



**UNIVERSITI PUTRA MALAYSIA**

**PLASMA PROGESTERONE (P4) PROFILES IN GOATS DURING  
INSERTION AND AFTER WITHDRAWAL OF PROGESTAGEN  
IMPREGNATED INTRAVAGINAL SPONGE FOR ESTRUS  
SYNCHRONISATION**

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ABDUL HALIM B. HAJI MANBAH

### ABSTRACT

Five cycling Kambing Katjang does were used in this study to determine the plasma progesterone profiles in an attempt to synchronize estrus in this species using the intravaginal sponge - 'Repromap'. Blood samples were taken before, during, and after the sponge was introduced. Only three does retained the sponge for a full period of twelve days treatment.

Heat was not detected from any of the does for the first three days after removal of the sponge. No mucus discharge, swelling of the vulva and vaginal erythema were observed. Plasma progesterone concentration was low ( $< 0.3$  ng/ml) during the period where the sponge was in the vagina. This plasma progesterone concentration was considered at basal level since sensitivity of the assay was  $0.27$  ng/ml. The sponge used in the experiment using a different set of sponge on group of three does also showed that the plasma progesterone concentration during the period when the sponge was in the vagina was also low ( $< 0.08$  ng/ml).

These results showed that the goats used in this experiment did not appear to respond to the treatment. This could be due to the low progesterone concentration in the sponge.

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## INTRODUCTION

In rural areas of Peninsular Malaysia goats are traditionally kept in small numbers by small holders and other low income-groups. The goat population of 312,000 heads (1980) can meet only 12% of the total demand for goat meat. Therefore in order to cope up with this demand, the goat population in our country should be increased.

Controlled breeding of goats and sheep have involved the artificial manipulation of ovarian activity using exogenous compounds such as intravaginal progestinated pessaries containing medroxy acetate progesterone (MAP : Repromap, Upjohn) and fluorogetone acetate (FGA : Cronolone). Sponges containing progestagen with or without follicle stimulating hormone (FSH) have also been used to synchronise estrus in goats but with mixed results (Barker, 1966; Dhindsa et al., 1969; Jones & Knifton, 1971; McGovern, 1971; Corteel, 1975; Smith, 1980). Since the first report on the use of progestagen impregnated vaginal pessaries for estrus synchronization in ewes, the method was been adapted and employed in does. Numerous studies have established the efficacy of sponge impregnated with the synthetic progestagen such as FGA to induce and synchronise estrus in ewes (Mauleon, 1979; Robinson, 1979). Although other synthetic progestagens have been used as alternatives, only medroxy acetate progesterone (MAP) has been used widely. MAP is administered in a manner similar to that used for FGA, inserted and retained in the vagina for 12 to 14 days. A number of reports indicated that

good responses followed by mating was obtained in ewes treated with sponge pessaries impregnated with 60 mg MAP (Roberts, 1966; Roberts et al., 1970; Gordon, 1975). Effective synchronization of estrus in does was achieved by the use of intravaginal pessaries impregnated with different quantities (25 to 45 mg) of cronolone during the breeding season (Corteel et al., 1972).

Many studies have been carried out to measure the plasma progesterone concentration during the estrus cycle in the goat. There was a temporal change in progesterone during estrus cycle in goat (Thorburn and Schneider, 1971). These changes are closely related to the morphological changes of the corpus luteum during the cycle in the doe. However, the effect of exogenous progestin on the corpus luteum function in the doe has not been studied. In ewes, progestin treatment given on the day of estrus either suppresses ovulation or suppresses the function of corpus luteum after ovulation (Bosuet al., 1978). On the other hand, progestagen treatment during the luteal phase has not demonstrable effect on the size and function of the corpora lutea (Smith and Robinson, 1969).

The aim of the present study was therefore consists of the following objectives:-

- i) to measure the plasma progesterone profile in the goat using progestagen-impregnated intravaginal sponge (Repromap).
- ii) to observe the physical signs of estrus following the removal of the progestagen-impregnated intravaginal sponge (Repromap).

## LITERATURE REVIEW

### Oestrus synchronization

It is possible to synchronise oestrus in does using progestagen-impregnated intravaginal sponges followed by the administration of PMSG. This method is applicable to the local non-descript breed of Malaysia (Bongso et al., 1982). Intravaginal progesterone-impregnated sponges have been widely used for oestrus synchronization in sheep (Hunter et al., 1971; Meaker et al., 1971; Van der Westhuysen et al., 1970; Van Zyl, 1976; Qgunbiyi et al., 1980). Van Zyl (1976) also reported that oestrus synchronization was possible through the use of prostaglandin, combined with intravaginal progesterone-impregnated sponges.

Although it is possible to synchronise groups of cyclic ewes using only progestagen-impregnated sponges, a better conception rate have been obtained when PMSG was administered following withdrawal of the sponges (Haresign, 1979).

McGovern (1971) reported that following removal of sponge, (containing 60 mg medroxyprogesterone acetate) after 17 days in the vagina, one goat showed oestrus within 24 hour and five goats showed oestrus within 48 hours. In another study, oestrus was synchronised in 88 goats in a similar manner followed by intramuscular injection of PMSG (30 i.u) at sponge removal and the numbers of does that came into oestrus in 0-24, 25-48, 49-72 and 73-96 hr periods were 41, 29, 12 and 6 (Bongsu et al., 1982). They also reported that the time of onset of oestrus ranged from

16 to 40 hr.

Fifteen does received intravaginal pessaries impregnated with 20 mg fluorogestone acetate (Cronolone) at various time for a period of 14 days and were treated with 15 mg of prostaglandin F<sub>2</sub> (Lutalyse) injected intramuscularly, 73% of the does showed oestrus within 2 days after withdrawal of progestin treatment (Serna et al., 1977).

FGA- and MAP-impregnated sponge pessaries which were left in the vagina for 14 days are equally effective in ewes in mating response, degree of synchronization and reproductive performance after 500 IU PMSG were injected at sponge removal (Ainsworth and Shrestha, 1983).

#### Progesterone concentration

During the oestrus cycle in the goat the changes in the progesterone concentration in the peripheral plasma are similar to those previously reported in the ewe (Thorburn et al., 1969) and the cow (Donaldson et al., 1970).

The plasma level of progesterone was very low, less than 0.5 ng/ml at the time of sponge insertion and remained low during the treatment period in the doe in which treatment was started on the last day of oestrus (Bosu et al., 1978).

The temporal change in the plasma concentration of progesterone during the oestrus cycle in goats have been described (Thorburn and Schneider, 1971). The mean concentration was extremely low on the day of oestrus (0.2 ng/ml) and was not significantly different from that found during anoestrus or in

ovariectomised animals. The concentration increased to a maximum of 4 ng/ml on about day 10 of the 21 day cycle and decreased rapidly during the last 3 days of the cycle (Thorburn and Schneider, 1972). However in another study, Thorburn et al., (1969) reported that in the first 4 days of the cycle, plasma progesterone concentration were below 0.4 ng/ml in the ewe, increasing to a mean level of 1.5-2.5 ng/ml between days 4 and 9 and remaining at this level for approximately 5 days declining rapidly in days 14 and 15 to reach a low level on the day before oestrus. They also reported that the progesterone concentration on the day of oestrus was extremely low (0.1 ng/ml).

Progesterone administered early in the oestrus cycle of the ewe shortened the life of corpora lutea (Woody et al., 1967; Lewis et al., 1968; Ginther, 1969).

The decline of concentration of progesterone at the end of cycle permits an increase in the release of estrogen (Hauger et al., 1977; Fogwell et al., 1978).

## MATERIAL AND METHOD

### EXPERIMENT 1

#### i) Experimental animals

Five cycling does of a local breed (Kambing Katjang), weighing between 20 to 30 kg were selected from the Universiti Pertanian Malaysia goat unit in Puchong. All the does were adult and had been kidded before. They were kept together in a confinement throughout the experiment and provided with water and cut grass ad libitum.

#### ii) Sponge insertion into vagina

Sponge containing 60 mg medroxyprogesterone acetate (Repromap : Upjohn) was inserted deep into the vagina of each does using a lubricated plastic speculum @ applicator and left in place for 12 days. Does were restrained in the normally accepted manner with hindlimb held up and forelimb on the ground during the insertion of the sponges. The sponge was removed from all the does on day 13 and on removal difficulty were encountered in two of the does. Therefore, a vaginal speculum and a long forcep have to be used to pull out the sponge from the vagina.

#### iii) Blood collection

Blood collection were taken from all the does via jugular vein before, during and after the introduction of sponge into the vagina of the goat. About 2 ml of blood were taken from each of the does using a syringe and transferred immediately into heparinised tubes. Two day before the introduction of the sponge,

blood collection were taken from each of the does. On the day the sponge was inserted into the vagina of the does (day 0), another blood collection were taken from each of the does. During the treatment, two blood samples were taken at 3 days interval (day 5 and day 9). After the sponge was removed from the does, blood collected were taken from each of the does and this followed by another five blood samples were taken with two days interval (Days 14, 16, 18, 21 and 24) after removal of sponges. All the blood samples taken were immediately centrifuged at 3000-3500 r.p.m. for 20 min and the plasma transferred into a new tube and stored in the freezer at -20 C until day of assay.

iv) Heat detection

Oestrus detection was carried out following sponge removal. A buck was left to run with the does in confinement and to observe for any mounting activity. Heat detection was carried out at 0800 hr and 1800 hr daily for half to one hour in the morning and evening. The does were examined for the vulva swelling, mucus discharge, vaginal erythema, mounting activity and tail wagging.

v) Radionamunoassay (RIA) of plasma progesterone

Plasma level of progesterone were measured by radio-immunoassay procedure as described by Abraham (1977) and Castellanos and Edquist (1978). Duplicate sample of plasma (200 ul) were extracted with 2 ml of petroleum ether by intensive shaking or vortex mixer for 5 minutes. This was followed by deep freezing the plasma phase at -20 C before the supernatant was transferred into assay tubes and dried with compressed

air/Nitrogen gas using semi-automatic drying device. To each tube was added with 500 ul buffer except for non-specific binding (NSB) and total count (TC) tubes. For the NSB and TC tubes, 600 ul and 850 ul of buffer were added respectively. This was followed by adding 100 ul H-progesterone (tracer) to all the assay tubes. Later 100 ul of Ab (antiserum) of 1:4000 dilution was added to all tubes except the NSB and TC tubes (Ab donated by Dr.Fletcher). After overnight incubation at 4°C in refrigerator, 250 ul of dextran-coated charcoal was added to each tube except that TC tube to separate Ab-bound and free hormone. Later, they were transferred to refrigerated centrifuge and were incubated until 15 min. Refrigerated centrifuge was than switch on for 7 min at 2500 r.p.m. Than the supernatant containing the antibody-bound was decanted into a scintillation vials after centrifugation. Finally 5 ml of toluene-PPO was added in liquid scintillation tubes and the radioactivity was counted in a liquid-scintillation spectrometer (Packard 300C). The progesterone content in the unknown samples was than calculated using a logit-log RIA programme on apple compatible computer (model lingo). The sensitivity of the assay was 0.27 ng/ml. The intra-assay coefficient of variation at the three plasma levels were 26.8%, 9.05% and 7.07% at  $0.21 \pm 0.05$  ng/ml (n=4),  $2.20 \pm 0.20$  ng/ml (n=4) and  $3.23 \pm 0.25$  ng/ml (n=4), respectively.

## EXPERIMENT 2

Analysis of progesterone concentration and its level in sponges used in experiment 1 (Batch No. I) and in another batch of sponge.

Sponges from the two batches were first soaked in the distilled water/buffer to dissolve the progesterone. Both of the sponges from this two batches contained 60 mg medroxyprogesterone acetate (Repromap : Upjohn). Serial dilution was done to dilute the progesterone concentration from 60 ng to 30 ng, 15 ng, 7.5 ng, 3.75 ng and 1.875 ng. The progesterone concentration from these two batches of sponge was than measured by a radioimmunoassay as described in experiment 1.

## EXPERIMENT 3

To determine the level of progesterone concentration when the sponge was in the vagina.

Three cycling does of local breed (Kambing Katjang) were chosen from UPM goat unit in Puchong. They weighed about 25 to 30 kg. All the does were kept in a confinement and provided with water and cut grass ad. libitum.

60 mg medroxyprogesterone acetate (MAP : Repromap) from batch No. 2 was used in this experiment. The sponges were inserted into the animals as described in experiment 1. The sponge was kept in the vagina of the does for 3 dys.

Blood samples were taken from the jugular vein using 5 ml heparinised tubes and than immediately centrifuged at 3000-3500 r.p.m. for 20 mins to seperate the plasma. All the plasma were stored at -20 C until assayed. Before sponge was inserted, blood

were taken from each of the does and another sample in the evening after sponge was inserted into the vagina followed by another two blood collection daily before the sponge was removed on the third day (day 3).

All the blood samples were assayed at the same time. The radioimmunoassay was carried out in similar manner as described in experiment 1. The sensitivity of the assay was 0.20 ng/ml.

## RESULTS

### EXPERIMENT I

#### Retention of sponge

Throughout the experiment, sponges (MAP : Upjohn) were retained in three of the five does for the entire 12 days period (Goat No. 1015, 1017 and 1020). In the other two does, sponge were expelled on the Day 2 after treatment (goat No. 1018) and on day 10 (goat no. 1022). However these sponges were replaced with a new sponges on the same day the sponges were expelled.

#### Oestrus detection

The first 3 days after the removal of the sponges i.e 24 hr, 48 hr and 72 hr respectively no physical signs of heat were observed in all the does. Mounting activity, vulva swelling, mucus discharge and vaginal erythema were not seen in all the five does. However, two does (goat No. 1018 and 1022) showed sign of restless.

### Plasma Progesterone profiles

There was no increase of progesterone level in all the five does after the sponge was introduced into the vagina. The mean ( $\pm$ S.D) plasma progesterone concentration before, during and after withdrawal of the sponge is as shown in Table 1 and Fig. I. The plasma progesterone concentration was also low before the introduction of the sponge in all of the does. However, overall results showed that all the five does having irregular level of plasma progesterone concentration and the level even not exceed 1 ng/ml during the period of 12 days. On the removal of the sponge, all the does showed a decreased level of progesterone level and followed by an increased two or three days later after the sponge was removed.

### EXPERIMENT II

#### Progesterone level in sponge

##### i) Sponge (batch No. I) used in experiment I

The plasma progesterone concentration in the sponge were as shown in Table 2. However the results obtained showed that the progesterone content in this sponge was still at the basal level (the sensitivity of the assay was 0.10 ng/ml).

##### ii) Sponge (batch No. 2) used in experiment III

The plasma progesterone concentration in this sponge is shown in Table 2. The progesterone content in this new batch of sponge also considered at the basal level (the sensitivity of the assay was 0.10 ng/ml).

### EXPERIMENT III

The mean (±S.D) plasma progesterone concentration of the three does is shown in Fig. 3. The level of the progesterone concentration was also low although there was an increased from the day the sponge was introduced until it was removed. The level of the progesterone concentration is still considered to be the basal level (the sensitivity of the assay was 0.20 ng/ml). The result of plasma progesterone concentration from the individual doe in this experiment is shown in Table 3.

### DISCUSSION

#### Rejection of sponge

The rejection of sponge during the treatment period (Goat No. 1018 and 1022) were probably due to the faulty insertion. Similar losses of MAP-impregnated sponges during the treatment of anoestrous milking ewes have been reported (Alifakiotis et al., 1982). The sponge rejection might also be due to the sponge that was not inserted deep enough into the vagina. Due to the contraction of the vagina, the sponge may be easily expelled.

#### Plasma progesterone profiles

No heat was detected from all the does after removal of the sponge. This failure of the animals to come into heat may be due to several reasons. Most probably the does were in long anoestrous period. Ovulation with corpus luteum formation but without signs of heat may occur in this period and this is called the spurious ovulation (Grant, 1934). However on laparoscopy done to all the does after the end of the experiment, two does showed the

## 12(a)

**Table 1 : Plasma progesterone concentration (ng/ml) in goats (Experiment I)**

No. of collection	Progesterone concentration (ng/ml) in goats					Mean $\bar{x} \pm$ S.D
	1015	1017	1018	1020	1022	
1	0.12	0.13	0.02	0.25	0.26	0.15 $\pm$ 0.1
2	0.04	0.22	0.17	0.21	0.83	0.29 $\pm$ 0.3
3	0.03	0.04	0.16	0.21	0.29	0.25 $\pm$ 0.2
4	0.04	0.00	0.13	0.20	0.35	0.14 $\pm$ 0.1
5	0.21	0.02	0.09	0.39	0.25	0.19 $\pm$ 0.1
6	0.18	0.32	0.07	0.27	0.17	0.21 $\pm$ 2.01
7	0.06	0.14	0.06	0.26	0.14	0.13 $\pm$ 0.1
8	0.00	0.15	0.11	0.15	0.22	0.13 $\pm$ 0.1
9	0.03	0.04	0.09	0.26	0.21	0.13 $\pm$ 0.1
10	0.00	0.09	0.09	0.16	0.18	0.10 $\pm$ 0.1
11	0.08	0.01	0.15	0.20	0.21	0.13 $\pm$ 0.1

- 
- 1 - before sponge insertion  
 2 - 5 - during sponge in the vagina  
 6 - 11 - after sponge withdrawal

**Table 2 : Progesterone level in the sponge  
(batch No. 1 and 2) in experiment**

Dilution (ng/ml)	progesterone contents (ng/ml)	
	Batch No. 1	Batch No. 2
60	0.11	0.06
30	0.13	0.08
15	0.04	0.07
7.5	0.00	0.06
3.75	0.01	0.10
1.875	0.05	

**Table 3 : The plasma progesterone concentration (ng/ml)  
in goats (experiment 3)**

No. of collection	Plasma progesterone concentration (ng/ml) in gat			Mean $\bar{x} \pm S.D$
	No. tag	1001	1004	
1	0.12	0.02	0.01	0.05 $\pm$ 0.1
2	0.04	0.13	0.06	0.07 $\pm$ 0.1
3	0.02	0.11	0.13	0.08 $\pm$ 0.1
4	0.05	0.13	0.03	0.07 $\pm$ 0.1

## EXPERIMENT I

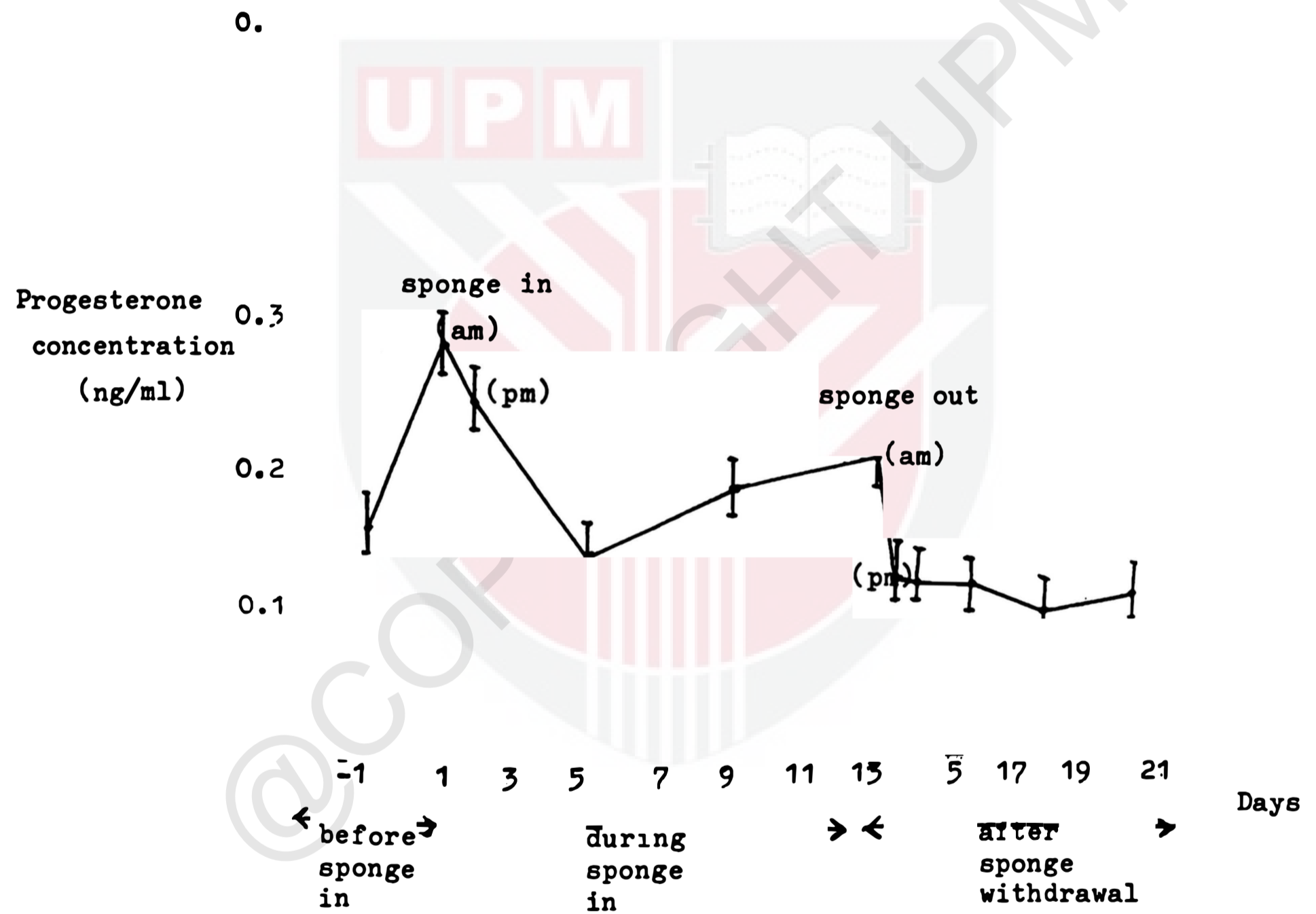


Fig. 1. The mean ( $\bar{x} + S.D$ ) plasma progesterone concentration of the five does before , during after withdrawal of sponge

EXPERIMENT II

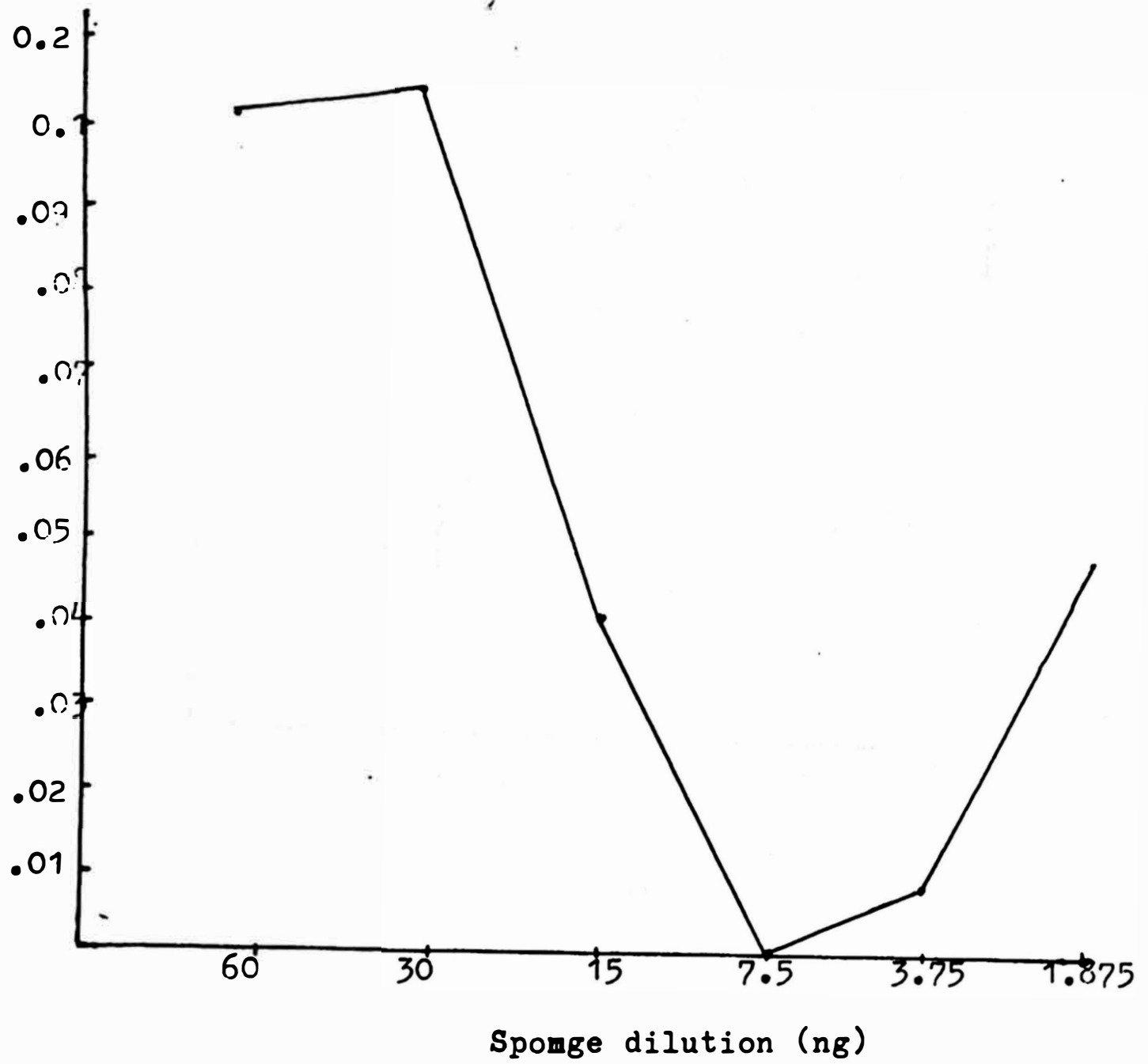


Fig. 2a. Progesterone concentration in sponge (batch no. 60)

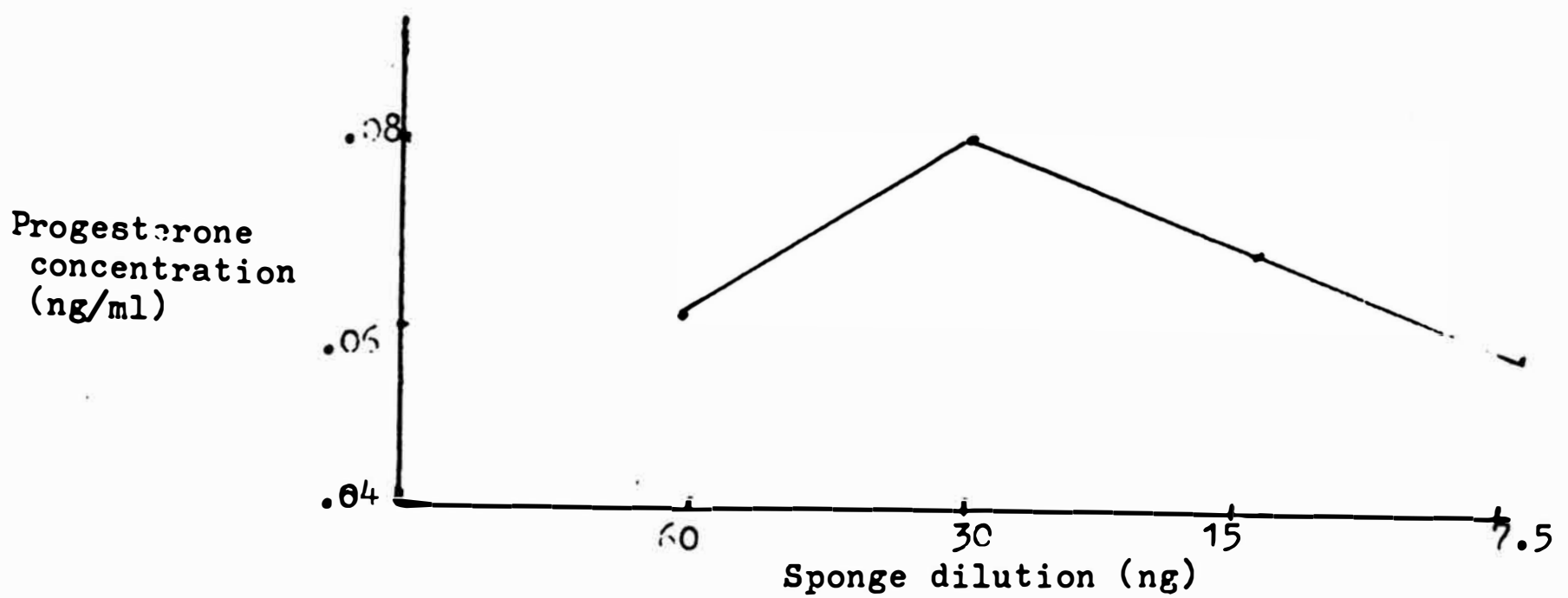


Fig. 2b. Progesterone concentration in sponge (batch no. 30)

## EXPERIMENT III

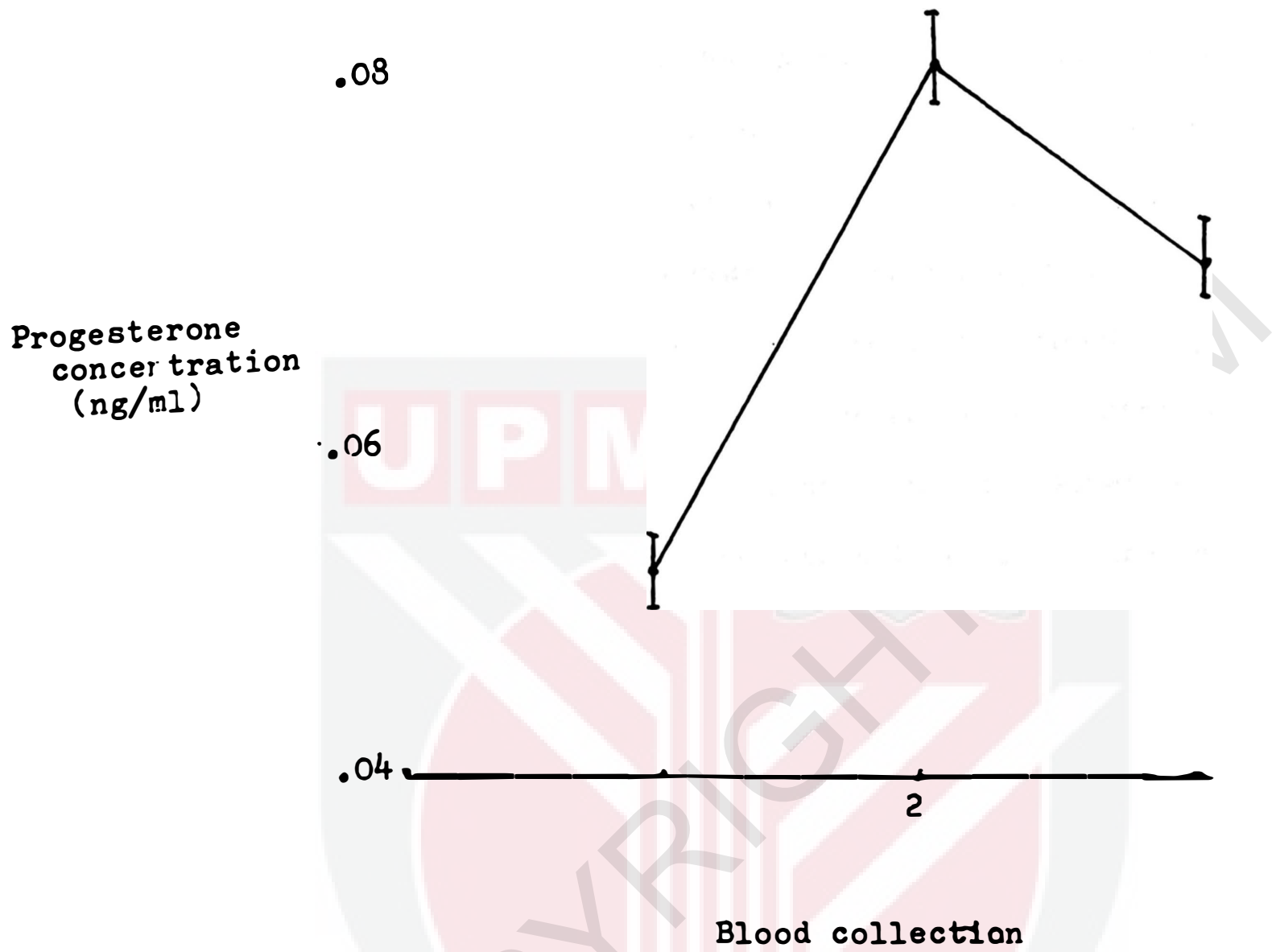


Fig. 3 The mean ( $\bar{x} + S.D$ ) plasma progesterone (ng/ml) of the three does

presence and formation of corpus luteum. The presence of a functional corpus luteum and the production of progesterone, inhibits the return to oestrous in this does by exerting a negative feed-back effect upon the anterior pituitary gland. The low plasma progesterone concentration during the treatment was probably due to low level of progesterone being released by the sponge. In experiment II, it showed that the sponge (batch No. 1 and 2) used in the experiment contained low level of the progesterone. However the progesterone level from the sponge could be higher if petroleum ether was used as the solvent instead of distilled water buffer as done in the experiment II. Besides this, there is also a possibility that the amount of progesterone from the sponge to be absorbed in the body fluid of the experimental animals could be reduced. This effect may also contributed to low level of progesterone concentration during the treatment. The mean ( $\bar{x} \pm S.D$ ) plasma progesterone concentration declined during the treatment as shown in Fig. 1. This might also suggest that the amount of progesterone being absorbed into the blood circulation during the period of treatment was very low.

There was also a possibility that the radioimmunoassay technique used as described by Abraham (1977) and Castellanos and Edquist (1978) is not able to detect the presence of progesterone in the plasma. Interference from other substances or other steroids may occurred during the assay. The distance of the sponge inserted into the vagina of the does may probably also influence the absorption of the hormone into the blood

circulation of the experimental animals. The secretion of the progesterone from the sponge which is inserted deep into the vagina may also probably higher when compared to the sponge that was inserted only midway of the vagina.

#### CONCLUSION

The overall results from this experiment showed that the plasma progesterone concentration did not response to the treatment during the Repromap. This may be due to several reasons:-

- i) The sponge may not inserted deep into the vagina.
- ii) Probably the sponge (Repromap : Upjohn) used in the experiment contain low progesterone level.
- iii) The RIA technique used in this experiment was unable to detect the progesterone level in the peripheral plasma.
- iv) The progesterone from the sponge most probably released low level of its content into the body fluid of the experimental animals.

Therefore, for the future study which involves the use of sponge, it is advisable to check the sponge first whether it has a high level of progesterone and to keep the sponge in the right place as indicated by the manufacturer. Due to the time factors, it is very ideal if more blood samples can be obtained before the introduction of the sponge and after the withdrawal of the sponge so that a complete progesterone profile can be seen.

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