

## Effect of Surgical and Immunological Castration on Haematological Variables, Reproductive Hormones and Ejaculate Characteristics in Mongrel Dogs

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**Summary:** Welfare concerns are growing regarding surgical castration (SC) in pets, necessitating the need for non-surgical alternatives. Administration of vaccines against gonadotropins releasing hormone (GnRH) have been reported as alternative to SC. This study determined the effect of surgical and immunological castrations (IC) on complete blood counts, plasma testosterone (T), luteinizing hormone (LH) concentrations and ejaculate characteristics in mongrel dogs. Ten intact male dogs were randomly divided into two groups (A & B). Dogs in group A were surgically castrated, while dogs in group B were immunologically castrated with single subcutaneous injection of GnRH vaccine (Improvac®). Blood and semen were collected before SC or IC and fortnightly until sixteen weeks. Blood was analyzed for packed cell volume (PCV), white blood cell count (WBC), haemoglobin concentration (Hb), absolute neutrophil (NEUT) and lymphocyte counts (LYMP), T and LH. Sperm volume (SV), concentration (C), motility (SM), live-dead ratio (LDR) and percentage of abnormal spermatozoa were determined for the semen. Data were presented as mean  $\pm$  standard deviation and compared using analysis of variance. The PCV and HB of dogs surgically castrated increased progressively up to 16th week after castration but only up to 10 weeks in dogs immunologically castrated. Both PCV and HB decreased progressively after 10 weeks in dogs immunologically castrated. Similarly, the WBC of dogs surgically castrated steadily increased from 2 weeks up to week 16, while it increased from 6 weeks up to 16 weeks in dogs immunologically castrated. However, PCV, Hb, WBC, NEUT and LYMP did not differ significantly ( $p > 0.05$ ) between SC and IC. In both groups, the SV, SC, SM, LDR and percentage of abnormal spermatozoa did not differ significantly. It was therefore concluded that there is no significant haematological or endocrinological changes between surgical and immunological castration and that immunological castration may provide safer alternative.

**Keywords:** Surgical castration, immunosterilization, dogs, GnRH vaccine, Testosterone, Luteinising hormone

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### INTRODUCTION

Dog overpopulation remains a serious problem in many developing countries including Nigeria despite local efforts to control population growth. In these countries, free roaming dogs are sources of ecological and social problems. They attack other animals and people, result in road accidents, frighten the public and contaminate the environment with urine and faeces (Ortega- Pacheco, 2006). Therefore, the development of effective population control measures has a high priority. Traditionally, surgical sterilization and mass euthanasia campaigns are used in developed countries but the impact has proved to be low in developing countries. Besides, the high cost of mass euthanasia campaigns is prohibitive and the often inhumane handling of the dogs is against international animal welfare regulations.

Surgical castration is the traditional method of gonadectomy in male dogs and cats (Reichler, 2008). However, welfare concerns are on the increase

regarding surgical method of castration in most domestic animals. Such concerns are that surgery is painful and places the animal at risk because it requires anaesthesia and that surgical removal of the testes is unnatural and objectionable. A study conducted in Brazil showed that the main reasons for the avoidance of surgical castration of adopted shelter dogs included compassion (56.5%), while 11.4% of the respondents believe the procedure is unnecessary (Soto et al., 2005). A study conducted in Nigeria showed that castration is seldomly performed because majority of dog owners believed it is cruelty against the dog (Ajadi et al., 2013). Another study carried out by Canadian Veterinary Private Practitioners found complication rates of 19% following castration in male dogs (Pollari and Bonnet, 1996). Serious complications such as infections, scrotal abscesses, rupture of the surgical wound, and chewed out sutures were reported at a 1-4% frequency, with surgical castration accounting for 10% of these complications (Lund et al., 2006). This

growing concern about surgical castration has necessitated the need for the development of alternative methods of castration which would be effective and acceptable to the animal owners in terms of welfare concerns.

The ideal non-surgical castration technique should produce permanent loss of fertility, permanent loss of sexual behaviour including displays of some forms of aggressive behaviour, requires single injection, safe, with no deleterious side effects for the target and non-target species (including humans) in case of accidental exposure or self-injection, has good efficacy (high success rate in treated animals), technically feasible, stable in formulation, allow for storage and handling under field conditions and should be affordable and cost effective (Kutzler & Wood, 2006). Advantages of non-surgical castration of dogs include less technical administration compared with surgery, lack of a requirement for anesthesia or surgery packs, and avoiding removal of the testes, which is widely viewed as unacceptable in some cultures.

Immunocastration is an immunological castration method which is currently undergoing evaluation for its efficacy and adverse effects. Immunocastration works as a vaccine, stimulating the immune system to produce antibodies against the gonadotropin-releasing hormone (GnRH) (Thompson, 2000). The antibody neutralizes endogenous GnRH resulting in suppression of secretion of luteinizing hormone (LH) and follicle stimulating hormones (FSH) (Walker et al., 2007). This will result in testosterone deprivation and subsequent impairment of spermatogenesis. Vaccine against GnRH also has the advantage of suppressing sexual behaviour in males and females (Kutzler & Wood, 2006). The main concern with immunocastration is the un-quantified side effects and achieving efficacy over long periods of time following single injection. The aim of this study is to compare the effect of surgical and immunological castration on complete blood counts, plasma testosterone and luteinizing hormone concentrations and ejaculate characteristics in mongrel dogs.

## **MATERIALS AND METHODS**

Ten adult male mongrel dogs mean weight of  $10.0 \pm 1.4$ kg, and age ranging between one and three years (mean age =  $2.4 \pm 0.3$  years) were used. They were sourced from households who used them either for security or hunting. The dogs were housed individually in concrete-floored kennels and allowed moderate exercise three hours daily. They were fed once daily on Indomie waste, supplemented with sufficient amount of proteins (fish) and palm oil, while water was provided ad libitum. They were dewormed with subcutaneous injection of 10% Levamisole hydrochloride at the dose of 10mg/kg, while external

parasites were treated by dipping in Diazintol (Animal Care, Nigeria) solution. In addition, the dogs were treated for any blood protozoan parasites with intravenous injection of 5% oxytetracycline (Oxytet®, Topsurf, Vancouver, Canada) at 10mg/kg for five days. The dogs were trained for eight weeks to get them accustomed to semen collection by masturbation method and their semen was analyzed to ensure that they were fertile prior to commencement of study. Also, the dogs were adjudged to be clinically healthy based on results of physical examinations, complete blood counts and faecal examinations before the commencement of the study. Ethical approval for this study was obtained from the Research Ethics Committee, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

### **Experimental Design**

This study was a controlled, randomized design involving two groups. Group A comprised of five intact male dogs surgically castrated, while group B comprised of five intact male dogs treated with a single subcutaneous injection of 400 µg of Improvac ((Pfizer laboratories (Pty), Sandton, South Africa).

### **Experimental procedure**

The dogs were weighed using puppy type weighing scale before the commencement of the study. Semen was obtained from the dogs before castration and thereafter fortnightly up to sixteen weeks of castration using digital manipulation method. In addition, blood was obtained from the cephalic vein fortnightly up to sixteen weeks for the determination of complete blood counts and plasma concentration of testosterone and luteinizing hormone.

### **Determination of haematological variables**

The Packed Cell Volume (PCV) and haemoglobin (Hb) concentration (g/dl) were determined using Mindray® BC-2800Vet automatic haematology analyser (Beckman Coulter, UK). The machine operated by sampling blood and suckling a standard amount through narrow tubing containing light detectors and electrical impedance, that determined the PCV and Hb concentration.

The total white blood cell count was determined using Mindray® BC-2800Vet automatic haematology analyser (Beckman Coulter, UK). The system made use of 10µL of blood. The red cells were lysed in the microcuvette by a haemolysing agent and the white blood cells stained by methylene blue. The camera in the analyser takes 37 images throughout the cuvette, and the cells were counted by image analysis and classified into each sub group. The total white blood cell, absolute neutrophil and lymphocyte count were then counted in less than five minutes and recorded in the range of  $\times 10^9/L$ .

#### **Determination of Plasma testosterone concentration**

The plasma concentration of testosterone was determined by radioimmunoassay technique using a fixed quantity of <sup>125</sup>I-labelled testosterone. All reagents were equilibrated to room temperature. Duplicate tubes for total counts (T), zero standard (Standard 1 = B0), standards (S2-6), control (C) and samples (Sx) were labelled. All reagents and samples were mixed thoroughly before use avoiding excessive foaming. 50 µl each of standards, control and samples were pipetted into the properly labelled tubes. 50 µl of tracer solution was pipetted into all tubes. 50 µl of the antiserum was pipetted into all tubes except T. All tubes except T were thoroughly vortex mix for 2-5 seconds. The tubes were covered with a plastic foil, and allowed to incubate at 37°C for three hours. T tubes were placed on a separate tube rack. The bottle containing magnetic immunosorbent was gently shaken until homogeneity. 500 µl was added to each tube except T. All tubes were thoroughly vortexed and incubated for five minutes at room temperature. The bound fraction was separated by centrifuging all tubes for 15 minutes at 1500 rpm or greater. The supernatant was gently aspirated taking care of not disturbing the precipitate. The radioactivity of each tube was counted for at least 60 seconds or longer in a gamma counter (Cobra II, Auto Gamma, Packard Instrument Company, USA). The concentration of hormone in the sample was calculated automatically by plotting a standard curve

#### **Determination of Plasma luteinizing hormone concentration**

The plasma concentration of luteinizing hormone was determined by radioimmunoassay technique using a fixed quantity of <sup>125</sup>I-labelled luteinizing hormone. All reagents were equilibrated to room temperature. Disposable tubes (12X75mm) were labelled in duplicate for total count (TC), nonspecific binding (NSB), zero standard (Bo), standards and samples. 200 µl of assay buffer was pipetted into NSB tubes and 100 µl assay buffer into Bo tubes. Starting with the most dilute, 100 µl of each standard (S1-7) was pipetted into the appropriately labelled tubes. 100 µl of unknown sample (Mx) was pipetted directly into appropriately labelled tubes. 100 µl antiserum was then added into all tubes except NSB and TC. The TC tubes was stoppered and set aside for counting. All tubes were thoroughly vortex mixed, covered and incubated for four hours at 37°C. 100 µl of tracer was then pipetted into all tubes. All tubes were again vortex mixed thoroughly, covered and incubated overnight at 37°C. 400 µl of the second antibody was added into each tube except the TC. All tubes were vortexed thoroughly and incubated at 37°C. The antibody bound fraction was then separated by decanting off the

solution and the tubes were left for 15 minutes. All tubes were then centrifuged at 4°C for 10 minutes at 1500 rpm. After which the tubes were carefully placed into suitable decantation racks, then poured off and supernatant discarded. The tubes were then inverted and place on a pad of absorbent tissues and allow to drain for 5 minutes. The radioactivity of each tube was counted for at least 60 seconds or longer in a gamma counter (Cobra II, Auto Gamma, Packard Instrument Company, USA). The concentration of hormone in the sample was calculated automatically by plotting a standard curve

#### **Determination of ejaculate parameters**

Both the volume and the colour of the first and second fractions of the ejaculate were determined. Thereafter the sperm motility, concentration, morphology and abnormalities were then determined. Sperm motility was assessed immediately after collection. A drop of semen was placed on a slide and examined at 100X magnification using light microscope. Both the total motility (% of spermatozoa that are moving) and progressive motility (% of spermatozoa that are moving in a straight line) were determined. The concentration of spermatozoa was measured on the collected ejaculate containing F1 and F2 fraction. The WBC Unopette system (Becton Dickinson, Rutherford NJ) was used. Semen was drawn up into the 20 microliter pipette and dispensed it into the 2 ml diluent container according to kit instructions. The solution was then discharge into both chambers of the Haemocytometer. The number of spermatozoa in the central 1 millimeter square (the square that fills the field using the 10X objective) was then counted. Sperm morphology was assessed by staining the semen sample with Giemsa stain (DiffQuik, Baxter Healthcare, Miami FL) and observing the cells under 1000X magnification (oil immersion). The Spermatozoa appeared purple on a clear background. Under oil immersion, 100 spermatozoa were examined and counted. The number of normal spermatozoa in 100 is the percentage morphologically normal spermatozoa. Total normal for the sample was calculated by multiplying total number of spermatozoa in the ejaculate by percentage morphologically normal. Also, the stained slide was examined for sperm abnormality. Abnormal spermatozoa were classified as primary defects (those that occur during spermatogenesis, including defects in head shape, bent midpiece, persistent proximal cytoplasmic droplet, and doubling of any portion of the spermatozoon) or secondary defects (those that occur during epididymal maturation or slide preparation, including detached heads, persistent distal cytoplasmic droplets, and bent tails).

**Statistical Analysis**

Data were presented as mean ± standard deviation. The packed cell volume, haemoglobin concentration, white blood cell counts, absolute neutrophil and lymphocyte counts, plasma concentration of testosterone and luteinizing hormone, sperm volume, percentage sperm motility and sperm concentration were compared between the different groups using analysis of variance (ANOVA) for repeated measures. Least square difference was used for post-hoc analysis. All statistical analyses were performed using SPSS 17.0 software.

**RESULTS**

The PCV and Hb of dogs surgically castrated increased progressively up to the 16th week after castration, while the PCV and Hb of dogs immunologically castrated first increased up to 10 weeks of castration and thereafter decreased progressively up to 16 weeks after castration (Fig. 1a & b). There was no significant ( $p > 0.05$ ) difference in the PCV and Hb of surgically or immunologically castrated dogs. Following surgical castration, the

WBC and NEUT of the dogs decreased at week 2 and thereafter steadily increased up to week 16 after castration. Similarly, WBC of dogs that were immunologically castrated also decreased up to week 6 after castration and thereafter steadily increased up to week 16 (Fig. 2 & 3). There was no significant difference ( $p > 0.05$ ) in the WBC & NEUT between surgically and immunologically castrated dog. However, the LYMP was significantly ( $p < 0.05$ ) lower in surgically castrated dogs than immunologically castrated dogs (Fig. 4).

Following surgical castration, the plasma testosterone of the dogs dropped significantly ( $p < 0.05$ ) from week 2 after castration and remained steady at that value up to week 16 after castration (Fig. 5). The plasma testosterone of dogs that were immunologically castrated also dropped from week 2 after castration, reached basal level at week 6 to week 16 after castration (Fig. 5). The plasma LH of both the surgically castrated and immunologically castrated dogs decreased progressively from the base line value up to week 8 post treatment at which point

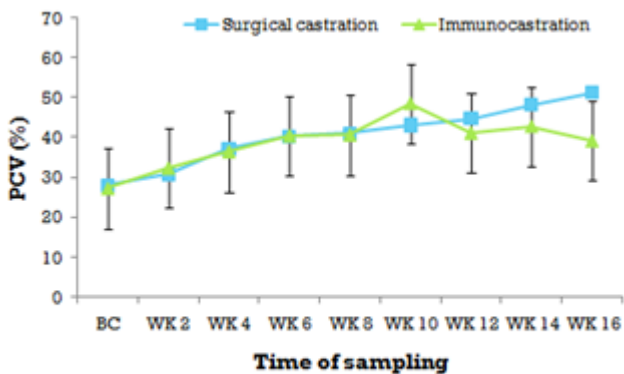


Fig 1a: Changes in packed cell volume following surgical or immunological castration in mongrel dogs

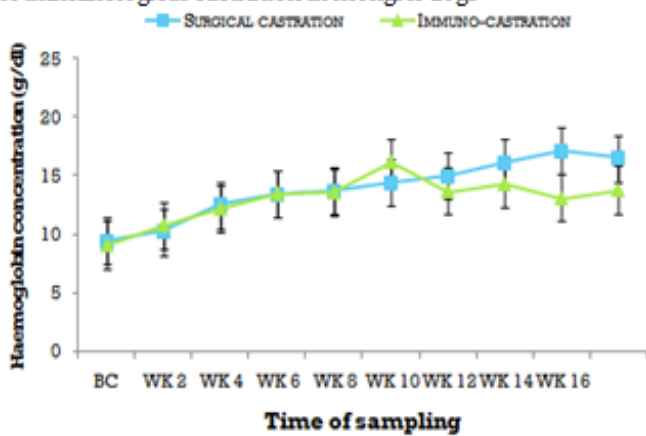


Fig. 1b: Changes in haemoglobin concentration following surgical or immunological castration in mongrel dogs

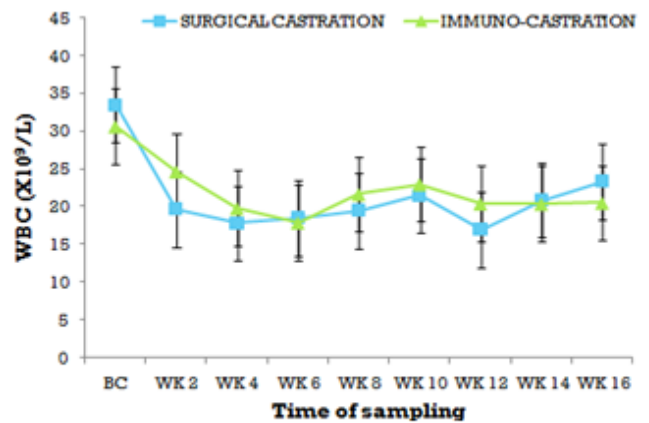


Fig. 2: Changes in total white blood cell count following surgical or immunological castration in mongrel dogs

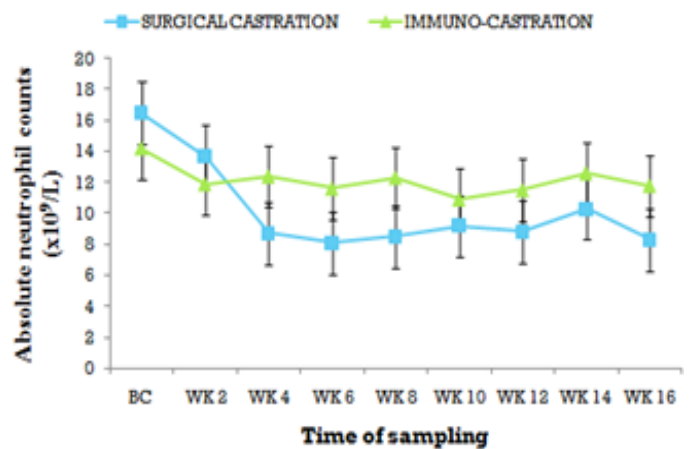


Fig. 3: Changes in absolute neutrophil counts following surgical or immunological castration in mongrel dogs

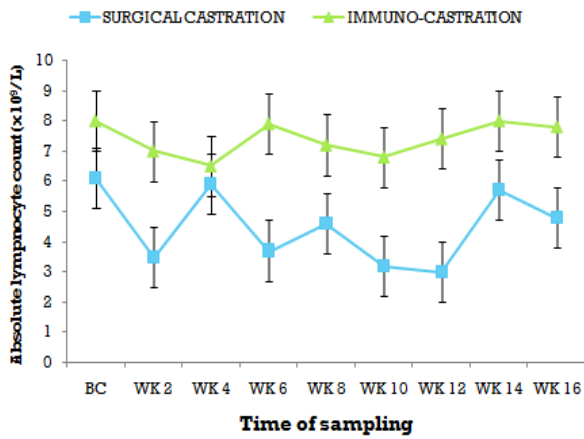


Fig. 4: Changes in plasma absolute lymphocyte counts following surgical or immunological castration in mongrel dogs

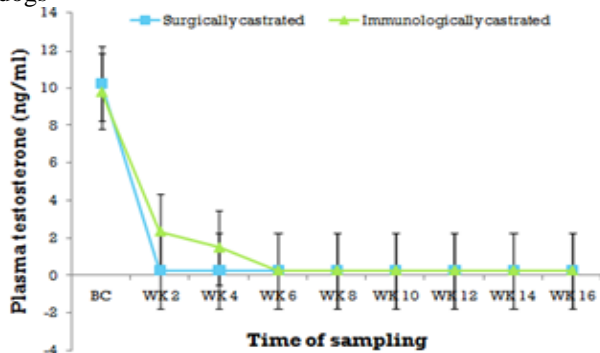


Fig. 5: Changes in plasma testosterone following surgical or immunological castration in mongrel dogs

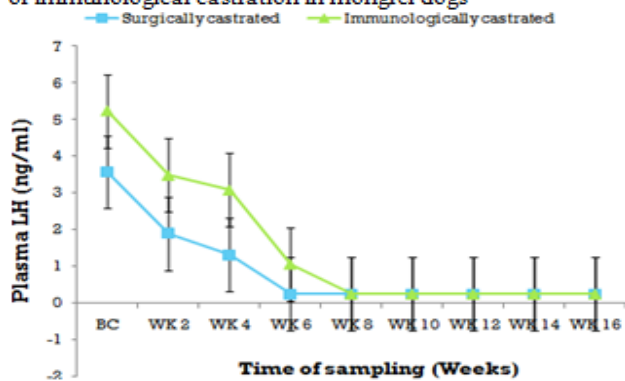


Fig. 6: Changes in plasma luteinizing hormone following surgical or immunological castration in mongrel dogs

Table 1. Mean Semen volume, sperm motility, sperm concentration, percentage live sperm, percentage abnormal sperm of mongrel dogs following surgical or immunological castration

Time of sampling (weeks)	Semen volume (ml)		Sperm motility (%)		Sperm concentration (x10 <sup>6</sup> /ml)		Percentage live sperm (%)		Percentage abnormal sperm (%)	
	SC (n=5)	IC (n=5)	SC (n=5)	IC (n=5)	SC (n=5)	IC (n=5)	SC (n=5)	IC (n=5)	SC (n=5)	IC (n=5)
BC	0.68 ± 0.15 <sup>a</sup>	0.63 ± 0.15 <sup>a</sup>	73.75 ± 5.5 <sup>a</sup>	80.00 ± 5.0 <sup>a</sup>	410.00 ± 37.0 <sup>a</sup>	484.25 ± 45.0 <sup>a</sup>	83.50 ± 7.5 <sup>a</sup>	88.20 ± 7.0 <sup>a</sup>	5.75 ± 1.5 <sup>a</sup>	4.50 ± 1.5 <sup>a</sup>
2	0.25 ± 0.23 <sup>b</sup>	0.28 ± 0.14 <sup>b</sup>	26.25 ± 6.5 <sup>b</sup>	41.50 ± 5.7 <sup>b</sup>	234.75 ± 53.0 <sup>b</sup>	248.00 ± 43.0 <sup>b</sup>	36.50 ± 6.5 <sup>b</sup>	41.50 ± 5.7 <sup>b</sup>	2.75 ± 1.3 <sup>b</sup>	2.50 ± 1.2 <sup>b</sup>
4	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>
6	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>
8	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>
10	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>
12	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>
14	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>
16	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>

BC: Before castration, SC: Surgical- castration group, IC: Immuno-castration group. Values with different superscript are significantly different at p < 0.05 along the same column.

Castration methods comparison in dogs

it reached the minimum value detectable, and thereafter the plasma LH became constant up to week 16 (Fig. 6). There was no significant difference (p > 0.05) in the plasma LH of surgically and immunologically castrated dogs (Fig. 6).

The colour of collected semen from dogs in both groups was cloudy white and devoid of blood. As shown in table 1, the mean semen volume, sperm motility, sperm concentration and percentage live sperms were significantly (p < 0.05) decreased 2 weeks post-surgical castration or administration of GnRH vaccine in immunological castration and the dogs were aspermic thereafter. The percentage abnormal sperm of the dogs ranged between 4.00 ± 1.4% and 5.75 ± 1.5%. and were significantly (p < 0.05) decreased in similar manner with the sperm characteristics (Table 1).

### DISCUSSION

The result of this study showed that both surgical and immunological castration in dogs resulted in increased packed cell volume and leukocyte parameters with a decrease in the plasma concentration of testosterone and luteinizing hormone, as well as the quality of the ejaculate. This effect occurred as early as two weeks following surgical castration or administration of GnRH vaccine with the dogs becoming aspermic thereafter. Also, the effect lasted for up to sixteen weeks after castration by either method. However, there was no significant difference in all these parameters between surgically and immunologically castrated dogs.

The hypothalamic-pituitary gonadal axis has been target for the development of non-surgical castration methods. Under normal condition, GnRH stimulates the release of luteinizing hormone (LH) by binding to its receptors on the anterior pituitary (Donovan et al., 2013). LH secretion is necessary for normal testosterone synthesis in the males. Following immunization with GnRH, antibodies are produced



which block the binding of GnRH to its receptors on the anterior pituitary thus inhibiting the secretion of LH and consequent synthesis of testosterone (Janett et al., 2009). This might have been responsible for the decreases in the plasma concentrations of the hormones beyond the detection limit. Decrease in testosterone secretion has been shown to result in testicular atrophy and disruption of normal spermatogenesis (Ghoneim et al., 2012). This may probably account for the disruption in normal spermatogenesis evidently leading to the progressive drop in the ejaculate parameters of the dogs until the dogs became aspermic.

It has been shown that testosterone is required for the maintenance of the seminiferous tubule epithelium in most mammals (Donovan et al., 2013). Withdrawal of testosterone secretion in the treated dogs might have caused severe reduction of spermatids in the treated dogs.

Sperm motility is an important characteristic in semen assessment. Spermatozoa gain motility during ejaculation as pH and bicarbonate concentration increases during mixing of sperm and seminal plasma (Vyt et al., 2004). The motility of the spermatozoa in the present study decreased progressively until the dogs became azoospermic in both groups. This is contrary to the findings in pigs (Bilskis et al., 2012) and may be related to the inability of the dogs to produce seminal plasma fluid.

There was no significant difference in the percentage of abnormal sperm cells in both groups. This result was expected because vaccination indirectly locks the release of FSH which is required for normal spermatogenesis, while surgically removal of the testis might have send a negative feedback to the hypothalamus to inhibit the secretion of LH and FSH. In conclusion, administration of single injection of GnRH vaccine to dogs resulted in decrease in the size of the testis and destruction of the germinal epithelium of the seminiferous tubules with resultant decrease in the ejaculate parameters until the dogs became azoospermic.

The advantage of the immuno-castration over surgical castration will be its virtual lack of any adverse effects and the ability to suppress semen production as early as two weeks following administration. In addition, relative cheap cost provides an additional advantage over surgical castration. The vaccine can be incorporated into the routine immunization plans for dogs and thus be very useful in developing countries where there is lack of surgical facilities. In conclusion, immuno-castration with GnRH vaccine appears to have advantage over surgical castration because of its lack of adverse effect and the lower cost when compared with surgical castration. However, the reported inability of the vaccine to produce a long lasting sterility may be a limitation especially where permanent sterility is required.

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