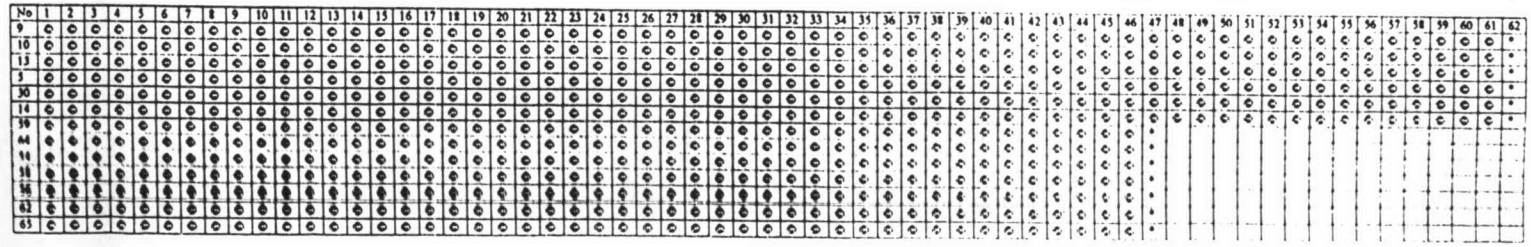


Table 13 Changes of vaginal cytology in ovariectomized female rats treated with 100 mg/kgBW of *Mucuna collettii*. © indicates vaginal leukocytes.



• indicates the sacrifice day of the rat

Experiment 3; Effects of *Mucuna collettii* on hormone-related testicular functions and reproductive organs in normal male rats.

3.1 Effects of *Mucuna collettii* on hormone-related testicular functions

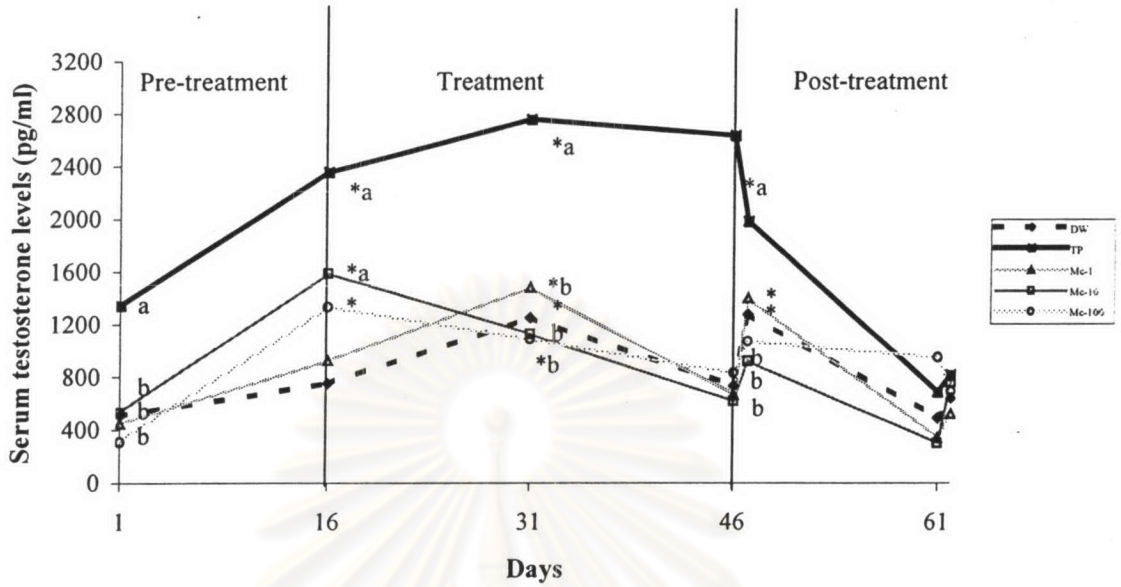
3.1.1 Serum testosterone levels

When compared to the pre-treatment levels (D_1), serum testosterone levels of DW and Mc-1 groups were significantly increased only at D_{31}-D_{47} (Figure 16). In Mc-10 and Mc-100 groups, serum testosterone levels were significantly increase at D_{16} and D_{16}-D_{31}, respectively. Serum testosterone levels of TP group were significantly increased during treatment period and recovered to the pre-treatment values after 2 weeks of TP withdrawal.

Comparison to the DW group, there were no significant differences in all of Mc treated groups throughout the study periods, except of Mc-10 group, serum testosterone levels were significantly higher at D_{16}. Serum testosterone levels of TP group were significantly higher than the DW group since D_1 and kept higher than the DW group throughout the treatment and early post-treatment periods (D_{16}-D_{47}).

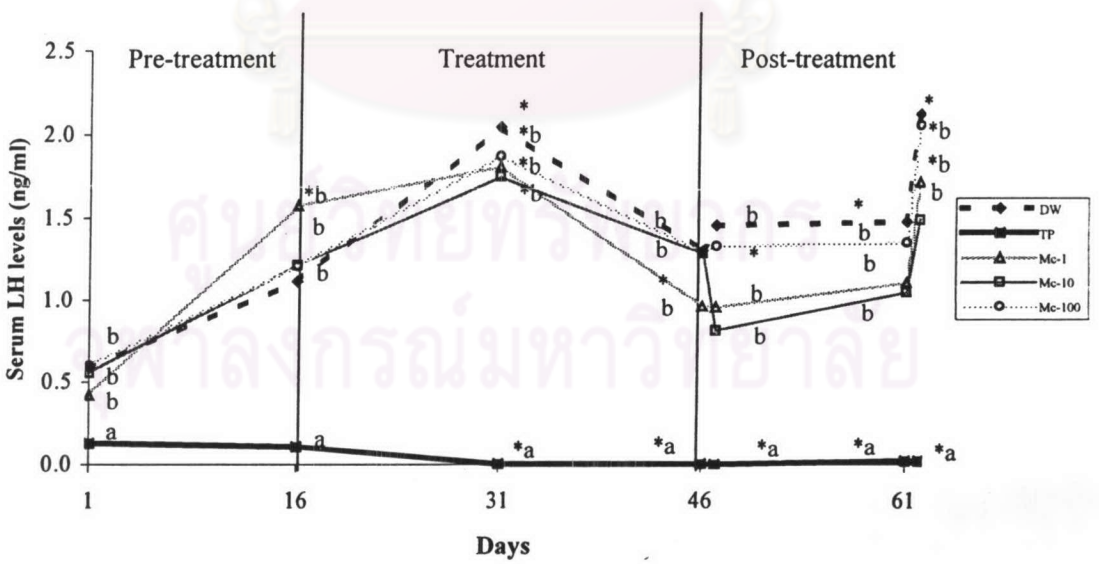
Comparison to the TP group, serum testosterone levels of all Mc treated groups were significantly lower since D_1 of study period and the serum testosterone levels were kept in the lower levels than the TP group throughout the treatment and early post-treatment periods (D_{16}-D_{47}).

Figure 16 Changes of serum testosterone levels in normal male rats treated with distilled water, testosterone propionate and *Mucuna collettii*.



* Significant difference compared to D_1 levels.
[a] Significant difference of TP and Mc groups compared to DW group.
[b] Significant difference of Mc groups compared to TP group.

Figure 17 Changes of serum LH levels in normal male rats treated with distilled water, testosterone propionate and *Mucuna collettii*.



* Significant difference compared to D_1 levels.
[a] Significant difference of TP and Mc groups compared to DW group.
[b] Significant difference of Mc groups compared to TP group.

3.1.2 Serum LH levels

When compared to the pre-treatment levels (D_1), serum LH levels of DW group were significantly increased during D_{31}-D_{62} (Figure 17). Serum LH levels of Mc treated groups were significantly higher than the pre-treatment levels in some points, Mc-1 group at D_{16}-D_{31} and D_{62}, Mc-10 group at D_{31} and Mc-100 group at D_{31} and D_{62}. Serum LH levels of TP group were significantly decreased after 15 days of TP administration until the injection was stop for 2 weeks.

Comparison to the DW group, serum LH levels of all Mc treated groups were no significant differences throughout the study periods. In contrast, serum LH levels of TP group were significantly lower than the DW group since D_1 to the end of study period (D_1-D_{62}).

Comparison to the TP group, serum LH levels of all Mc treated groups were significantly higher since D_1 until the end of study period (D_1-D_{62}).

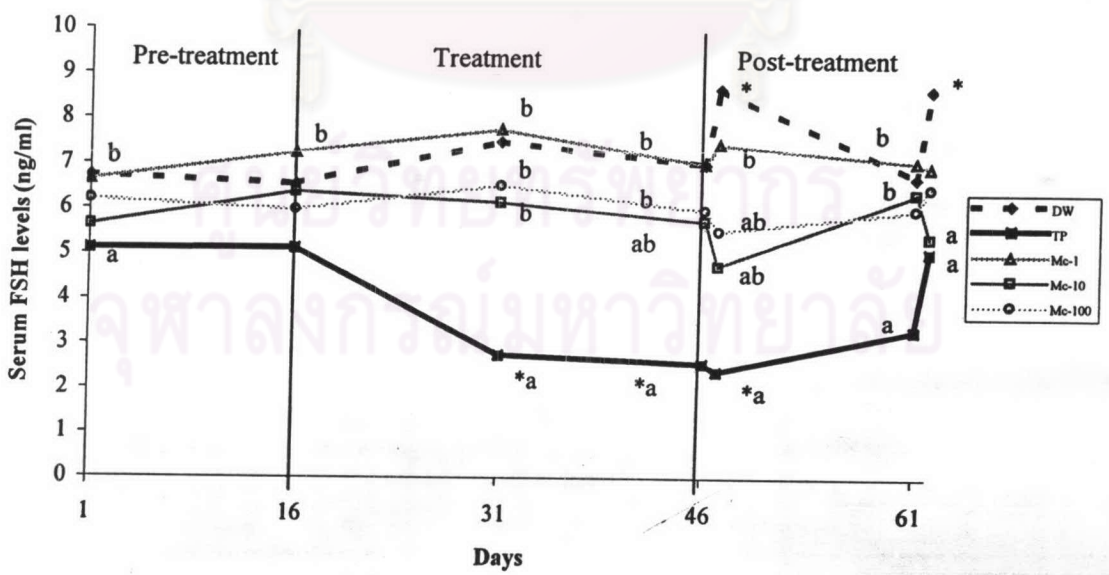
3.1.3 Serum FSH levels

When compared to the pre-treatment levels (D_1), serum FSH levels in DW and all Mc groups were no significant differences throughout the study periods (Figure 18), except in the DW group, the levels were significantly increased at D_{47} and D_{62}, but serum FSH levels of TP group were significantly decreased during D_{31}- D_{47}.

Comparison to the DW group, serum FSH levels of Mc-1 group were no significant differences throughout the study periods. Serum FSH levels of Mc-10 group at D_{46}, D_{47} and D_{62} were significantly lower and serum FSH levels of Mc-100 group only D_{47} were significantly lower. In TP group, serum FSH levels were significantly lower throughout the study period (D_1-D_{62}), except at D_{16}, serum FSH levels were not significant differences.

Comparison to the TP group, serum FSH levels of Mc-1 group during D_1-D_{61} were significantly higher. Serum FSH levels of Mc-10 group during D_{31}-D_{61}, and Mc-100 group during D_{31}-D_{47} were significantly higher and recovered to the TP levels at D_{62} and during D_{61}-D_{62}, respectively.

Figure 18 Changes of serum FSH levels in normal male rats treated with distilled water, testosterone propionate and *Mucuna collettii*.



* Significant difference compared to D_1 levels.
[a] Significant difference of TP and Mc groups compared to DW group.
[b] Significant difference of Mc groups compared to TP group.

3.2 Effects of *Mucuna collettii* on testes, epididymis and seminal vesicle

When the weights and absolute weight of testes, epididymis and seminal vesicle in each group were compared between 2 periods of at the end of treatment and at the end of post-treatment, epididymal weights of DW group were significantly increased, but absolute testicular weight was significantly decreased (Table 14A and 14B). The weights and absolute weights of testes, epididymis and seminal vesicle of Mc treated groups were no significant differences within each group. Except for Mc-10 group, the absolute weight of epididymis was significantly decreased. In TP group, the weights and absolute weights of testes, epididymis and seminal vesicle were significantly decreased.

Comparison to the DW group, the weight and absolute weight of testes, epididymis and seminal vesicle of Mc treated groups were no significant differences. Except epididymal weight of Mc-1 group was significantly higher at the end of treatment period and decreased to the DW levels at the end of post-treatment period. Testicular and epididymal weights and absolute weight of epididymis of Mc-10 group were significantly higher at the end of treatment period and recovered to the DW levels at the end of post-treatment period. In TP group, testicular weight and its absolute weight were no significant differences in both at the end of treatment and at the end of post-treatment periods, except testicular weight at the end of post-treatment period were significantly lower. In addition, the weight and absolute weight of epididymis and seminal vesicle of TP group were significantly higher in both at the end of treatment and at the end of post-treatment periods. Except epididymal weight at the end of post-treatment period were significantly lower.

Comparison to the TP group, Testicular weight of Mc treated groups were significantly higher at the end of post-treatment period. The absolute weight of testes of Mc treated groups were no significant differences in both at the end of treatment and at the end of post-treatment periods. Epididymal weight and its absolute weight of all Mc treated groups were significantly lower at the end of treatment period and increased to the DW levels at the end of post-treatment period. Except epididymal weight of Mc-1 group was significantly higher at the end of post-treatment period. In addition, seminal vesicle weight and its absolute weight of Mc treated groups were significantly lower in both at the end of treatment period and at the end of post-treatment period. Except seminal vesicle of Mc-1 group were increased at the end of post-treatment period.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 14A Changes of the weights of testes, epididymis and seminal vesicle at the end of treatment and at the end of post-treatment periods in normal male rats treated with distilled water, testosterone propionate and *Mucuna collettii*.

Group	Organs weight (grams)					
	Testes		Epididymis		Seminal vesicle	
	Treatment	Post-treatment	Treatment	Post-treatment	Treatment	Post-treatment
DW	3.48±0.12	3.90±0.08*	0.95±0.06	1.25±0.07*	1.04±0.10	1.39±0.13
TP	3.59±0.07	2.89±0.08*[a]	1.67±0.05[a]	1.07±0.03*[a]	3.66±0.14[a]	1.84±0.07*[a]
Mc-1	3.68±0.07	3.63±0.32[b]	1.16±0.07[ab]	1.26±0.05[b]	1.22±0.16[b]	1.63±0.12
Mc-10	3.88±0.01[a]	3.87±0.13[b]	1.25±0.03[ab]	1.13±0.05	1.24±0.13[b]	1.47±0.10[b]
Mc-100	3.69±0.12	3.85±0.13[b]	1.07±0.07[b]	1.24±0.07	1.31±0.16[b]	1.32±0.07[b]

Table 14B Changes of absolute weights of testes, epididymis and seminal vesicle at the end of treatment and at the end of post-treatment periods in normal male rats treated with distilled water, testosterone propionate and *Mucuna collettii*.

Group	Absolute weight ($\times 10^{-3}$ grams)					
	Testes		Epididymis		Seminal vesicle	
	Treatment	Post-treatment	Treatment	Post-treatment	Treatment	Post-treatment
DW	10.66±0.23	9.18±0.29*	2.90±0.14	2.91±0.10	3.14±0.22	3.27±0.31
TP	10.20±0.21	8.23±0.37*	4.76±0.19[a]	3.03±0.09*[a]	10.40±0.44[a]	5.23±0.18*[a]
Mc-1	10.11±0.38	8.33±0.68	3.17±0.17[b]	2.90±0.09	3.29±0.32[b]	3.79±0.28[b]
Mc-10	10.74±0.13	9.54±0.49	3.47±0.09[ab]	2.77±0.12*	3.45±0.37[b]	3.61±0.27[b]
Mc-100	10.04±0.26	9.08±0.35	2.92±0.15[b]	2.93±0.15	3.55±0.40[b]	3.12±0.17[b]

* Significant difference compared to at the end of treatment period.
[a] Significant difference of TP and Mc groups compared to DW group.
[b] Significant difference of Mc groups compared to TP group.

3.3 Effects of *Mucuna collettii* on histological changes in testes, epididymis and seminal vesicle

3.3.1 Histological changes in testes

The testicular histology of all groups at the end of treatment period revealed the intact structure of seminiferous tubules, Leydig's cells, Sertoli cells and spermatogenic cells. The spermatogenesis and spermatogenic lineage were normally found in seminiferous tubules (Figure 19A-E). In addition, the testes treated with TP showed more abundant spermatozoa in the lumen of seminiferous tubules than DW group.

The microanatomy of testes of all groups at the end of post-treatment period presented the intact structure of seminiferous tubules, Leydig's cells, Sertoli cells and spermatogenic cells. The spermatogenesis and spermatogenic lineage were normally developed in seminiferous tubules. The density of spermatozoa was the same in all groups (Figure 19F).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

When the testicular tissue study at the end of treatment period groups were compared to post-treatment period groups, it revealed no changes of testicular structure in all groups, except the spermatozoa of TP group at the end of treatment period was more abundant than that at the end of post-treatment period.

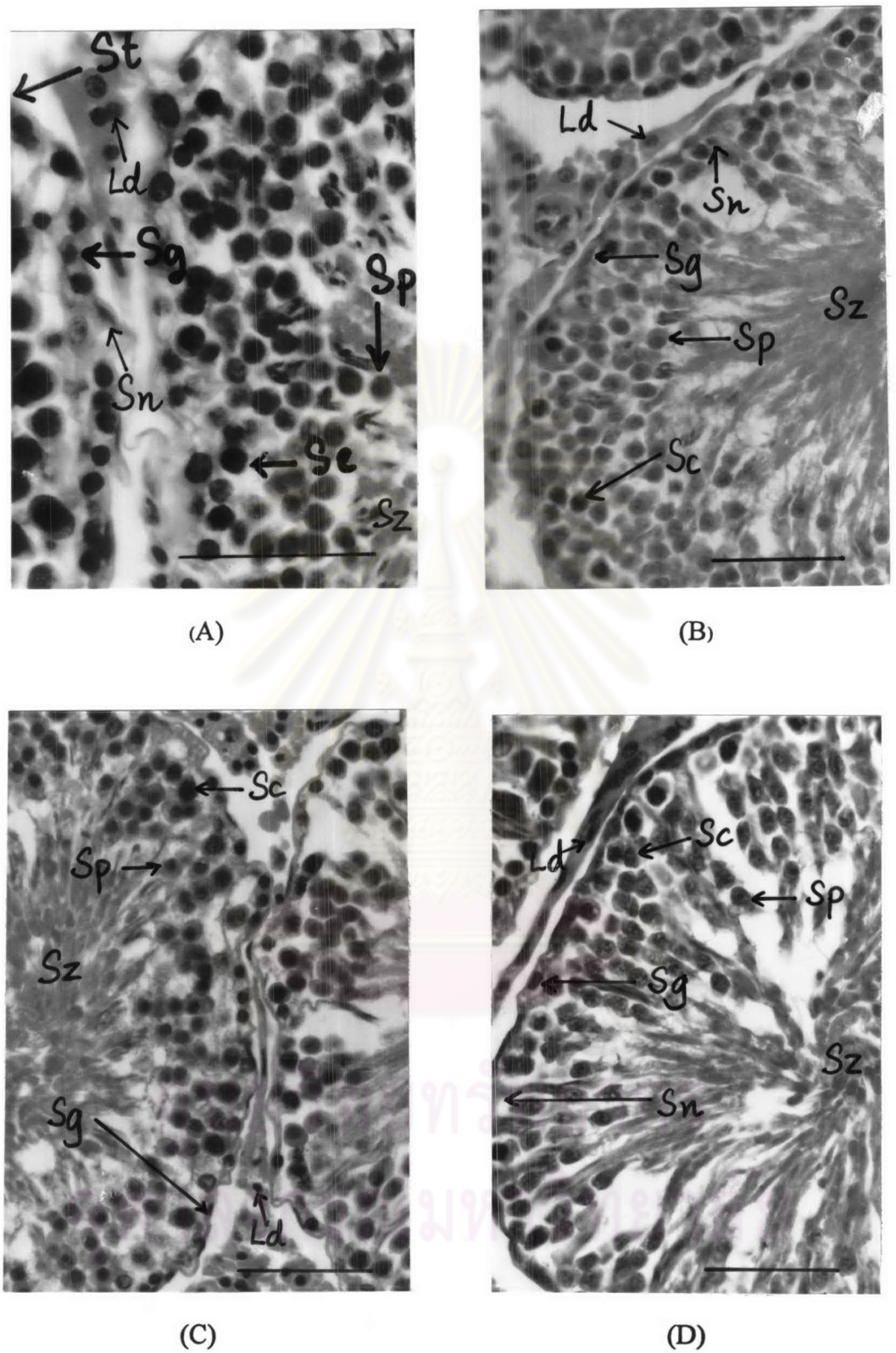
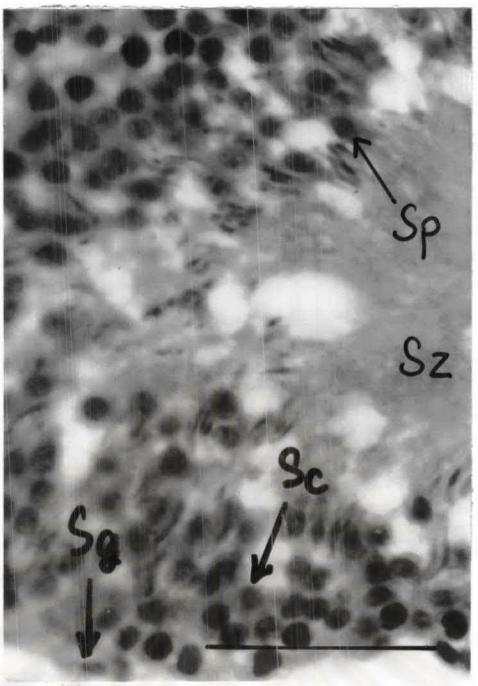
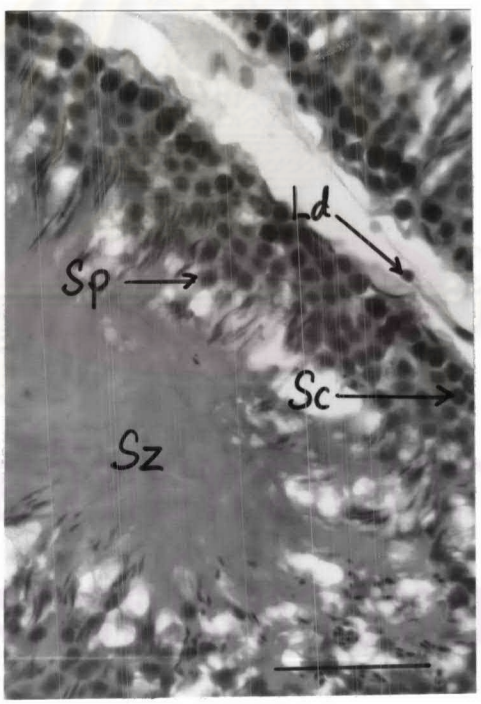


Figure 19 Testicular histology of normal male rats treated with distilled water (A), Mc-1 (B), Mc-10 (C), Mc-100 (D) and TP at the end of treatment (E) and TP at the end of post-treatment periods (F) with H & E staining in seminiferous tubules (St), Leydig's cells (Ld), Sertoli cells (Sn), spermatogonia (Sg), spermatocytes (Sc), spermatids (Sp) and spermatozoa (Sz). Bar scale = 50 μm



(E)



(F)

Figure 19 (continued) Testicular histology of normal male rats treated with distilled water (A), Mc-1 (B), Mc-10 (C), Mc-100 (D) and TP at the end of treatment (E) and TP at the end of post-treatment periods (F) with H & E staining in seminiferous tubules (St), Leydig's cells (Ld), Sertoli cells (Sn), spermatogonia (Sg), spermatocytes (Sc), spermatids (Sp) and spermatozoa (Sz). Bar scale = 50 μm

3.3.2 Histological changes in epididymis

The structure of epididymis of all groups at the end of treatment period demonstrated the cuboidal shape of epididymal epithelial cell lining with stereocilia in head, body and tail regions of epididymis. The diameter of epididymal tubules tended to be wider in the latter regions and the height of epididymal epithelial cell lining tended to be lower. The spermatozoa tended to be abundant in the latter parts of the epididymal tubules. In addition, the numbers of spermatozoa in TP group tended to be higher than DW group (Figure 20A-E).

The epididymal histology of all groups at the end of post-treatment period presented the cuboidal shape of the epithelial cell lining with stereocilia in head, body and tail regions of epididymis and diameter of epididymal tubules tended to be wider in the latter region and the height of epididymal epithelial cell lining tended to be lower. The numbers of spermatozoa were no different between groups.

The epididymal tissue at the end of treatment period groups were compared to at the end of post-treatment period, it showed no differences of the histological structure in all groups, except the numbers of spermatozoa of TP group at the end of treatment period tended to be increased than that of at the end of post-treatment period.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

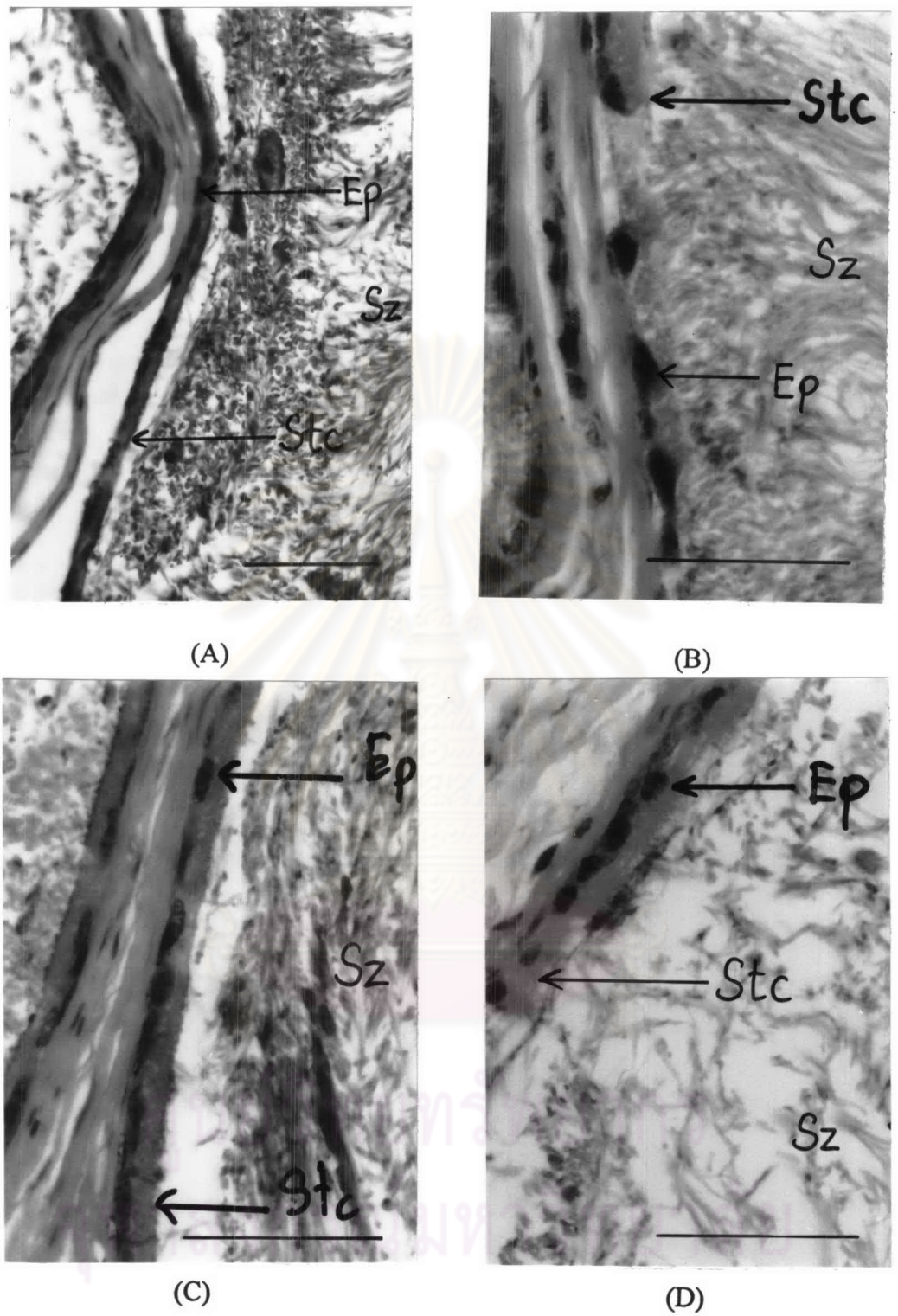
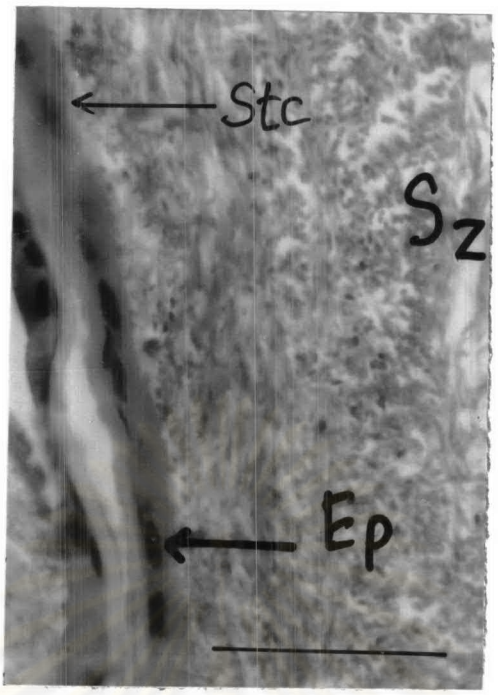
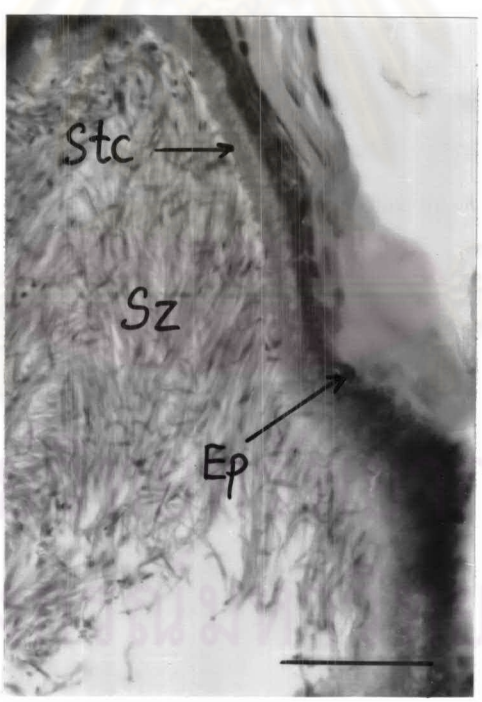


Figure 20 Tail region of epididymis of normal male rats treated with distilled water (A), Mc-1 (B), Mc-10 (C), Mc-100 (D) and TP at the end of treatment (E) and TP at the end of post-treatment periods (F) with H & E staining in epithelial cells (Ep), spermatozoa (Sz) and stereocilia (Stc). Bar scale = 50 μm



(E)



(F)

Figure 20 (continued) Tail region of epididymis of normal male rats treated with distilled-water (A), Mc-1 (B), Mc-10 (C), Mc-100 (D) and TP at the end of treatment (E) and TP at the end of post-treatment periods (F) with H & E staining in epithelial cells (Ep), spermatozoa (Sz) and stereocilia (Stc). Bar scale = 50 μm

3.3.3 Histological changes in seminal vesicle

The seminal vesicle of all groups at the end of treatment period consisted of normal papillary pattern and simple cuboidal shape of the epithelial cell, except for TP group, the papillary of the epithelial cell lining increased folding than DW group (Figure 21A-E).

Seminal vesicle of all groups at the end of post-treatment period appeared to be normal papillary pattern with simple cuboidal shape of the epithelial cell lining, except for TP group, the papillary pattern of the epithelial cell lining consisted of numerous folding and more branching than DW group (Figure 21F).

There were no changes of the seminal vesicle histology between at the end of treatment period and at the end of post-treatment period, except the papillary pattern of the epithelial cell lining of TP group at the end of treatment period consisted of more folding pattern than that of at the end of post-treatment period.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

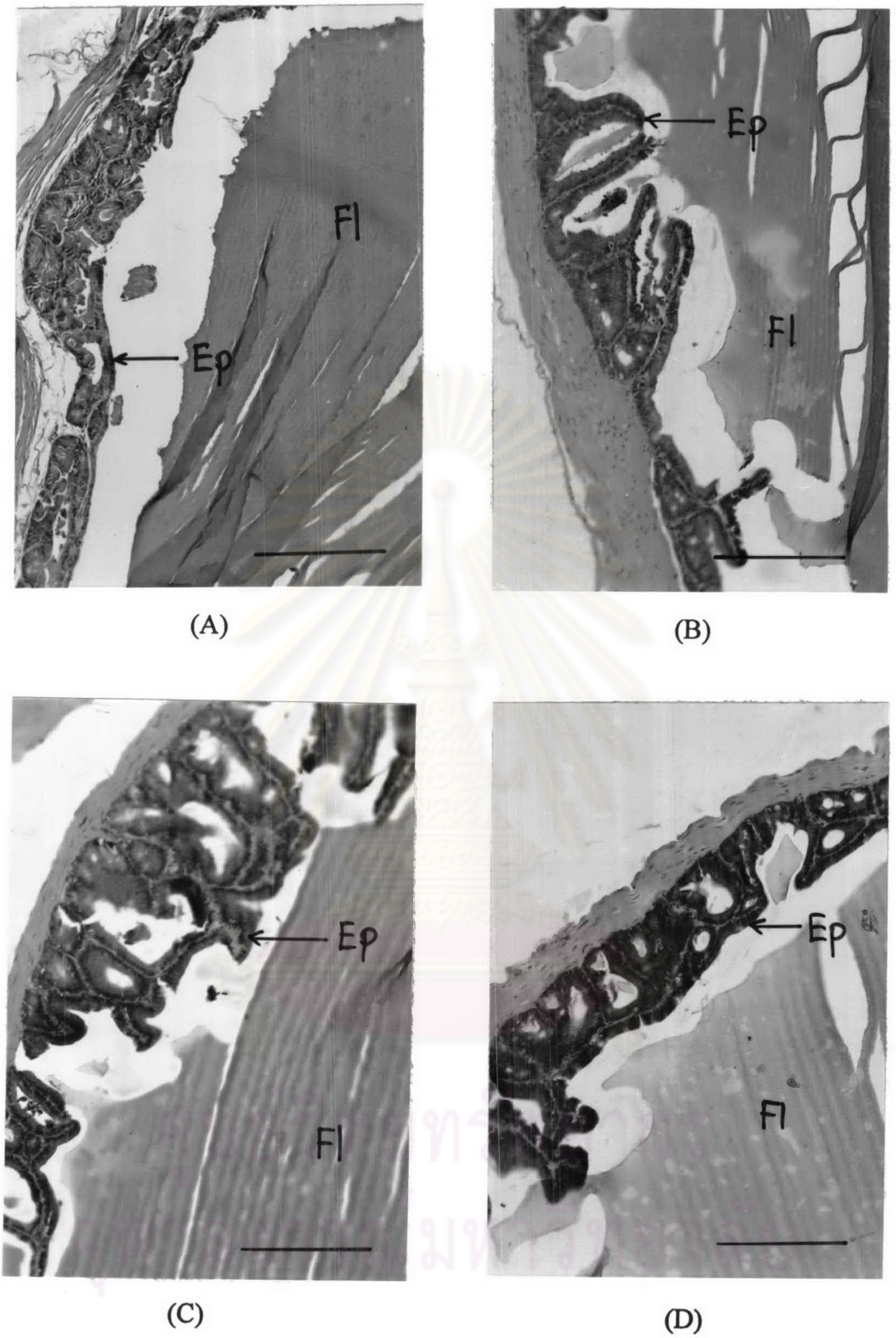
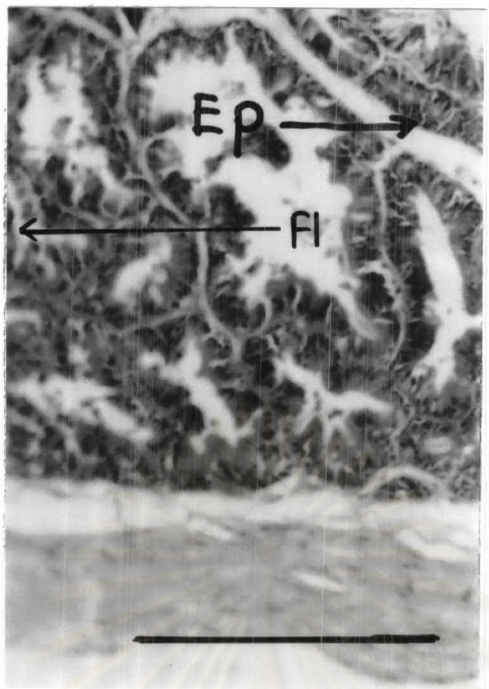
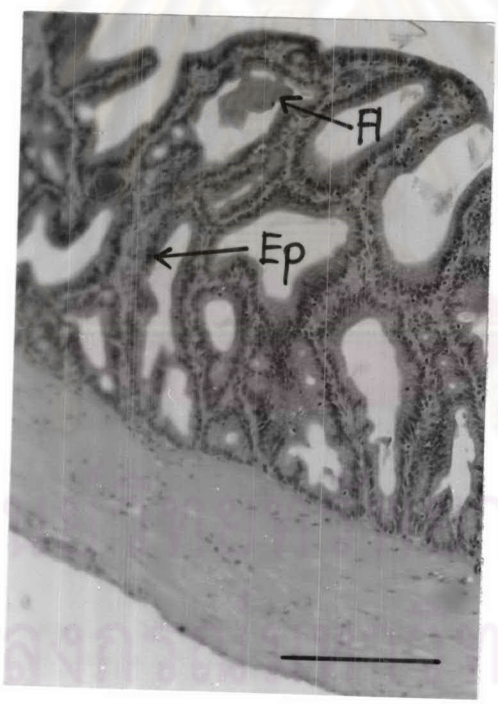


Figure 21 Seminal vesicle of normal male rats treated with distilled water (A), Mc-1 (B), Mc-10 (C), Mc-100 (D) and TP at the end of treatment (E) and TP at the end of post-treatment periods (F) with H & E staining in epithelial cells (Ep) and fluid (Fl). Bar scale = 200 μm



(E)



(F)

Figure 21 (continued) Seminal vesicle of normal male rats treated with distilled-water (A), Mc-1 (B), Mc-10 (C), Mc-100 (D) and TP at the end of treatment (E) and TP at the end of post-treatment periods (F) with H & E staining in epithelial cells (Ep) and fluid (Fl). Bar scale = 200 μm

Experiment 4; Effects of *Mucuna collettii* on hormone-related testicular functions and reproductive organs in orchidectomized male rats.

4.1 Effects of *M. collettii* on hormone-related testicular functions

4.1.1 Serum testosterone levels

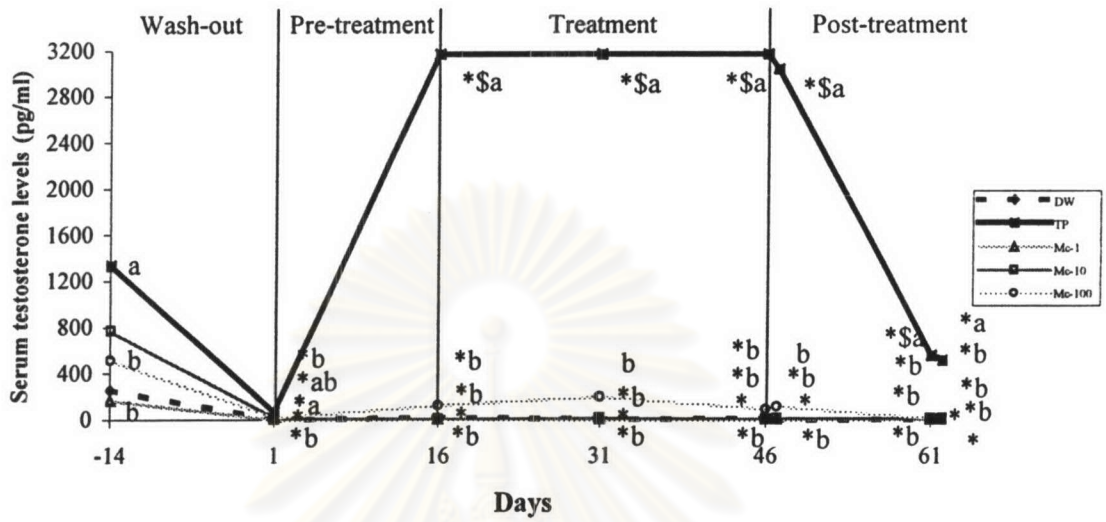
Serum testosterone levels in all 5 groups were significantly dramatically decreased after orchidectomy at D_1 (607.79±210.09 and 21.03±13.20 pg/ml at D_{-14} and D_1, respectively as shown in Figure 22). Serum testosterone levels in male rats after orchidectomy (D_1) were significanty lower than the normal male rats (21.03±13.20 and 628.97±183.47 pg/ml in ODX rats and normal male rats, respectively). Serum testosterone levels of DW and all Mc groups were kept in the low levels throughout the study periods when compared to the day of ODX levels (D_{-14}). No significant differences were found between that 3 periods. In TP group, serum testosterone levels were significantly increased soon after the TP injection (D_{16}) and kept high levels throughout the treatment period (D_{16}-D_{46}). The levels after TP withdrawal for 2 weeks (D_{47}-D_{61}) were still higher than the level at D_1.

Comparison to the DW group, serum testosterone levels of all Mc treated groups were no significant differences throughout the study periods, except for Mc-100 group, serum testosterone levels were significantly higher than DW at D_1. In contrast, serum testosterone levels of TP group were significantly higher since D_{-14} and kept in the higher levels than that of DW group throughout the study periods.

Comparison to the TP group, serum testosterone levels of all Mc treated groups were significantly lower during D_1 -D_{62}.

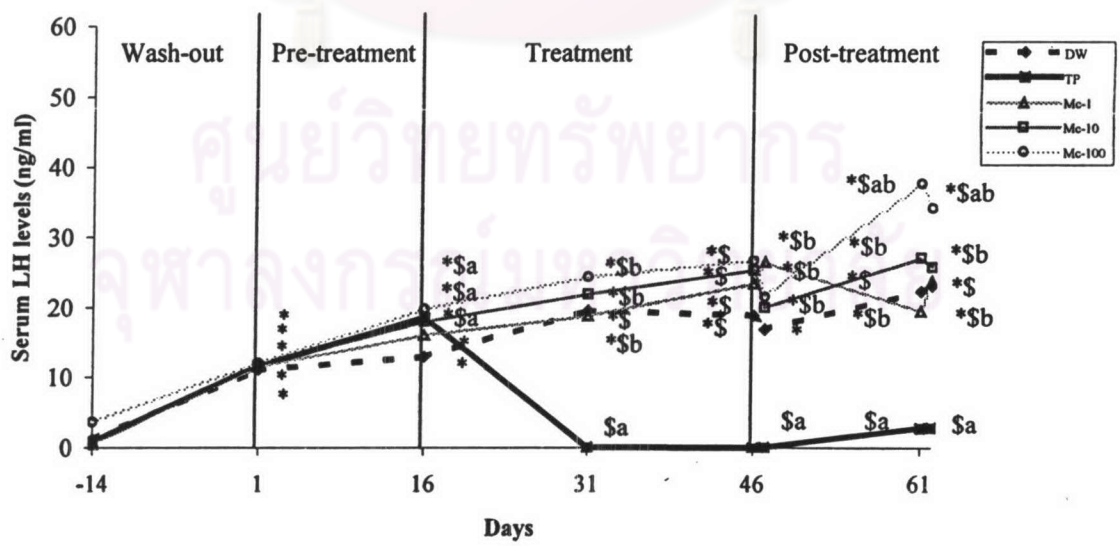
ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Figure 22 Changes of serum testosterone levels in orchidectomized male rats treated with distilled water, testosterone propionate and *Mucuna collettii*.



* Significant difference compared to D_{-14} levels.
$ Significant difference compared to D_1 levels.
[a] Significant difference of TP and Mc groups compared to DW group.
[b] Significant difference of Mc groups compared to TP group.

Figure 23 Changes of serum LH levels in orchidectomized male rats treated with distilled water, testosterone propionate and *Mucuna collettii*.



* Significant difference compared to D_{-14} levels.
$ Significant difference compared to D_1 levels.
[a] Significant difference of TP and Mc groups compared to DW group.
[b] Significant difference of Mc groups compared to TP group.

4.1.2 Serum LH levels

In agreement with the low levels of testosterone, serum LH levels in all 5 groups were significantly increased after orchidectomy (1.35±0.58 and 11.63±0.16 ng/ml at D_{-14} and D_1, respectively). Basal serum of LH levels in male rats after orchidectomy (D_1) were significantly higher than the normal male rats (11.63±0.16 and 0.46±0.09 ng/ml in ODX rats and normal male rats, respectively) as shown in Figure 23. When compared to D_{-14}, serum LH levels of DW and all Mc treated groups were gradually increased since D_1 until the last day of study (D_{16}-D_{62}). Except serum LH levels of DW group at D_{16} and D_{47} and Mc-1 group at D_{16} were no significantly differences. Serum LH levels of TP group were significantly increased at D_1 and D_{16} and returned to D_{-14} levels at (D_{31}-D_{62}).

Comparison to the DW group, serum LH levels of Mc-1 group were no significantly differences throughout the study periods. However, serum LH levels of Mc-10 group at D_{16} and Mc-100 group at D_{16} were significantly higher than the DW group, these higher levels were agreed with the non-different levels between D_1 and D_{16} of the DW group. Serum LH levels of TP group were significantly higher only D_{16}, while the LH levels were significantly lower than the DW group (D_{31}-D_{62}).

Comparison to the TP group, serum LH levels of all Mc treated groups were significantly higher after Mc administration for 15 days (D_{31}-D_{46}) and the higher levels were still remained after the cessation of treatment (D_{47}-D_{62}).

4.1.3 Serum FSH levels

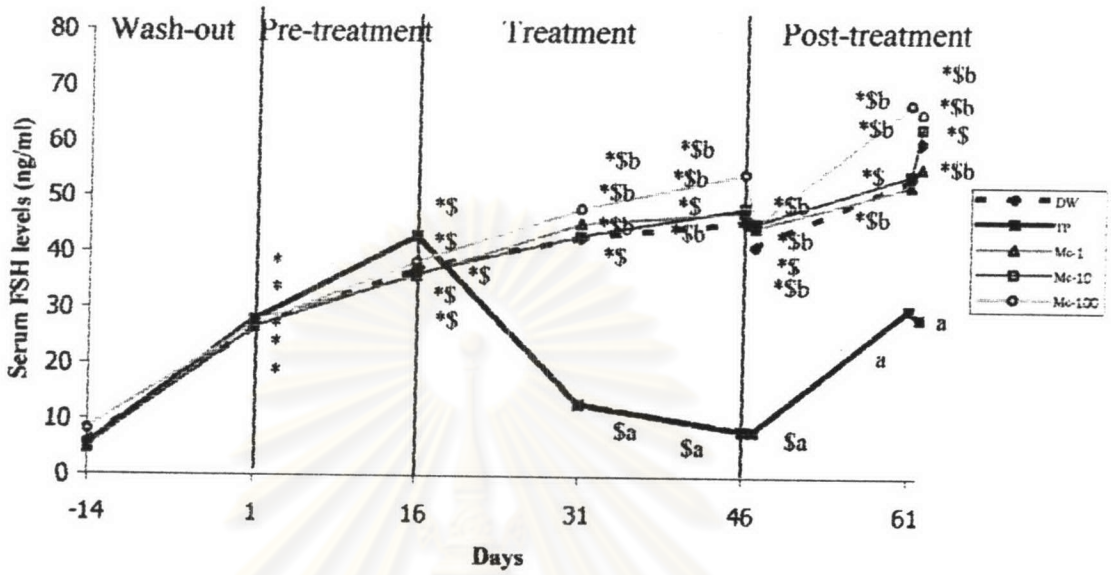
In agreement with the high levels of LH, serum FSH levels in all 5 groups were significantly increased after orchidectomy (5.74±0.65 and 27.15±0.28 ng/ml at D_{-14} and D_1, respectively as shown in Figure 24). Basal serum of FSH levels (D_1) in male rats after orchidectomy were significantly higher than the normal male rats (27.15±0.28 and 6.06±0.31 ng/ml in ODX rats and normal male rats, respectively). Serum FSH levels of DW and all Mc treated groups after ODX were abruptly increased since D_1 and kept in the higher levels until the last day of experiment (D_{62}). Serum FSH levels of TP group were significantly increased at D_1-D_{16}, while the FSH levels were significantly decreased during D_{31}-D_{47}.

Comparison to the DW group, serum FSH levels of TP group were significantly lower after TP administration for 15 days (D_{31}-D_{46}) until the study was over (D_{47}-D_{62}). Serum FSH levels of all Mc treated groups were no significant differences from the DW group throughout the study periods.

Comparison to the TP group, serum FSH levels of Mc treated groups were significantly higher during D_{31}-D_{62}.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Figure 24 Changes of serum FSH levels in orchidectomized male rats treated with distilled water, testosterone propionate and *Mucuna collettii*.



* Significant difference compared to D_{-14} levels.
$ Significant difference compared to D_1 levels.
[a] Significant difference of TP and Mc groups compared to DW group.
[b] Significant difference of Mc groups compared to TP group.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

4.2 Effects of *Mucuna collettii* on epididymis and seminal vesicle

When compared the weights and absolute weights of epididymis and seminal vesicle at the end of post-treatment period to the end of treatment period, no significant differences were found in DW and all Mc treated groups (Table 15). In TP group, seminal vesicle weight and its absolute weight at the end of treatment period were significantly higher than at the end of post-treatment period.

Comparison to the DW group, the weights and absolute weights of epididymis and seminal vesicle of Mc treated groups were no significant differences in both periods of treatment. In TP group, the weights and absolute weights of epididymis and seminal vesicle were significantly higher at the end of treatment period and still remained at the end of post-treament periods.

Comparison to the TP group, the weights and absolute weights of epididymis and seminal vesicle of all Mc treated groups were significantly lower than the TP group in both periods of treatment.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 15 Changes of the weights and absolute weights of epididymis and seminal vesicle at the end of treatment and at the end of post-treatment periods in orchidectomized male rats treated with distilled water, testosterone propionate and *Mucuna collettii*.

Group	Organs weight (grams)				Absolute weight (x 10^{-3} grams)			
	Epididymis		Seminal vesicle		Epididymis		Seminal vesicle	
	Treatment	Post-treatment	Treatment	Post-treatment	Treatment	Post-treatment	Treatment	Post-treatment
DW	0.26±0.03	0.29±0.05	0.09±0.01	0.08±0.01	0.71±0.82	0.79±0.14	0.24±0.02	0.22±0.02
TP	0.83±0.09[a]	0.60±0.04[a]	3.17±0.14[a]	1.44±0.20*[a]	2.07±0.20[a]	1.57±0.15[a]	8.04±0.36[a]	3.79±0.51*[a]
Mc-1	0.25±0.04[b]	0.35±0.02[b]	0.08±0.01[b]	0.08±0.10[b]	0.65±0.09[b]	0.87±0.05[b]	0.21±0.03[b]	0.20±0.02[b]
Mc-10	0.30±0.05[b]	0.33±0.01[b]	0.11±0.01[b]	0.08±0.00[b]	0.82±0.13[b]	0.88±0.05[b]	0.27±0.04[b]	0.20±0.01[b]
Mc-100	0.26±0.03[b]	0.33±0.02[b]	0.09±0.01[b]	0.07±0.10[b]	0.65±0.09[b]	0.83±0.05[b]	0.21±0.02[b]	0.18±0.02[b]

* Significant difference compared to at the end of treatment period.
[a] Significant difference of TP and Mc groups compared to DW group.
[b] Significant difference of Mc groups compared to TP group.

4.3 Effects of *Mucuna collettii* on histological changes in epididymis and seminal vesicle

4.3.1 Histological changes in epididymis

The epididymal tissue of all groups at the end of treatment period revealed a few number of spermatozoa remaining throughout the regions of epididymis. However, spermatozoa of the TP group showed more density than DW and Mc treated groups. The diameter of head, body and tail of epididymis was narrow and the thickness of simple cuboidal epithelial cell lining with stereocilia was increased, 3-4 layers, but the height of epithelial cell lining tended to be lower (Figure 25A-E).

The histology of epididymis of DW and Mc treated groups at the end of treatment period revealed a few number of spermatozoa remaining throughout the epididymal tubules. The diameter of head, body and tail of epididymis was narrow and the thickness of epithelial lining was increased, 3-4 layers. In contrast, the diameter of epididymis of TP treated group tended to be wider in the certain regions of epididymal tubules, but the height of epithelial cell lining tended to be lower (Figure 25F).

The epididymis of rat at the end oftreatment period groups were compared to at the end of post-treatment period, there were no changes of epididymal tissue in all groups.

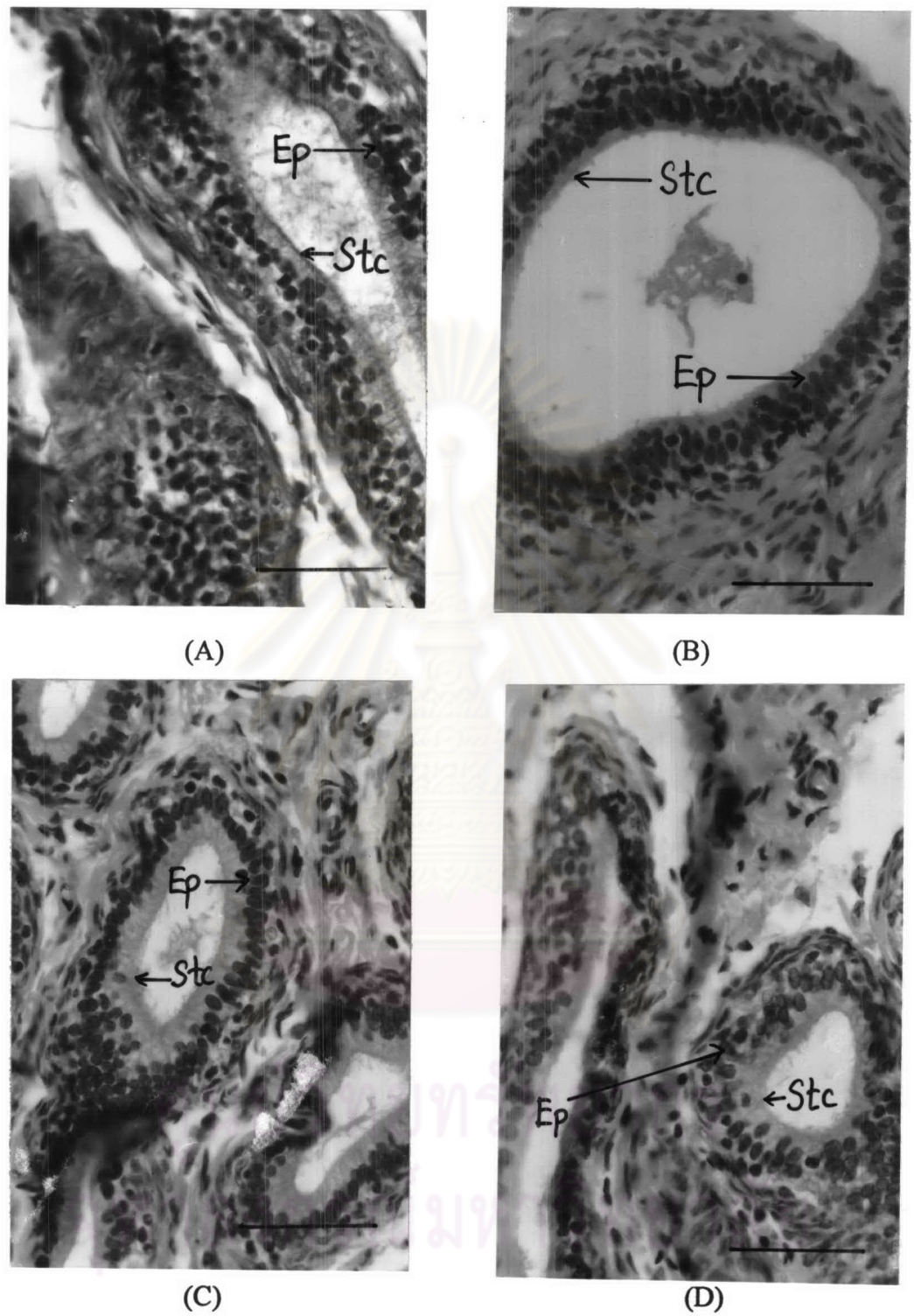
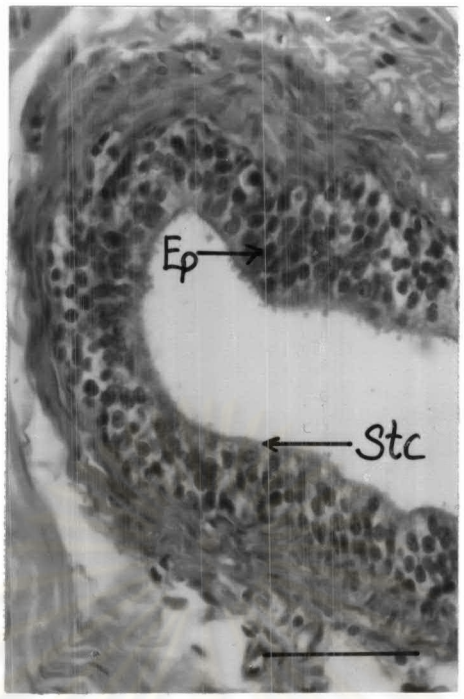
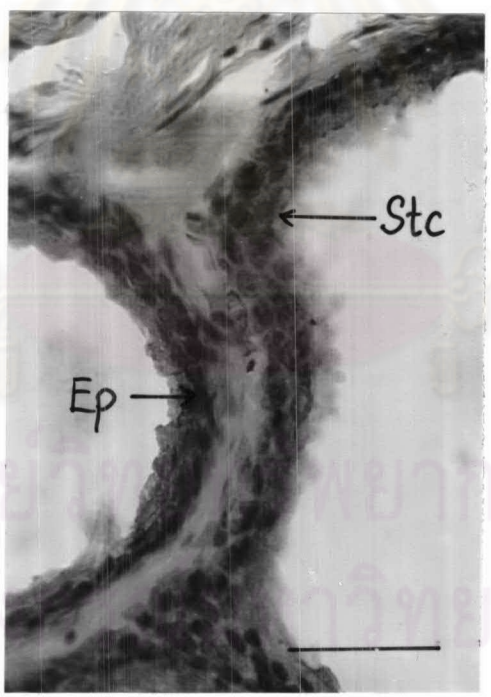


Figure 25 Tail region of epididymis of orchidectomized male rats treated with distilled-water (A), Mc-1 (B), Mc-10 (C), Mc-100 (D) and TP at the end of treatment (E) and TP at the end of post-treatment periods (F) with H & E staining. Epithelial cells (Ep) and stereocilia (Stc). Bar scale = 50 μm



(E)



(F)

Figure 25 (continued) Tail region of epididymis of orchidectomized male rats treated with distilled-water (A), Mc-1 (B), Mc-10 (C), Mc-100 (D) and TP at the end of treatment (E) and TP at the end of post-treatment periods (F) with H & E staining. Epithelial cells (Ep) and stereocilia (Stc). Bar scale = 50 μm

4.3.2 Histological changes in seminal vesicle

The histology of seminal vesicle of all groups at the end of treatment period showed a simple folding pattern with simple cuboidal epithelial cell lining, except for TP group, the papillary pattern of the epithelial cell lining consisted of the increasing folding and the amount layer of seminal fluid were also higher than DW group (Figure 26A-E).

The seminal vesicle of all groups at the end of post-treatment period also appeared a simple folding pattern with simple cuboidal epithelial cell lining, except the papillary pattern of the epithelial cell lining of TP group was increased folding than DW group (Figure 26F).

There were no changes of the seminal vesicle histology at the end of treatment period when compared to the rat at the end of post-treatment period, except the papillary pattern of the epithelial cell lining of TP group at the end of treatment period was increased folding pattern than that of the rat at the end of post-treatment period.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

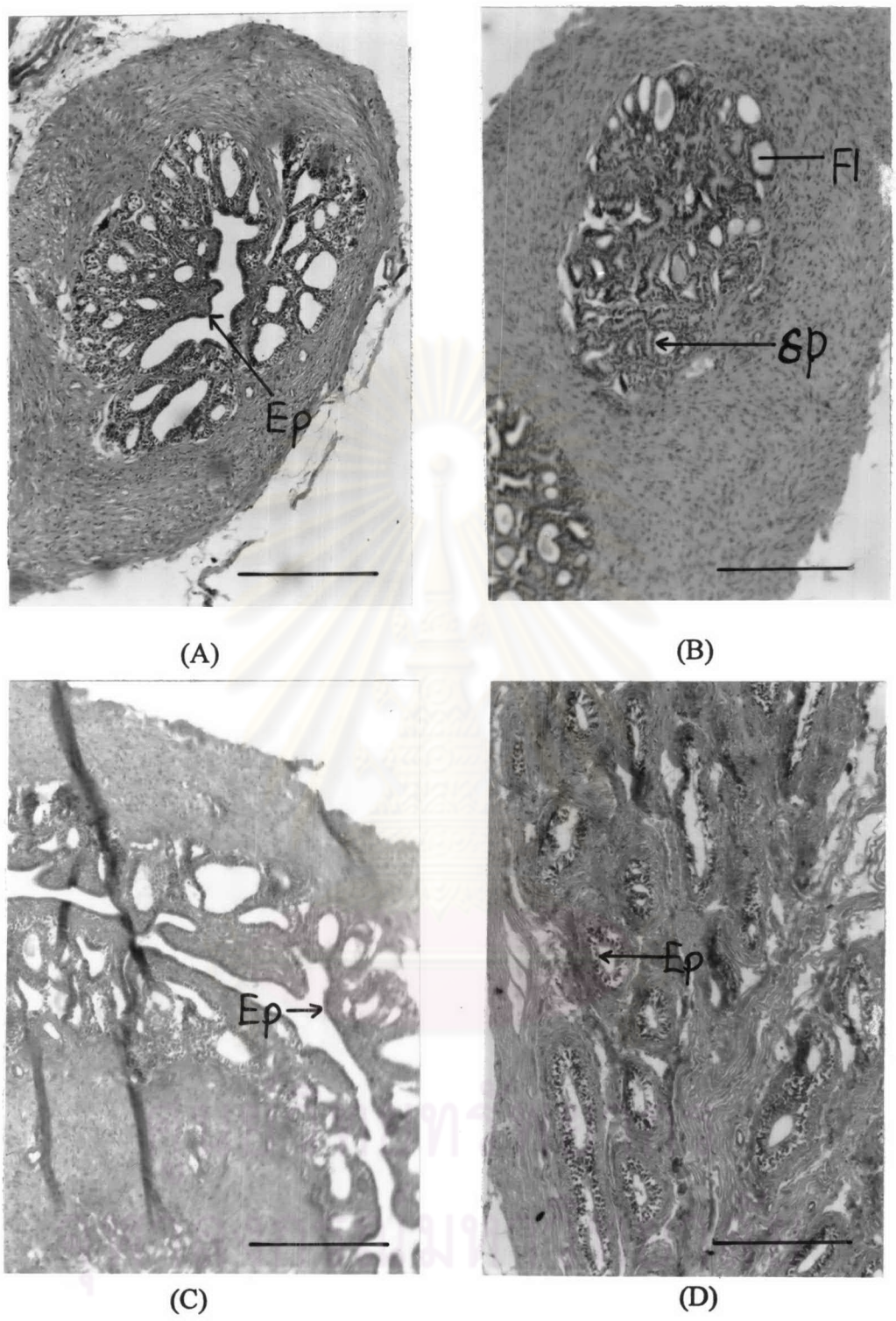
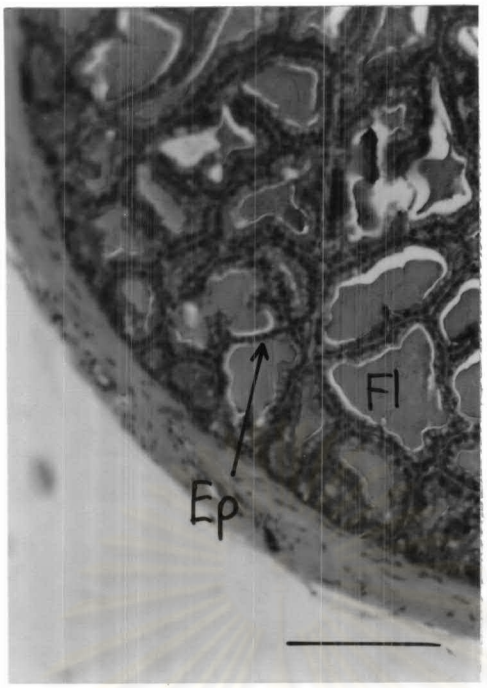
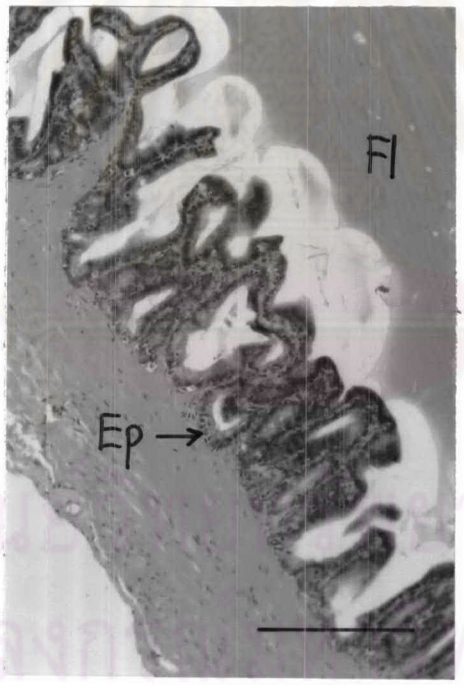


Figure 26 Seminal vesicle histology of orchidectomized male rats treated with distilled-water (A), Mc-1 (B), Mc-10 (C), Mc-100 (D) and TP at the end of treatment (E) and TP at the end of post-treatment periods (F) with H & E staining in epithelial cells (Ep). Bar scale = 200 μm



(E)



(F)

Figure 26 (continued) Seminal vesicle histology of orchidectomized male rats treated with distilled-water (A), Mc-1 (B), Mc-10 (C), Mc-100 (D) and TP at the end of treatment (E) and TP at the end of post-treatment periods (F) with H & E staining in epithelial cells (Ep). Bar scale = 200 μm