



Effect of laparoscopic adrenalectomy on estrogen, progesterone and cortisol hormones in bitch

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Abstract

The effect of laparoscopic left adrenalectomy on adult bitches' estrogen, progesterone, and cortisol levels was studied. Fifteen adult stray bitches were used. The average weight was 20 kg, age was 27 months. The study animals were randomly divided equally into three groups, each group containing five bitches. The first group underwent unilateral laparoscopic left adrenalectomy (ULLA), the second group underwent left adrenalectomy with bilateral ovariectomy, and the third group was left as a control group. Blood samples were taken from all animals on days 0, 7, 14, and 30 after surgery to estimate estrogen, progesterone, and cortisol levels. The result of using sternal decubitus laparoscopic left adrenalectomy was a practical, feasible, and rapid technique with a short average surgical time of 72 minutes and easy dissection; therefore, it is considered a standardized technique. The results also showed that the estrogen concentration on the 30th day of the three groups was 141.2, 56.8, and 137.8, respectively, with a significant difference in the second group compared to the rest groups and the progesterone concentration on the 30th day of the three groups was 3.8, 3.6 and 4.5 respectively with a significant difference among the groups, and in another context the cortisol concentration on the 30th day of the groups was 128.4, 82 and 129, respectively, with a significant difference in the second group compared to the rest groups. In conclusion, the possibility of laparoscopic adrenalectomy with sternal recumbency in dogs is a standardized method, and unilateral adrenalectomy does not affect the levels of estrogen, progesterone, and cortisol due to the compensation mechanism.

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Introduction

The adrenal glands are positioned at the craniomedial pole of the kidneys in a retroperitoneal position at the roof of the abdomen (1,2). The gland comprises two functionally and structurally distinct endocrine tissues, the outer layer, is the cortex, and the inner layer is the medulla (2). responsible for the production of various hormones like aldosterone (3,4). Cortisol, and steroid hormones (progesterone and estradiol) during the estrous phase (3-6). Adrenal glands can be affected by disorders such as Cushing's disease, Addison's disease, and adrenal medullary tumors and cause hormonal disturbances like cortisol, progesterone, estrogen, and

aldosterone (7-9). Cushing's disease and adrenal medullary tumors need surgical intervention. The laparoscopic adrenalectomy lateral or sternal recumbence considers the treatment of choice such cases with low intraoperative and postoperative death due to the benefits of laparoscopic surgery (10-12) such as a minimum pain, lower complications, shorter time, and rapid recovery compared with laparotomy (12) as well as the sternal positioning of the animal provide better visualization of the adrenal gland through displacement of abdominal organ away from it as a result of gravity (13). The domestic bitch is a monoestrous kind who is reproductive cycle comprises a follicular phase with spontaneous ovulations, a pregnancy that lasts between

58 and 65 days, or a non-pregnant luteal phase that lasts around 75 days, and a non-seasonal anestrus that takes between two and ten months (14). Pituitary hormones, including follicle stimulating hormone (FSH), luteinizing hormone (LH), and adrenocorticotropic hormone (ACTH), regulate the synthesis and release of progesterone and estradiol (15). The estradiol is in charge of sexual behavior (estrus signs) during proestrus. It rises from 5-10 pg/ml basal levels to peaks of 45-120 Pg/ml, which typically happen 1-3 days before the pre-ovulatory LH peak (14). The Progesterone hormone is produced by the ovaries and placenta, and It helps to sustain pregnancy by inducing the development of an intricate net of glands in the uterus called endometrial glands, which serve to nourish the developing fetus and develop into the maternal side of the placenta. Additionally, progesterone helps maintain the uterine muscle layers relatively quietly manner so as not to disturb (6). Cortisol, a steroid hormone, is created in the zona fasciculate section of the adrenal cortex and can pass through the cytoplasmic membrane due to its lipid-soluble properties (16). The cortisone is considered one of the biochemical parameters' characteristics of adrenocortical enlargement after unilateral adrenalectomy as compensatory growth (2,17) to keep the residual gland's physiological functions close to normal levels (2). The adrenal gland is also the primary source of progesterone and estradiol during the estrous phase (3-6).

Due to the prescience of different diseases like Cushing's disease and adrenal medullary tumors that need adrenalectomy, as well as, the small size of adrenal glands and their location deeply in the abdomen, reaching and removing them normally requires a large incision (laparotomy) and effects of these diseases on the body hormone like cortisol, progesterone, and estrogen. Therefore, the project is designed to standardize laparoscopic adrenalectomy with sternal recumbence and investigate the effects of adrenalectomy on the levels of estrogen, progesterone, and cortisol.

Materials and methods

Ethical approve

Ethical approval was granted through the local committee on animal care and use at the College of Veterinary Medicine within the University of Mosul. number U.M.VET.2023.110 dated 03/09/2023.

Animals

In the current study, fifteen stray mature female dogs were used. The average weight is 20 ± 1.3 (kg), and the age 27 ± 1.5 (months). All animals were healthy, housed in the animal house at the College of Veterinary Medicine, University of Mosul, and exposed to the same environment, including climate, and nutrition. They adapted to the place for 15 days before the surgery. The dogs were treated for

common gastrointestinal diseases and external parasites by giving ivermectin subcutaneously at 0.2 mg/kg body weight (18,19). The ultrasound and gross examination of the vulva and vagina were utilized to detect a non-pregnant addition to estimating the estrous cycle stage of bitch (diestrus).

Experimental design

The study animals have been divided into three groups randomly, each group including 5 bitches. The first group underwent unilateral laparoscopic left adrenalectomy. In contrast, the second group underwent unilateral laparoscopic left adrenalectomy along with the ovaries, while the third group was left as a control group. Every animal had a blood sample collected under aseptic conditions on days 0, 7, 14, and 30 after operative the operation 5 ml of blood was collected from the jugular vein and put in a clean glass tube to get the serum, then sent to a laboratory to measure the estrogen, progesterone, and cortisol hormones.

Anesthesia

Before general anesthesia, all batches received atropine sulfate (Anova, Vietnam) at 0.05mg/kg BW/SC as a pre-anesthetic agent (20,21). Then, all bitches were given general anesthesia by intramuscular injection as a mixture of ketamine 10% HCl (Alfasan, Holland) at 15 mg/kg, and xylazine 2% (Nitafarm, Russia) at 2 mg/kg B.w. (22-24).

Surgical technique

After ensuring that the animals were in the surgical stage of general anesthesia, the animals were placed in the sternal recumbent position, preventing visceral compression as determined by the operating table gravity-enhanced visualization of the adrenal gland and surrounding structures. The skin and subcutaneous incisions were made with a surgical blade to insert a Verses needle, 4 cm posterior to the last rib and 8 cm ventral to the transverse process of the third lumbar vertebra described by Kirsten *et al.* (25). pneumoperitoneum was established by insufflation of Carbon dioxide into the abdominal cavity until the intra-abdominal pressure reached 12 mmHg, as reported by Al-Badrany *et al.* (26). After distension of the abdominal cavity, the Verses needle was replaced with a 10 mm trocar- cannula to insert the telescope into the abdominal cavity. Exploring the abdominal cavity to identify the inadvertent injury to internal organs reported by Al-Qattan *et al.* (27), the second port was used to insert a 5-mm trocar-cannula into the lumbar fossa, and the third port was used to insert a 10-mm cannula behind the last rib, approximately 3 cm ventral to the transverse process of the second lumbar vertebra, to insert the clips applicator (Figure 1).

By using artery forceps and unipolar electrocautery, the gland was dissected from the surrounding tissues through the second port. The clips applicator with titanium clips was inserted from the third to ligate the phrenico-abdominal vein as described by Kirsten *et al.* (25) (Figure 2). The adrenal

becomes free after dissecting the phrenico-abdominal vein. ligating, and cutting. It is then extracted from the abdomen using tissue extractor or artery forceps via the third port. Through the same steps and ports, the ovaries are removed after the ligation of an ovarian artery by titanium clips, as described by Al-Badrany *et al.* (26) (Figures 3 and 4). All the animals were left in the recovery room until they fully recovered and then their cages were moved.

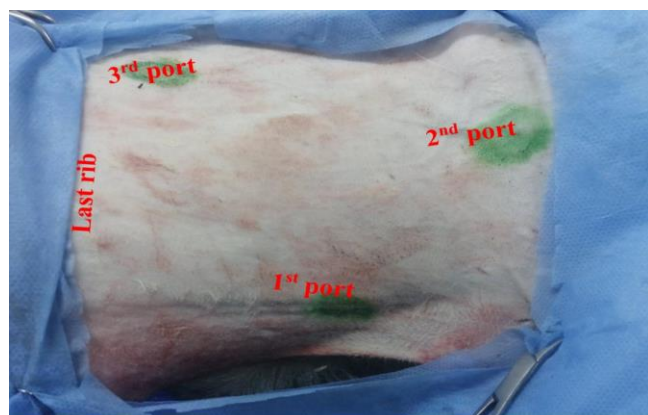


Figure 1: Showing the position of ports in laparoscopic adrenalectomy and ovariectomy.

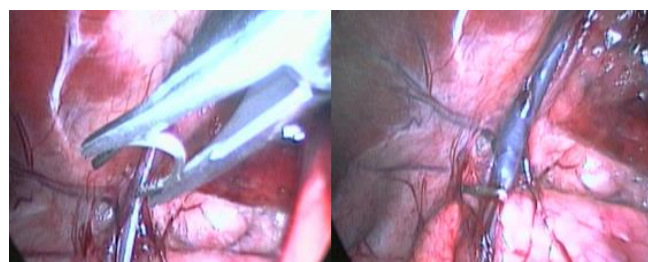


Figure 2: Showing applying titanium clips on the phrenico-abdominal vein.

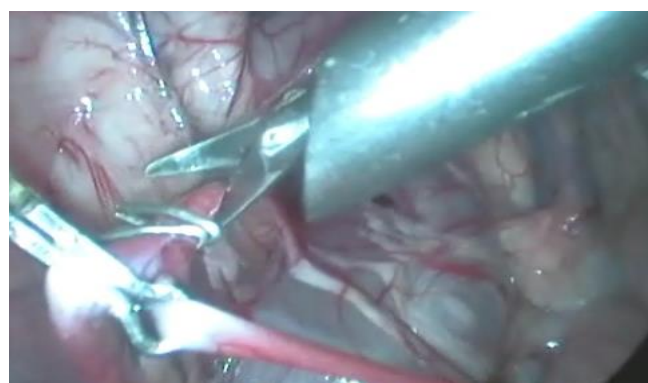


Figure 3: Showing applying titanium clips to ligate the ovarian artery.

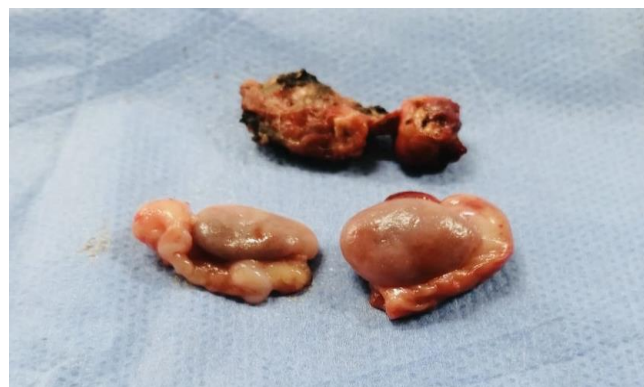


Figure 4: Showing adrenal gland, and ovaries after exteriorized from the abdomen.

Postoperative care

The penicillin-streptomycin combination (kanavet, Canadian) was administered intramuscularly (I.M.) to the experimental animals over three days (19). The dogs were physically examined in the first three days following surgery, with particular attention to their activity levels and food intake. Eight days following the procedure, the skin sutures were removed.

Biochemical examination

Blood samples were taken from all bitches at 0,7,14,30 postoperative days to estimate levels of estrogen, progesterone, and cortisol in serum. The laboratory examination was done by using a Cobas device/roach company.

Statistical analysis

The study's results were expressed as Mean \pm S.E. using normal distributions, and data were compared using ANOVA. Duncan's Multiple Range Test was used to find significant variances. Sigma Plot 12.5 was used for statistical analysis (Sigma stat, Jandel Scientific Software V3.1), and $P<0.05$ was considered statistically significant (28,29).

Results

Laparoscopic adrenalectomy in sternal recumbence was a practical and feasible technique for removing the adrenal gland with fewer complications, with a short duration surgical time mean of 72 ± 2.1 minutes. In addition, the dissection of the adrenal gland from surrounding tissues was fast and precise and provided better visualization of the abdominal cavity, although the anatomical position of the adrenal gland is deeply in the abdomen; therefore, we can consider it as an alternative surgical approach to the classical invasive open adrenalectomy. The results of the hormonal assay in the 1st group showed that the estrogen concentration at 0, 7th, 14th, and 30th postoperative days (P.O.D.s) was

123.8, 143.8, 146, and 141.2 Pg/ml, respectively, with a significant difference between 0 day and the remaining days. In contrast, progesterone concentration was 3.9, 3.8, 3.7, and 3.8 ng/ml, respectively, with no significant difference. The cortisol concentration was 130.6, 122.5, 126, and 128.4 nmol/L, respectively, with a significant decrease at the 7th P.O.D. compared to 0 days, then returned to the average level on days 14th and 30th (Table 1).

In another context, the results in the 2nd group reveal that the estrogen concentration at 0, 7th, 14th, and 30th post-surgery was 150.4, 139.2, 126.2, and 56.8 Pg/ml, respectively, with a significant difference at 7th and 14th P.O. Ds compared with the rest of the days. The progesterone concentration was 4.5, 4.0, 3.8, and 3.6 ng/ml at 0, 7th, 14th, and 30th P.O. Ds, respectively, with a significant difference at all times. The cortisol concentration was 92.9, 73, 80, and 82.6 nmol /L at 0, 7th, 14th, and 30th P.O. Ds, respectively, with a significant difference at 14th and 30th as compared with the rest of the days, as shown in (Table 2).

While in the 3rd group, the results reveal estrogen concentration at 0, 7th, 14th, and 30th days post-surgery was 150, 149.6, 146.2, and 137.8 Pg/ml, respectively, with no significant difference. The progesterone concentration was 4.3, 4.4, 4.4, and 4.5 ng/ml, respectively, with no significant difference at all times. While cortisol concentration was 130, 132, 131, and 129 nmol/L, respectively, with no significant difference between days (Table 3).

The results also showed that the estrogen concentration at 30th P.O. Ds for the three groups was 141.2, 56.8 and 137.8 Pg/ml, respectively, with a significant difference between the second group and the rest of the groups. The progesterone concentration at 30th P.O. Ds for the three groups was 3.8, 3.6, and 4.5 ng/ml, respectively, with a significant difference between the groups. The cortisol concentration was 128.4, 82.6, and 129 nmol/L, respectively, with a significant difference in the second group compared with the first and third groups (Table 4).

Table 1: Concentration of serum estrogen, progesterone, and cortisol in first group

Hormones	Mean ± Standard error			
	0 day	7 days	14 days	30 days
Estrogen (Pg/ml)	123.08±8.20 ^a	143.80±10.2 ^b	146.00±1.70 ^b	141.20±0.50 ^b
Progesterone (ng/ml)	3.90±0.02 ^a	3.80±0.04 ^a	3.70±0.05 ^a	3.80±0.07 ^a
Cortisol (nmol/L)	130.60±2.00 ^a	122.50±2.00 ^b	126.00±1.60 ^a	128.40±0.64 ^a

Different letters mean there is a significant difference at P<0.05.

Table 2: Concentration of serum estrogen, progesterone, and cortisol in second group

Hormones	Mean ± Standard error			
	0 day	7 days	14 days	30 days
Estrogen (Pg/ml)	150.40±2.00 ^A	139.20±1.40 ^B	126.02±0.50 ^B	56.80±2.70 ^C
Progesterone (ng/ml)	4.50±0.10 ^A	4.00±0.03 ^B	3.80±0.02 ^C	3.60±0.07 ^D
Cortisol (nmol/L)	92.90±1.60 ^A	73.00±1.10 ^B	80.00±1.10 ^C	82.60±1.30 ^C

Different letters mean there is a significant difference at P<0.05.

Table 3: concentration of serum estrogen, progesterone, and cortisol in third group

Hormones	Mean ± Standard error			
	0 day	7 days	14 days	30 days
Estrogen (Pg/ml)	150.00±3.60 ^A	149.60±3.40 ^A	146.20±2.50 ^A	137.80±2.70 ^A
Progesterone (ng/ml)	4.30±0.10 ^A	4.40±0.30 ^A	4.40±0.02 ^A	4.50±0. 7.00 ^A
Cortisol (nmol/L)	130.00±1.70 ^A	132.00±1.80 ^A	131.00±1.70 ^A	129.00±1.00 ^A

Different letters mean there is a significant difference at P<0.05.

Table 4: concentration of serum estrogen, progesterone, and cortisol on 30 days in all groups

Hormones	Mean ± Standard error		
	First group	Second group	Third group
Estrogen (Pg/ml)	141.20±0.50 ^A	56.80±2.70 ^B	137.80±2.70 ^A
Progesterone (ng/ml)	3.80±0.07 ^A	3.60±0.07 ^B	4.50±0.70 ^C
Cortisol (nmol/L)	128.40±0.64 ^A	82.60±1.30 ^B	129.00±1.60 ^A

Different letters mean there is a significant difference at P<0.05.

Discussion

The effectiveness and feasibility of using laparoscopic adrenalectomy techniques for removing the gland with fewer complications intra and postoperative results were similar to the results found by Jiménez *et al.* (10), Collivignarelli *et al.* (12), Kirsten *et al.* (25) and Balsa and Culp (30). The mean time of 72 minutes of laparoscopic adrenalectomy with sternal recumbence, this result was in line with findings (12,13) that suggest that laparoscopic adrenalectomy with sternal recumbence was a shorter time than open or conventional laparoscopic adrenalectomy. In addition, Rodrick *et al.* (31) and Turner *et al.* (32) suggest that laparoscopic adrenalectomy has a shorter duration than conventional open adrenalectomy due to laparoscopic reaching deep organs in the abdomen. Dissection of the gland from its position in the abdomen was fast and precise and made it considered a surgical approach of choice to the invasive open adrenalectomy. The laparoscopic adrenalectomy with sternal recumbence has advantages allowing gravity to the displacement of the abdominal organs, providing better visualization of the adrenal gland, and simplifying and shortening dissection compared with conventional laparoscopy or open adrenalectomy (13,25).

The results of the study in the 1st group showed that the estrogen concentration at 0, 7th, 14th and 30th P.O.D.s was 123.8, 143.8, 146, and 141.2 Pg/ml, respectively, with a significant increase at 7th, 14th and 30th P.O.D.s compared with 0 day due to the compensatory mechanism after adrenalectomy because the adrenal gland produces an amount of estrogen. While the progesterone concentration was 3.9, 3.8, 3.7 ng/ml and 3.8, respectively, with no significant difference due to the ovaries producing the progesterone, this result agrees with (6). While the cortisol concentration was 130.6, 122.5, 126 and 128.4 nmol/L, respectively, with a significant decrease on the 7th day compared to 0 day, the reason may be due to the insufficiency of the remaining adrenal glands to produce cortisol after the removal of the other adrenal gland, The reduced cortisol could be due to corticosteroid deficiency after unilateral adrenalectomy (33). On the other hand, cortisol returned to the normal level on days 14th and 30th, due to the compensating strategy remnant gland. The serum cortisol level increased and returned nearly to control on the 7th day after unilateral adrenalectomy (2,34). The unilateral adrenalectomy rarely causes permanent adrenal insufficiency because the remnant adrenal gland has a compensatory mechanism to compensate for the lost functions of the removed gland (35,36).

The results of the 2nd group also found estrogen concentration at 0, 7th, 14th and 30th was 150.4, 139.2, 126.2 and 56.8 Pg/ml, respectively, it shows a decrease at all times, with a significant difference at 7th and 14th P.O.D.s compared with the rest of the days, this decrease due to cessation of estrogen production by the ovaries, in addition to the

inability of the residual gland to compensate for the secretion of the hormone in sufficient quantity. The result agrees with Hanan *et al.* (6) and Sontas *et al.* (37). The progesterone concentration was 4.5, 4.0, 3.8 and 3.6 ng/ml, respectively, with a significant decrease at all times as a result stopped production of progesterone by the ovaries, in addition to the inability of the resting gland to compensate secreting of progesterone in sufficient quantity, this result agrees with Hanan *et al.* (6). The cortisol concentration was 92.9, 73, 80 and 82.6 nmol/L, respectively, with a significant decrease at 7th P.O.D.s as compassion with 14th and 30th and the rest of the days, with a significant decrease at 7th, 14th and 30th compared to 0 day, the reason may be due to the insufficiency of the remaining adrenal glands to produce cortisol after unilateral adrenalectomy, in contrast, cortisol returned to a level close to average on days 14th and 30th, as a result to the compensatory mechanism of the other adrenal gland (2). The results of the 3rd group showed that the estrogen concentration at 0, 7th, 14th and 30th post-surgery was 150, 149.6, 146.2 and 137.8 Pg/ml, respectively, with no significant difference. The progesterone concentration was 4.3, 4.4, 4.4 and 4.5 ng/ml, respectively, with no significant difference at all times due to the presence of the main source of hormone production (14). The cortisol concentration was 130, 132, 131 and 129 nmol/L, respectively, with no significant difference between days due to the presence of the main source of hormone production (adrenal gland) (2).

The results also conducted estrogen concentration in 30th days for the three groups was 141.2, 56.8 and 137.8 Pg/ml, respectively, with a significant difference at the second group in comparison with the rest groups and the progesterone concentration at 30th days for the three groups was 3.8, 3.6, and 4.5 ng/ml, respectively, with a significant difference among the groups, In another context the concentration of cortisol at 30th days for the three groups was 128.4, 82 and 129 nmol/L, respectively, with a significant difference at the second group in comparison with the rest groups, the reason for the decrease in this hormone at the second group is due to the lack of sources that produce these hormone (ovaries and adrenal gland), this result comes with Hanan *et al.* (6) and Sontas *et al.* (37).

Conclusion

We conclude that the possibility of removing the adrenal gland by laparoscope in sternal recumbence in dogs and consider it a standardized method through providing better visualization and dissection of the gland and unilateral adrenalectomy does not affect estrogen, progesterone, and cortisol levels due to the compensatory mechanism of the remnant gland.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper

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تأثير استئصال الغدة الكظرية باستخدام الجراحة المنظارية على الأستروجين والبروجيسترون والكورتيزول في أنثى الكلاب

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الخلاصة

تم دراسة تأثير استئصال الغدة الكظرية اليسرى بالمنظار على مستويات هرمون الأستروجين والبروجيسترون والكورتيزول لدى اناث الكلاب البالغة. تم استخدام خمسة عشر انثى ضالة بالغة. كان متوسط الوزن ٢٠ كجم، وكان العمر ٢٧ شهراً. تم تقسيم الحيوانات المدروسة عشوائياً بالتساوي إلى ثلاث مجموعات، كل مجموعة تحتوي على خمس اناث. خضعت المجموعة الأولى لاستئصال الغدة الكظرية اليسرى بالمنظار من جانب واحد، وخضعت المجموعة الثانية لاستئصال الغدة الكظرية اليسرى مع استئصال المبيضين، وتركت المجموعة الثالثة كمجموعة تحكم. تم أخذ عينات دم من جميع الحيوانات في الأيام ٠ و ٧ و ١٤ و ٣٠ بعد الجراحة لتقدير مستويات هرمون الأستروجين والبروجيسترون والكورتيزول. كانت نتيجة استخدام استئصال الغدة الكظرية اليسرى بالمنظار من خلال وضع الاستلقاء القصي تقنية عملية وقابلة للتطبيق وسريعة بمتوسط وقت جراحي قصير يبلغ ٧٢ دقيقة وتشريح سهل؛ لذلك، تعتبر تقنية معيارية. وأظهرت النتائج أيضاً أن تركيز هرمون الأستروجين في اليوم الثلاثين للمجموعات الثلاث كان ١٤١,٢ و ٥٦,٨ و ١٣٧,٨ على التوالي مع وجود فرق معنوي في المجموعة الثانية مقارنة بمجموعات الراحة وتركيز هرمون البروجيسترون في اليوم الثلاثين للمجموعات الثلاث كان ٣,٦ و ٤,٥ و ١٢٨,٤ على التوالي مع وجود فرق معنوي بين المجموعات، وفي سياق آخر كان تركيز الكورتيزول في اليوم الثلاثين للمجموعات ٨٢ و ١٢٩ و ١٢٨,٤ على التوالي مع وجود فرق معنوي في المجموعة الثانية مقارنة بمجموعات الراحة. وفي الختام، فإن إمكانية استئصال الغدة الكظرية بالمنظار مع الاستلقاء القصي في الكلاب هي طريقة موحدة، واستئصال الغدة الكظرية من جانب واحد لا يؤثر على مستويات هرمون الأستروجين والبروجيسترون والكورتيزول بسبب آلية التعويض.