



Indo - Asian Journal of Multidisciplinary Research (IAJMR) ISSN: 2454-1370

EFFECTIVENESS OF ADRENALECTOMY ON OVARIECTOMIZE BITCH ON LEVEL PROGESTERONE HORMONE

Hanan G. Hashem, Tahir A. Fahid and Abdulbari A. Alfari*,

Department of Obstetrics & Surgery, College of Veterinary Medicine, Basrah University, Basrah, Iraq.

Abstract

The present study was conducted about on bitches to affect the adrenal ectomy on ovariectomized to evaluated the level progesterone. Ten adult female dogs were used in the present study. The present study was done in October of 2015 - 2016 in December, the animals divided into two equal groups as: Control group and Unilateral adrenalectomy - ovariectomy group. All grouped examination at first month to determine pregnancy and estrous phase then treated according to dividable groups in proestrous phase, then the animals leaved to eight weeks later, after that the animal ware killed using over dose of anesthesia. The result revealed the average number of day of estrous of cycle of bitches (9.00 ± 1.00) in proestrus phase with change colour of vulva slightly deep - deep pink). Average number of estrous phase (9.00 ± 1.00) with change color vulva to deep pink and slight odema. The appetite and activity of animals observation before and after operation which normal before operation and show simple alterative in some days after operation but restore normal after operation. The level of progesterone hormone recorded significant ($p \leq 0.005$) decrease in period 1 - 2 - 4 - 5 - 7 - 8 weeks in UADX - UOVE & Control group. The level of progesterone hormone in all period show significant ($p \leq 0.005$) decrease compare with control group. The levels of progesterone hormone were significant ($p \leq 0.005$) decrease in 3 to 6 weeks to compare UADX- UOVE & control group.

Key words: Adrenalectomy, Ovariectomize bitch, Progesterone and Adrenal gland.

1. Introduction

The reproductive cycle of the domestic bitch, a mono-oestrous species is characterized by a follicular phase with spontaneous ovulations, followed by pregnancy lasting 58 - 65 days or a non-pregnant luteal phase of about 75 days and a non seasonal anoestrus of 2 - 10 months (Sokolowski *et al.*, 1977). The synthesis and release of progesterone and estradiol was controlled by pituitary hormone [follicle stimulating hormone (FSH), luteinizing hormone (LH), adrenocorticotrophic hormone (ACTH), there is evidence that the response of hormone-secreting

cell was modulated by neural information arriving to the ovaries and adrenal and present asymmetry, Unilateral ovariectomy (OVE) and unilateral adrenalectomy (UADX) (Berga and Naftolin, 2012). Irregularity in the ovaries morphology, physiology and regulatory structures is well established. The degree of asymmetry between gonads fluctuates along the estrous cycle (Dominguez *et al.*, 2008). So, it is a useful tool for study the mechanisms involved in the asymmetric responses of the ovaries endocrine regulating heat cycle in bitch and the level of estrogen and progesterone hormones (Dominguez *et al.*, 2008). Ovariectomy was term used for ovarian removal. It could be unilateral (partial) or bilateral (complete) when one ovary or both ovaries are

*Corresponding author: Abdulbari A. Alfari

E.mail: vetedu2013@gmail.com

Received: 01.08.2016; Revised: 05.09.2016;

Accepted: 02.10.2016.



removed respectively (Elnaz *et al.*, 2014). Also called gonadectomy is one of the most frequent performed surgical techniques in veterinary practice because it is the most reliable mean of pet population control (Duta, 2010).

The adrenal gland also produce small amount of estrogen, some study refer that the adrenal gland was principle source for produce progesterone in estrous phase. So, adrenalectomy is refer to removal of adrenal, it may Unilateral when one adrenal was remove or bilateral when complete. Progesterone is a hormone produced by the ovaries and the placenta, that helps to maintain pregnancy, it was main effect on tissues inside the body include induction of an elaborate network of glands (endometrial glands) in the uterus to help provide nutrition to the early concept us and become the maternal side of the placenta, during pregnancy; it helps keep the uterine muscle layers relatively quiet so as not to disrupt (Catharina *et al.*, 2010). Progesterone would be produce in substantial amount around the time of ovulation in the bitch and continue to rise into the first half of pregnancy (Sudson, 2011). The hormonal analysis is so important in determine estrous cycle and reproductive states in bitch and also to estimate the changes in estrogen and progesterone hormone after UADX and UOVE in local bitch. The aims of study was effect of unilateral adrenalectomy on ovariectomize bitch on progesterone hormone & clinical observation and behavior of bitch.

2. Materials and Methods

Ten bitches were used with an average body weight (20 - 25 kg) and their ages between (1 - 3) year old. The animals were housed in individual cages measuring (1 × 1 × 1 m) in dog's house in Collage of Veterinary Medicine College/University of Basra. All animal were exposed to the same environmental including climate, management feeding and acclimatize, adaptive on place for one month before treatment. The dogs were treated for common gastrointestinal and ectoparasites by giving a single dose of Ivermectin (200 microgram/kg body weight subcutaneous) and examined for any infection by

give coarse of systemic antibiotic to make sure that they were healthy before the beginning of the study. All animals diagnosis for pregnancy. Then determine estrous cycle stage of bitch according to the grossly examination of vulva and vagina and progesterone profiles as following to determine the period of hormones examination.

To diagnostic of pregnancy, no blood or urine test exists to confirm pregnancy in dog, pregnant dogs typically not hormonally are distinct from non-pregnant animals in post estrus period. Dogs do not have an estrous cycle every 3 - 4 week like humans do. Thus, they cannot be observed for aimed cycle, methods of pregnancy diagnosis used include abdominal palpation, radiography and ultrasound. At approximately 28 days post LH peak in the dog (range 21 to 35 day) as the uterus gets larger, the individual gestational sacs can be palpated. As the uterus get larger, the individual sacs can no longer be differentiated. The larger uterus can often still be palpated.



Fig - 1: Ultrasound in non pregnant ovary of bitch

Ultrasound is an excellent way to determine if an animal is pregnant, if the fetuses are viable and what the gestational age is. Ultrasound generally is not an accurate method of determining litter size, fasting the patient before the pregnancy check. Ultrasound exam is often not necessary.



Breeders should be informed ahead of time that the animal will be shaved, as some will object to this. We usually begin the exam in the caudal midabdomen, imaging the urinary bladder first, then examining dorsally for the uterine body. Then follow the Left uterine horn cranially, then proceed to follow the right uterine horn caudally (Fig - 1).

The following attributes were observed in the bitches during experimental per bitch: Keen interest in the adjacent kennel where the male, frequent micturition, deviation of the tail to the side (flagging), vaginal discharge are odematous and soft vulva lips. The animals are divided into two groups: Control group, intact animal left without treatment until the end of the experiment, and Unilateral ovariectomy & unilateral adrenalectomy group. The animal treated with removal right ovary & removal right adrenal gland, using open surgery to remove right adrenal gland and right ovary. All the animals of these group underwent estimation of hormone (progesterone), were recorded at one hour before operation then 24 hrs post operation and continued to estimate the hormone weekly for 8 weeks. In this time, all the animals underwent clinical observation include weight of animals, appetite and activity of animals, then the animals of all groups were killed and observe the internal organs.

Food and water withheld for 12 hrs prior to anesthesia to ensure that the stomach empty to avoid vomiting (Bulus *et al.*, 2013). The dogs were anesthetized generally by using a mixture of ketamine hydrochloride (15 mg/kg) B.W. and xylazine hydrochloride (5 mg/kg) B.W. intramuscularly. The area of operation was prepared by clipping and shaving, then washing by soap and water dried and disinfected with iodine. The approach to the right adrenal was made through an 10 to 15 cm oblique incision, starting close to the first lumbar transverse process and running parallel to the last rib. A lobe of the liver directly covered the gland. Also, the gland was attached to the vena cava (Fig - 2). Therefore must be a ligation between the adrenal gland and vena

cava. The gland was carefully dissected and freed from surrounding tissue and cut between ligation. After that the gland was excised, as in (Fig - 3). The muscular and subcutaneous layers were closed separately using a continuous suture pattern chromic catgut, curved cutting 35 mm and the skin was closed by using an interrupted suture pattern, silk 2 curved cutting 40 mm, 75 cm, U.S.P. (non absorbable). A gauze and iodine dressing was placed over the wound to keep it out of the dirt. The antibiotic (Procaine penicillin) was administered intramuscularly immediately post - operatively and daily for seven days following operation.

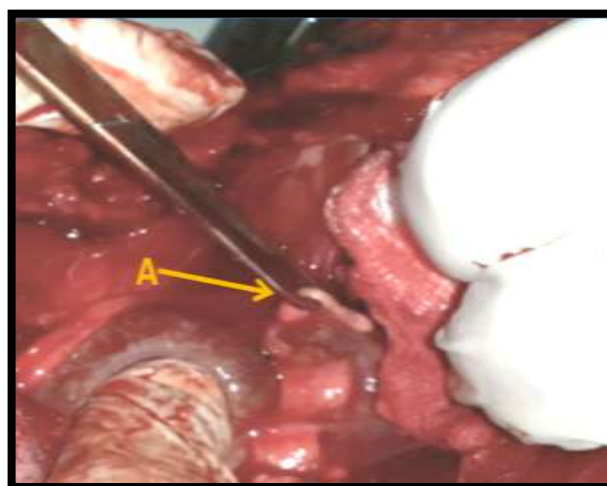


Fig - 2: Show right adrenal gland.

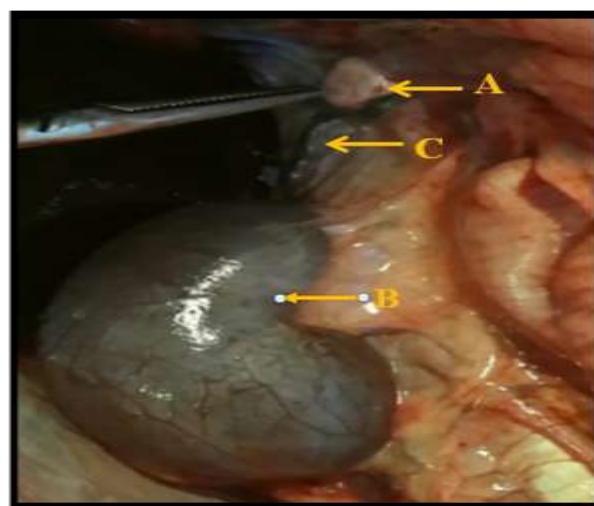


Fig - 3: Show, A) Right adrenal gland , B) Vena cava , C) Right kidney



Surgical technique OVE was started by a median celiotomy extending from the umbilicus to approximately halfway between umbilicus and os pubis, depending on dog size. In deep-chested or obese dogs, it was sometimes necessary to enlarge the incision cranially to allow sufficient exposure of the ovarian pedicle. The ovary was located, and retracted caudally to expose the suspensory ligament and ovarian pedicle. The suspensory ligament was stretched, by electrocoagulation or scissors; Figure - 4 to improve manipulation and observation of the pedicle. The arteriovenous complex within the pedicle, arising from the ovarian artery and vein was ligated with absorbable suture material (chromic catgut), after which it was transected. The uterine artery and vein were ligated at the cranial tip of the uterine horn, 5 mm caudal to the proper ligament, using absorbable suture material, and transected at the proper ligament. After excision, the ovarian bursa was opened and the ovary inspected to confirm complete ovarian removal. OVH was also performed through a median celiotomy. Although, based on the dog's size and body condition, the incision was lengthened in a caudal direction. After the ovarian pedicles were ligated and severed, the broad ligament was examined. If it is vascular, it is ligated with 1 or 2 ligatures using absorbable suture material before it is cut or torn. A clamp was placed on the uterine body just cranial to or on the cervix. The uterine arteries were individually ligated proximal to the clamp using absorbable suture material and the uterus was ligated circumferentially in the crushing groove that remains after removal of the clamp using absorbable suture material. After inspection for potential bleeding at the ligate, the muscular and subcutaneous layers were closed separately using a continuous suture pattern. Chromic catgut and the skin was closed by using an interrupted suture pattern, silk 2 curved cutting (non absorbable). A gauze and iodine dressing was placed over the wound to keep it out of the dirt. The antibiotic (Procainpencilline) was administered intramuscularly immediately post – operatively and daily for seven days following operation.

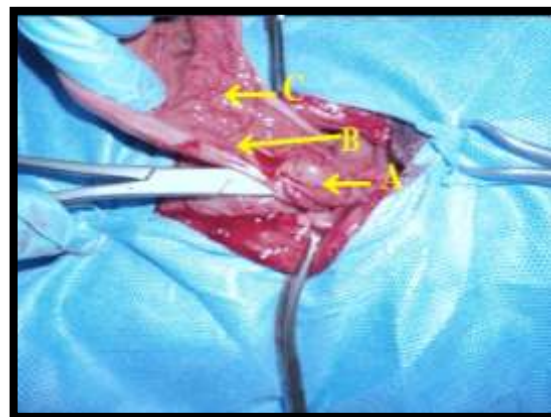


Fig - 4: This figure shows (A) the right ovary, (B) Uterine tube, (C) Broad ligament

The blood samples were collected using is possible syringe from the sapheunes vein before operation and after 24 hrs of operation and repeated each week for 8 weeks later, separation of serum by centrifuging of sample at 5000 rpm for 30 minutes and the serum were aspirated by appetite and transferred to poly ethylene tubes at (-20 °C) in deep freezer for hormonal test. All blood sample were collected between 8 - 10 hrs in order to standardize the time of collection. The hormonal test performed using the serum which was extracted from blood sample, collected without anticoagulant and freezed until the time of the examination. The hormonal test includes progesterone, using a device called ELISA Micro wells machine hormones assay with the kit of Monobind Inc. of USA.

3. Results

The present study beginning in October on bitches. When we brought the animals attempt to determine estrus phase were identified using the colour of mucous membrane of varied from slight pink during proestrus to deep pink during oestrus. It was pale during diestrus phase (Table - 1). The size of vulva lips increased from slightly oedematous at the start of proestrus to grossly edematous and firm to touch at the end of proestrus. During estrus the odema was still maintained but become soft to touch thus, making the orifice of the vulva more accessible. At diestrus phases, the odema was lost and the vulva



returned to their small sized as before proestrus. The colour of vaginal discharge was bright red, seroanguinous with increasing volume at proestrus while it was dark red (chocolate) with a reduced volume at oestrus. At diestrus the vaginal discharge was whitish sticky or cheesy and the volume was very small. The more visible clinical characteristics during estrous of Basrah local bitches were, keen interest in the kennel where the studs were kept, interest and acceptance of the stud (male), frequent micturition, deviation of the tail (flagging), chocolate red coloured vaginal discharge, odematous and flaccid vulva lips.

The clinical and physical observations include appetite, activity of animals were normal before operation and showed simple alternative in some days after operation, but restore normal observation in different period of time after operation as in Table - 2.

The Table - 3 shows the level of progesterone hormone recorded significant ($p \leq 0.005$) decrease in period 1 - 2 - 4 - 5 - 7 - 8 weeks in UADX and UOVE group, compare with UOVE group. The level of progesterone hormone in all period show significant ($p \leq 0.005$) decrease compare with control group. The level of progesterone hormone were significant ($p \leq 0.005$) decrease in 3, 6 weeks compare UADX & UOVE group.

The left adrenal gland of bitch in control group appeared normal encased in a thick connective tissue capsule. The adrenal cortex consists of three zones which are not sharply separated from each other. The Zona glomerulosa (ZG) is the outer zone, the cells are arranged in ovoid groups or clumps and surrounded by numerous sinusoidal capillaries, the cytoplasm of these cells stains pink in hematoxyline eosin stain and contains few lipid droplets. The cell of Zona fasciculata (ZF) are arranged into vertical columns or radial plates direction. Because of the increased amount of lipid droplet in their cytoplasm, the cell of the (ZF) appear light or vacuolated after a normal slid preparation. The Zona reticularis (ZR) is the innermost zone of adrenal gland cortex, the

cell of this zone form anastomosing cords surrounded by sinusoidal capillaries (Figure - 5). Section in left adrenal gland of control group after same period of treatment (8 weeks) show capsule, ZG, ZF, ZR. The Fig - 6 shows left adrenal gland of bitches treated with UADX & UOVE there was thickening the capsule, excessive vacuolated and enlargement in ZG. In Figure - 7, there was thin capsule with hemorrhage in ZF. And in Figure - 7 was left adrenal gland treated with UADX & UOVE, Hemorrhage in medulla with clear vacuolated.

4. Discussion

The results observed in the present study as represented in the Table - 1 in results which similar with the results that reported by Nelson and Couto (2003), in other breeds of dog, it was discovered that there were changes in the colour of the mucous membrane of the vulva from deep pink during late proestrus to very pale during anoestrus period and this was consistent in all the bitches in this study. This can be used to identify the phases of the oestrous cycle in the bitch, though qualitatively. The changes in colour of the mucous membrane might be due to the amount of blood supply to the reproductive organs and the effect of estrogen on their smooth muscle at the different phases of estrous cycle. It was also discovered that the changes in the sizes of the vulva lips through the four phases of the estrous cycle was consistent in all the bitches and throughout all the estrous cycle studied. The quantity, colour and consistency of the vaginal discharge throughout the oestrus cycle in all bitches are consistent with the reports of some previous workers on other breeds of dogs (Daval, 2000; Beimborn *et al.*, 2003).

The different behavioral characteristics that were observed in the local bitches used for this study during estrus were keen interest in male, acceptance of the male, frequent micturition and flagging of the tail which varied from bitch to bitch. These inconsistencies have been observed by some worker on other (Sokolowski *et al.*, 1977).



Table - 1: Change in vaginal discharge, vulva lips and the average number of day of oestrous cycle of bitches

Phases	Colour of vulval mucous membrane	Consistency vulval lip to touch	Quantity,colour consistency of vaginal discharge	Average number of days for different phase
Proestrus	Slightiy-deep pink	Odematous and firm to touch	Serosanguinous quantity-large	9.00±1.00
Estrus	Deep pink	Slight odema and soft	Chocolate red quantity-small	9.00±3.00
Diestrus	Pale	No odema	Whitish quantity-springly	62.00±3.00
Anestrous	Non detect	Non detect	Non detect	Non detect (end of the study)

Table - 2: Clinical observation in UADX and UOVE group

Parameters	Time 0	1 day P.o.	3 days P.o.	5 days P.o.	7 days P.o.	21 days P.o.
Appetite of animal	Normal	Sub Normal	Sub Normal	Sub Normal	Sub Normal	Normal
Activity of animal	Normal	Sub Normal	SubNormal	Sub Normal with bloody diarrhea	Normal	Normal

Table – 3: Level of Progesterone hormone recorded

Treatment Parameters			Normal	Ovarectomy	Adrenalectomy-Ovarectomy
Proestrus	0 week #	Progesterone	1.09 ±0.28 A	1.59±0.70 A	0.39±0.02 B
	0 week *	Progesterone	1.13±0.32 NS	1.15±0.34 NS	1.21±0.047 NS
	1 week	Progesterone	1.23±0.027 A	1.15±0.024 A	0.45±0.048 B
Estrus	2 week	Progesterone	1.33±0.018 A	1.00±0.022 B	0.75±0.017 C
	3 week	Progesterone	1.11±0.017 AB	0.76±0.046 B	1.20±0.019 A
Diestrus	4 week	Progesterone	1.00±0.023 NS	0.90±0.021 NS	0.73±0.033 NS
	5 week	Progesterone	1.13±0.020 A	1.01±0.023 AB	0.79±0.016 B
	6 week	Progesterone	1.22±0.22 A	0.99±0.22 AB	0.77±0.39 B
	7 week	Progesterone	1.33±0.12 A	1.00±0.19 B	0.70± 0.29 C
	8 week	Progesterone	0.98±0.32 NS	0.81±0.044 NS	0.76±0.031 NS

*n = 5, large later effor to significant ($p \leq 0.05$)

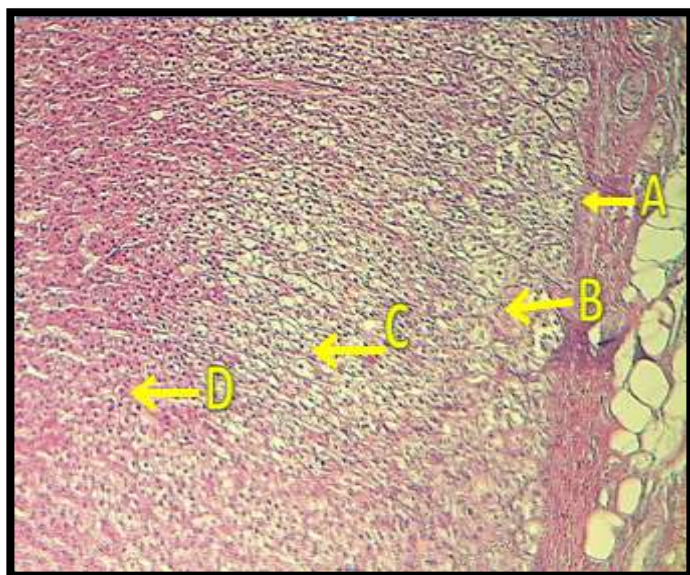


Fig - 5 : Left adrenal of control group after same period of treatment (8 weeks) show, A) capsule, B) ZG, C) ZF, D) ZR, stain (H & E), X10

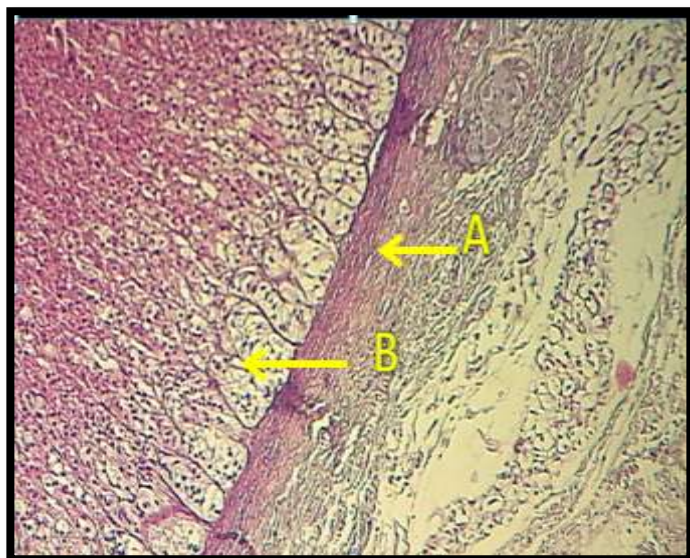


Fig - 6: Left adrenal gland of bitches treated with UADX & UOVE (A) thickening the capsule, (B) excessive vacuolated & enlargement in ZG. Stain (H & E) X10

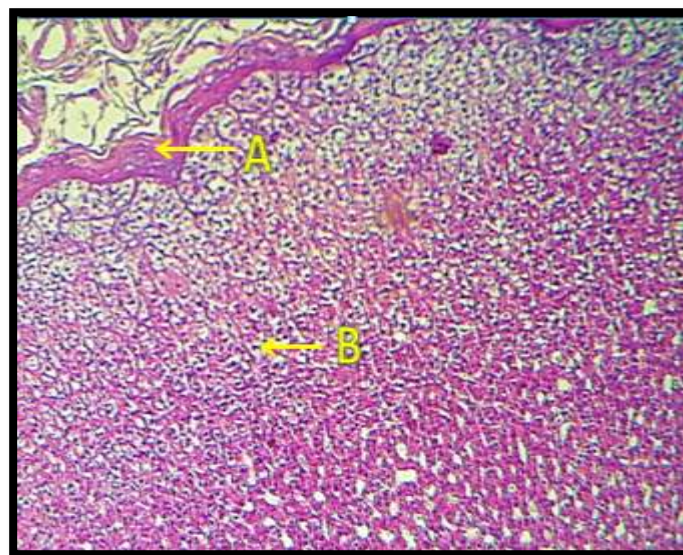


Fig - 7: Left adrenal gland of bitch treated with UADX & UOVE, (A) thin capsule, B) hemorrhage in ZF stain (H & E) X 10

The other physical characteristics observed during estrus in this study were odema of the vulva and change in the quantity and colour of the vaginal discharge. These were more constant characteristics during estrus in all the bitches as was reported in other breeds. There also exists a relationship between behavioral events, hormonal and physiologic change, which occur due to changes in level of estrogen (Wildt *et al.*, 1979).

No complication in this due to understanding of the anatomy, experience of surgeon and good post operative care make facilitate in recovery all bitches after operation and survival to end period of study, when we attempted to killed them for histopathological study. Most common difficult in this group there the bitch biting there suture and cause open the skin incision, so we cleaning, repair and suture the site of operation later and give antibiotic. Always E-collar is true choice for this condition. The animals show loss of apitite after operation and continue for up 7 days less than 21 days, due to increase stress after operation and return gradually after that when bitches recovery this agree with Ready (2000), also the loss of appetite post



operation agree with (Christen *et al.*, 2013). Although, bitches developed a mild and short episode of bloody diarrhea after 5 days of surgery as in Table – 2. The surgery is stressful to the body, and the stress induce diarrhea can self – limiting. But, the diarrhea is not always due to stress, if the animals had normal stools for at least a week - 10 days of surgery and then develops diarrhea, on their activity and appetite decrease, while they are having diarrhea, or the diarrhea is protracted lasting longer than 5 - 7 days.

This indicates that the bitches diarrhea is not due to stress but some other medical allergic. Most medication cause bloody diarrhea was Asparin, pencilline and ectra (Angelica *et al.*, 2008). Also loss of activity of bitches post operation that adrenal ectomy cause fatigue to body condition agree with (Root Kustritz, 2012). While, Mandle (1951) show there is significant decrease in progesterone and this work does not there for propose a longer period of sex hormone estimation following ovariectomy as a result obtained six week, post operative did not differ significantly from the one obtained before 84 hrs. The results observed in the present study confirm with the result that the reported mice and rats have established that the remaining ovary undergoes compensatory hypertrophy and compensatory ovulation after 8 week of surgery. Although, follicular in hamster ovary distribution of follicle in the intact or semi-spayed hamster, the follicle destined to ovulate at any one estrous are set aside by 3rd day of cycle. But in rat, the peak of follicular degeneration occur between the onset of oestrus and ovulation.

5. References

- 1) Angelica, F., Jacqueline Velasco, Alma, I., Fernando, D., Pamela, M., Maria, C and Reberto, D. 2011. Acute effects of unilateral sectioning the superior ovarian nerve of rats with unilateral ovariectomy on ovarian hormone (Progesterone, testosterone and estradiol) level vary during the estrous cycle. *Reproductive Biology and Endocrinology*, 9 (1): 34.
- 2) Angelica, F., Alma, I., Jacqueline, V., Fernando, D., Cristina, M., Pamela, M., Maria, E and Reberto, D. 2008. The acute effects of bilateral ovariectomy or adrenal ectomy on progesterone, testosterone and estradiol serum level depend on the surgical approach and the day of the estrous cycle when they are performed. *Reproductive Biology and Endocrinology*, 6: 48.
- 3) Beimborn, K., Tarpley, H., Bain, P. and Latimer, K. 2003. The canine estrous cycle staying using vaginal cytology examination.
- 4) Berga, S. and Naftolin, F. 2012. Neuroendocrine control of ovulation. *Gynecol Endocrinology*, 28 (1): 9 - 13.
- 5) Bulus, H., Uslu, H., Karakoyun, R and Kocak, S. 2013. Compassion of laparoscopic and open adrenal ectomy. *Acta Chirp Belg*, 113 (3): 203 - 207.
- 6) Catharina, B., Edward Philip, Alejandro, E. Coralli, J. 2010. Serum 17 α – hydroxyl progesterone concentration during the reproductive cycle in healthy dogs with *Hypoadreno corticism*. *Javama*, 11: 1208 - 1214.
- 7) Christen, W., Edward, H and Roce, I. 2013. Reported on all old dog spayed before 6 months of age that developed signs of estrous. *Reproduction of Domestic Animal*, 81 (16): 193 - 198.
- 8) Davol, P. 2000. Canine Reproduction Part 1. Reproduction and the bitch pdavol @ labbies.com.
- 9) Dominguez, R. Morales, L and Cruz, M. E. 2008. Ovarian asymmetry. *Annual Reviews of Biomedical Science*, 5: 95 - 104.
- 10) Duta, S. 2010. Suprarenal gland-arterial supply: an embryological and applied importance. *Romanian Journal of Morphology and Embryology*, 51 (1): 137 - 140.
- 11) Elnaz, S., Jalal, B., Alireza, K and MIR, N. 2014. Comparison between two portal laparoscopy and open surgery for ovariectomy in dogs. *Veterinary Research Forum*, 5: 219 - 223.
- 12) Nelson, R and Couto, C. 2003. Small Animal Internal Medicine 3rd Ed. Mosby 11830 Wet



line Industrial Drive St. Louis Missouri, :847 - 933.

- 13) Root Kustritz, M. 2012. Effect of surgical sterilization on canine and feline health and society. *Reproductive Biology and Domestic Animals*, 47 (4): 214 - 222.
- 14) Sokolowski, J., Stover, D and Van Ranvenswaa, F. 1977. Seasonal Incidence of estrus and interestrous interval for bitches of Seven Breeds. *Journal of American Veterinary Medical Association*, 171(3): 271 - 273.
- 15) Sokolowski, J., Stover, D and Van-Ranvenswaa, F. 1977. Seasonal Incidence of estrus and interestrous interval for bitches of Seven Breeds. *Journal of American Veterinary Medical Association*, 171(3): 271 - 273.
- 16) Sudson, S. 2011. Control of estrus and ovulation in dog and cat: An update. 41: 65 - 68.
- 17) Wildt, D., Panko, W., Chakraborty, P and Seager, S. 1979. Relationship of serum estrone estradiol-17 B and progesterone to LH, sexual behavior and time of ovulation in the bitch. *Biological Reproduction*, 20: 648 - 658.

