

## CHAPTER 4

### Results

Eighteen animals from six litters were used in this study; three animals in each litter are randomly divided into 3 groups; implanted deslorelin at the age 4 months old (n= 6), implanted deslorelin at the age 7 months old (n=6) and control group (n=6). Finally, the observation period of 4 month of age-deslorelin or placebo implantation dog was 36 weeks and 7 month of age-deslorelin implantation dog was 26 weeks. One dog from the 4 month of age-treatment group died at week 11 after implantation due to endocarditis resulting from septicemia. Results of this dog were included in the analysis up to week 10.

#### 1. Site of implantation

There was no allergic or inflammatory reaction observed at the site of implantation at 10-day observation period or at any time of the study in all groups. There were no swellings or systemic reaction as a result of implantation.

#### 2. Determination of oestrus

All 4 month of age-GnRH implantation (6/6) showed no sign of estrous for the total experimental period (36 weeks) with no appearance of a serosanguineous vaginal discharge or vulvar swelling, and no change in vaginal cytology and progesterone level (Figure 7-12 and Table 5). 5/6 animals in the control group showed first oestrus between 12-28 weeks after experiment had started (at 7-10 month old of age) (Figure 7-12 and Table 5).

All the animals showing oestrus in the control group were diagnosed by vulvar swelling, serosanguineous vaginal discharge, increased in the percentage of superficial cell on vaginal cytology and increase of plasma progesterone more than 2 ng/ml (Table 6).

Of the dogs seven month of age at GnRH implantation 6/6 showed vulvar swelling and serosanguineous vaginal discharge within 1-2 weeks after implantation (Figure 7-12 and Table 5). 4/6 dogs in this group had been observed for the increasing of percentage of superficial cell on vaginal cytology and progesterone level more than 2 ng/ml. Of the other two dogs one had been showed only increasing of percentage of superficial cell on vaginal cytology, the other showed neither increasing of percentage of superficial cell on vaginal cytology nor progesterone level (Table 5).

Table 5. Incidence (number of dogs) of detecting 1<sup>st</sup> oestrus or not in treatment and control group after implantation with deslorelin containing or placebo implants within the experiment period.

Group	No. showing 1 <sup>st</sup> oestrus within 1 month after experiment started	No. showing 1 <sup>st</sup> oestrus after experiment started > 1 month	No. showing no sign of oestrus along the experiment
Control	0/6 (0%) <sup>a</sup>	5/6 (83.3%) <sup>b</sup>	1/6 (16.7%) <sup>a</sup>
4 month of age deslorelin implantation group	0/6 (0%) <sup>a</sup>	0/6 (0%) <sup>a</sup>	6/6 (100%) <sup>b</sup>
7 month of age deslorelin implantation group	6/6 (100%) <sup>b</sup>	0/6 (0%) <sup>a</sup>	0/6 (0%) <sup>a</sup>

a-b differ significantly ( $P < 0.001$ ).

Table 6. Incidence of the dogs observed along the experiment with signs of proestrus-estrus (vulvar swelling, serosanguineous vaginal discharge), increasing of percentage of superficial cell, serum progesterone concentrations > 2 ng/ml.

Group	<i>n</i>	Vulvar swelling	Sanguineous vaginal discharge	Increasing of percentage of superficial cell	Serum progesterone > 2ng/ml
Control	6	5 <sup>a</sup>	5 <sup>a</sup>	5 <sup>a</sup>	5 <sup>a</sup>
4 month of age deslorelin implantation group	6	0 <sup>b</sup>	0 <sup>b</sup>	0 <sup>b</sup>	0 <sup>b</sup>
7 month of age deslorelin implantation group	6	6 <sup>a</sup>	6 <sup>a</sup>	5 <sup>a</sup>	4 <sup>a</sup>

a-b differ significantly ( $P < 0.01$ ).

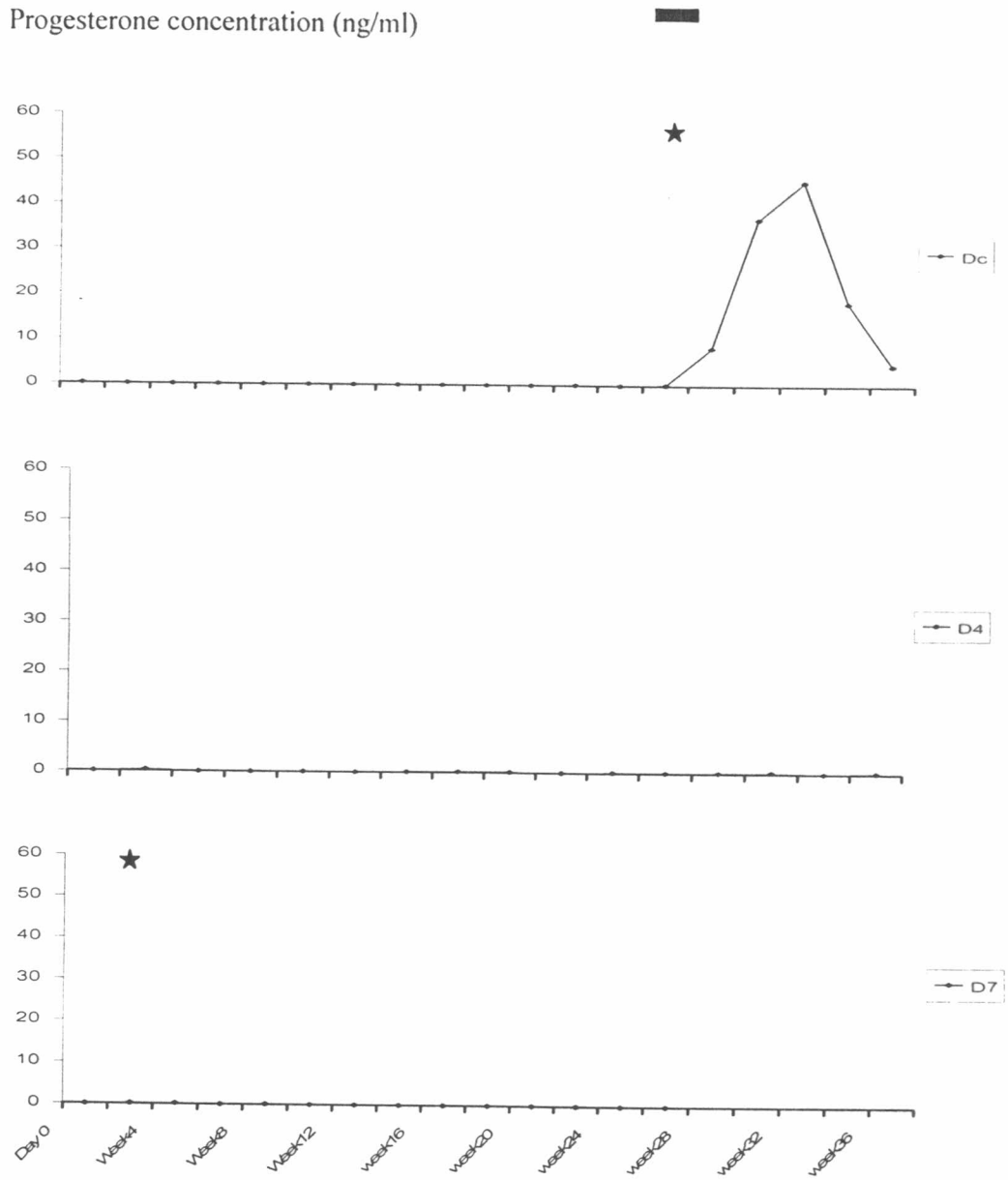


Figure 7. Changes in progesterone concentrations in litter 1 (4 month and 7 month of age-GnRH implantation dogs and the control dog before (week 0) and after implantation with deslorelin containing or placebo implants (week 2-36).

- ★ Vulvar swelling and serosanguineous vaginal discharge.
- Cornified cells in vaginal smear

## Progesterone concentration (ng/ml)

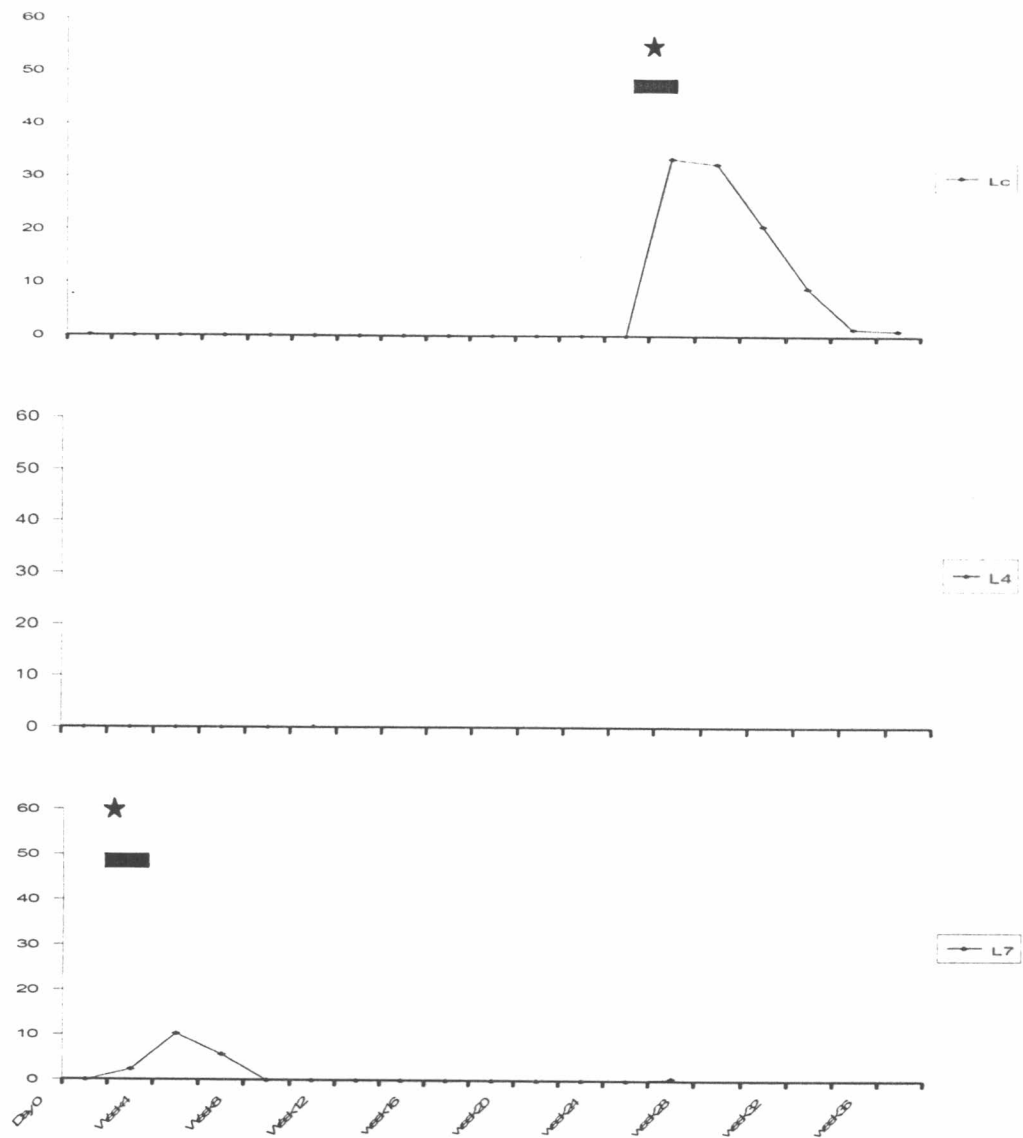


Figure 8. Changes in progesterone concentrations in litter 2 (4 month and 7 month of age-GnRH implantation dogs and the control dog before (week 0) and after implantation with deslorelin containing or placebo implants (week 2-36).

★ Vulvar swelling and serosanguineous vaginal discharge.

■ Cornified cells in vaginal smear

Progesterone concentration (ng/ml)

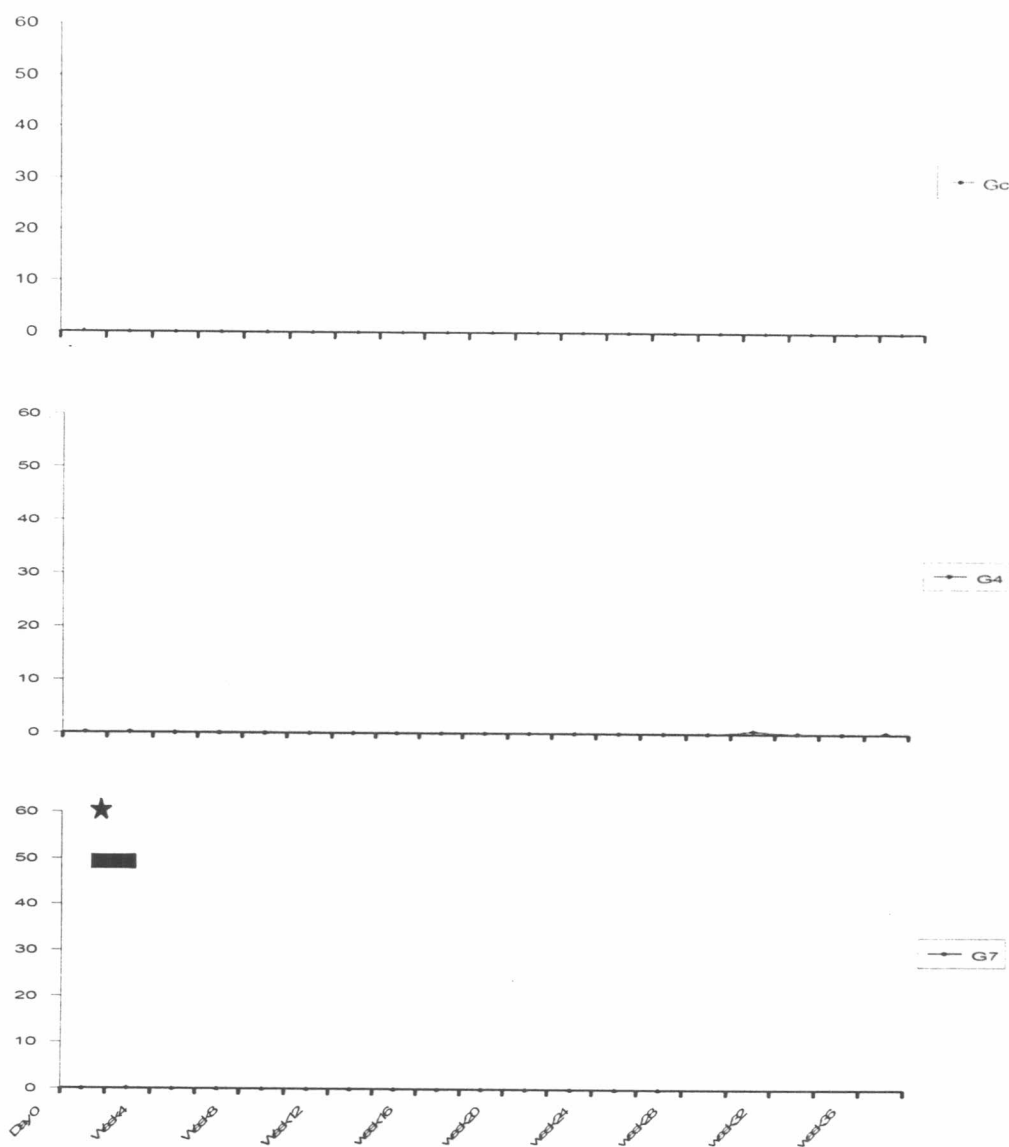


Figure 9. Changes in progesterone concentrations in litter 3 (4 month and 7 month of age-GnRH implantation dogs and the control dog before (week 0) and after implantation with deslorelin containing or placebo implants (week 2-36).

- ★ Vulvar swelling and serosanguineous vaginal discharge.
- Cornified cells in vaginal smear

Progesterone concentration (ng/ml)

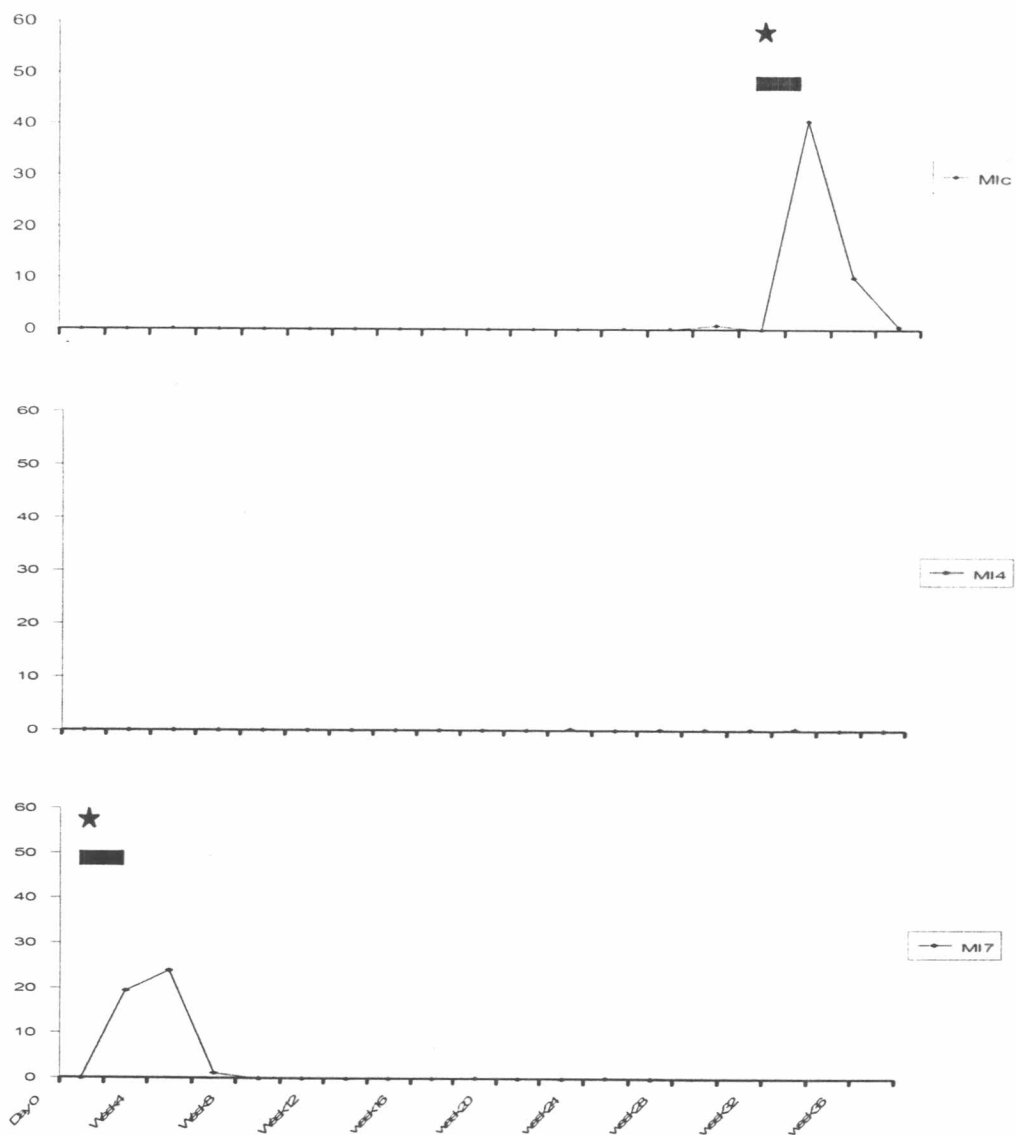


Figure 10. Changes in progesterone concentrations in litter 4 (4 month and 7 month of age-GnRH implantation dogs and the control dog before (week 0) and after implantation with deslorelin containing or placebo implants (week 2-36).

- ★ Vulvar swelling and serosanguineous vaginal discharge.
- Cornified cells in vaginal smear

## Progesterone concentration (ng/ml)

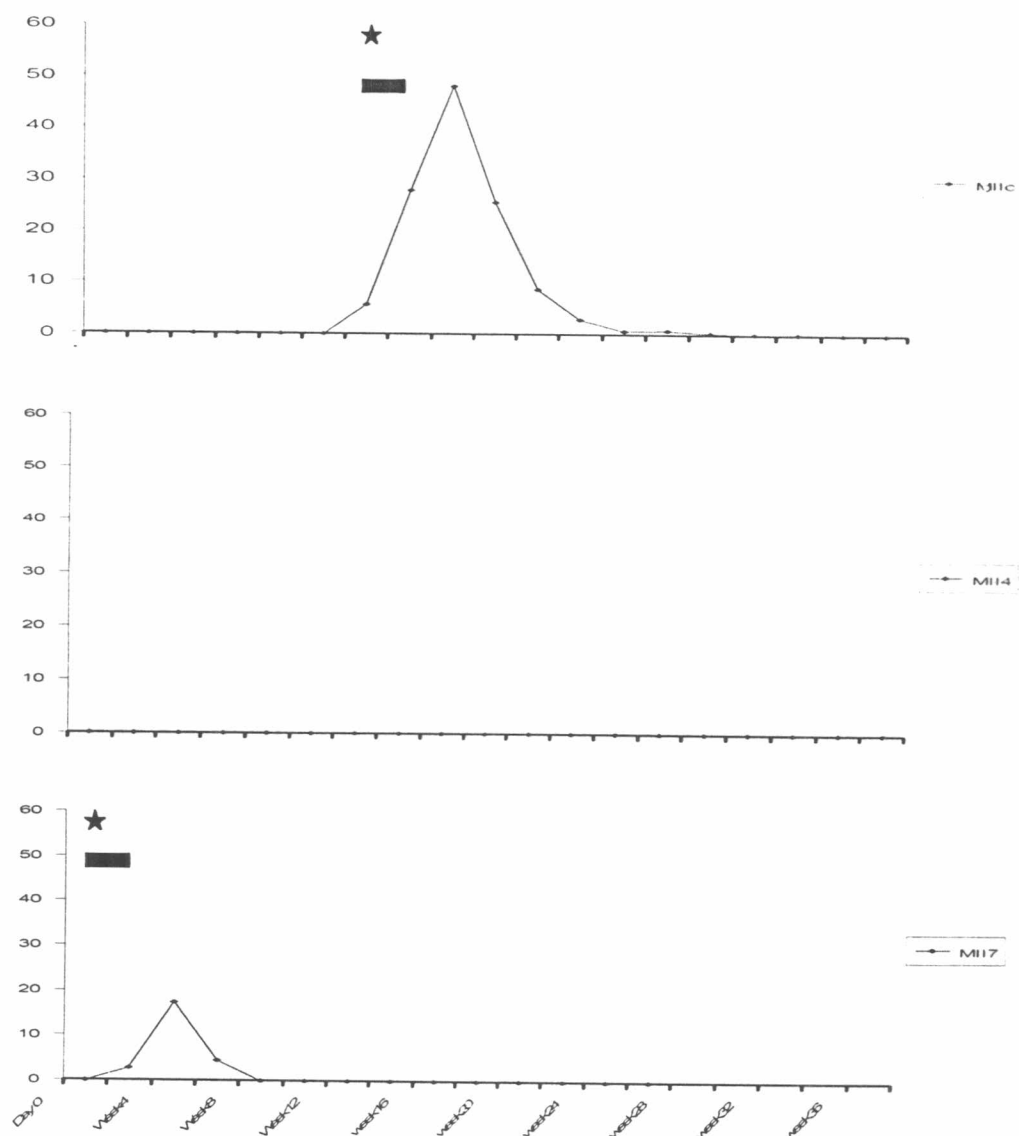


Figure 11. Changes in progesterone concentrations in litter 5 (4 month and 7 month of age-GnRH implantation dogs and the control dog before (week 0) and after implantation with deslorelin containing or placebo implants (week 2-36).

★ Vulvar swelling and serosanguineous vaginal discharge.

■ Cornified cells in vaginal smear

## Progesterone concentration (ng/ml)

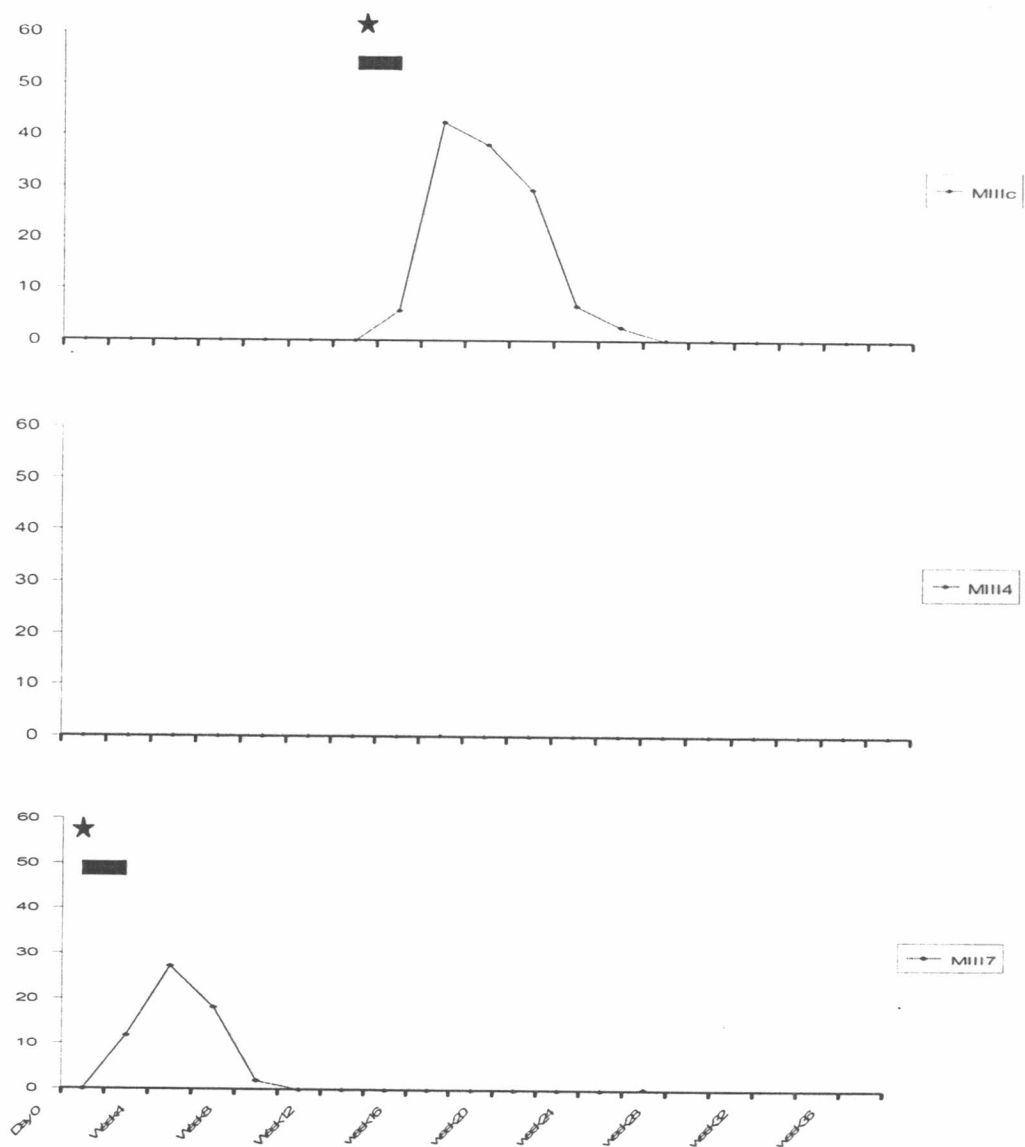


Figure 12. Changes in progesterone concentrations in litter 6 (4 month and 7 month of age-GnRH implantation dogs and the control dog before (week 0) and after implantation with deslorelin containing or placebo implants (week 2-36).

★ Vulvar swelling and serosanguineous vaginal discharge.

■ Cornified cells in vaginal smear



According to Figure 7-12 and Table 7 had shown the period (weeks) before 1<sup>st</sup> oestrus shown by 7 month of age treatment compared with control was lower ( $1.3 \pm 0.7$  versus  $22.5 \pm 9.8$  [mean  $\pm$  S.D.];  $P < 0.003$ ).

Table 7. Duration (weeks) of non-oestrous period pre- and post-1<sup>st</sup> oestrus detected in Treatment and Control group. Superscripts (a,b) within the same column differ ( $P < 0.003$ ).

Group	Monitoring time (weeks)	Non-oestrous period (weeks)
		Pre-1 <sup>st</sup> oestrus (Mean $\pm$ SD)
Control	36	$22.5 \pm 9.8^a$
4 month of age deslorelin implantation group	36	$36 \pm 0$
7 month of age deslorelin implantation group	26	$1.3 \pm 0.7^b$

Table 8 shows the comparison of the duration of prooestrous, oestrous and dioestrous between the dogs which showed oestrus in the 7 month of age-GnRH implantation group and the control group. The data of 4 month of age-GnRH implantation group can not included in this table because there were no signs of oestrous for the duration of the experimental period. The prooestrous duration of the 7 month of age-GnRH implantation group was not different to the control group as  $6.5 \pm 2.3$  days:  $6.4 \pm 1.3$  days (mean  $\pm$  SD). Oestrous duration was  $7.8 \pm 1.8$  days:  $7.0 \pm 0$  days for the 7 month of age-GnRH implantation group and the control group. The dioestrous period of the implantation group was  $31 \pm 0$  days when compared with control group which was  $57.6 \pm 15.5$  days.

Table 8. Pro-oestrous, oestrous, di-oestrous duration of dogs in the 7 month of age-GnRH implantation group and the control group which had been detected the sign of oestrous within the experiment.

	GnRH implantation group								Control group							
	Dog L <sub>7</sub>	Dog D <sub>7</sub>	Dog G <sub>7</sub>	Dog MI <sub>7</sub>	Dog MII <sub>7</sub>	Dog MIII <sub>7</sub>	$\bar{x}$	SD	Dog L <sub>7</sub>	Dog D <sub>7</sub>	Dog G <sub>7</sub>	Dog MI <sub>7</sub>	Dog MII <sub>7</sub>	Dog MIII <sub>7</sub>	$\bar{x}$	SD
Proestrous duration(day)	7	4	4	7	10	7	6.5	2.3	7	7	-	7	4	7	6.4	1.3
Estrous duration(day)	7	-	7	11	7	7	7.8	1.8	7	7	-	7	7	7	7.0	0.0
Diestrus duration(day)	31	-	-	31	31	31	31.0	0.0	67	60	-	42	77	42	57.6	15.5

- Complete oestrus not exhibited.

### 3. Anatomy and Histology of reproductive organ

Table 10 shows the relationship between vulvar dimensions (width x height) and GnRH implantation. Vulvar width of the both GnRH implantation at the age of 4 and 7 months was not smaller than control group ( $P > 0.05$ ;  $P > 0.11$ ). Similarly vulvar height, showed no difference between both implantation and control group ( $P > 0.11$ ;  $P > 0.14$ ).

The histological preparation was done on 11 samples, 4 samples from control group, 3 samples from 4 month of age-GnRH implantation group, 4 samples from 7 month of age-GnRH implantation group. There were no lesion of the ovary or uterus, such as ovaritis or endometritis, in any animal (Table 11) (see also figure 13 and 14). Follicular atrophy (figure 15) was found in 2 dogs from 4 month of age-GnRH implantation group and one dog from 7 month of age-GnRH implantation group. Multiple island of corpus lutea were found in the ovary (figure 16), endometrial hyperplasia and subendometrial haemorrhage in uterus (figure 17) were found in 2/3 dogs in control group. The subendometrial congestion in uterus (figure 18) was observed in one 4 month of age and one 7 month of age- GnRH implantation group.

Table 10. Measurement of vulva (width x height) (cm.) of treatment and control dogs

Group	Control	4 month of age deslorelin implantation group	7 month of age deslorelin implantation group
Litter 1	2.1 x 2.7	1.9 x 2.2	2.0 x 3.0
Litter 2	3.0 x 3.2	1.3 x 1.5	1.9 x 2.0
Litter 3	3.4 x 3.4	nd	2.4 x 4.1
Litter 4	1.7 x 2.3	1.3 x 2.0	1.4 x 1.7
Litter 5	2.3 x 4.5	2.3 x 4.2	2.1 x 2.6
Litter 6	1.9 x 3.5	1.2 x 1.2	0.9 x 1.2

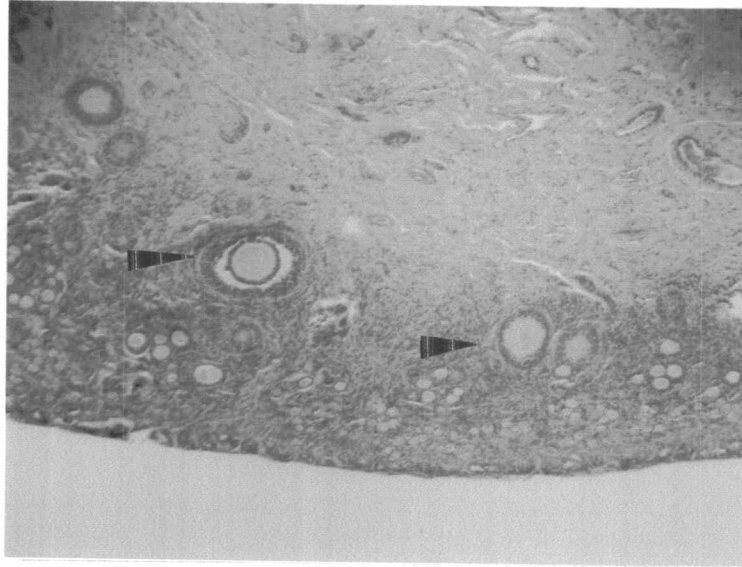
nd Not determined

Table 11. Histological appearance of ovary and uterus of treatment and control dogs.

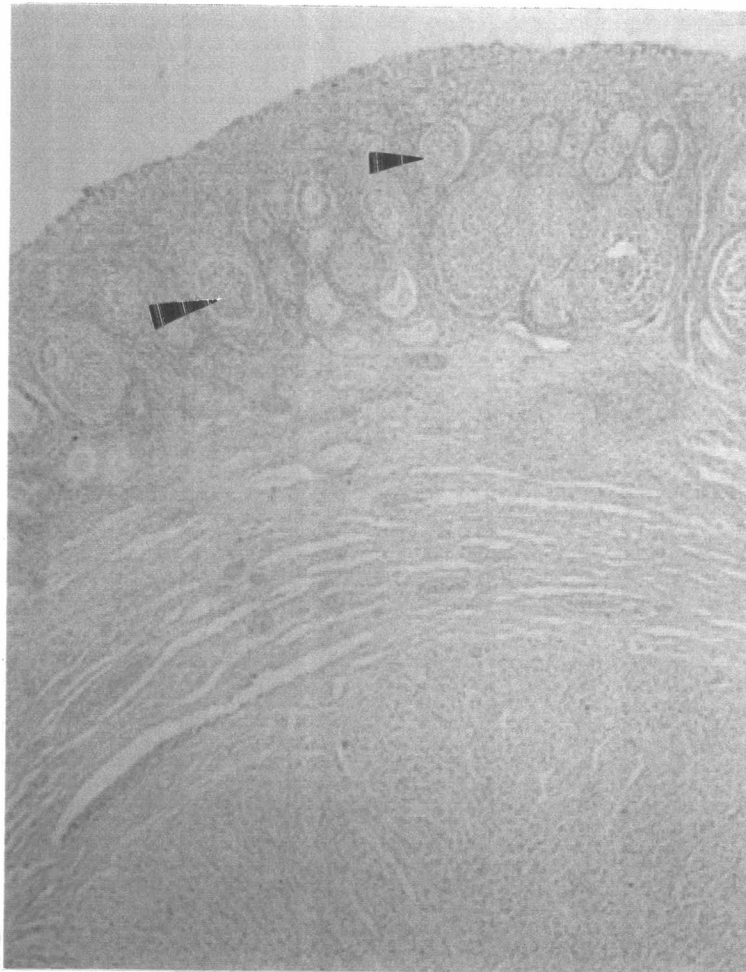
Group	Histological appearance of ovary	Histological appearance of uterus
	NRC	NRC
Control	Multiple island of CL	subendometrial haemorrhage, endometrial hyperplasia
	Multiple island of CL	subendometrial haemorrhage, endometrial hyperplasia
	NRC	nd
4 month of age deslorelin implantat ion group	NRC	NRC
	Follicular atrophy	subendometrial congestion
	Follicular atrophy	nd
7 month of age deslorelin implantat ion group	NRC	NRC
	NRC	NRC
	NRC	subendometrial congestion
	Follicular atrophy	nd

NRC No remarkable change

nd Not determined

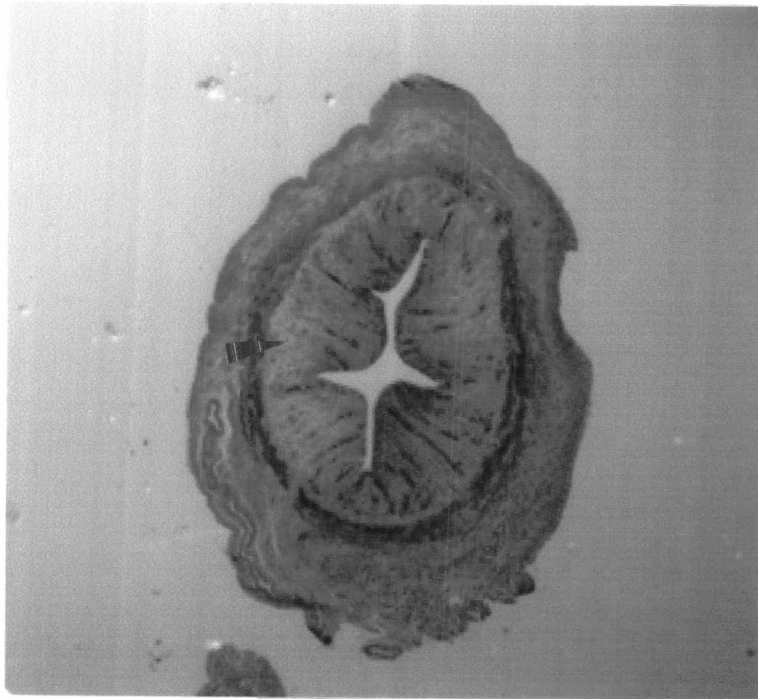


a.

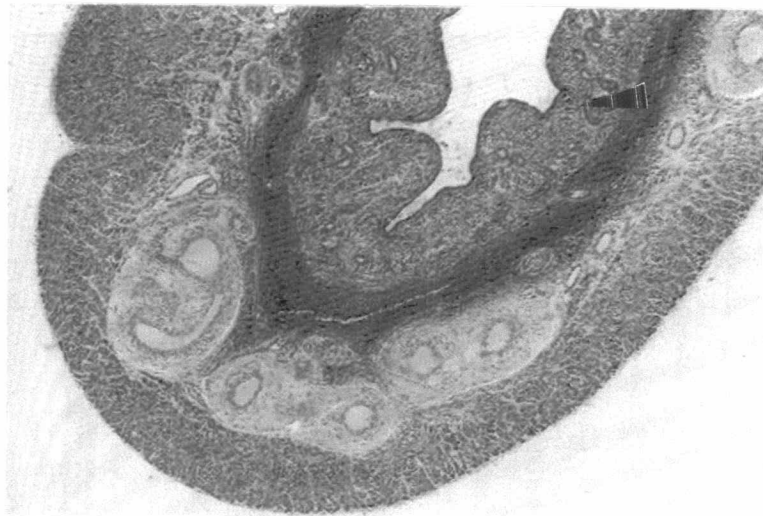


b.

Figure 13. Ovary show several follicles in the cortex of surface epithelium (straight arrow) : Normal structure, No remarkable change (HE, x 100).  
a. present study b. Buergelt,1997.



a.



b.

Figure 14. Uterus : Normal structure, No remarkable change in endometrium (straight arrow) a. present study (HE, x 90) b. Bacha and Wood (1990) (HE, x 100).

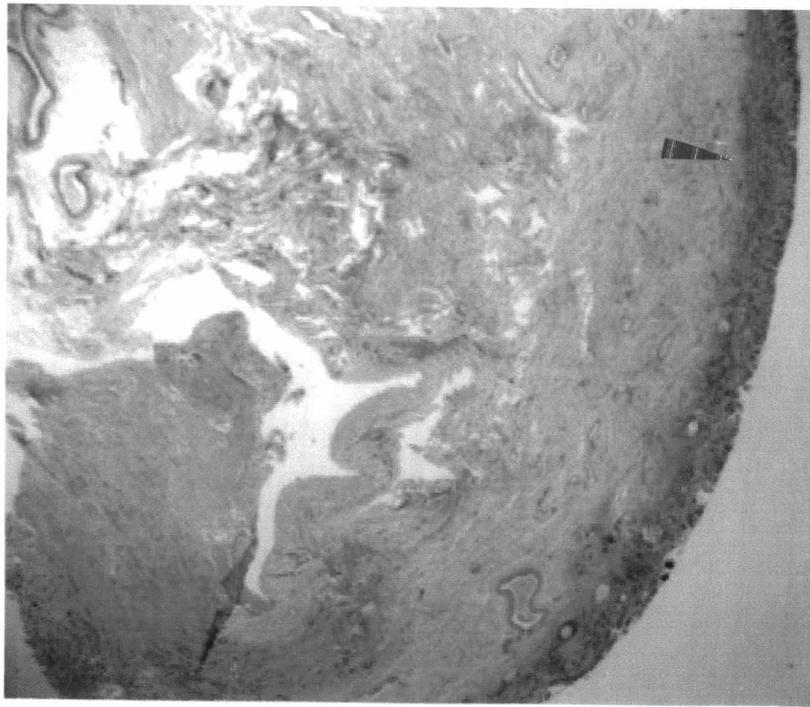


Figure 15. Ovary with no follicle in the cortex of surface epithelium (straight arrow) ; Follicular atrophy (HE, x 90).

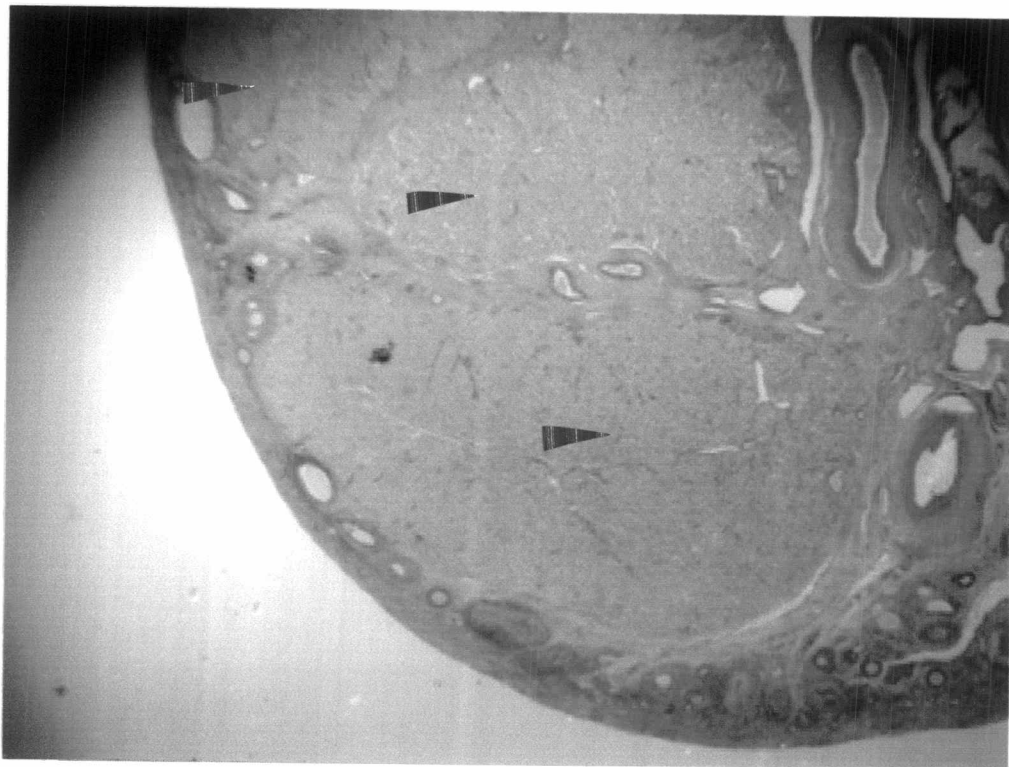


Figure 16. Ovary; Multiple island of corpus luteum (straight arrow) (HE, x 90).

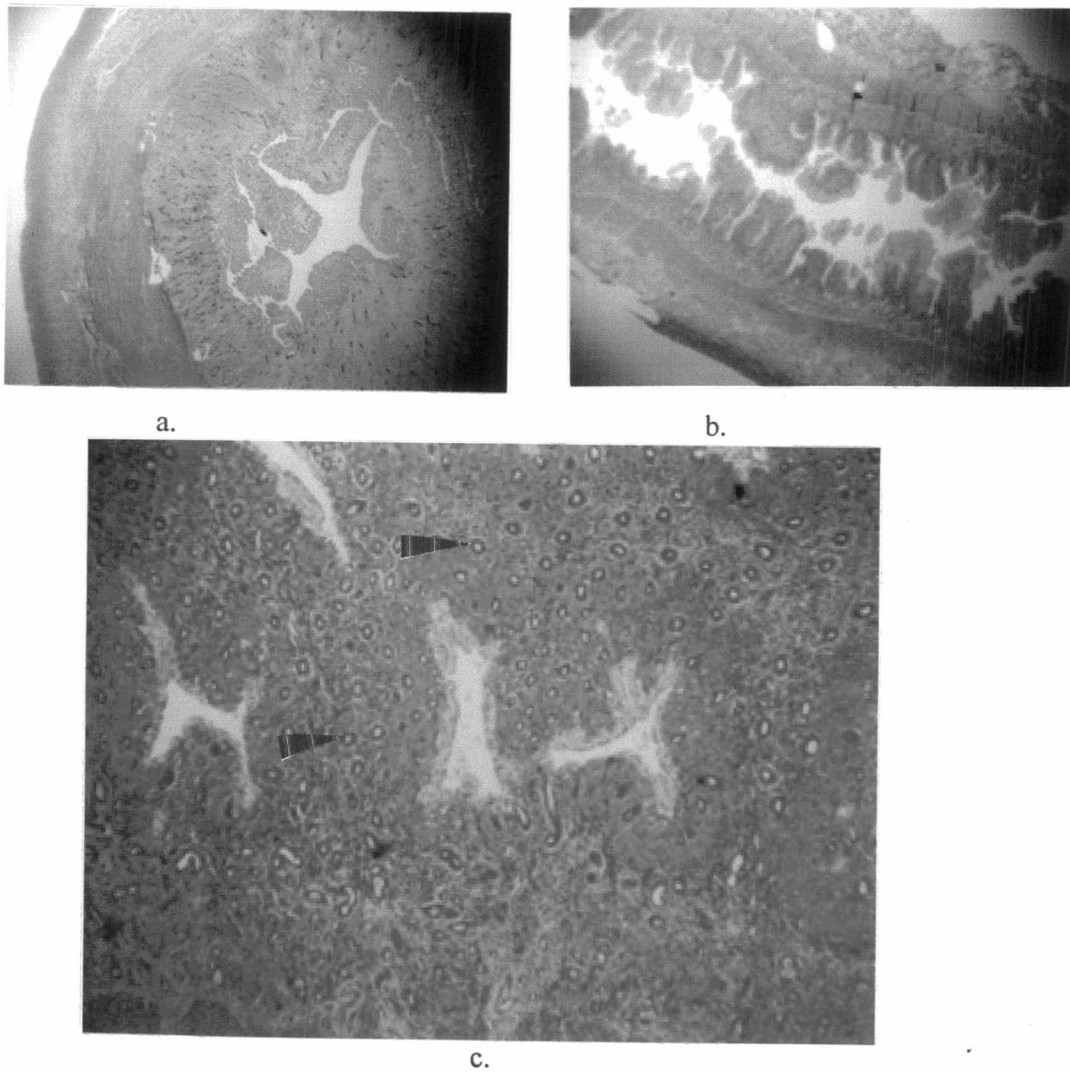
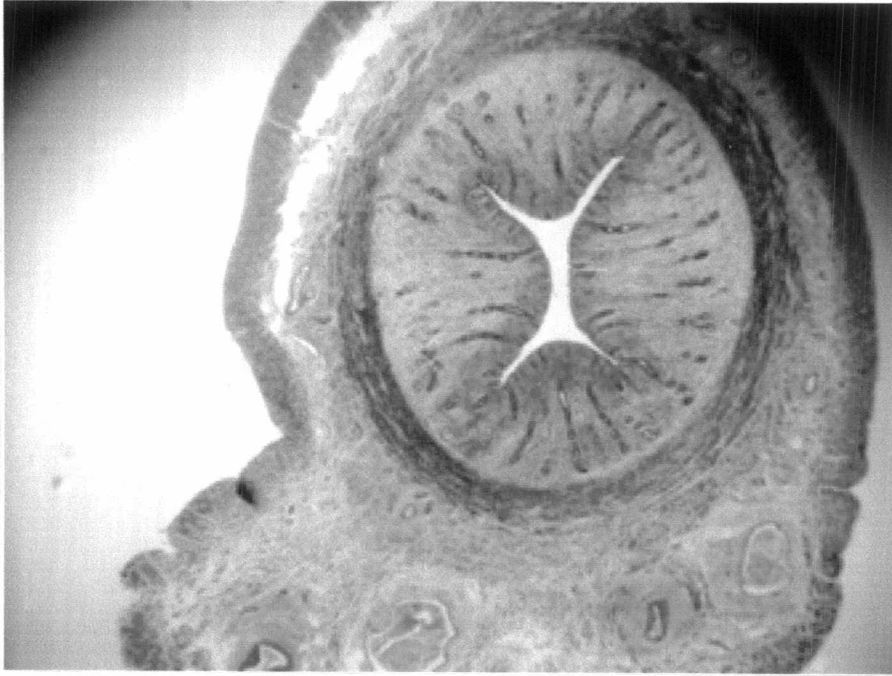
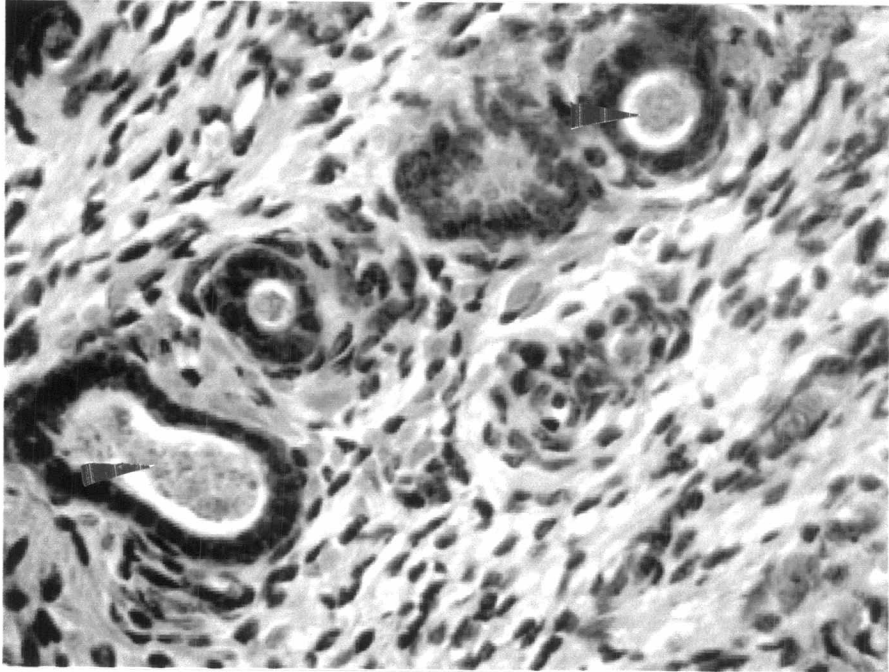


Figure 17. Uterus with fully development of endometrial glands (straight arrow)  
: endometrial hyperplasia and subendometrial haemorrhage  
a and b : HE, x 90 c : HE, x 100.





a.



b.

Figure 18. Uterus ; subendometrial congestion (straight arrow)

a; HE, x 90 b; HE, x 400.