EVALUATING AUTOLOGOUS COMPOSITE COLOPERITONEAL GRAFTS IN THE REPAIR OF URINARY BLADDER DEFECTS IN DOGS

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FACULTY OF VETERINARY MEDICINE

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August, 2018

DECLARATION

I declare that the work in this thesis entitled "**Evaluating Autologous Composite Colo-peritoneal Grafts in the Repair of Urinary Bladder Defects in Dogs**"has been carried out by me in the Department of Veterinary Surgery and Radiology, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

Sa'idu Tanko MUHAMMAD _____ _____

Name of student **Signature** **Date**

CERTIFICATION

This thesis, entitled: "**Evaluating Autologous Composite Colo-peritoneal Grafts in the Repair of Urinary Bladder Defects in Dogs**" by Sa'idu Tanko MUHAMMAD, meets the regulations governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University, Zaria, and is approved for its' contribution to knowledge and literary presentation.

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ABSTRACT

The efficiency of autologous composite colo-peritoneal pedicle grafts in the surgical repair of experimental urinary bladder (UB) defects was evaluated in four Nigerian indigenous dogs (NIDs), age range of 0.75-1 year. Evaluation was done based on demonstration of the suitability of the sero-muscular pedicle flap and peritoneal free flap as composite grafts to repair UB defects.Anatomical evaluations through ultrasonography and radiography of the UB architecture, and histological assessment of the repaired UB tissue were performed.Physiological evaluations werecarried outbased on pre-and post-surgery vital parameters, urinalysis, haematological, serum biochemical, and plasma cytokine assessments. Surgical procedures were performed under general anaesthesia. The dorsal aspect of the UB served as the recipient site, while sero-muscular colonic pedicle flap of the descending colon along with free peritoneal sheath formed a composite tissue (donor). Dextrose, dextrose-saline solution, pentazocine injection and metronidazole infusion were used forpost-surgical management of the dogsfor 5 days. Bland diet was gradually introduced for one week, before resumption ofnormal feeding. There wereslight deviations in vital parameters between pre-surgical and respective post-surgical (first week) values. The base-line rectal temperature(38.6 ± 0.16 ^{o}C) wasnot significantly higher than values recorded after the surgery on days 1 (39.23 ± 0.28 ^{o}C), 2 (39.23 ± 0.34 ^{o}C), 4 (38.78 ± 0.17 ^{o}C), and 6(38.83 ± 0.12 ^{o}C) post-surgery. Thepre-surgical pulse rateof 105.04 ± 3.26 beats per minute was obtained, andit fluctuated5 days post-surgery within the range values of 106.00 ± 7.12 - 130.00 ± 15.69 beats per minute,but wasnot significant ($P > 0.05$).The mean pre-surgical respiratory rate(21.36 ± 1.78 cycles perminute)increased relatively post-surgery on days 1, 2 and 3 (34.75 ± 3.86 cycles per minute), (36.00 ± 5.42 cycles per minute) and (26.50 ± 4.72 cycles per minute), respectively. The difference in the values was notsignificant ($P > 0.05$). Wound adhesion(apposition) of the laparotomy incisions wasobserved between 5 and 15 days

post-surgery. Post-surgical complications observed were straining, incisional hernia and constipation.Post-surgical cystosonographic assessments on day 8 showed thickened bladder wall at the point of the graft, which decreased markedly on day 21.Cystographic evaluations revealed gradual transition of the UB architecture at the point of graft with clear delineation and minimal blurring at week 2 post-operation,which gradually disappeared at weeks 10 and 14. However, at 108^{th} week post-surgery there was evidence of diverticulum (radiographically and grossly) at the point of patch in one of the subjects, and omental adhesion to the reconstructed site. There was evidence of "graft take" histologically at the site of anastamosis.Post-surgicalurinalysis showed decrease in specific gravity on days 7 (1.006 ± 0.002), 10 (1.005± 0.005)and 13 (1.010 ± 0.004), which fluctuated between 1.008 ± 0.002 and 1.020 ± 0.004 up to the termination of sampling at day 42. Base-line urine pH value(6.81 ± 0.50) fluctuated between 5.75 ± 0.48 and 7.50 ± 0.29 post-surgically. Post-surgical proteinuria was observed for the first 7 days, but disappeared on day 12. Haematuria was observed within the first 16 days post-surgery. The values of the haematological, serumboichemical, plasma cytokines (IL-6 and IL-10) fluctuated immediately post-surgery, but remained within the limits of base-line data (findings) by the end of observation period. In conclusion, autologous composite colo-peritoneal tissue graft wassuccessfuly formed and effectively used in the repair of` extensive urinary bladder defects in dogs.

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LIST OF ABBREVIATIONS

%	Percentage
µg/kg	Microgram per kilogram
A.A	Ascorbic acid
ABU	Ahmadu Bello University
ABUCAUC	Ahmadu Bello University Committee on Animal Use
ABUTH	Ahmadu Bello University Teaching Hospital
ALP	Alkaline phosphatase
ALT	Alanine amino transaminase
ANOVA	Analysis of variance
AST	Aspartate amino transaminase
BASO	Basophil
Bil.	Bilirubin
Bld	Blood
Ca^{2+}	Calcium ion
CIC	Clean intermittent catheterisation
Cl^{-}	Chloride ion
Cm	Centimetre
CTT	Composite tissue transplantation
EC	Enterocystoplasty
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme-linked immunosorbent assay
F.Cath	Foley catheter
g	Gravity
GUTB	Genitourinary tuberculosis
H & E	Haematoxylin and eosin
Hb	Haemoglobin
HCO_3^{-}	Bicarbonate ion
IL	Interleukin
IL-10	Interleukin 10
IL-6	Interleukin 6
IVP	Intravenous Pyelography
K.B	Ketone bodies
K^{+}	Potassium ion
Kg	Kilogram
Leu.	Leucocyte
LYMP	Lymphocyte
Mg	Milligram
mg/kg	Milligram per kilogram
Ml	Millilitre
mmol/L	Millimole per litre
Na^{+}	Sodium ion

NEG	Negative
NEUT	Neutrophil
NIDs	Nigerian Indigenous Dogs
Nit.	Nitrate
NORM	Normal
^{o}**C**	Degree celcius
PCV	Packed cell volume
pg/ml	Picogram per millilitre
PO_3^-	Phosphate ion

CHAPTER ONE

1.0 INTRODUCTION

1.1 Preamble

Deformities of organs/tissues may lead to extensive damage or loss of the organ/tissue functions, necessitating a reconstructive surgery or an eventual replacement of the organ (Atala *et al*., 2006). The defects could be due to either congenital malformation or acquired injury as a result of trauma and surgical resection in which the patient's own tissues can be best used for the repair or reconstruction, known as autologous transplantation (Atala *et al*., 2006). Extensive urinary bladder defect is one of the most severe forms of soft tissue loss, which leads to devastating injury with tremendous impact on the patient's survivability and quality of life (Petruzzo and Dubernard, 2011). Congenital defects are believed to occur due to deviation from normal embryologic development of the pronephros, mesonephros, or metanephros during urogenital development as a result of intrinsic or extrinsic factors (Dean *et al*., 1988). Modernism, social conflicts, human and natural disasters, and pelvic surgical complications were reported to be the cause of acquired bladder defects (Morehouse, 1988; Dobrowolski *et al.,* 2002). Acquired urinary bladder injuries include bladder neoplasm, urolithiasis, tuberculosis and neurogenic bladder dysfunction as well as traumatic rupture among others. The congenital bladder defects on the other hand include exstrophy, diverticula and hypoplasia (Caldamone, 1987; Neuman *et al*., 1991; Cilento and Nguyen, 2001; Nabi *et al*., 2003).

To understand the complexity and severity of urinary bladder injuries, it is important to review in details the anatomy, the nature and causative agents of urinary bladder injuries, previous medical and surgical interventions and materials used in the attempts at resolving

urinary bladder injuries. The urinary bladder and urethra plus the caudal segment of each ureter constitute the lower urinary tract, with functions determined ultimately by innervation (Fletcher *et al*., 2010). The urinary bladder (UB) is the storage house for urine, filtered through the kidneys and transported to it via the ureters (Dyce *et al*., 2003).

The urinary bladder (*vesica urinaria* or urocyst) in animals according to Dyce *et al*. (2003)is a hollow musculo-membranous organ that forms part of the lower tract of the urinary system. It is connected to the renal pelvis via the ureters that transport urine produced by the kidneys. The ureters are the caudal continuation of the remaining parts of the renal pelvis and each ureter has a fairly even caliber. On reaching the pelvic cavity the ureter bends medially to enter the genital fold in the male and the broad ligament in the female; this carries the ureter over the dorsal surface of the bladder, into which it opens near the neck of the bladder (Evans and De lahunta, 2010). In males, the ureter passes dorsal to the corresponding *ductus deferens* (Fletcher *et al*., 2010). The ureter penetrates the bladder obliquely (Dyce *et al*., 2003).The urinary bladder functions in storing urine until it is disposed of through the urethra (Evans and Christensen, 1993; Dyce *et al*., 2003). The arterial supply of the bladder are derived from the branches of umbilical (cranial vesical) and urogenital (caudal vesical) arteries (Fossum *et al*., 2002), while the nerve supply are composed of hypogastric and pelvic nerves which originates from the sympathetic and the parasympathetic trunks (Fossum *et al*., 2002).

According to Stief *et al*. (2003), the urinary bladder could be described histologically as being made up of approximately four layers, namely; the inner mucosa, *lamina propria*, *muscularis*, and the *adventitia*. The *muscularis* (*tunica muscularis)* is further made of three layers of unstriped muscular fibres; an external layer *detrusor urinae* muscle whose fibres are longitudinally arranged; and a middle layer whose fibres are arranged in a circular manner and made up of very thin and irregular fibres, which are scattered all over the middle layer.

The internal layer has generally a longitudinal arrangement (Wilfred *et al*., 1975). The bladder is anatomo-physiologically endowed with transitional epithelium (urothelium) which is highly elastic (connective tissue at the submucosal level) and thus capable of holding large volume of urine produced by the kidneys (Dyce *et al*., 2003). The urothelium comprises multiple layers of cells (the exact number depends upon the species) that functions as a primary barrier (Khanderlwal and Apodaca, 2009), thus a first responder to different physiological interventions or stress including changes to transmural bladder pressure; generation of lateral tension in the urothelium or bladder wall; torsion, movement of visceral organs and urine composition or tonicity (Birdar *et al*., 2012).

The urinary bladder has an apex, body and a neck (Lipscomb, 2012). The bladder lies in the pelvic abdominal cavity and dorsal to the ventral abdominal wall in animals that makes the organ seldom injured (Dyce *et al*., 2003), but, once injured, the extent of the injury can be devastating. The organ associated with the large intestines, the uterus in females and other argans are held in place by triangular cystic ligaments (Fletcher and Clarkson, 2011). Once the bladder is full or distended, it lies along the ventral abdomen and micturation reflexes are triggered to initiate voiding. When the bladder is empty, it is positioned at the pelvic inlet and is always presented as a thick-walled collapsed sac, with prominent vascular network (Dyce *et al*., 2003; Fossum *et al*., 2002).

Any aberration of the anatomic and physiologic endowment of the urinary bladder is diagnosed as a urinary bladder defect (Anon[2], 2012). Urinary bladder defects or injuries to the urinary bladder constitute some of the most devastating urologic diseases involving the lower urinary tract (Lynch *et al*., 2003). A urinary bladder defect ranges from congenital to acquired which can lead to extensive damage or loss of the organ, necessitating a repair or an eventual replacement of the organ (Atala *et al*., 2006).

Some commonly encountered congenital urinary bladder anomalies are bladder exstrophy, diverticula, bladder ears, cystic hypoplasia, megacystis, agenesis, bladder duplication and septa, which have been reported in young male and female animals as well as humans (Caldamone, 1987; Cilento and Nguyen, 2001; Savanelli, *et al*., 2003).

Traumatic injuries of the urinary tract in small animals often occur concurrently with abdominal and pelvic trauma, and are the most common cause of uroperitoneum (Rieser, 2005). Acquired urinary bladder defects may be iatrogenic or accidental in origin resulting from abdominal surgeries or complications, due to female pelvic surgery (Gueye *et al.,* 1992), some vaginal operations (Neuman *et al.*, 1991; Natta *et al*., 1993), caesarean section and hysterectomy (Meirow *et al*., 1994; Bencheckroun *et al*., 1997). Some urologic, vascular and orthopaedic operations near the bladder have also resulted in extensive urinary bladder damage (Lynch *et al.,* 2003). Other forms of acquired urinary bladder injury could be due to trauma and these traumatic injuries could either be blunt (common multisystem trauma) or penetrating traumas (Sagalowsky and Peters, 1998; Srinivasa *et al*., 2009), which can result in extraperitoneal and or intraperitoneal ruptures (Gambini *et al.*, 2001). Extraperitoneal bladder perforations have accounted for 50%-71% of urinary bladder injuries (Corriere, 1996), while intraperitoneal and combined perforations accounted for 25%-43% and 7%-14 % respectively of urinary bladder injuries (Brown *et al*., 1998).

The body naturally heals itself conservatively (without any adjunctive therapy) provided the patient is stable, underlying cause of the trauma has been addressed, no evidence of devitalisation and bladder tonicity has not been compromised, which all rely on the extent and severity of the bladder injury (Osman, 2005; Sura, 2011). Non-surgical intervention of traumatically ruptured urinary bladder employed the use of indwelling urinary bladder catheter (Osman, 2005).

Repair of such injuries have previously utilized different tissues such as fascia, skin, peritoneum and omentum among others with minimal success rates (Gleeson and Griffith, 1992). The basis of all modern reconstructive (and plastic) surgeries is tissue transplantation (Lee and Butler, 1997; Petit *etal*., 2003). Composite tissue transplantation offers the potential to provide more aesthetic, functional, and durable tissue replacements to defective or severely damaged tissue/organ (Barth and Bartlett, 2007). The composite tissue pedicle flap is aimed at addressing complex tissue grafts, such as repair of extensive urinary bladder defects, for the treatment of devastating injuries (Petit *etal*., 2003; Atala *et al*., 2006).

The first application of a free tissue graft for urinary bladder replacement was reported in 1917 as cited by Greenwell *et al* (2001), where fascia was used to augment the urinary bladder in dogs. Following that first report, surgical correction of urinary tract abnormalities with different forms of free graft have been used experimentally and clinically, including peritoneum (Shaul *etal*., 1996; Nanni *etal*., 2001), omentum (Aslan *etal*., 2004), pericardium (Moon *etal*., 2011), sero-muscular grafts (Cheng *et al*., 1994), small intestinal submucosa (Vaught *et al*., 1996), dura mata (Mavrich *etal*., 1998), placenta, and skin, (Gleeson and Griffith, 1992; Probst *et al*., 1997). The current techniques for bladder augmentation with or without urinary continence procedures include cystoplasty using stomach (Adams *et al*., 1988; Mingin *et al*., 1999), large bowel (Arikan *et al*., 2000), small bowel (Bramble, 1982; Mundy and Stephensons, 1985; Reidmiller *et al*., 1989; Krishner *et al*., 1995), ileo-caecal (Mayo and Chapman 1988), ureter (Dewan and Anderson 2001) bladder submucosa and engineered tissue (Atala *et al*., 2006, 2011).

Surgery is often implored to manage critically devitalised portions of the urinary bladder with intensions of 75% prognosis (Caroll and McAninch 1984; Husmann, 1996). Some major indications for performing a urinary diversion or a bladder reconstruction/replacement using

transposed intestinal segments are congenital anomalies, extensive bladder rupture, bladder cancer, neurogenic bladder dysfunction, idiopathic detrusor overactivity and chronic inflammatory conditions (Nabi *et al.,* 2003). Some surgical techniques and approaches that have been used to solve/reconstruct bladder defects include enterocystoplasty (Bramble, 1990; Gough, 2001), gastrocystoplasty (Greenwell *et al.*, 2001) and ureterocystoplasty (Bellinger, 1993; Buson*etal*., 1994).

Severe traumas such as pelvic fractures (Cass, 1984) have accounted for high morbidity (90%) and mortality rates of 12-22% (Caroll and McAninch, 1984; Cass and Luxenberg, 1987). The probability of bladder injury varies according to the degree of bladder distention at the time of trauma; hence, a full bladder is more likely to become injured than an empty one (Lynch *et al*., 2003). The incidence of traumatic injuries in humans recorded is worldwide due to current level of aggression, wars, natural disasters, some sporting events and often, high-energy accidents (Hochberg and Stone, 1993; Summerton *et al*., 2015).

Other acquired urinary bladder problems include infections such as tuberculosis (Narmada *et al*., 2008), cystolithiasis, neoplasia, detrusor over activity, incontinence and chronic inflammatory disorders (Nabi *et al.,* 2003). Gambini *et al*. (2001) reported that, it is possible in 85% of the urinary bladder injuries to have anuria, macro- and micro-haematuria. The diagnosis of urinary bladder diseases has been a perpetual challenge due to the last stage presentation of the patient, resulting to high morbidity rate of 100% and mortality rate of 69% (Steiner *et al*., 1995; Bomalaski *et al*., 1999).

Early clinical presentation with thorough and appropriate examination has resulted in successful diagnosis and treatment (Lee *et al*., 1991; Husmann, 1996).With the increasing availability of advanced diagnostic imaging modalities, the urinary system is receiving wider attention, and prompt diagnosis is possible (Sura, 2011). Some reliable diagnostic procedures

available for detection of urinary bladder defects include cystoscopy, cystography, ultrasonography, Computed Tomography and exploratory laparotomy (Husmann, 1996). Once urinary bladder injuries are diagnosed, treatment modality and decision becomes easy.

So far, ingenious attempts have been made towards assisting patients suffering from diseased, defective or injured urinary bladder in both humans and animals but persistent difficulties presented by the various forms of bladder defects also leave significant challenges in the minds of medics and urologic surgeons despite the fact that treatment, medical or surgical, varies with the severity of the disease (Muhammad *et al.,* 2016a).

The repair process following surgery is initiated immediately by the release of various growth factors, cytokines, and low-molecular weight compounds from the serum of injured blood vessels and from degranulating platelets (Sabine and Richard, 2003). Cytokines and growth factors are polypeptides/glycoproteins produced by diverse cell types at the site of injury, whose relatively rapid appearance after injury reflect active gene transcription and translation by the injured or stimulated cell (Clark, 1996; Spink and Cohen, 1997; Lin *et al*., 2000). The cytokines are important in cellular signalling present in serum, plasma and wound fluids, generally either to augment (pro-inflammatory) or attenuate (anti-inflammatory) the inflammatory response following surgery in humans and dogs (Hallaq *et al*., 2015).

1.2 Statement of Research Problem

The urinary bladder defects are most often associated with embryologic development (for example, bladder hypoplasia), congenital abnormalities such as exstrophy, diverticula and bladder ears (Gearhart and Robert, 1998; Cilento and Nguyen, 2001; Shokeir, 2002) and pelvic trauma caused by various accidents connected with modern life, including road traffic accidents, industrial and criminal activities (Dobrowolski *et al.*, 2002). Others are either iatrogenic or due to other forms of disorders (Shokeir, 2002; Muhammad *et al.,* 2016a).

Medical treatment to these defects, anomalies or disorders is mostly limited thereby necessitating surgical intervention as augmentation, repair or eventual substitution of the organ (Atala *et al*., 2006; Andersen *etal*., 2012; El-Taji *etal*., 2015).

Several attempts have been made by many investigators using a variety of autologous, homologous and heterologous tissue grafts in the surgical treatment of defective urinary bladder with the outcome strongly suggesting the utilisation of autologous intestinal colonic segment (Shokeir, 2002; Cody *etal*., 2012; Muhammad, 2013). Autologous enterocystoplasty has been a major advancement and a common method in the surgical management of hereditary/congenital and some acquired abnormalities of the urinary bladder (Cody *etal*., 2012; Subramaniam *et al*., 2012; El-Taji *etal*., 2015). However, the benefits of the autologous enterocystoplasty (colocystoplasty) are challenged by complications, including mucus production, stone formation, chronic low-grade infection, metabolic disturbance and the potential for malignancy due to the long-term exposure of the bowel mucosa to urine (Greenwell *et al*., 2001; Gurocak *et al*., 2007; Husmann, 2009; Muhammad, 2013; El-Taji *etal*., 2015).

Alternative to the full thickness colocystoplasty is colonic sero-muscular flap cystoplasty (Lima *et al*., 2004; Subramaniam *et al*., 2012), in which colonic epithelial regrowth, fibrosis and contraction of the sero-muscular flap were the main set-backs reported (Dewan *et al*., 1997; Jednak *et al*., 2000; Fraser *et al*., 2004; Vilar *et al*., 2004; Gurocak *et al*., 2007; Subramaniam *et al*., 2012). Tissue engineering strategies on urinary bladder are currently under study which appeared to be highly challenging as it posses various draw backs based on the origin of the graft (Orabi *etal*., 2013) which include: increase patient morbidity associated with graft harvest (autologous materials), increased cost in allograft and xenograft materials production, risk of disease transmission and difficulty in ascertaining pathologic

changes from the materials (allograft and xenograft) (Subramaniam *et al*., 2011; El-Taji *etal*., 2015). Other obstacles associated with the engineered tissues for bladder reconstruction are time taken for tissue culture and propagation (requires *invitro* studies), cell and tissue incompatibility, requirement for immunosuppressive therapy, survivability following transplantation and potential for tumourigenicity (Aboushwareb and Atala, 2008; Harrington *et at*., 2008;Baker *etal*., 2009; El-Taji *etal*., 2015).

1.3 Justification for the Study

Various options for urinary cystoplasty technique have been widely used clinically by reconstructive urologic surgeons and researchers in the surgical management of defective urinary bladder (Aslan *etal*., 2004), involving alternative tissue interposition (Gurocak *et al*., 2007). Reconstructive urologic surgery emphasises successful restoration of form and function of any defective urologic organ/tissue utilising the autogenous tissue, which appears to be grossly inadequate in cases of extensive congenital or acquired defects (Atala *et al*., 2006; Jessop *etal*., 2015;Muhammad *et al.*, 2016a).

Currently, the common and major advancement in the surgical management of congenital and some acquired abnormalities of the urinary bladder involves the use of autogenous intestinal segment, as alternative tissue due to the absence of other superior option, for reconstruction in order to restore bladder storage and voiding capability (Fraser *et al*., 2004; Cody *etal*., 2012; El-Taji *etal*., 2015). Colonic segment is one of the most frequently utilised tissues in urinary bladder reconstruction due to its proximity to the bladder, thick muscular wall, large lumen, ability to withstand distension and abundant mesentery which guarantee adequate bladder capacity and manoeuvrability, features peculiar and appropriate for urinary bladder, (Greenwell *et al*., 2001; Moon *etal*., 2013; El-Taji *etal*., 2015). However, colocystoplasty is associated with relatively common and minimal post-surgical complications, including mucus

production, infection and metabolic disturbance (Greenwell *et al*., 2001; Gurocak *et al*., 2007; Muhammad, 2013). The complications are largely related to the physiologic activities of the epithelium (rather than the smooth muscle) of the interposed intestinal segment, an absorptive and mucus secreting epithelium, in contact with urine (Filmer and Spencer, 1990; Gurocak *et al*., 2007; Muhammad, 2013). The use of seromuscular colonic segment takes an advantage of "spare part concept" to minimise morbidity and can be extremely beneficial where demucosalized, vascularized tissue flap was used for bladder reconstruction to avert the complications associated with the intestinal mucosa (Johnathon *et al*., 2015). Despite the merit of colonic seromuscular pedicle flap in addressing the complications associated with the epithelium, the problems of the demucosalised tissue (seromuscular flap) frequently encountered are the epithelial regrowth, fibrosis and tissue shrinkage/contracture (Lima *etal*., 1995; Gurocak *et al*., 2007;Subramaniam *et al*., 2012).

Free graft of the peritoneal tissue has been used as substitute to urinary bladder mucosa in the repair of hypospadias, subsequently transformed closely into uroepithelium without sign of fibrosis or contracture (Shaul *etal*., 1996; Nanni *et al*., 2001). The parietal peritoneum is abundant, easy to harvest and resistant to fluids (for example, ascitic fluid in cases of renal failure) very close to urine in composition (Shaul *etal*., 1996), and its adaptability within the lining of the urinary tract was demonstrated (Nanni *et al*., 2001). In addition, it was also reported that free peritoneal graft was compatible with excellent graft take following its application on seromuscular and muscular layers (Yin, 2005).

To address the possible complications (mucus production, infection and metabolic disturbance) based on the factors mentioned above, we hypothesise a new procedure/technique of bladder reconstruction by means composite (colo-peritoneal) tissue transplantation (CTT) in dogs (*in-vivo*), as cells/tissues (*in-vitro*) alone cannot provide

solutions to the complications. This approach is believed to facilitate the restoration of function and structural reliability, and is considered a quality-of-life intervention (Hettiaratchy *et al*., 2004; Petruzzo and Dubernard, 2011). Dogs were preferred based on the relative thickness of the intestinal wall that makes harvest of sero-muscular grafts easier. The anatomy of their lower urinary tracts and urodynamic profile of micturition are also similar to that of human being, as the work was also aimed at solving similar problems in humans (Zapadnyuk *et al*., 1983; Van Asselt *et al.*, 1995; Neuhaus *et al*., 2001; Hassan *et al*., 2005).

1.4 Aim and Objectives

1.4.1 Aim of the study

The aim of this study was to evaluate the use of autologous composite colo-peritoneal pedicle grafts in the repair of experimentally created urinary bladder defects in dogs.

1.4.2 Objectives

The objectives of this study were to:

i. demonstrate the suitability or otherwise of the seromuscular pedicle flap and peritoneal free flap as composite grafts to repair urinary bladder defects.
ii. comparatively evaluate ultrasonographical and radiographical image of the urinary bladder architecture post repair with the composite coloperitoneal grafts.
iii. compare the pre- and post-surgical chemical composition of urine by urinalysis post-colo-peritoneo-cystoplasty.
iv. demonstrate haematological alteration following colo-peritoneo-cystoplasty.
v. comparatively evaluate some plasma cytokines (IL-6 and IL-10) post-repair of urinary bladder defects with the composite coloperitoneal grafts.

vi. correlate plasma levels of IL-6 and IL-10 with each other, and with haematological and vital parameters in dogs.

vii. evaluate serum biochemical changes occurring in autologous colo-peritoneo-cystoplasty.

viii. evaluate histologically, the composite tissue viability and tissue integration post-repair.

1.5 Research Questions

i. Is peritoneal sheath and sero-muscular colonic pedicle flap “composite tissue” a suitable graft in the repair ofmassive urinary bladder defects in dogs?

ii. What will be the outcome of the ultrasonographic and radiographic evaluations of the urinary bladder architecture post repair with the composite graft?

iii. Are there differences in urine chemical composition between pre- and post-colo-peritoneo-cystoplasty urinalysis in dogs?

iv. What are the haematological alterations following colo-peritoneo-cystoplasty in dogs?

v. How does plasma cytokine (IL-6 and IL-10) respond to surgical reconstruction of urinary bladder defect in dogs?

vi. Are there correlations between plasma IL-6 and IL-10 with each other, and with hematological and vital parameters in dogs?

vii. Are there serum biochemical changes that may accompany autologous composite colo-peritoneo-cystoplasty in dogs?

viii. What will be the histological appearance of the composite tissue in the urinary bladder and tissue integration post-repair?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical Perspectives of Lower Urinary Tract Disorders

Lower urinary tract disorders occurring in young animals and humans may result from genetic (heritable) or acquired processes that affect differentiation and growth of the developing urinary tract (Bartges and Kruger, 2011). Understanding of the congenital defects of urinary bladder begins with detailed comprehensive knowledge on the embryogenesis of the organ (Haraguchi *etal*., 2007). Vertebrates in general have one of three distinct excretory organs: the pronephros, mesonephros, or metanephros, which appear in succession during urogenital development, and parts of each may be retained in a developing embryo (Owen, 1973; Dean *et al*., 1988). In mammals, only the duct of the pronephros is retained as the mesonephric duct (Owen, 1973). According to Sadler (2010), uro-rectal septum divides the cloaca into dorsal and ventral aspects; which marks the division between the urinary system and the genital system. The division of the cloaca leads to the formation of urogenital sinus that is divided into three component parts. The first of these is the cranial portion which continued with the allantois and forms the bladder proper. The pelvic part of the sinus forms the prostatic urethra and epithelium as well as the membranous urethra and bulbo-urethral glands in the male and the membranous urethra and part of the vagina in females. Thirdly, the caudal portion, or definitive uro-genital sinus, forms the penile urethra in males and the vestibule in females. However, the trigone portion is formed by the caudal ends of the mesonephric ducts. As the bladder expands, the mesonephric ducts begin to become incorporated into the wall of the bladder dragging the ureters along with them. Further growth causes the ureters to eventually have their own opening into the bladder. The mesonephric duct, which is temporarily active in canine foetuses, is retained as the

ductusdeferens in males but degenerate and becomes vestigial in females (Owen, 1973). Any deviation from normal embryologic development can lead to a variety of anomalies, including persistent cloaca, recto-vesical fistula, recto-urethral fistula, ectopic ureter, bladder exstrophy, diverticula, hypoplasia, megacystis, agenesis etc (Caldamone, 1987).

2.2 Urinary Bladder Defects

Bladder injuries do occur and are broadly classified as acquired and congenital defects (Jessop, *etal*., 2015) based on the extent of injury, nature, aetiology and location (Gomez *etal*., 2004). Acquired urinary bladder injury may be caused either by external (blunt or penetrating) or iatrogenic trauma with similar frequency (Carlin and Resnick, 1995). The incidence of modern transportation preferences and increasing reliance on motor vehicles with advances in mobile technology, domestic or professional accident and violence of any sort may contribute to the increasing frequency of bladder injury (Dobrowolski *et al.*, 2002). Diagnosis of acquired bladder injury is primarily by history of anuria with abdominal distension (Sagalowsky and Peters, 1998), and presenting clinical signs in which the two most common signs and symptoms are gross haematuria (82%) and abdominal tenderness (62%) in the patients (Carroll and Mc Aninch, 1984). The standard and most accurate diagnostic procedure for diagnosing bladder rupture is the retrograde cystography (Baniel and Shein, 1994; Sandler *et al*., 1998) with accuracy rate of 85-100% (Sandler *et al*., 1998; Deck *et al*., 2000). The diagnosis is usually made easily on cystography when the injected contrast is identified outside the bladder (Lynch *et al.*, 2003).

Pearson and Gibbs (1973), Agut *et al.* (2002), Savanelli *et al*. (2003) and Awasum (2010) reported that urinary tract abnormalities are of significance and morbidity mayoccur in many species of animals, namely cattle, dogs, cats, and humans (Beasley *et al*., 1992; Metts *et al*., 1997; Cascio *et al*., 1999).Urinary bladder defects such as bladder exstrophy, diverticula,

hypoplasia, megacystis, agenesis, bladder ears, duplication and septa are of congenital origin, while most acquired injuries result from complications of pelvic surgeries and vaginal operations (Caldamone, 1987; Neuman *et al*., 1991; Cilento and Nguyen, 2001). Bladder neoplasm, neurogenic bladder dysfunction, idiopathic detrusor over-activity, chronic inflammatory conditions (such as tuberculosis, interstitial cystitis, schistosomosis and post-radiation bladder contraction) and urolithiasis also affect the anatomy and physiology of the urinary bladder (Nabi *et al.*, 2003). This range of conditions can damage or compromise bladder function, necessitating eventual organ replacement or repair.

Several approaches had been employed to manage different urinary bladder conditions with limited successes, especially those associated with severe and extensive tissue devitalisation (Powell and Kreder, 2009; Muhammad, 2013).The surgeries employed on the lower urinary tract could be urinary diversion, urinary bladder reconstruction or replacement with either autologous urologic tissues or non-autologous urologic tissues (Probst *et al*., 1997; Mingin *et al*., 1999; Dewan and Anderson 2001; Atala *et al*., 2006).

A number of reviewed articles suggest that the following indications for urinary bladder surgery may benefit from colo-peritoneal graft as the operation associated to the indications reviewed from literature requires extensive and invasive surgical intervention. The indications and management are discussed below:

2.2.1 Urinary bladder exstrophy

Bladder exstrophy is a congenital malformation or evasion of the urinary bladder in which the bladder is opened and the inner surface (urothelium) is exposed on the lower abdominal wall, thereby establishing a contact between the bladder and the external abdominal wall (Andiran and Tanyel, 1999; Kumar *et al*., 2009).

Embryologically, the potential causes of bladder exstrophy occur mainly due to the failure of the cloacal membrane to be reinforced by ingrowths of the mesoderm resulting in abnormal or lack of caudal development of the lower abdominal musculature and genital hillocks (Bonitz and Hanna, 2016), abnormal over-development of the cloacal membrane and insertion of the body stalk, failure of interposed mesenchymal tissue in the midline (Beasley *et al*., 1992; Mathur *et al*., 2002). Diagnosis is usually made at delivery by the typical appearance of the bladder urothelium exposed on the lower abdominal wall (Bonitz and Hanna, 2016). Incidence is higher in males than females in ratio 1.3: 1, and often associated with other birth defects (Gurocak *et al*., 2007; Coker *et al*., 2008).

Several surgical treatment options of bladder exstrophy are available depending upon the severity of the condition, which include modern staged reconstruction (bladder pelvic closure, urethral reconstruction and bladder neck surgeries) and urinary diversion (Coker *et al*., 2008; Mushtaq *etal*., 2014). However, the management (modern reconstruction) is directed at stabilising the patient for the closure of the bladder and abdominal wall to establish physiologic urinary continence (Wild *etal*., 2011). The reconstruction has a success rate of 71-75% when performed in the first 48 to 72 hours of life; the failure of the treatment is due to post-surgical complications, which include bladder prolapse, bladder calculi, renal calculi and wound dehiscence (Gearhart, 2002).

2.2.2 Bladder diverticula

Bladder diverticulum is an out-pouching or herniation of the bladder mucosa and submucosa through the bladder musculature caused by congenital bladder muscular anomalies (Gerridzen and Futter, 1982; Russell *et al*., 2000; Yovchevski and Kostov, 2009). The diverticula vary greatly in size with narrow or wide rings having functional implications as dictated by the size of the detrusor defect (Pieretti and Vanmarccke, 1999); for

example,narrow-mouthed diverticula often empty poorly and stasis of urine within diverticula can also lead to stone formation or epithelial dysplasia (Lakshminarayana *etal*., 2010). Bladder diverticula can be unilateral or bilateral and are common in humans and animals (Pieretti and Vanmarccke, 1999; Gearhart, 2002).

The defect is a multi-factorial disease process of congenital or acquired origin (Pearson and Gibbs, 1973; Pieretti and Vanmarccke, 1999; Coker *et al.*, 2008).The congenital bladder diverticulum is more common in paediatric populations due to congenital deficiency or weakness in Waldeyer fascial sheath, a dense rectosacral fascia that begins at the level of the fourth sacral body and extends anteriorly to the rectum, covering the sacrum and overlying the vessels and nerves, more solitary located at the junction of bladder trigone and detrusor, ureterovesical junction or posterior urethral valve (Pieretti and Vanmarccke, 1999; Coker *et al.*, 2008). In dogs and cats, the congenital bladder diverticula occur as a result of urachal abnormalities (such as persistent urachus and urachal cyst) at the vesicular apex and in the trigonal area (Klauschner *et al.*, 1983; Osborne *et al*., 1987), where urachal closure is incomplete or fails (Lobetti and Goldin, 1998).

The acquired bladder diverticulum generally occurs in adult male humans and dogs as a result of urethral stenosis or obstruction of urine flow through the urethral orifice due to space occupying tumours, foreign body lodgment, infection or iatrogenic causes (Klauschner *et al*., 1983; Prakash *et al*., 2010). It is thought that increased intravesical pressure results from the underlying pathology, which causes the bladder mucosa to insinuate itself between vesical muscle bundles.It results in the development of mucosal extravasational sac or sacule, which further leads to the formation of a diverticulum (Gerridzen and Futter, 1982; Russell *et al*., 2000). Sometimes, urinary tract infection, improper bladder emptying, cystolithiasis, epithelial dysplasia, haematuria, neoplasia or peritonitis due to bladder rupture may be

observed in some cases of large size bladder diverticulum (Micic and Ilic, 1983; Melekos *et al*., 1987). Imaging modalities employed include ultrasonography, contrast radiography (intravenous pyelography, and voiding cysto-urethrogram) and computed tomographic scan are some of the diagnostic tools and techniques used in the diagnosis of bladder diverticulum (Cilento *et al*., 1998).

Management protocols to bladder diverticulum in patients may be either medical or surgical (Powell and Kreder, 2009). In patients with diverticulum secondary to obstruction, for example, in stenosis of urethra, the first line of therapy is surgery (Fox *et al*., 1962; Haecker *et al*., 2005). In cases of diverticulum, which is larger than the bladder itself, the emptying will be poor and stasis of urine within the pouch can also lead to stone formation, thereby rendering the bladder to atrophy and less useful. Hence, it necessitates immediate diverticular excision with subsequent bladder augmentation or patch (Zia-Ul-Miraj, 1999; Berrocal *etal*., 2002). Whereas in urinary tract obstruction due to urethroliths, medical intervention is often employed.Exceptions occurs in chronic situations where there is calcification in which the stones cannot be dislodged or dissolved, medical intervention may also be carried out or when bladder diverticula cause obstruction, recurrent urinary tract infections, vesico-ureteral reflux, malignant transformation or favours stone formation, then such intervention (diveticulectomy) becomes ultimate (Scheepens and L'Eplattenier, 2005; Powell and Kreder, 2009). Cystoplasty, transurethral resection, urethroplasty fulguration or diverticulectomy are the surgical management protocols (Micic and Ilic, 1983; Melekos *et al*., 1987; Myer and Wagner, 2007). The success rate to the management is almost 100 % (Silberman and Jeanmonod, 2011).

2.2.3 Megacystis

Megacystis is a congenitally enlarged bladder, believed to be secondary to overfilling of the foetal bladder during embryonic development, associated with vesico-ureteral reflux (Burbige and Hensle, 1986; Robyr *etal*., 2005). The anomaly is believed to occur due to massive reflux of large percentage of voiding bladder volume into the upper tracts, thereby causing constant recycling of the urine between the bladder and ureters, resulting in progressive dilation of both (Burbige *et al*., 1984; Bornes *etal*., 2013). Megacystis can occur or be observed with other conditions such as sacro-coccygeal teratoma, pelvic neuroblastoma and urethral diverticulum (Levard *et al*., 1989; Osborne *et al*., 2011). It can best be diagnosed using voiding cysto-urethrogram test, to demonstrate the enlarged bladder and massive refluxing megaureters (Burbige *et al*., 1984).

Clean intermittent catheterisation may be considered in those patients with incomplete bladder emptying or frequent urinary tract infections (Madersbacher, 2002). Surgically, urethral re-implantation (for vesico-urethral reflux), reduction cystoplasty and vesicostomy are employed in the management of the condition, to decompress the urinary tract and to preserve renal function (Christman and Casale, 2012). Surgical excision of the overstretched urinary bladder with subsequent utilisation of the gastro-intestinal tract for its reconstruction has been advocated in most patients (Puri and Shinkai, 2005; Muhammad, 2013).

2.2.4 Urinary bladder agenesis

Urinary bladder agenesis is an extremely rare congenital anomaly seen mainly in infants that have other urogenital tract anomalies as well as neurologic and orthopaedic disorders, and it is generally incompatible with life (Cilento and Nguyen, 2001). Cases and literature on urinary bladder agenesis have rarely been documented or available in domestic animals (Metts *et al*., 1997). The cause of agenesis of the bladder is uncertain (Teresa *et al.*, 2002). It

was proposed that bladder agenesis may be the result of secondary loss of the anterior division of the cloaca. This may occur due to a lack of distention with urine, caused by failure of incorporation of the mesonephric ducts and ureters into the trigone; thus, preventing urine from accumulating in the bladder (Krull *et al.,* 1988). In suspected cases or patients with bladder agenesis, full evaluation is best performed with voiding cystourethrography (VCUG) or intravenous pyelography (IVP) (Berrocal *et al*., 2002). In most cases of bladder agenesis, the ureters have the same course as normal ureters with ectopic vestibular openings and there is usually some preservation of renal function, thereby necessitating urine exit or reservoir (Berrocal *etal*., 2002). In a situation where the infant is alive, the only management option for the condition is surgical urinary diversion and subsequent neo-bladder reconstruction (for example, continence urinary reservoir),utilising intestinal tissue (Cilento and Nguyen, 2001; Nabi *etal*., 2003; Gurocak *et al*., 2007; Muhammad, 2013; Muhammad *etal*., 2016a).

2.2.5 Urinary calculus

The significance of urolithiasis in small animals is increasing daily, and may be influenced by a variety of factors, including: species, breed, sex, age, diet, anatomical abnormalities, urinary tract infection, medication, and urine pH (Ettinger and Feldman; 2000). The most common minerals found in canine uroliths are magnesium ammonium phosphate (struvite), oxalate, cystine, and ammonium urate (Stevenson, 2002). Muhammad *et al*. (2016a) reported bladder rupture as a severe complication sequel to magnesium ammonium phosphate (Struvite) and calcium oxalate in dogs. History, physical examination, urinalysis, quantitative urine culture, ultrasonography, and radiography are required to confirm urolithiasis (Doreen and Andrew, 2009).

Management protocol of urolithiasis may involve the relief of obstruction to urine outflow; elimination of existing uroliths; eradication of urinary tract infection; increase in the

solubility of crystalloids in urine; and prevention of recurrence of uroliths (Fossum *et al*., 2002). Specific therapy to prevent recurrence depends on the type of urolith, and analysis is thus the cornerstone to formulation of urolith management, which can either be medical or surgical (Fossum *et al*., 2002).Two objectives of medical management of most types of urolith, including prevention of further urolith growth and causing the urolith to dissolve, which can be achieved by dietary therapy, through consumption of calculolytic feed with large quantity of water; alteration of urine pH, and administration of binding agents that can prevent potentially calculogenic metabolites (crystals) from growth and aggregation (Abdullahi *et al.,* 1984). Administration of antibiotics is highly indicated in cases of concurrent infection or "infection stone" (Markwell *et al*., 2000). Surgery is currently the only method to remove non-typed urolith, especially if there are concurrent or predisposing anatomical abnormalities (for example, urachal diverticula), or calcium oxalate uroliths from the urinary tract and medical protocols should be considered post-operatively to minimise recurrence or to prevent growth of uroliths remaining in the urinary tract (Fossum *et al*., 2002).

Management option of urolithiasis (medical or surgical) is according to the size, type, duration and location of the urolith within urinary tract. Where the urolith is lodged in the urethra blocking urine outflow, the urinary bladder will become distended beyond its elastic limit, later degenerate and rupture, thus necessitating replacement of the organ or its damaged part (Muhammad *et al*.,2016a). In situations of coarseness nature of the surface of the calculus, lodging in the bladder and remains for long duration, there is high possibility of various degrees of erosion and ulceration of the urothelium with consequent perforation of the bladder, hence the need to patch the perforated area (Cohen *etal*., 1997).

2.2.6 Blunt trauma

Blunt trauma occurs when extensive external force exerted on the abdominal wall displaces the bladder to an unbearable elastic (stretch) limits such that the bladder wall gets weakened and sometimes may lead to tears (Muhammad *et al*., 2015). Blunt trauma injuries have been reported by Carroll and McAninch (1984) to account for 67-86% of bladder ruptures. It may be classified as either extraperitoneal with leakage of urine limited to the perivesical space, or intraperitoneal, in which the peritoneal surface is being disrupted with concomitant urinary extravasation (Muhammad *et al*., 2015).Aumann *etal*. (1998) also observed the most common cause for uroperitoneum to be blunt trauma (85%), often associated with motor vehicle accidents.

When extraperitoneal blunt trauma occurs as a consequence of blunt pelvic trauma, it resultsinto lacerations or punctures of the bladder by bone fragment(s) and shearing of ligamentous attachment(s) due to pelvic and other long bone fractures, the consequences are grievous (Dreitlein *et al*., 2001). Moreover, in the intraperitoneal blunt trauma, which occurs due to high velocity blunt lower/caudal abdominal trauma and high intravesical pressure with bladder rupture (injuries) at dome, it is characterised by high rate of associated intra-abdominal escape of urine and mortality (Dreitlein *et al*., 2001).

The intraperitoneal bladder rupture can be handled by exploration and primary bladder closure or laparoscopic repair (Gunnarsson and Heuman, 1997), while the extraperitoneal bladder injury is predominantly managed conservatively, with 2 weeks of indwelling Foley catheter drainage (Allen *et al*., 2001). Surgical intervention is generally recommended in animals, but the decision in humans is based on the site and extent of the lesion as well as any concomitant injuries (Stafford and Bartges, 2013). Depending on the time of presentation, management (by exploration and primary bladder closure) of intraperitoneal bladder rupture

has a lesser success rates compared to laparoscopic repair, apparently, due to late presentation, lack of specialised equipment and diagnostic ability, length of hospital stay and infection risk (Suad *et al*., 2011).

Many parameters have been found to aid in determining surgical suitability, but the evidence of the parameters is conflicting (Peterson *etal*., 2015). However, in a situation of severe contusion to the bladder following severe mechanical tissue (blunt) injury that occurs at the time of the trauma with subsequent localised degeneration of the bladder, partial cystectomy may be required and can be repaired using alternate tissue (Jones *etal*., 2014).

2.2.7 Iatrogenic bladder injury

The intimate association of the urinary bladder and other visceral organs supports the report by Christopher *et al.* (2011) that iatrogenic bladder injuries are occasional complications of surgeries performed in the pelvis or caudal abdominal region. This is due to the close proximity of the bladder to the rectum in male and the uterus in females; thus, there is the potential for iatrogenic injury occurring during surgeries of the afore-mentioned structures. Iatrogenic bladder injuries which occur as complications in gynaecologic surgeries are of high incidence (Gambini *et al*., 2001; Dobrowolski *et al*., 2002), especially in ovarian cystectomy (Mteta *et al.,* 2006), caeserean section, hysterectomy, hernioplasty, ovariohysterectomy, and other emergency surgeries close to the urinary bladder, which could render the bladderto be subjected to surgical reconstruction (Christopher *et al.*, 2011).

Early diagnosis and repair offer the best outcomes in the management of iatrogenic bladder injuries (Muhammad *et al*., 2015). Delayed diagnosis often leads to the development of local infection, which usually progresses to sepsis, thereby complicating repair, impairing wound healing and producing poor outcomes (Gómez *et al*., 2004). Continuous bladder

drainageshould, however, be employed following itspatch/repair until cystography confirms the integrity of the bladder repair (Dobrowolski *et al*., 2002).

2.2.8 Bladder neoplasms

Neoplasm of the urinary system in animals and humans can affect the kidneys, ureters, urinary bladder, prostate, or urethra (Dahm and Gschwend, 2003). The urinary bladder is the location most frequently affected with cancer in small animals (Higuchi *et al*., 2013), and it comprises about 1% of all cancers in dogs and cats (Philips, 1999). The most common cancer of the urinary bladder in dogs is invasive transitional cell carcinoma (TCC) of intermediate to high grade which develops from the bladder transitional epithelial cells, also called urothelial carcinoma (Knapp *et al*., 2013). It causes obstruction to the flow of urine from the kidneys to the bladder or from the bladder to the outside of the body (Mutaers *et al*., 2003).The exact cause of TCC in an individual dog is usually not known. However, canine TCC results from a combination of several factors, including genetic predisposition (sex, age and breed) and environmental factors (including pesticides, insecticides and chemotherapy) (Glickman *et al*., 2004; Raghavan *et al*., 2004). In most cases, bloody urine, incontinence, frequent urination, and straining to urinate are the most frequent signs of TCC in dogs (Knapp *et al*., 2013). Inability to pass urine may be caused by obstruction of the urethra (Wilson *et al.*, 2007). A diagnosis of TCC requires a contrast radiography, ultrasound and tissue biopsy, obtained by surgery, cystoscopy or in some cases with a urinary catheter (Childress *et al*., 2011; Naughton *et al*., 2012). The vast majority of TCC cases are treated chemotherapeutically (McMillan *etal*., 2011; Hahn *etal*., 2012). However, in cases of tumour that occurs in a location and has not spread beyond the bladder where surgery is possible, surgical excision (partial cystectomy, permanent urinary catheter placement, urethral stenting, ureterocolonic anastomosis or laser ablation with chemotherapy) could be considered as the treatment of

choice (Childress *et al*., 2011). Following such surgical intervention, tissue replacement may be required. Radiation therapy has been used to successfully control TCC growth in the bladder of dogs, if surgery is not possible (Mutaers *et al*., 2003).

2.2.9 Bladder tuberculosis

Genitourinary tuberculosis (GUTB) is still a major health problem in many developing countries and had been declared by World Health Organisation (WHO) as 'public health emergency' in 1993 (Anon[1], 2007). The GUTB is the second commonest extra-thoracic form of tuberculosis that occurs due to metastatic spread of tubercle bacilli through the bloodstream, during the initial infection, affecting males and females equally (Narmada *et al*., 2008).The involvement of the urinary bladder is usually secondary to renal tubercular involvement that generally results in patchy cystitis due to inflammation of the urothelium by the tubercle bacilli (*Mycobacterium tuberculosis*) with resultant granulomatous inflammation, caeseous necrosis. Final healing by fibrosis may lead to marked contracture of the urinary bladder within about one year (Lambie *etal*., 2005; Wong-You–Cheong *etal*., 2006; Narmada *et al*., 2008). Urinary bladder tuberculosis affects the mucosa near the ureteral orifice, which, in advanced infection, the bladder becomes small, irregular, contracted (Muttarak *etal*., 2005) and calcified. Eventually,it may be complicated by fistulas/sinus tract formation, leading to non-functional urinary bladder (Goel *et al*., 2002; Wong-You–Cheong *etal*., 2006). Surgery continues to play a key role in the management of bladder tuberculosis, despite the availability of effective anti tubercular therapy (Rizzo *et al*., 2004). This is because once the tubercular bladder shrinks to a very small size (reduced elasticity and compliance) - tubercular thimble bladder - the process may no longer be reversible and reconstructive surgery in the form of augmentation cystoplasty becomes a must in order to relieve the

irritating lower urinary tract symptoms as well as prevent upper tract deterioration (Gow and Barbosa, 1984; Gupta *et al*., 2008).

2.3 Overview of Some Conventional Urinary Bladder Repair Techniques

Urinary bladder can be damaged by disease, trauma or rendered useless where an alternative tissue may be sourced in order to repair/replace/reconstruct the organ. This need has posed challenge to urological surgeons over the years (Nabi *et al*., 2003). Many principles guiding the urinary bladder surgical procedures and techniques are currently being used to repair the defective bladder both anatomically and physiologically.

2.3.1 Principles of urinary bladder surgery

Proper understanding of anatomy and physiology of the bladder are among essential considerations in the preparation for the surgical repair of the organ (Giot *etal*., 2011). As a general rule in both principle and practice, all animals and human patients must be fully evaluated prior to surgery to determine the nature and extent of the disease (if it exists) (Stoffman *etal*., 2003). A complete history, thorough physical examination and routine haematology, biochemistry and urinalysis are important to identify underlying renal disease and recognise patients with fluid, electrolyte and metabolic imbalances or other conditions, which are likely to increase the risks associated with anaesthesia and surgery (Fossum *et al*., 2002; Feely *etal*., 2013). The underlying problem of the urinary bladder needs to be evaluated and the consequence of such identified problem to the patient should be ascertained (Feely *etal*., 2013). By attendingto medical emergencies first, it relatively stabilises the candidate to be fit for the intended surgical procedure (Feely *etal*., 2013).

According to Brown *et al.* (1997), surgery which involves entry into the bladder or urethra, in the absence of infected urine, is classed as a clean-contaminated procedure. The infection rate

for this type of surgery is approximately 5 percent and the administration of prophylactic antibiotics does not reduce this rate. In cases of urinary tract obstruction, urine leakage, extensively traumatised tissues or renal failure, or if the surgery is likely to exceed 90 minutes, a prophylactic antibiotic is recommended. A broad-spectrum, bactericidal, non-nephrotoxic, intravenous antibiotic is recommended to be given at the induction of anaesthesia, repeated every two hours during surgery and discontinued at the end of surgery. If a urinary tract infection is present, a course of therapeutic antibiotics is indicated post-operatively, ideally based on the results of urine culture and sensitivity testing. Antibiotics can be withheld until intraoperative cultures have been taken. Suitable empirical choices of therapeutic antibiotics include enrofloxacin and trimethoprim sulphur (Nelson and Couto, 1998).

The location of a urinary tract rupture must always be identified using contrast radiography prior to surgery (McLoughlin, 2000). A positive contrast cystogram should reveal the presence of a bladder rupture, identify radiolucent calculi and outline any bladder masses (Feeney and Anderson, 2011). Small bladder tears may be difficult to visualize on initial radiography and serial radiographs may be required to demonstrate the presence of free contrast material within the peritoneal cavity (Muhammad *etal*., 2015). It is helpful not to dilute the contrast material and to administer more contrast if the bladder is not fully distended at the first attempt (Muhammad, 2013). A double contrast cystogram produces enhanced mucosal detail and is recommended for highlighting the bladder wall lesions and cystic calculi (Allen *et al*., 2001).

Cooley (1999) has described the effects of indwelling catheter and tube cystotomy in dogs where the demonstration of the catheterisation is as follows: Cystostomy tubes are usually placed surgically via a laparotomy. A Foley catheter or mushroom-tipped catheter (8 to 14

French gauges) is commonly used. Foley catheters are retained in the bladder by inflating the distal balloon with saline, whereas mushroom tipped catheters are maintained by virtue of the button-shaped distal tip. The catheter is introduced into the abdomen through a stab incision in the body wall, lateral to the caudal ventral midline. A purse-string suture using a synthetic absorbable material is placed in the bladder. A small stab incision is made in the bladder within the purse-string suture. The distal end of the tube is placed into the bladder and the purse-string suture is tightened around the tube. Three or four tacking sutures are placed from the bladder in the region surrounding the tube to the abdominal wall. These sutures do not penetrate the bladder lumen and engage the internal muscular layer of the body wall. The external part of the tube is secured to the body wall. Cooley (1999) stated further that the catheter is either attached to a continuous closed collection system or the bladder can be intermittently drained. The catheter must remain in place for at least seven days to allow time for a secure adhesion to form between the bladder and the body wall.

Fossum *et al*. (2002) recommended that an incision is generally made on the dorsal or ventral surface of the bladder away from the urethra and should be sutured water tight. The goal of the bladder closure is to obtain a water-tight seal that will not promote calculi formation. Monofilament suture material will cause less tissue drag in the delicate lower urinary tract tissues than multifilament suture materials. The bladder and urethra heal quickly compared with other organs, regaining 100 percent of normal tissue strength in 14 to 21 days and complete re-epithelization occurs in 30 days. Polyglactin 910 (Vicryl; Ethicon) loses 50 per cent of its tensile strength by 14 days, which corresponds well to the time needed for lower urinary tract healing. Poliglecaprone (Monocryl; Ethicon) loses 50 per cent of its tensile strength by seven days. There is evidence that absorbable synthetic suture materials lose their tensile strength very quickly when in contact with alkaline infected urine (Schiller *et al*.,

1993). Commonly used suture sizes for bladder surgery in dogs are 3/0 or 4/0, depending on the size of the patient (Rawlings *et al*., 2003).

2.3.2 Cystotomy

Fossum *et al*. (2002) described cystotomy (surgical incision into the urinary bladder) as follows: A midline laparotomy incision is made from the umbilicus to the pubis. The bladder is isolated from the rest of the abdomen with moistened laparotomy swabs and a stay suture is placed in the apex. A stab incision is made in the ventral surface with a blade and the incision is extended cranially and caudally along the mid-line with scissors. A dorsal midline incision in the bladder is an alternative approach, but a ventral cystotomy is easier and is preferred, if the ureteral openings need to be identified and catheterised. The bladder is emptied of urine and blood, as necessary, using suction. Further stay sutures can be placed at the incision edges to gently hold the cystotomy open. A transurethral catheter can be placed prior to surgery or transabdominal is employed temporarily for seven days and attached to an extension set to allow for urine draining or flushing the bladder temporarily for seven days.

A large number of suture patterns have been reported in the veterinary literature for closing cystotomy incisions, many of which are appropriate; the choice of suture pattern is, in part, down to individual surgeon preference. One- or two-layer, interrupted or continuous, appositional or inverting suture patterns may be used to close the bladder. Continuous suture patterns provide more precise submucosal apposition in between sutures and better haemostasis. Inverting patterns that can be used include Cushing or Lembert patterns, both of which penetrate the submucosa, but not the lumen (Fossum *et al*., 2002). The overall risk of this surgery is low. The major risks are those of general anesthesia, bleeding (hemorrhage), postoperative infection, urine leakage, and wound break-down (dehiscence) over the incision

(Fossum *et al*., 2002). Overall complication rate is low, but serious complications can result in death or the need for additional surgery (Nelson and Couto 1998).

2.3.3 Cystectomy (Partial cystectomy)

Surgical removal of portion of urinary bladder (cystectomy) is easiest performed when a lesion is located at the bladder apex (Fossum *et al*., 2002). Neoplastic lesions are ideally resected with 1 to 2 cm of normal tissue margins. Up to 75 per cent of the bladder can be resected without any major problems, provided the trigone region is left intact: a return to normal bladder volume and function as expected over a period of months (Waldron, 2003). Closure of cystectomy is performed in similar manner to that described earlier for cystotomy. If a large amount of bladder is excised, a temporary cystotomy tube or indwelling urethral catheter is placed to allow the bladder to be kept decompressed in the immediate post-operative period. Total cystectomy has been described for an extensive bladder lesion or lesion involving the bladder trigone, but this requires a urinary diversion procedure (Fossum *et al*., 2002).

2.3.4 Urinary bladder augmentation

Bladder augmentation is an important tool in the management of individuals requiring reconstructions for urinary incontinence or preserving of the urinary tract in congenital malformations (Gurocak *et al.,* 2007). Natural tissues that have been used in the procedure include gastro-intestinal organs (such as stomach, ileum, caecum, colon as tubular or detubularised, simple or complex segments and reversed sero-muscular bowel grafts). Others are the peritoneum, omentum, lyophilised human dura, skin, preserved bladder, pericardium and even placenta (Elbahnasy *et al.,* 1998). Initially, candidates were generally diverted from augmentation procedures due to the retention problems, until the efficiency of clean intermittent catheterisation (CIC) to empty the bladder was proved in 1972 (Lapides *et*

al.,1972). Bladder augmentation with gastro-intestinal tissue is the armamentarium of the urologists, and enterocystoplasty is considered to be the best of all augmentations (Gurocak *et al.,* 2007). However, this form of reconstruction with intestinal segment is associated with some complications, as well as the morbidity of harvesting bowel segment (Vilar *et al*., 2004; Gurocak *et al*., 2007; Subramaniam *et al*., 2012).

The synthetic materials that have been tried include: gelatine sponge, Teflon, polyvinyl sponge, resin coated paper, poly-amino membrane, collagen/polyglactin membrane and Silastic (Greenwell *et al*., 2001). All have been followed by a variety of associated problems including metaplastic bone formation, recurrent or persistent urinary tract infection (UTI), stone formation, fistulae, contracture and fibrosis (Barrett and Donovan 1984; Elbahnasy *et al.,* 1998; Subramaniam *et al*., 2011; El-taji *etal*., 2015).

2.3.5 Enterocystoplasty

Small and large intestines have been used for more than a century either to enlarge the bladder in rare conditions such as bladder exstrophy, or as a bladder replacement or substitution in patients with tuberculosis (Gouch, 2001). Complications such as infection and upper urinary tract damage were common at that time (Greenwell *et al*., 2001; Gurocak *et al*., 2007). However, with the introduction of clean intermittent catheterisation together with antibiotics, these complications were reduced (Dounis *et al*., 1980; Lemke *et al*., 2005).

Intestinal segments from the jejunum through to the sigmoid have been used for enterocystoplasty; however, jejunum quickly fell into disrepute because of particular problems with water re-absorption. Ileum can be used in the form of a cup (Bramble, 1982; Mundy and Stephensons 1985; Reidmiller *et al*., 1989; Krishner *et al*., 1995) or simply as a detubularised segment (Reidmiller *et al.,* 1989). The ileocaecal, caecum and the sigmoid colon (Mayo and Chapman 1988) are all alternatives better than jejuno-cystoplasty and gasto-

cystoplasty (Adams *et al*., 1988). The possible surgical complications of enterocystoplastic procedures are anastomotic leakage, stricture, intestinal obstruction or bladder rupture (Bertschy *et al.*, 2000; Shekarriz *et al*., 2000; Gouch, 2001).

2.4 Composite Tissue Transplantation

Composite tissue transplantation (CTT) is defined as transplantation of histologically different tissues in order to facilitate the restoration of function and structural reliability and is considered a quality-of-life intervention (Hettiaratchy *et al*., 2004; Petruzzo and Dubernard, 2011). Limited reconstructive surgery options using own tissue from other parts of the body to correct defect necessitate transplanting tissue from other animal of either the same or different species to offer an effective way to reconstruct the defect (Horner, 2008). Clinical interest in the reconstructive surgery is currently transforming from autogenous free tissue transfers to vascularised composite tissue grafts and is also gaining momentum due to the recent successes tissue/organ (for example, facial and hand) transplantation (Barth and Bartlett, 2007). Experimental CTT has been performed in different models, including rodents, large animals, and non-human primates to test immunologic response, functional recovery, and technical feasibility (Siemionow and Klimczak, 2010).The success in CTT is the culmination of progress in two disparate surgical disciplines - reconstructive surgery and transplantation (Swearingen *et al*., 2008).The first successful reconstruction using a composite tissue allotransplant was a hand transplant performed in France in 1998 (Horner, 2008). The initial success of this transplant paved the way for more procedures, including hand transplants (Lanzetta *et al*., 2007), abdominal wall transplants (Levi *et al*., 2003), scalp (Jiang *et al*., 2005), knee transplants and face transplants (Kanitakis *et al*., 2006). The CTT is currently accepted as an alternative therapy and retains great potential for the reconstruction

of various parts of the body in selected cases of plastic/reconstructive surgeries, despite its complexity (Siemionow and Klimczak, 2010).

For urinary bladder reconstruction with composite tissue, the concept of composite cystoplasty was successfully piloted by Fraser *etal.* (2004) in a surgical mini-pig model using autologous cultured urothelium, combined with vascularised uterine smooth muscle. The same concept was employed for composite enterocystoplasty utilising vascularised sero-muscular intestinal segment, in which autologous urothelium propagated *invitro* is combined with a segment of de-epithelialised bowel at the time of surgical reconstruction (Fraser *etal.*, 2004). Moreover, this offers significant advantage over other tissue engineering strategies as it exploits the patient's vascularised smooth-muscle tissue and requires only urothelium to be engineered *invitro* (Turner *etal*., 2011). The composite cystoplasty was the product of previous research on the development of reliable cell-culture systems for generating clinically relevant areas of normal human urothelium *invitro* (Southgate *etal*., 1994). In the most recent concept/description of the composite cystoplasty as described by Turner *etal*. (2011), urothelium harvested from the urinary bladder by open biopsy was expanded in culture and subsequently propagated to develop sheets of non-differentiated or differentiated urothelium, thus, require time for tissue culture and propagation (that is*invitro* studies) (Baker *etal*., 2009; El-taji *etal*., 2015). The sheets were transplanted onto a vascularised, de-epithelialised, sero-muscular intestinal segment at the time of bladder augmentation (Turner *etal*., 2011).

2.4.1Peritoneal tissue flap

The understanding of the normal anatomy and function of the peritoneum is essential to the understanding of abdominal surgery and transplantation of the peritoneal tissue. The peritoneum (derived from the mesoderm lining the body cavity of the primitive embryo) is a heterogeneous, serous and semi-permeable membrane, which lines the innermost surface of

the abdominal wall and the majority of the abdominal organs (Healy and Reznek, 1998; Blackburn and Stantom, 2014). It is described to have a parietal layer, lining the body wall, and a visceral layer, which lies over the abdominal organs (Blackburn and Stantom, 2014).

The parietal peritoneum cranially covers the ventral abdominal wall, in a series of folds, which overly the obliterated urachus (the median fold), obliterated umbilical arteries (the medial fold)and cranial epigastric vessels (the lateral unbilical fold). The peritoneum continues caudally and is reflected over the dome of the bladder. The parietal peritoneum passes up the dorsal abdominal wall before being reflected over the root of the mesentery and the small bowel. Laterally at this level, the peritoneum overlies the retro-peritoneal portions of the descending and ascending colon and is reflected over the colon.

The vascular supply to the parietal peritoneum is by intercostal, subcostal, lumbar, and iliac vessels; and innervated by somatic segmental spinal nerves supplying the overlying muscle (Healy and Reznek, 1998; Borley, 2008). Histologically, the peritoneal tissue is made up of layers of mesothelial, submesothelial (elastic lamina, a thicker layer of connective tissue), and adipose tissue layers (Knudsen, 1991). The mesothelium is a single layer of flat, simple, stratified squamous epithelial cells (linked by intercellular junctions) on a basal laminar (Borley, 2008; Blackburn and Stantom, 2014). The elastic lamina is composed of a network of elastic fibres situated immediately beneath the basement membrane of the peritoneal mesothelium, which varies in its density (Blackburn and Stantom, 2014).

The sub-mesothelial layer is thinner in areas that can undergo expansion (for example, caudal abdominal wall), and thicker in more fixed areas like pelvic fascia (Borley, 2008; Blackburn and Stantom, 2014). These account to some functional properties of the peritoneum that are of interest to reconstructive surgeons (Blackburn and Stantom, 2014). Normal peritoneal fluid contains water, electrolytes, solutes, proteins, and cells with specific gravity of 1.010-1.020,

pH of 7.5-8.0 and as part of the local defence. The secretion, circulation and absorption of other fluidsare of relevance with respect to peritoneal tissue harvest as graft, insufflations and gas re-absorption in laparoscopic surgery or laparotomy, and peritoneal dialysis (Dunn *etal*., 1985).The more recent "three-pore model" describes capillary–peritoneal flow in more detail. The three pores are the frequent "small pores," which allow passage of water and small solutes; very infrequent "large pores," which are permeable to protein; and the "ultra-small pores" (quaporin-1), which are permeable to water only. Free peritoneal tissue graft, successfully used in the lower urinary tract reconstruction (Shaul *etal*., 1996; Nanni *et al*., 2001), was found to be compatible with excellent graft take following its application on seromuscular and muscular layers (Yin, 2005).

2.4.2 Colonic seromuscular tissue transplantation

Standard procedures for colonic seromuscular pedicle (tissue) harvest and transplantation have been described (Subramaniam *etal*., 2012). The de-epithelialisation process of the colonic segment could be through injection of saline between the layers of the mucosa and the submucosa (Lima *etal*., 1995), demucosalisation from within the lumen by combination of sharp and blunt dissection (Fraser *etal*., 2004) and insertion of Foley catheter (as mucophilic device) inside the lumen to facilitate demucosalisation by incising the seromuscular layer only, then separated from the *laminapropria* (Lima *etal*., 2004). In general, the colon is identified and the segment closest to the bladder wall should be surrounded with gauze, desired length identified along with its vascular arcades within the mesentery (Lima *etal*., 1995). Stay sutures placed on the antimesenteric seromuscular border of the colon just beyond the dissection limits (Fraser *etal*., 2004). The dissection limit of the bowel and mesentery is defined by marking with a surgical marker pen (Lima *etal*., 2004). The isolated segment is de-tubularised by opening along its antemesenteric border to produce

a rectangular seromuscular colonic pedicle flap for transplantation or composite tissue formation (Fraser *etal*., 2004; Subramaniam *etal*., 2012;Jednak, 2014). For the pedicle flap transplantation, a defective (recipient site) dome or lateral walls of the bladder is prepared to accommodate the harvested colonic seromuscular grafting colonic segment, and sutured by continuous suture pattern using 2/0 to 4/0 synthetic absorbable sutures (Subramaniam *etal*., 2012;Jednak, 2014).

2.4.3 Composite colocystoplasty

The technique of the composite colocystoplasty, as described by Fraser *etal.* (2004) and Turner *etal*. (2011) involves extramucosal colonic dissection to develop a vascularised, de-epithelialised, seromuscular segment of appropriate pre-determined size. The intended colonic segment is identified, exteriorized and isolated on its vascular pedicle. A Foley catheter is inserted rectally and the balloon filled with sterile water within the portion of sigmoid bowel to be dissected. The colonic segment is de-tubularised by seromuscular incision along its antemesenteric border and de-epithelialized using a combination of sharp and blunt dissection, to produce a flat rectangular 'plate'. The remaining bowel is re-anastomosed in a single layer with 3/0 or 4/0 polydioxanone. The autologous tissue-engineered urothelial sheet in complex with Vicryl mesh is sutured to the de-epithelialised colonic surface using 6/0 or 8/0 Vicryl sutures with the cellular face of the mesh-cell support in contact with the stroma, thereby forming a composite tissue. The dome and lateral walls of the bladder are incised to accommodate the augmenting composite colonic segment, which is sutured to the bladder by a continuous suture pattern using 2/0 polydioxanone as in a Clam cystoplasty. Before completing the augmentation, a silicone balloon is inserted, and this serves to gently appose the urothelial mesh graft to the de-epithelialised stroma. A suprapubic

catheter is used for bladder drainage, tunnelled subcutaneously to exit on the lateral abdominal wall (Turner *etal*., 2011).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Experimental site

This study was carried out at Ahmadu Bello University (ABU), Zaria, located in the Northern Guinea Savannah zone of Nigeria (11^{o} 04 N, 7^{o} 42 E) (Anon[3], 2016).

3.1.2 Ethical approval

Ethical clearance was sought and obtained in accordance with the statutory regulations guiding animal care and use as approved by Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC). An approval number(ABUCAUC/2016/010) was thereafter issued.

3.1.3 Experimental animals

A total of fourNigerian indigenousdogs (NIDs) comprising 2 male and 2 female and varying in weight (15-20 kg) and age (0.75-1 years) acquired from Zaria and its environs, were used for the study. They were humanely transported by road in a vehicle (car) to the Faculty of Veterinary Medicine. Upon arrival at the faculty, the dogs were housed in the kennels of Veterinary Teaching Hospital and the Department of Veterinary Surgery and Radiology, ABU, Zaria, and conditioned for two weeks. They were fed maize, rice, beans, blood meal, soft bones, fish and palm oil, and given access to water *ad libitum*. During the conditioning period, the dogs were evaluated clinically through physical and laboratory examinations, including erythrocyte and leucocyte counts, urinalysis, blood chemistry, haemoparasitic and helminthic screening.

3.1.4 Surgical materials

The materials used in the study (APPENDIX I) were obtained from the Departments of Veterinary Surgery and Radiology; Veterinary Pathology, Veterinary Parasitology and Entomology; Veterinary Medicine; Chemical Pathology of Ahmadu Bello University Teaching Hospital (ABUTH) and ABU Veterinary Teaching Hospital, Zaria. Some items were, however, imported from China (such as ELISA Cytokine Kits) and local Pharmaceutical shops in Zaria.

3.1.5 Consumables

The consumables used in the study include suture materials, antibiotics, anaesthetic agents, pre-medications and laboratory reagents (APPENDIX I), food, feeding and watering items (APPENDIX II).

3.1.6 Kennel and laboratory preparations

Seven days prior to the arrival and housing of the experimental animals to their individual kennels, all the kennels were washed, cleaned, disinfected and sprayed with acaricide, while rodenticide (Warfarin) was spotted around the kennels to repel rodents. The foodstuff (APPENDIX II) were purchased, and kept in a kitchen adjacent to the kennel for preparation and feeding of the dogs.

3.2 Methodology

3.2.1 Location of the experimental study

The study was carried out in the Faculty of Veterinary Medicine, ABU, Zaria. The Small Animal Surgical theatre and dog kennel of the Veterinary Teaching Hospital and the Department of Veterinary Surgery and Radiology were used for the surgical procedures, pre- and post- operative management, throughout the study period. Laboratory examinations of all samples collected were performed in the relevant laboratories, including Protozoology, Helminthology, Entomology, Clinical Pathology, Histopathology, Radiology and Necropsy laboratories all within the Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, and the Department of Pathology (Chemical Pathology) ABUTH, Zaria.

3.2.2 Experimental animal screening and distribution

Four adult (2 males and 2 females) NIDs were housed in the dog kennel of Veterinary Teaching Hospital and were acclimatised for two weeks prior to the commencement of the study. They were fed maize, beans, soft bones, beef, blood meal, cooked with palm/vegetable oil and water provided *ad libitum*. During the conditioning period, the dogs were examined clinically by physical and laboratory examinations. The dogs were screened for ecto- and endo-parasites. Ticks and fleas were sent to entomology laboratory for parasites identification. Blood samples (whole, plasma and serum) collected through cephalic venopuncture were sent to protozoology (for haemoparasites screening) and clinical pathology laboratories for haemogram; faecal samples collected per rectum were sent to helminthology laboratory for enteroparasites screening; while urine samples collected by cystocentesis were sent to Clinical Pathology Laboratory for urinalysis. Dogs with underlying problems (Table 4.1) were treated accordingly using acaricides (Diazinon dimpylate, DIAZINTOL$^{(R)}$ - Animal Care Services Konsult Ltd., Nigeria; Amitraz (AMITIX$^{(R)}$ - JA

Woerden, Holland) and pyrethrin (ECTOCIDE Soap(R) – Lovet Med Agric Veterinary Nigeria Enterprises, Nigeria); diminazene aceturate (Diminazene(R)- Vetinda Pharmaceuticals Ltd., India) at 4 mg/kg; oxytetracycline L.A(R) (20 mg/kg), sulphadimidine sodium (Sulfanor(R)- Shijiazhuang Guanghua Pharmaceutical Co. Ltd., China) at dose rate of 100 mg/kg, ivermectin (IVERMEC SUPER(R)- Essential Animal Care Co. Ltd., Longquan China) at dosage of 400 µg/kg and praziquantel (PRIZILCIDE(R)- Yanzhou Xier Kangtai Pharmaceutical Co. Ltd., Shangnong China) at 10 mg/kg.

Regular acaricide spraying of the kennels, the environment and the dogs, together with systemic chemotherapy, and acclimatising the dogs before the commencement of the research were performed during the pre-conditioning.

3.2.3 Experimental design

Table 3.1: Identification, description of the surgical techniques and observation of the experimental dogs

Identity	Activity	Observations/Intervention
	Pre-surgical base-line data	Daily rectal temperature, and pulse and respiratory rates. Urinalysis, complete blood count, plasma cytokines and serum biochemistry were evaluated at 3 days interval, for one week.
EC_1 (male) **EC_2 (female)** **EC_3 (male)** **EC_4 (female)**	**Surgical procedure**	For each dog, 3 cm x 5 cm dimension each of colonic sero-muscular pedicle flaps and peritoneal sheath were harvested and used in the formation of composite colo-peritoneal pedicle graft. The composite colonic pedicle graft (15 cm^2) was then used in the repair of a 2 cm x 4 cm (8 cm^2), surgically created urinary bladder defect on the dorsal aspect of the bladder.
	Post-surgical evaluations	Daily physical examination, rectal temperature, and pulse and respiratory rates for the first three weeks post-operatively, then weekly for the subsequent seven more weeks. Urinalysis, complete blood count and serum biochemistry were conducted twice weekly for three weeks, then weekly for the next three weeks and fortnightly for eight more weeks post-operatively. Plasma cytokines were assayed daily for seven days post-operative and then every-other-day until four weeks post-operatively. Ultrasonography (at weeks 1 and 3 post-operative) and cystography (at weeks 2, 10, 14 and 108 post-operative) were performed. Histology of the composite colo-peritono-cystoplastic site post-operative at weeks 14 and 108.

Keys: EC = Enterocystoplasty; 1, 2, 3 and 4 = Identity of each dog

3.2.4 Pre-surgical considerations

Food and water were withheld for 36 and 12 hours, respectively prior to the surgery. Pre-operative (prophylactic) antibiotic, metronidazole (Trimetro IV(R) - J B Chemicals and Pharmaceuticals Ltd., India) at dose rate of 25 mg/kg intravenously and 25 mg/kg of paracetamol injection (Gocee(R)- Shandong Xier Kangtai Pharmaceutical Co. Ltd., China) intramuscularly were administered, 24 and 6 hours, respectively before commencement of every operative procedure.

3.2.5 Sterile preparation of caudal abdomen

In preparation for surgery, the ventral abdominal region from the xiphoid extending to the perineum, including the stifle joints and 20 cm on either side of the ventral mid-line of each dog was shaved, and scrubbed clean with soap and water (Plates 1A and IB).



Plate IA : Aseptic prapation of caudal abdomen of male dog



Plate IB : Aseptic prapation of caudal abdomen of female dog

3.2.6 Anaesthesia

Each dog was aseptically prepared and anaesthetised (general anaesthesia) for invasive surgery. The surgical procedures were performed under general anaesthesia through a cannulated cephalic venopuncture. Premedication with 0.05 mg/kg of atropine sulphate (Amopin(R) – Yanzhou Xier Kangtai Pharma. Co., Ltd., China) and chlorpromazine hydrochloride (Clomazin(R)-Maxheal Pharmaceuticals, Karnal, India) at 4 mg/kg. Thiopentone sodium (Thiopental(R)- Rotex Medica Trittau, Germany) at a dosage of 15 mg/kg (IV) was used for induction of anaesthesia. The dogs were intubated with size 7.5 endotracheal tubes post-induction to ensure a patent airway and also to prevent aspiration pneumonia. Halothane (Halothane(R) - Nicholas Pharmal India Limited, India) at 2% concentration was employed for maintenance of anaesthesia. The dogs were placed in dorsal recumbency with fore- and hind- limbs extended and restrained on the surgical table (Plates IIA and IIB). The surgical site was fine-swapped with 0.05% chlorhexidine gluconate (Purit(R) – Saro Life Care Ltd., Lagos, Nigeria) and 10% povidone iodine solutions (Wosan(R)-Jawa International Ltd., Lagos, Nigeria) and draped in a rectangular pattern (Plates IIIA and IIIB). Aseptic conditions were adhered to in all cases and throughout the procedures. Intrasurgically, pentazocine injection (2 mg/kg) was administered intravenously.



Plate IIA: A maledog anaesthesised, intubated and connected with anaesthetic machine, restrained in a dorsal recumbency and fine scrubbed ventral abdomen ready to for draping



Plate IIB: A femlale dog anaesthesised, intubated and connected with anaesthetic machine, restrained in a dorsal recumbency and fine scrubbed ventral abdomen ready to for draping



Plate IIIA: Surgical site of a male dog rectangularly draped and instrumentation ready for laparotomy



Plate IIIB: Surgical site of a female dogrectangularly draped and instrumentation ready for laparotomy

3.2.7 Surgical technique/procedure

The abdominal cavity was accessed via caudal mid-ventral abdominal incision (laparotomy), extending cranio-caudally from the umbilicus to the pelvic brim (Plate IVB)as described by Desch and Wagner (1986) and Hassan and Hassan (2003). The incision in males (Plate IVA) was paramedian at the level of prepuce (Muhammad, 2013). Each animal was reclined on the surgical table at an angle 20° C cranially, to allow for abdominal viscera to gravitate cranially (Muhammad, 2013).



Plate IVA: Midline and paramedian abdominal incision in a male dog

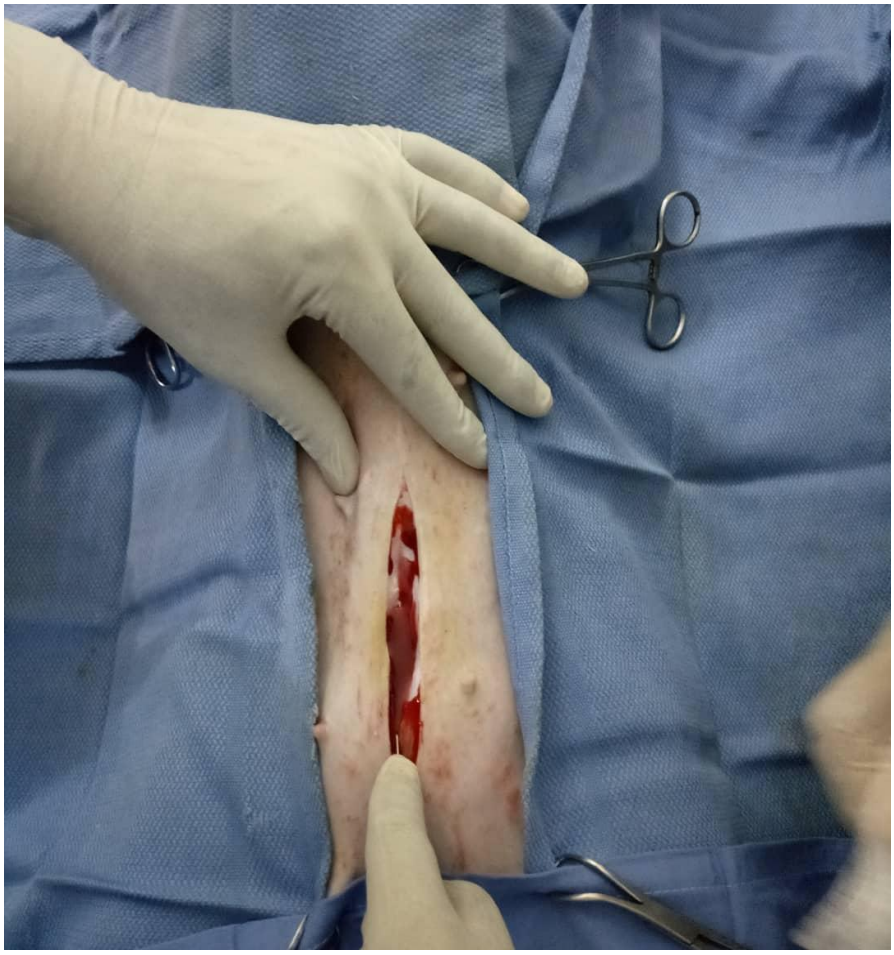


Plate IVB: Midline abdominal incisionin a female dog

3.2.7.1 Preparation of colonic pedicle flap

A size 24 Fr Foley catheter was inserted rectally and advanced to the proposed site of sero-muscular flap harvest in the colon(Plate VA). The cuff of the Foley catheter was inflated with physiologic saline solution to a considerable size to provide support and firmness to the colon (Plate VB). The pre-determined sero-muscular layer of the colon (PlatesVC and VD) was incised lengthwise and transversely (using a sterile No. 15 scalpel or a micro-blade) from the ante-mesenteric border(Plate VIA and VIB), four stay sutures preplaced at the 4 different angles of the incised sero-muscular layer (Plate VIC) and bluntly detached (using a blunt-blunt scissors) from the *lamina propria* as shown in Plates VIIA and VIIB. Saline-adrenaline soaked gauze was used to wrap the pedicle flap (Plate VIIIB). The sero-muscular edges from the remaining bowel were then apposed and sutured (using size 2/0 Ethicon-Vicryl$^{(R)}$), ‘‘intussuscepting’’ the deseromuscularised mucosa(Plate VIIIA). The mesenteric defect was also closed (with 2/0 Ethicon-Vicryl$^{(R)}$) to prevent internal hernia.



Plate VA: Colon preparation for sero-muscular harvest. Insertion of Foley catheter per rectumwhich was advanced to the selected colonic segment

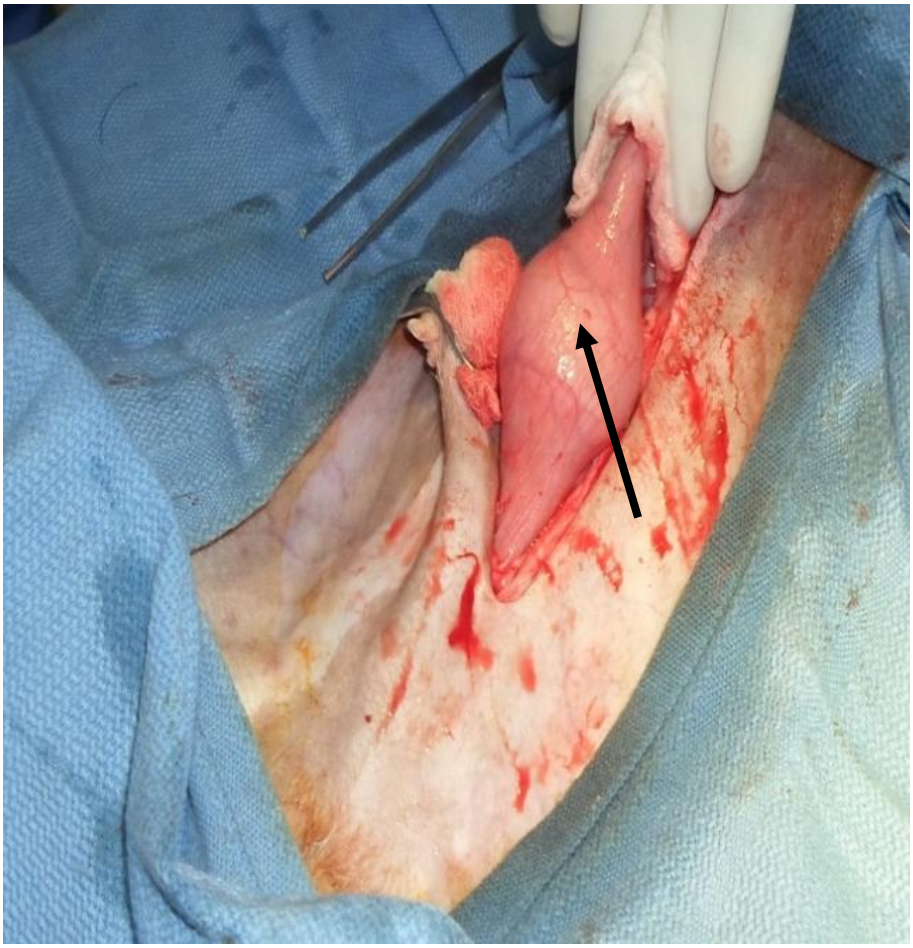


Plate VB: Selected colonic segment, inflated (arrowed) to provide firmness to the colon during measurement, marking and sero-muscular harvest

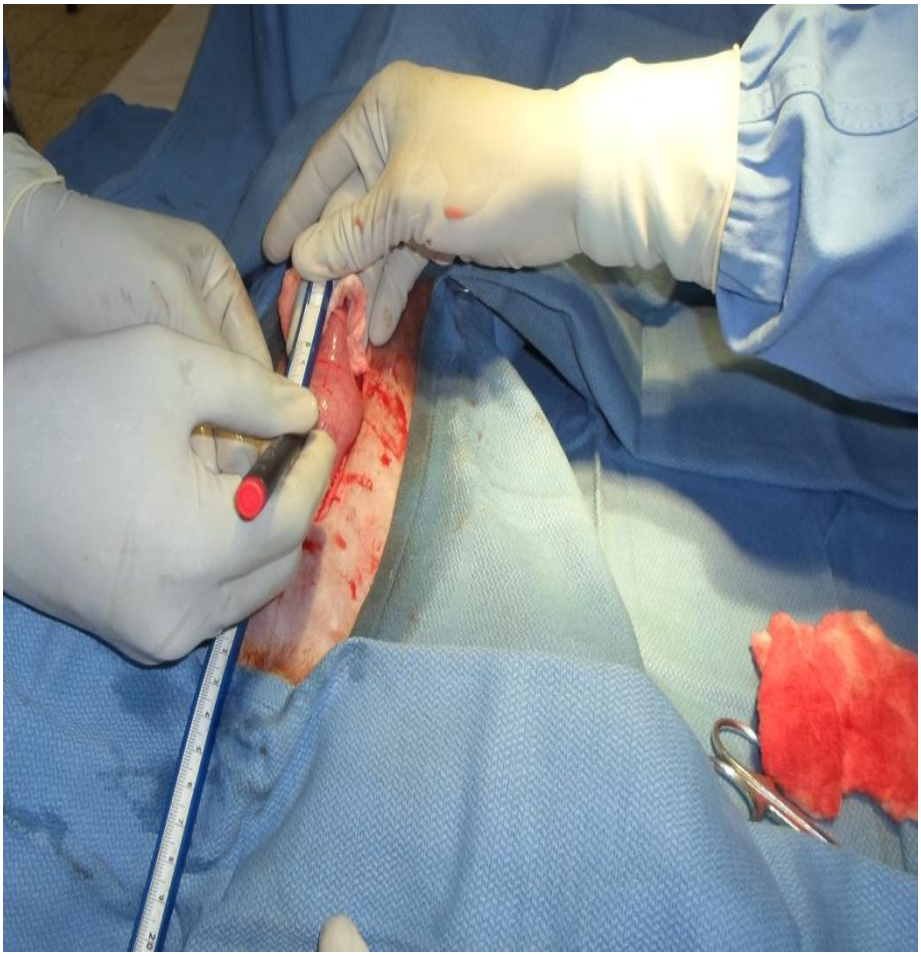


Plate VC: Measurement and marking of thecolonic sero-muscular layer to be harvested

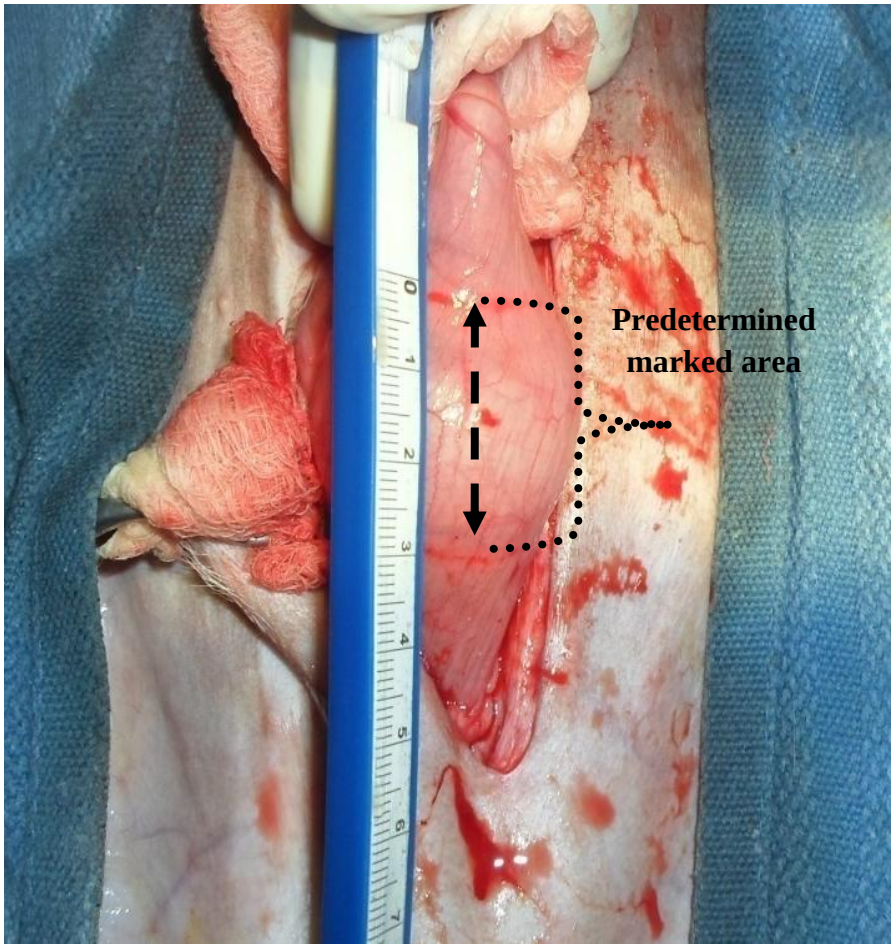


Plate VD: Measured and marking colonic sero-muscular tissue in preparation for sero-muscular harvest

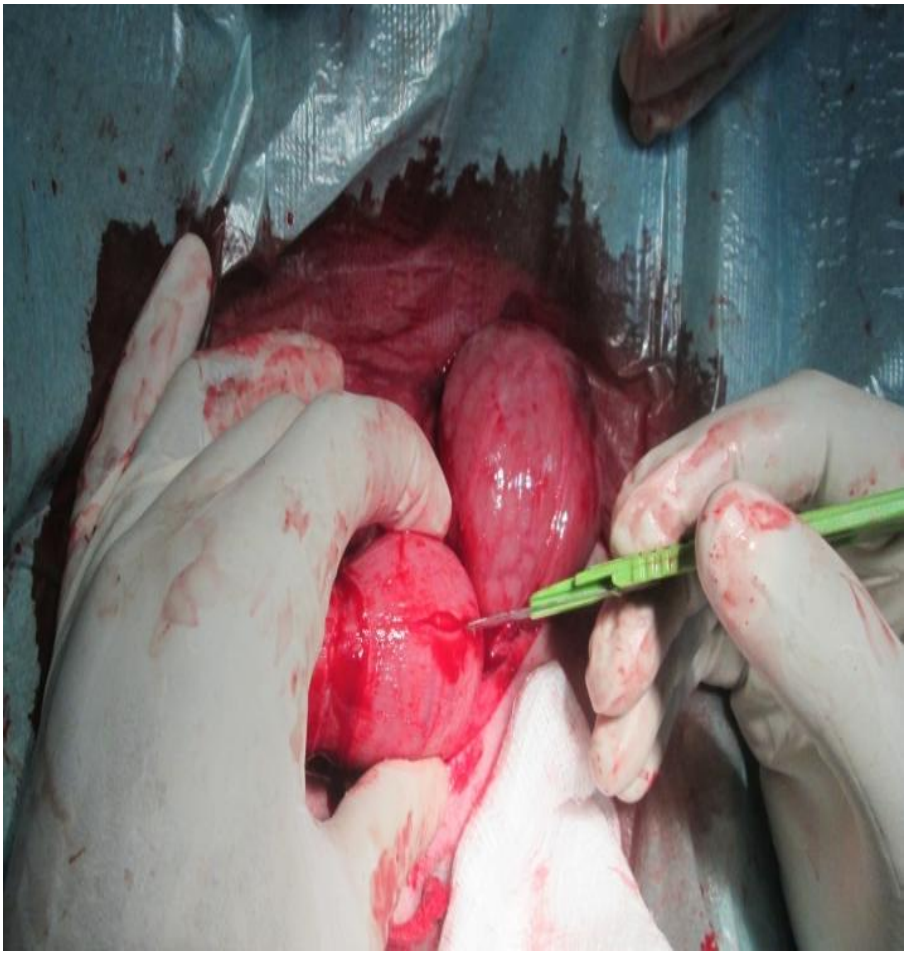


Plate VIA: Seromuscular incisions, incising colon along the marked borders

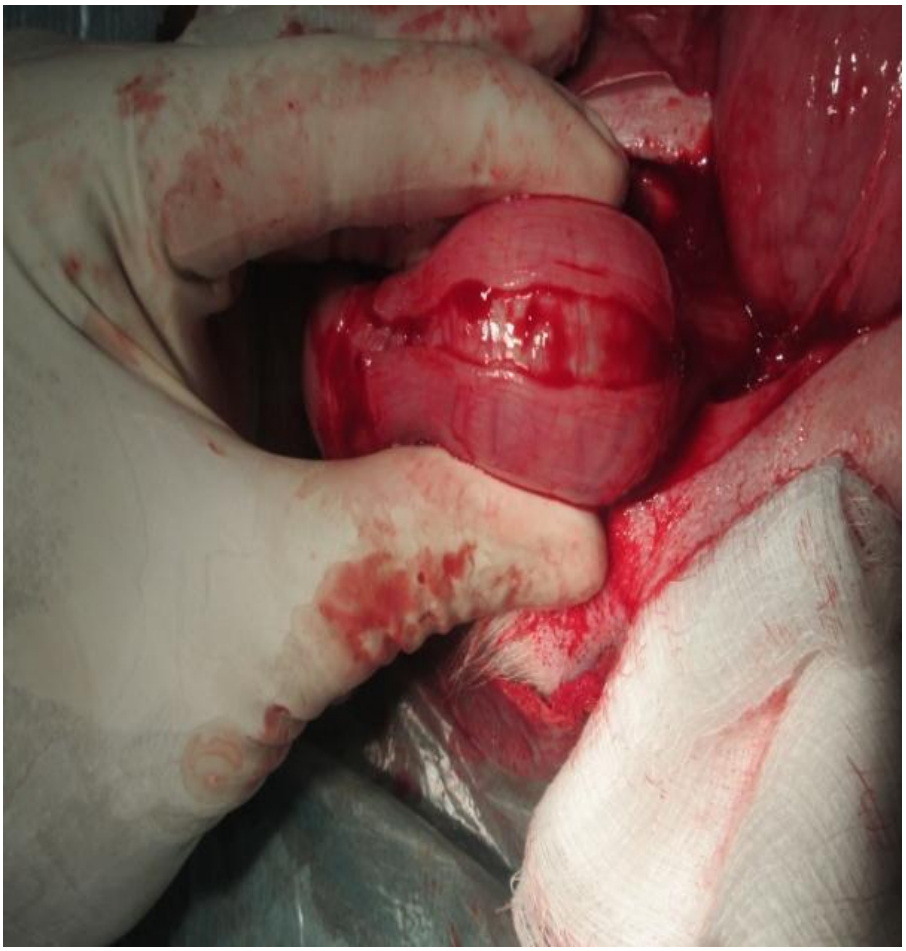


Plate VIB: De-tubularisation of the predetermined colonic sero-muscular segment at the antemessenteric border



Plate VIC: Preplaced stay sutures at the 4 different angles on the ante-mesenterc surface of the incised sero-muscular layerof the colon to aid harvest.

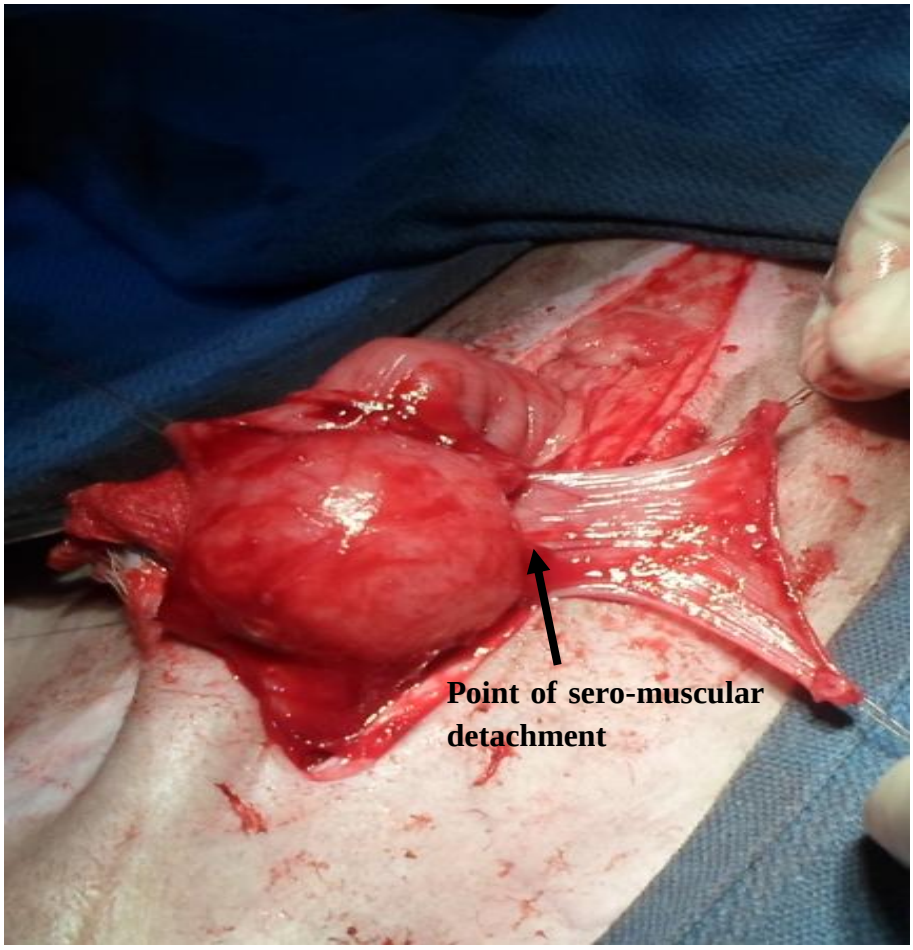


Plate VIIA: Blunt separation (dissection) of the colonic sero-muscular layer from its *lamina propria*

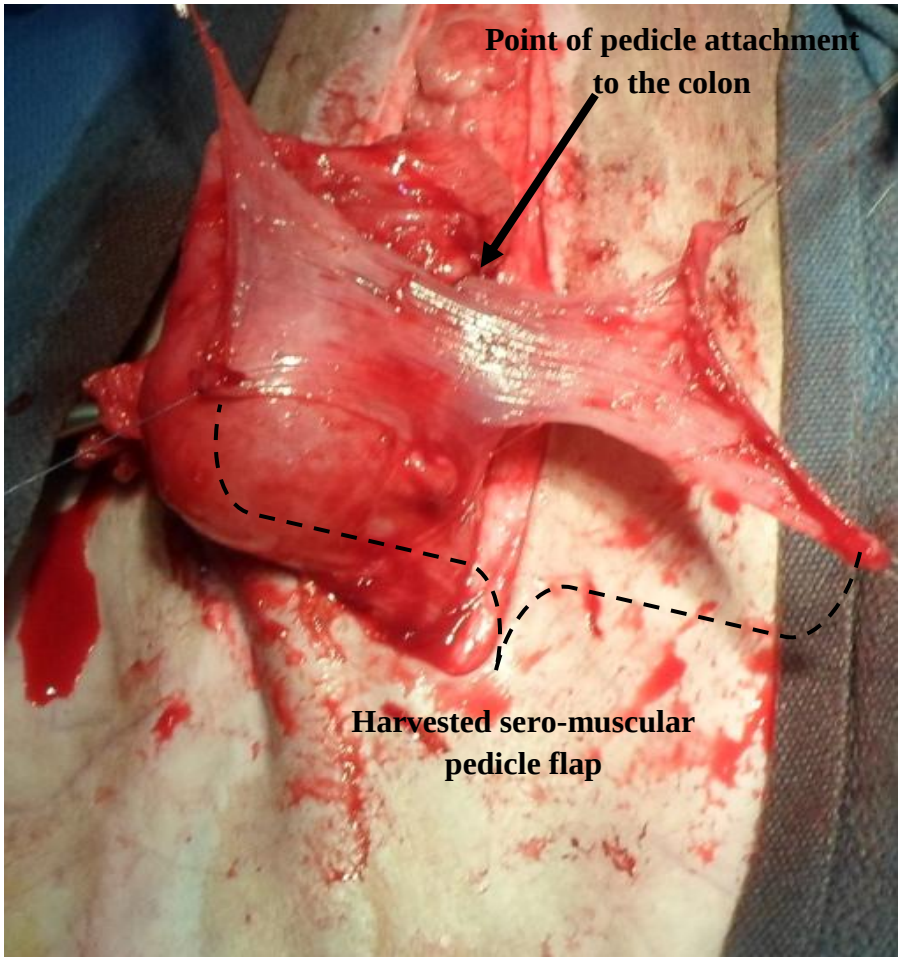


Plate VIIB: Harvested colonic sero-muscular layer with its preserved vascular supply

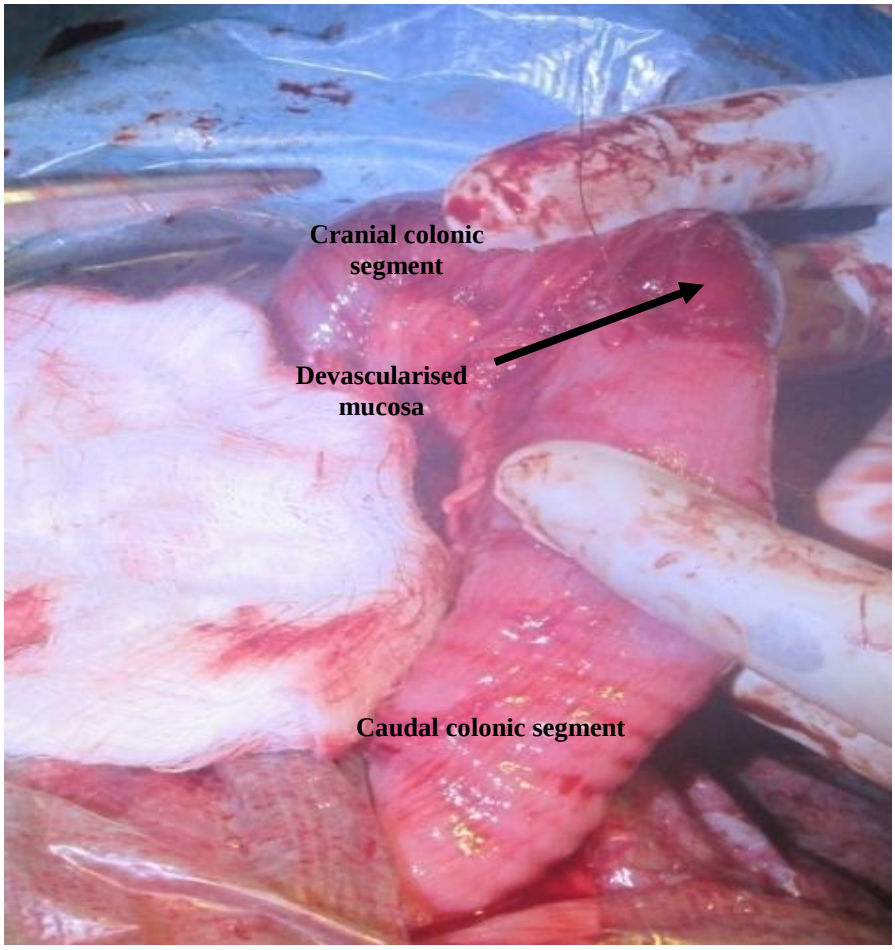


Plate VIIIA: Apposition of sero-muscular edges of the remaining (cranial and dcaudal) colonic segments using size 2/0 Ethicon-Vicryl(R), ‘‘intussuscepting’’ the desero-muscularised mucosa

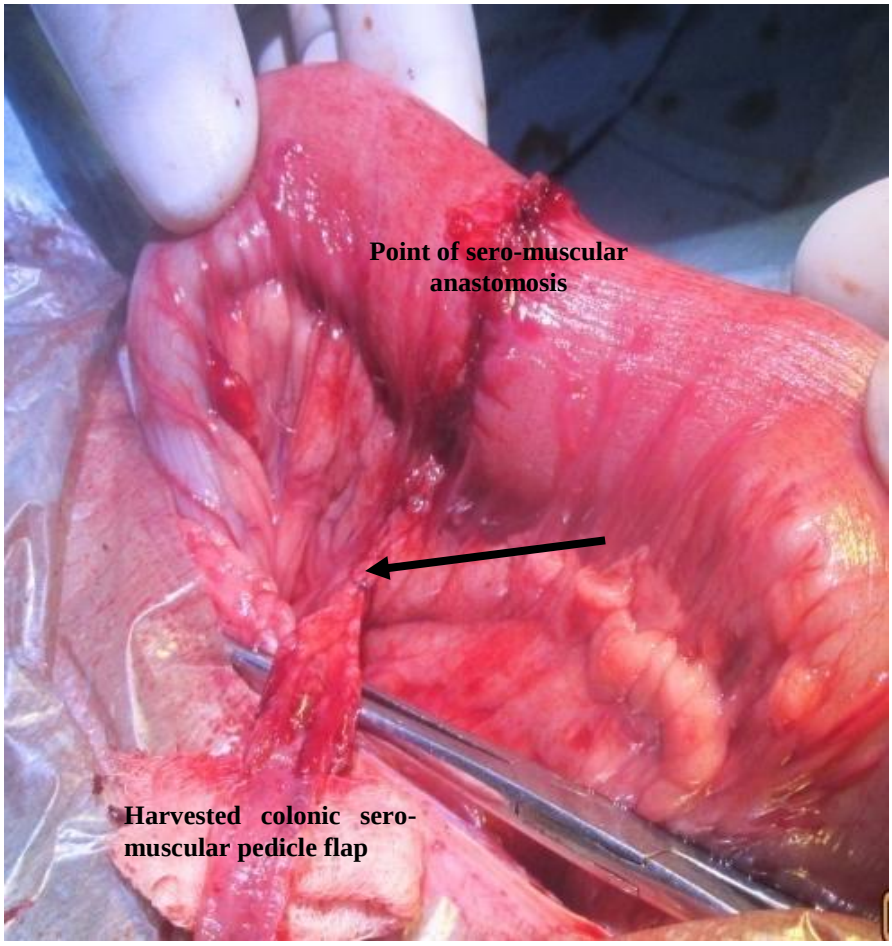


Plate VIIIB: Harvested and preserved colonic sero-muscular pedicle (arrowed) flap, wrapped around saline-soaked gauze

3.2.7.2 Preparation of free peritoneal flap

The desired graft size of the parietal peritoneum caudal to the umbilicus was exposed, measured and marked with a surgical pen (Plate IXA). Stay sutures were then preplaced (at 4 different angles) connecting the marked borders of the graft site. Linear incisions (joining the marked borders), using a No. 15 scalpel or a micro-blade, were made on the marked borders and bluntly advanced laterally (using curved iris scissors), thereby lifting the peritoneum off the underlying muscular tissue and finally resected (Plate IXB). The harvested, full-thickness, free peritoneal graft was immediately placed on saline-soaked gauze. A section was taken and preserved in 10 % buffered formalin (for histology). Larger section was immediately transplanted to the harvested sero-muscular colonic pedicle flap on the same dog to form a composite tissue (3.2.6.3). The donor site was reconstructed during abdominal (laparotomy) closue. The peritoneum was apposed along during closure of the *linea alba*, thereby overlaying the donor (exposed abdominal muscle) site.



Plate IXA: Measured and marked desired parietal peritoneum (3 cm x 5 cm) with a surgical pen

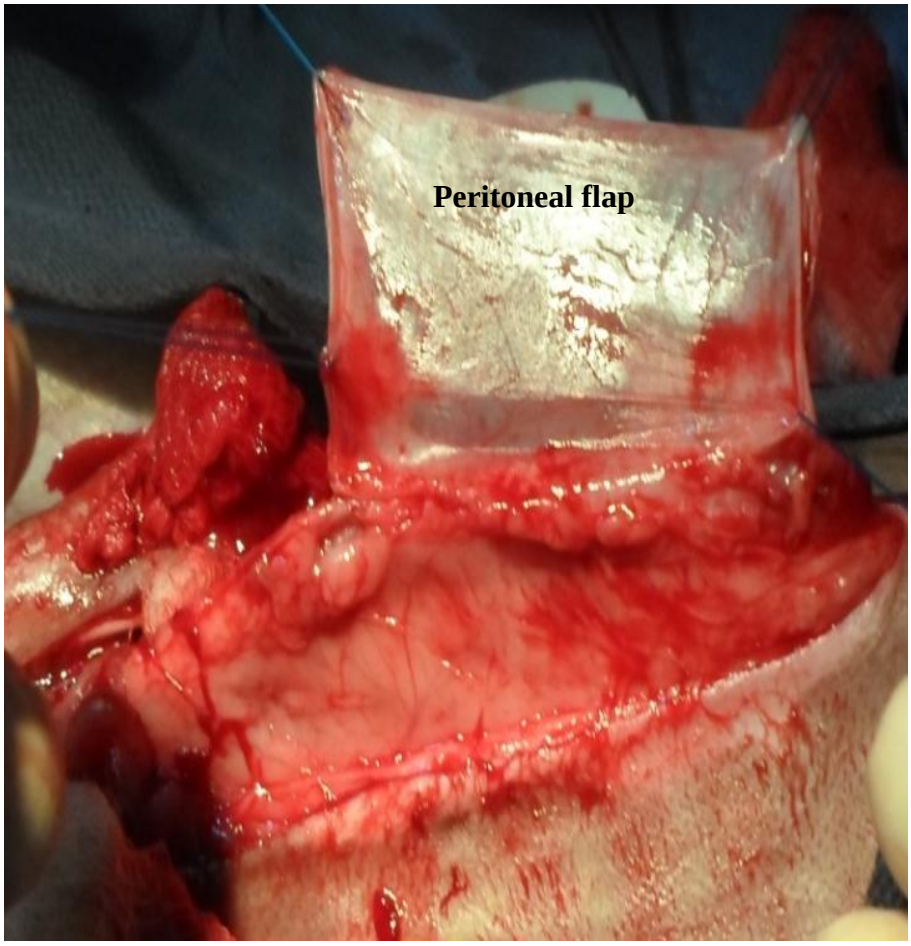


Plate IXB: Preplaced stay sutures (at 4 different angles) on the marked borders and harvest of the peritoneal graft

3.2.7.3 Formation of composite colo-peritoneal graft

The muscular surface of the sero-muscular pedicle flap was apposed (in contact)with the muscular surface of the pre-harvested free parietal peritoneal flap (Plate XA) held together with stay sutures and tissue forceps, as composite (Plate XB). The apposed composite tissue wasthen sutured (secured) using size 4/0 Ethicon-Vicryl$^{(R)}$ in an interrupted pattern to form the composite colo-peritoneal pedicle flap in preparation for grafting (Plate XC). The composite pedicle tissue was then wrapped with saline-soaked gauze.

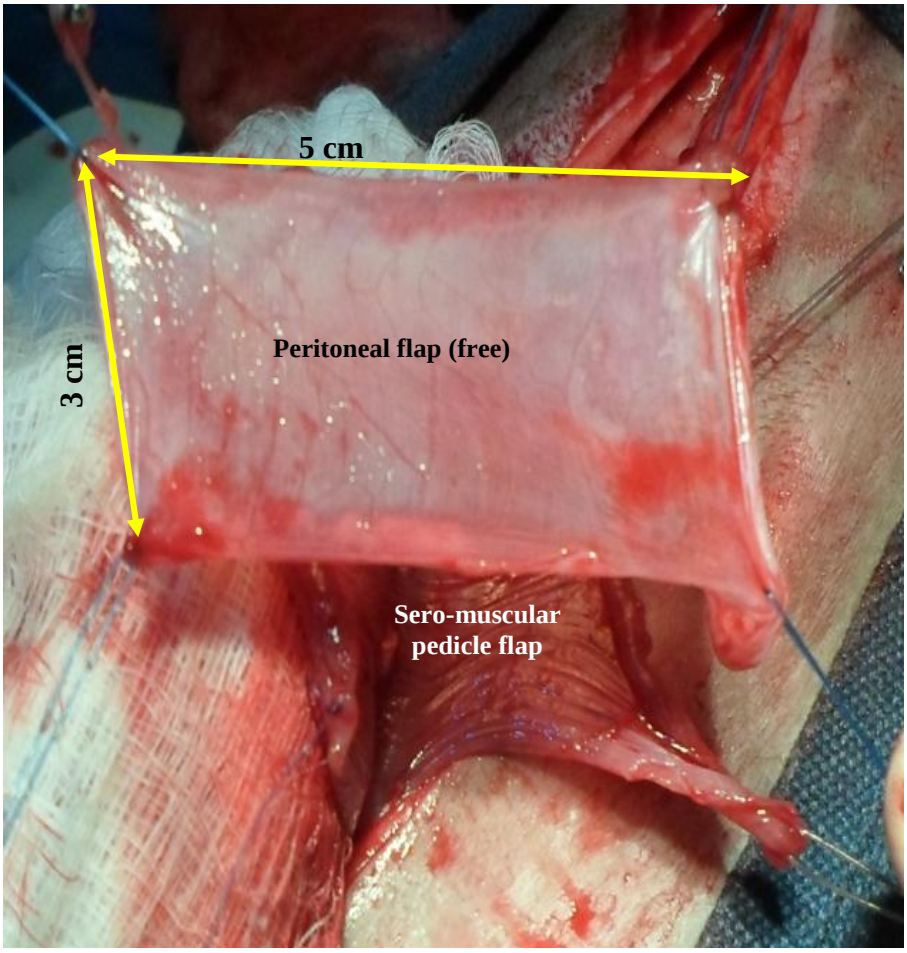


Plate XA: Formation of composite colo-peritoneal pedicle flap: Muscular surfeac of seromuscular pedicle flap in contact with the muscular surface of the pre-harvested peritoneal flap

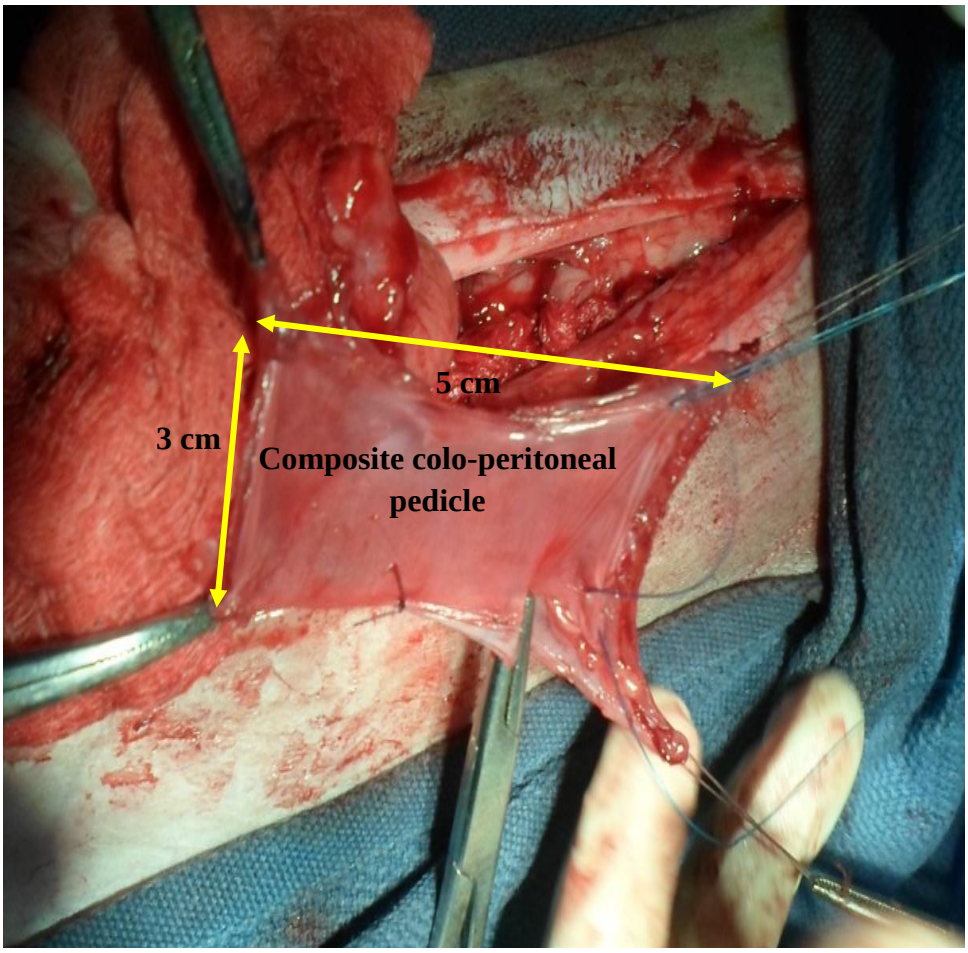


Plate XB: Peritoneal sheath apposed with sero-muscular colonic pedicle flap in preparation for placement of interrupted sutures to hold the tissues as "a composite colo-peritoneal" pedicle graft

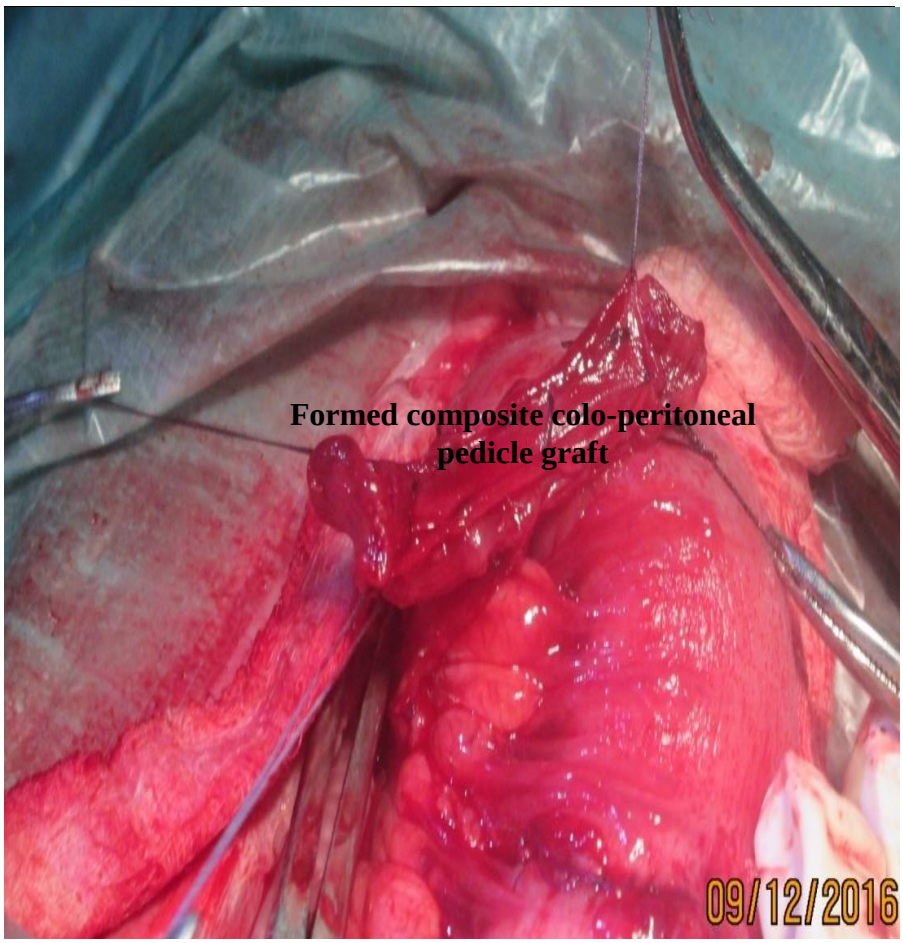


Plate XC: Formed composite colo-peritoneal pedicle flap in preparation for grafting

3.2.7.4 Preparation of urinary bladder graft bed

The urinary bladder was then isolated, exteriorised and positioned to expose dorsal surface (Plate XIA). Thereafter, stay sutures were placed on the apex and the body (Plate XIB) as described by Desch and Wagner (1986), a predetermined 2 cm x 4 cm dimension (Plate XIC) of a full thickness rectangular flap was excised (Plate XID) on the dorsal surface (partial cystectomy).The bladder was then emptied of urine and blood using an electric suction machine during bladder wall resection. The urinary bladder was packed with moist saline soaked gauze to control haemorrhage and spillage of urine. The excised piece was preserved in 10% formalin for histological evaluation.

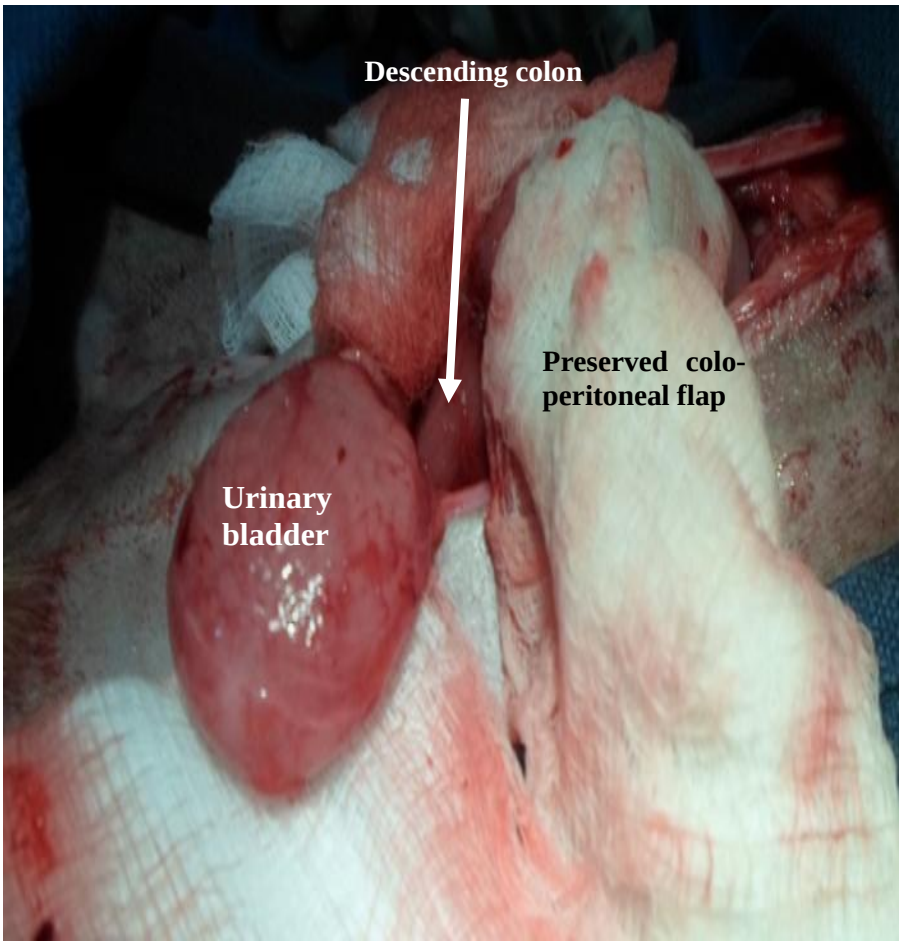


Plate XIA: Urinary bladder exteriorised and the dorsum exposed

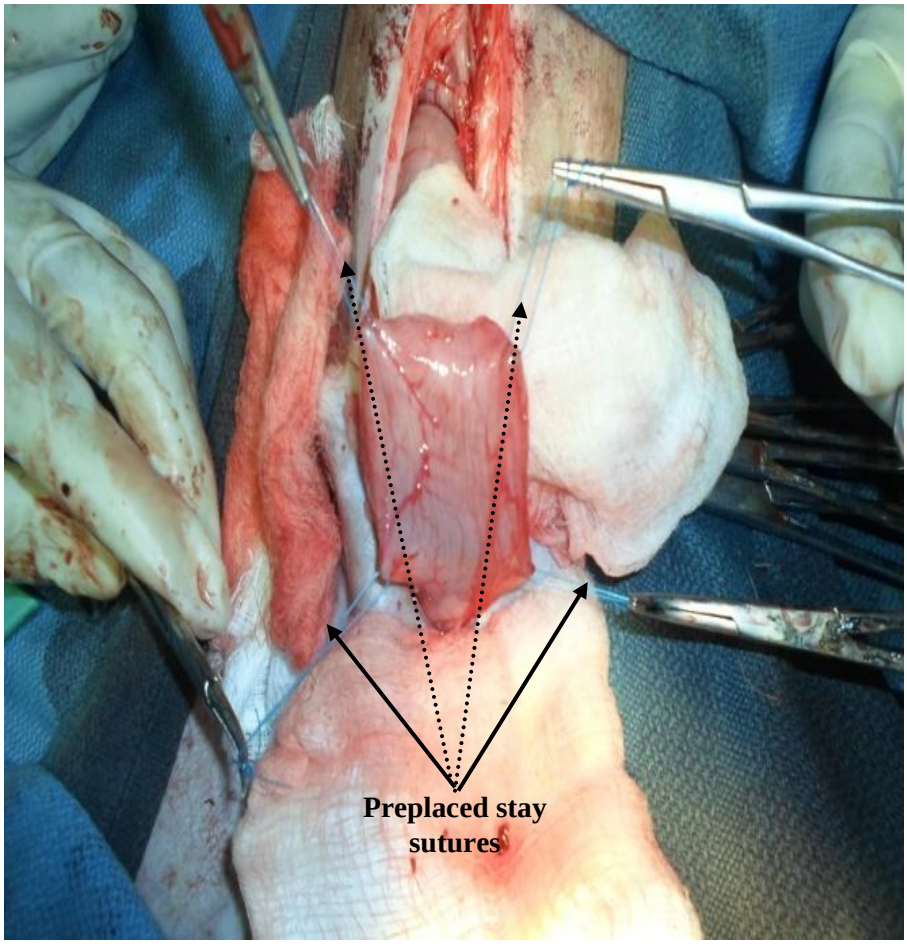


Plate XIB: Preplacement of 4 stay sutures on the exposeddorsum of the urinary bladder

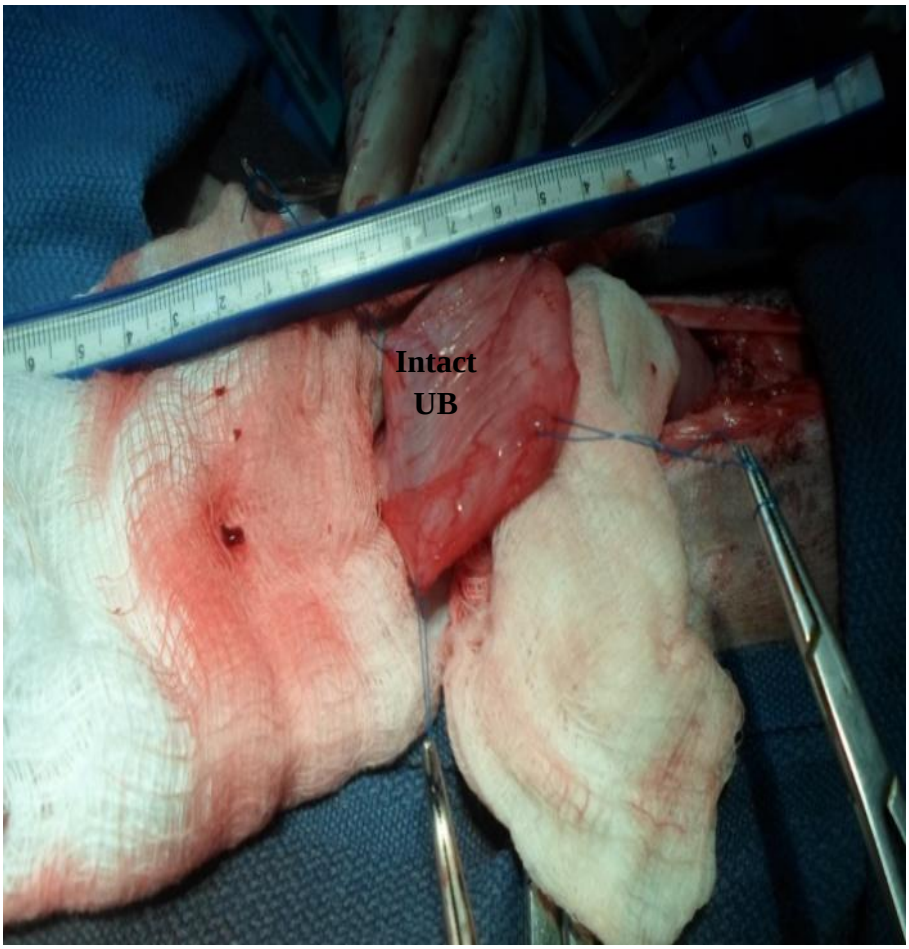


Plate XIC: Measured 2 cm x 4 cm rectangular dimension

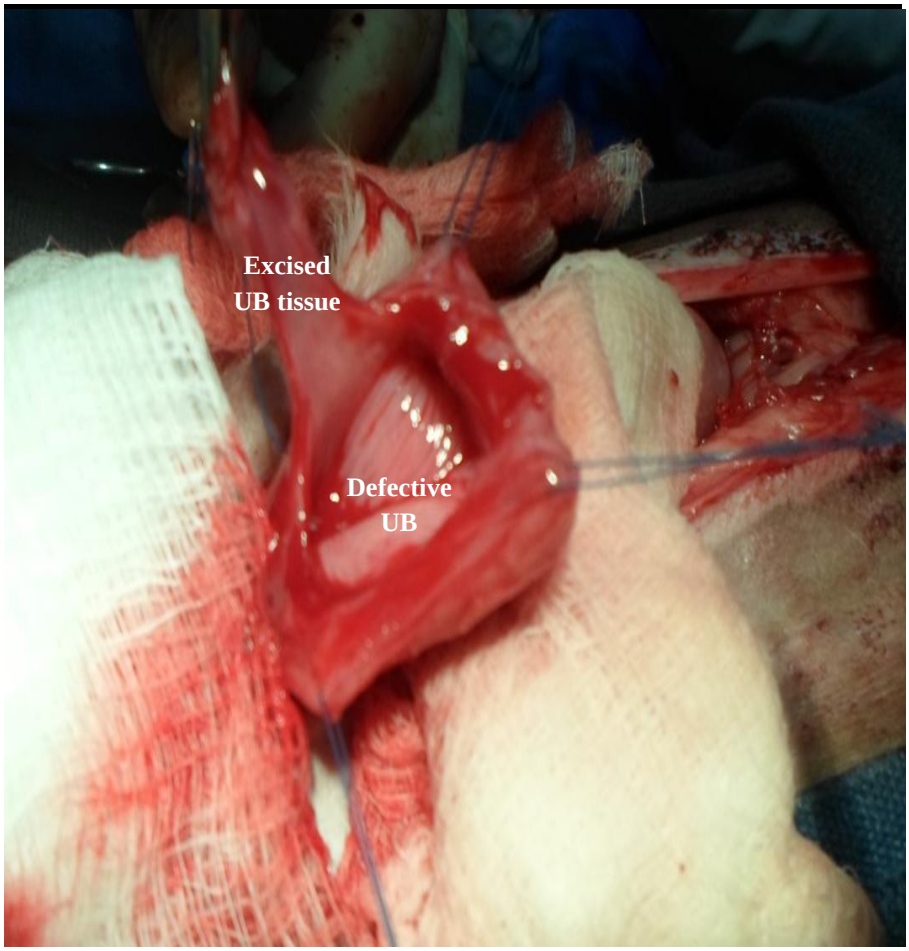


Plate XID: Partial cystectomy: Rectangular (12 cm perimeter) urinary bladder defect in preparation for reconstruction (Colo-peritoneo-cystoplasty)

3.2.7.5 Urinary bladder repair

The composite colo-peritoneal pedicle (accompanied by its mesenteric supply)flap wasfinely ‘tailored’ and grafted to the defective site of the urinary bladder to correct/repair the defect by the initial placement of 4 interrupted sutures anchoring the colo-peritoneal tissue at the 4 right angles of the defect in preparation for the bladder reconstruction (colo-peritoneo-cystoplasty) using size 4/0 Vicryl$^{(R)}$ (Plate XIIA). The moistened gauze was then removed and simple continuous (interlocked after every 3 bites) suture pattern using size 4/0 Vicryl$^{(R)}$ was employed to close the bladder defect (Plate XIIA). The urinary bladder of each dog was catheterised transabdominally (Plate XIIB) before the final closure of the defect with a 2-way standard Foley catheter (10FR) (Becton$^{(R)}$- Zhanjiang Star Enterprise Co. Ltd., Zhanjiang, China). The cuff of the catheter was inflated to occupy the urinary bladder cavity and make the bladder wall turgid, in order to drain the bladder post-surgery(Plate XIIC).The suturing was continued (Plate XIID)and the bladder defect closed into its initial water-tight state (Plates XIIIA and XIIIB). The Foley catheter was connected to the tube of sterile urine collection bag (U-MEC, DRAINAGE BAG$^{(R)}$ - Jiangsu Kaishou Medical Apparatus Co. Ltd., China) for periodic urine collection(14 to 28 days) after surgery (Plate XIV) and occasional bladder flushing.

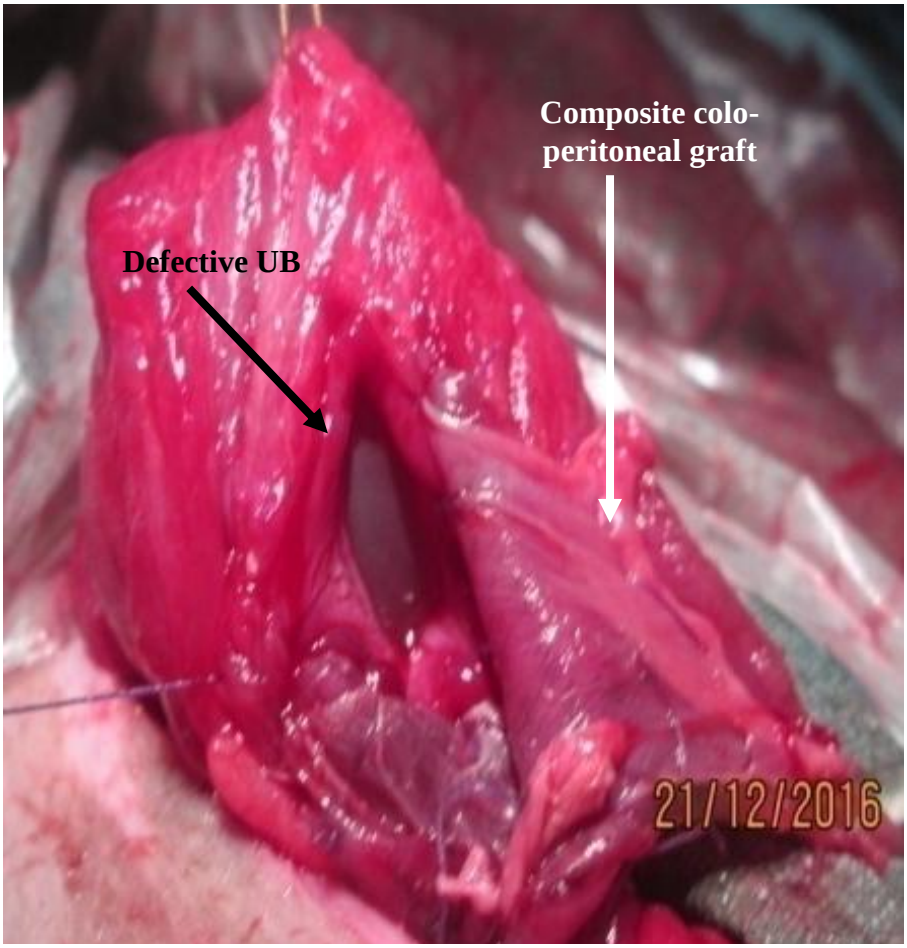


Plate XIIA: Colo-peritoneo-cystoplasty: Reconstruction of 2 cm x 4 cm urinary bladder defect with 3 cm x 5 cm composite colo-peritoneal pedicle tissue using size 4/0 Vicryl$^{(R)}$

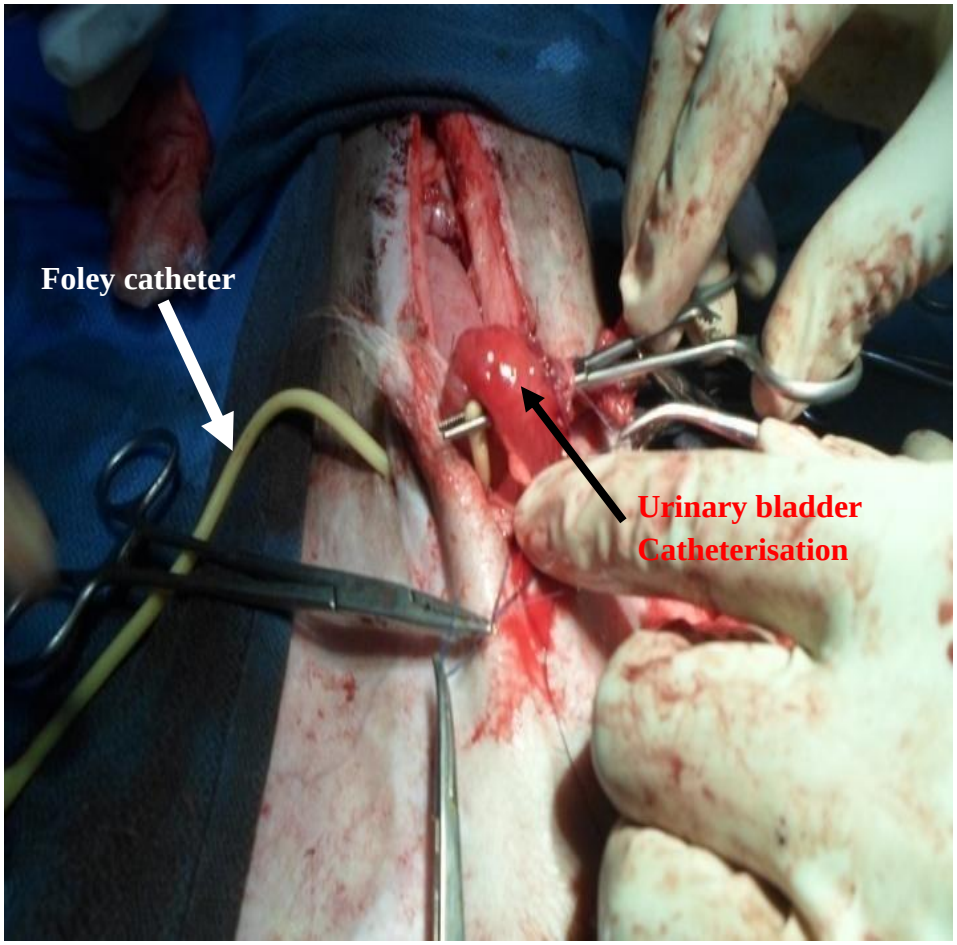


Plate XIIB: Transabdominal catheterization using in-dwelling, 2-way Foley catheter (10FR)

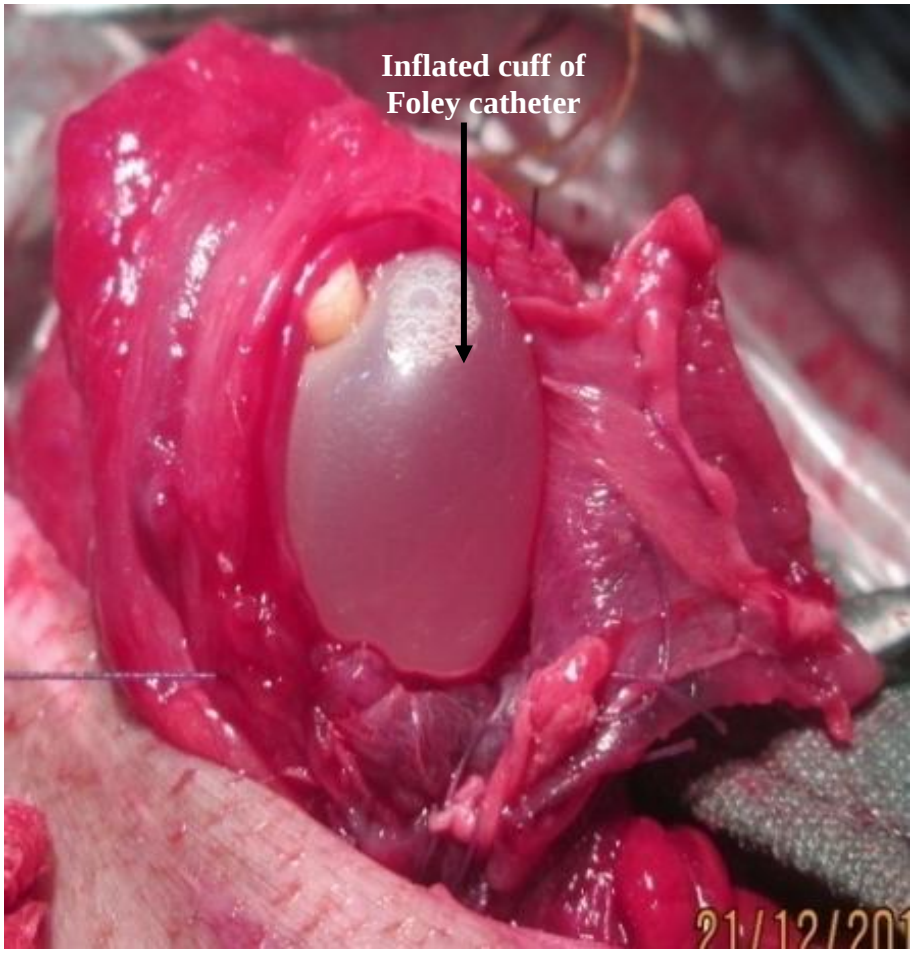


Plate XIIC: Inflated cuff of the in-dwelling, 2-way Foley catheter (10FR) intra-cystic making the bladder wall turgid

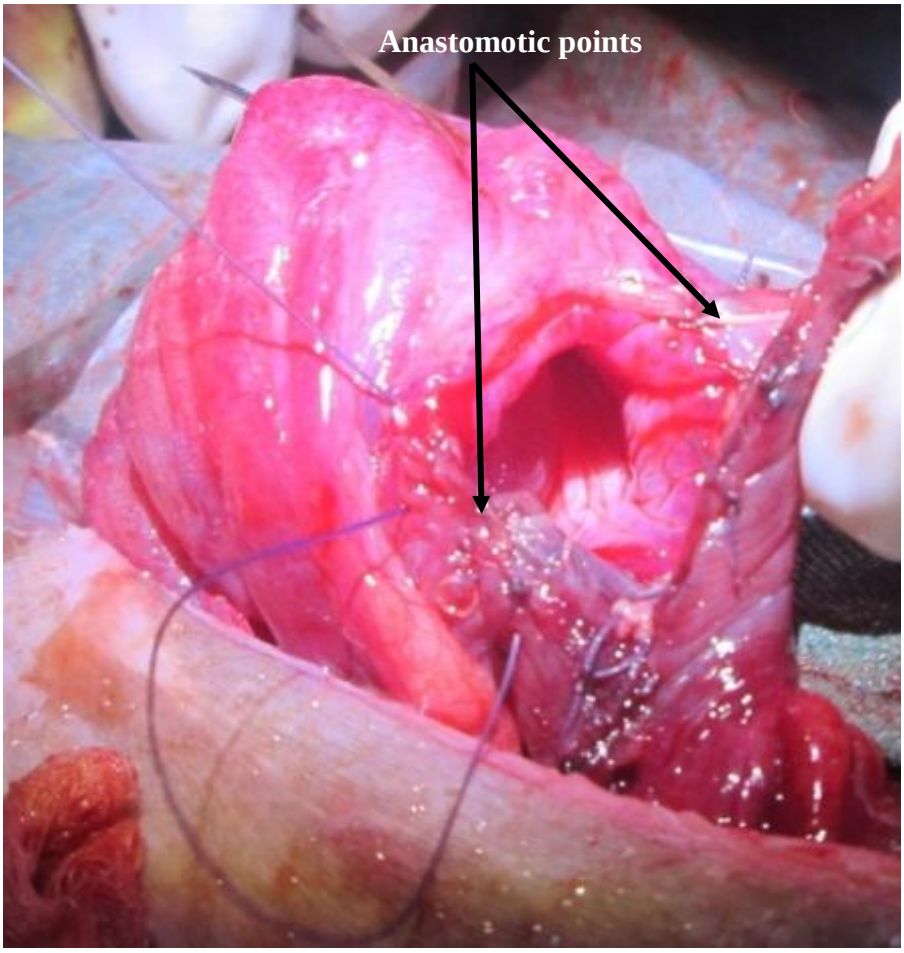


Plate XIID: Anastomotic point of the composite colo-peritoneo-cystoplasty (reconstructed urinary bladder)

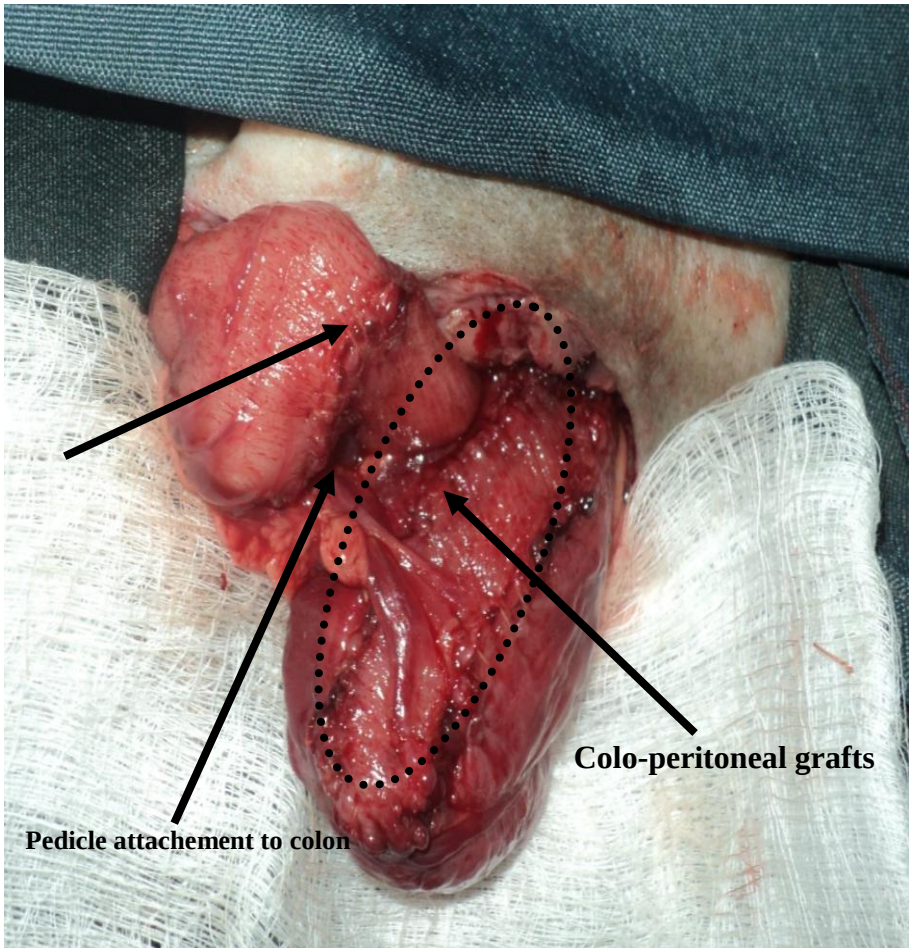


Plate XIIIA: Reconstructed of 2 cm x 4 cm urinary bladder defect with 3 cm x 5 cm composite colo-peritoneal pedicle graft in a female dog, seen engorged with inflated Foley catheter (10FR) intra-cystic and anastomotic point of the colonic harvest (arrowed) on the colon

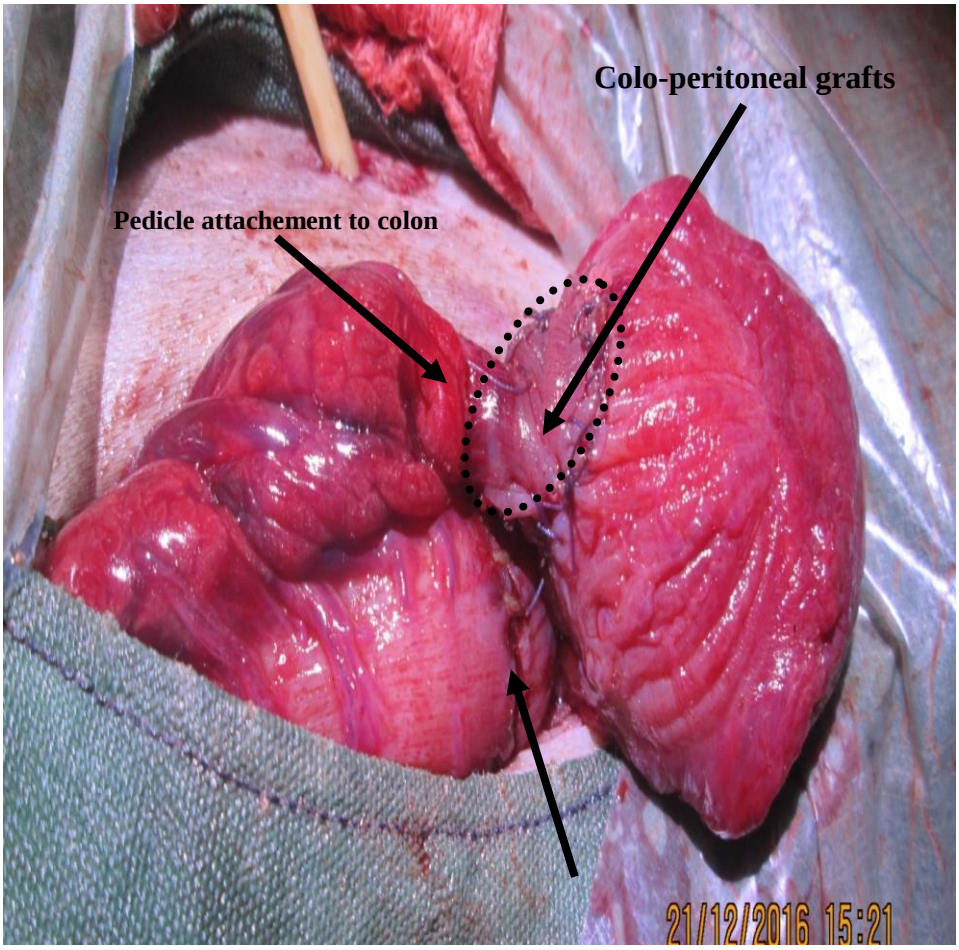


Plate XIIIB: Reconstructed of 2 cm x 4 cm urinary bladder defect with 3 cm x 5 cm composite colo-peritoneal pedicle graft in a male dog, seen engorged with inflated Foley catheter (10FR) intra-cystic and anastomotic point of the colonic harvest (arrowed) on the colon

Abdominal closure was performed in three layers using size 1/0 Ethicon-Vicryl(R) (Polyglactin 910- Johnson-Johnson Jult, Belgium), size 1 Ethicon-Vicryl(R) (Polyglactin 910- Johnson-Johnson Jult, Belgium) and size 1 Nylon (OGOTEX, NYLON MONOFILAMENT(R)- Shenzhen Runch Industrial Corporation China) for the *lineaalba*, subcutis and skin, respectively (Plate XIV). The dogs were monitored until recovery from anaesthesia (Plates XVA and XVB), after which they were moved to their individual kennels for post-operative recovery and monitoring (Plate XVIA and XVIB).

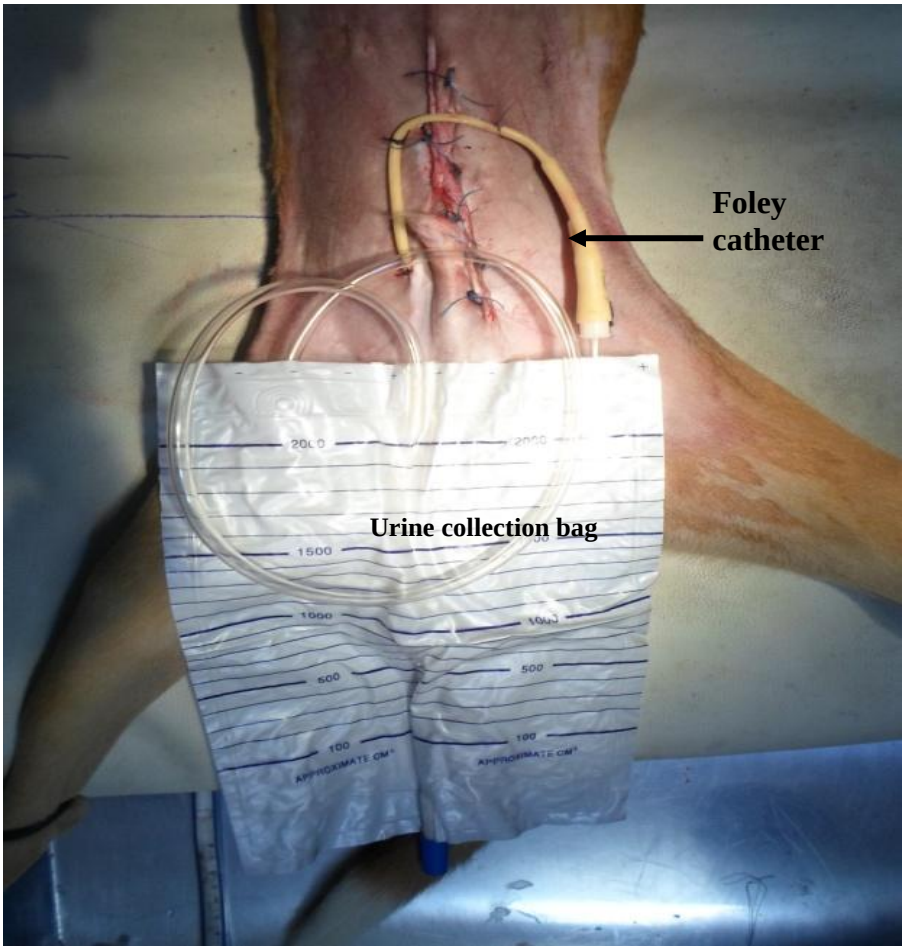


Plate XIV: Sutured laparotomy defect and appropriate anchorage placement of Foley catheter to the abdominal wall



Plate XVA: A female dog being monitored for full recovery from anaesthesia after the colo-peritoneo-cystoplasty



Plate XVB: Adog (male) being monitored for full recovery from anaesthesia in a recovery area



Plate XVIA: A male dog in the kennel after recovering from anaesthesia for post-operative recovery and monitoring



Plate XVIB: The female dog in thekennel after recovering from anaesthesia for post-operative recovery and monitoring

3.2.8 Post-surgical care and evaluation

An Elizabethan collar (improvised) was attached to the collar belt for each dog to prevent interference with the surgical site. The animals were maintained daily on 50% dextrose (DANA Pharmaceuticals Ltd., Nigeria), dextrose-saline (DANA Pharmaceuticals Ltd., Nigeria) solution and metronidazole (Trimetro IV$^{(R)}$ - J B Chemicals and Pharmaceuticals Ltd., India) at dose rate of 25 mg/kg intravenously for a period of 5 days intravenously. Feeding with bland diet (a paste composed of water, ground millet and guinea corn, sugar, blood meal and fried fish) commenced on the 5th day. Pentazocine injection (2 mg/kg) was administered intramuscularly for 5 days post-surgery. Urine collected in the urine-collection bag was emptied every 12 hours for 2 weeks, and the urine-collection bag was changed every other day. The Foley catheters were removed between weeks 2 and 4 post-surgery. The dogs were monitored daily and evaluated twice weekly for the first 3 weeks post-surgically by physical examination, urinalysis, complete blood count, cytokine, blood chemistry and weekly diagnostic imaging evaluations. The evaluations were done once weekly for three weeks consecutively, and then subsequently once fortnightly for additional 8 weeks. Tissue section (biopsy) was taken at week 14 post-reconstruction.

3.2.8.1 Clinical examination

Post-surgically, the physical examination included the visual evaluation of each dog through observation of the general body condition, behaviour, attitude, posture, ambulation, and respiratory pattern for the first three weeks daily. The examination was subsequently done weekly for seven more weeks. During this time, each dog was placed on the examination table and vital parameters (rectal temperature, pulse and respiratory rates) were recorded using standard procedures.

3.2.8.2 Urinalysis

Five millilitres of urine was aseptically collected from each dog twice weekly for 3 weeks post-surgically, into a clean sterile sample bottle for analysis by catheterisation. The sampling was thereafter done weekly for the next three weeks and subsequently once in two weeks for further 8 weeks. The urine sample was evaluated immediately within 60 minutes of collection or stored at 4 ^{0}C. The sample was gently mixed, then transferred into the conical tube and evaluated for gross appearance. Two different urine analysis test strips, namely: Medi-Test Combi 9$^{(R)}$ (Macherey-Nagel GmbH, Germany) and ACCU-ANSWER$^{(R)}$ Uric 11V (Fas Test Tech Limited, London) were used for the urinalysis according to the manufacturer's instructions. The urinalysis test strip (reagent area) was immersed completely into the urine specimen for 1-2 seconds, after which it was drawn out immediately across the brim of the conical tube to remove excess urine and timed for 30-60 seconds. The reagent area was then compared to its corresponding colour blocks on the colour scale/chart on the urine analysis test strips' container, and values were recorded (Michael, 2011).

3.2.8.3 Blood Evaluations

Eight milliliters of blood collected from each dog through cephalic venopuncture was divided into three (3) sterile screw-capped test tubes with two of the tubes containing potassium ethylenediaminetetraacetic acid (EDTA) and the other was plain. Two milliliters of whole blood collected in one of the tubes was used for haematology. For plasma harvest and cytokine assay, 3 ml of whole blood was used. Serum was harvested from the 3-ml blood, which was allowed to clot from the plain test-tube for serum biochemical tests.

Haematology*:* Haematological parameters evaluated were packed cell volume (PCV) using Wintrobe's haematocrit tube method, haemoglobin (Hb) concentration by Sahli's acid haematin method, total leucocyte count (TLC) by haemocytometer, and differential leucocyte count by Leishman's stain (Miller *etal*., 1989).

Cytokineassay:For the plasma harvest, 3 ml of anticoagulated blood was centrifuged at 2000 x g for 10 minutes using automated centrifuge. The plasma harvested from each blood sample was carefully extracted into 2-ml sterile plastic tubes, and stored at -20^{0}C until used for cytokine assay (Masih, 2011). The cytokine (IL-6 and IL-10) assay in the plasma was carried out using enzyme-linked immunosorbent assay kits according to the manufacturer's instructions (Wuhan Fine Biotech Co., Ltd., China).

Assay procedure: The materials were first equilibrated; plasma samples were placed in 96 well plates strips (pre-coated with specific Canine cytokine antibody for IL-6 or IL-10), and prepared reagents and standards were kept under standard room temperature (25 -26 ^{o}C) prior to commencement of the procedure. Serially diluted standards (100µl each) were dispensed into individual 8 wells (of the 96 wells' plate), while the first 2 wells were left as blank. Micropipettes were used to dispense 100µl of the plasma sample separately, into the remaining 86 wells. The plates were then covered and incubated at room temperature for 150

minutes for the sample cytokine to bound immobilised antibody. The wells were washed four times with 1X buffer solution and blot-dried with paper towels.

Prepared biotinylated anti-canine cytokine antibody (100µl) was then added to each well and incubated at room temperature for 60 minutes,and thereafter washed four times as above. After washing away unbound biotinylated antibody, prepared 1X HRP (horseradish perosidase)-conjugated, streptavidin (100µl) was pipetted to the wells and incubated at room temperature for 45 minutes. The wells were again washed four times as above. 100µl of TMB (3,3',5',5'-Tetramethylbenzidine) substrate solution was then added to the wells and incubated at room temperature for 30 minutes to allow colour development in proportion to the amount of cytokine bound. Stop solution (50µl) was dispensed into each well to change the colour from blue to yellow, and the intensity of the colour was measured using spectrophotometer at wavelength of 450 nm. The absorbance (λ) obtained was used in extrapolating the concentration of the corresponding cytokine from graphical standard curve plots (Figure 3.1).

The plasma interleukin 6 (IL-6) and interleukin 10 (IL-10) assayed from each dog during this study was pooled and mean values were calculated and recorded as mean ± standard error of the mean (SEM).

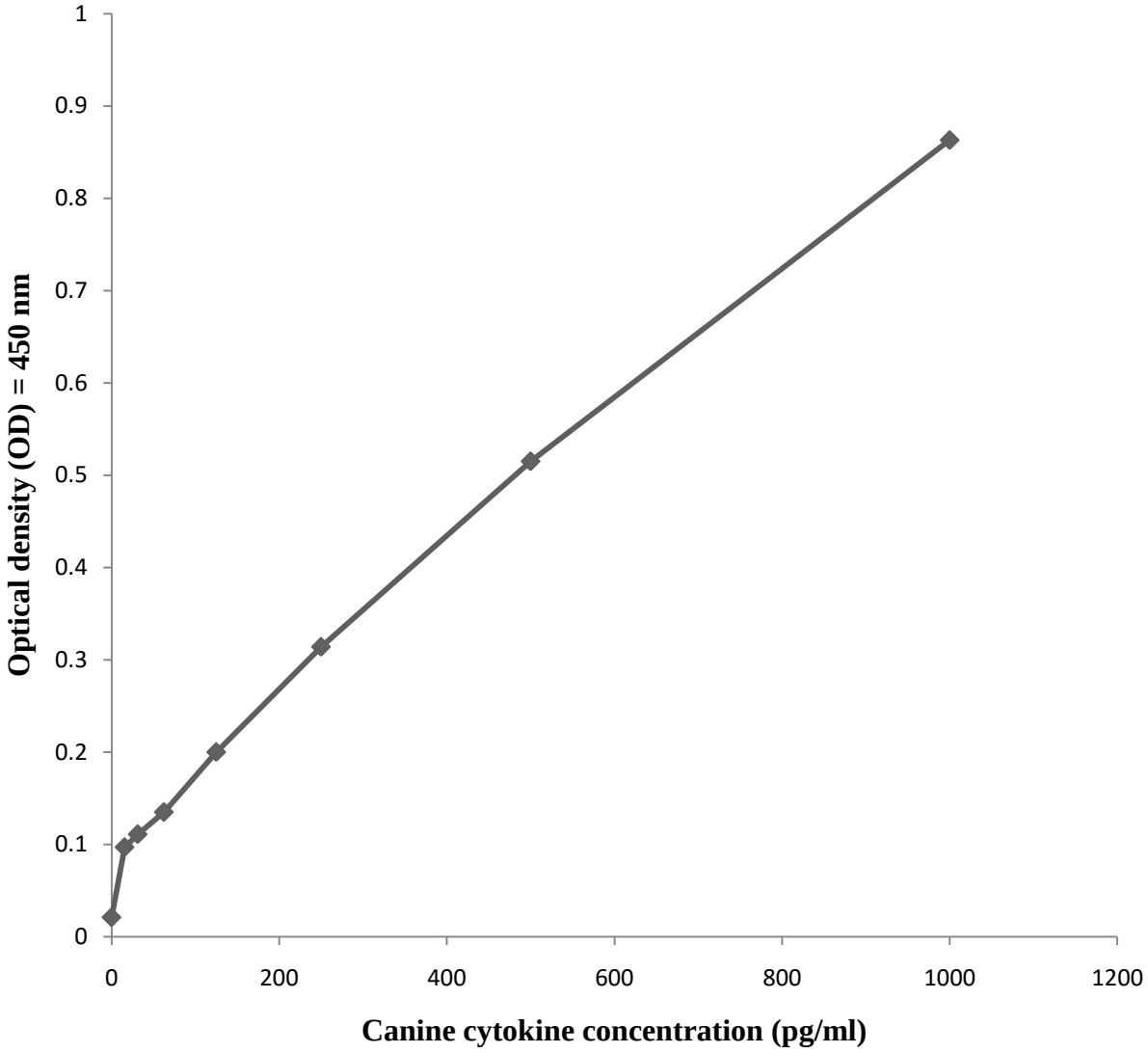


Figure 3.1: A Typical standard curve

Serum biochemistry: For serum biochemical evaluations, 3 ml of blood sample in labeled sterile plain test tube was kept in a slanting position and allowed to clot at room temperature (25-26 ^{o}C) for 1 h. The serum was then separated from the blood cells by centrifugation at 2000 x g for 10 minutes and kept frozen at -20^{o}C until analysis (Ramnath *et al.*, 2008; Zuhoor, 2013). Serum sodium (Na^{+}), potassium (K^{+}), calcium (Ca^{2+}), phosphate (PO_4^{-}), bicarbonate (HCO_3^{-}), chloride (Cl^{-}), urea, creatinine, total protein, albumin, glucose, cholesterol concentrations, and activities of aspartate aminotransferase (AST), alkaline phosphataes (AP) and alanine aminotransferase (ALT) were estimated using audiocomb serum auto-anlyser (Kerr, 1989; Pati *et al*., 2015).

3.2.8.4 Imaging

Cystosonographic scan: For ultrasonography, urine produced and stored in the bladder before its void, was utilised to distend the bladder before carrying out the ultrasonographic examination. Where the bladder was empty,normal saline solution was used to distend the urinary bladder through catheterisation.The bladder was imaged from the caudal ventral abdominal wall (adopting a para-preputial approach in the male dog) following the application of aquasonic gel. A high-frequency (6-7.5 MHz) transducer/probe was placed just cranial to the pubic brim, moving cranially until the bladder was identified. The bladder of each dog was imaged twice(weeks 1 and 3 post-surgery) in bothtransverse (Plate XVIIA) and sagittal (Plate XVIIB)planes of section.

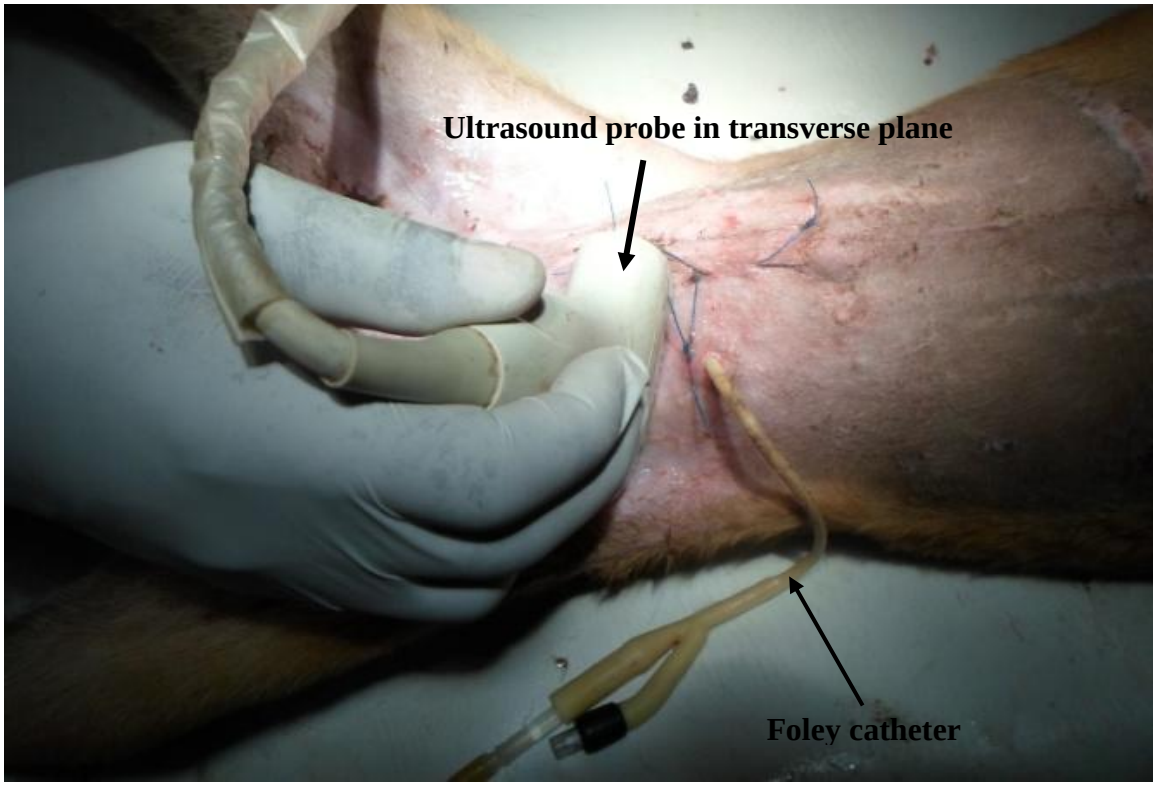


Plate XVIIA: Trans-abdominal ultrasonographic scanning of the urinary bladder in transverse plane



Plate XVIIB: Trans-abdominal ultrasonographic scanning of the urinary bladder in sagittal plane.

Cystography: Cystography (contrast radiography) was performed at the 2nd, 10th and 14th weeks post-surgery. Each dog was fasted for 24 hours before comencement of the contrast study. Diazepam injection (0.5mg/kg IM) was administered for sedation, and the dog was restrained in a lateral recumbency, with the upper leg held away from the prepuce. The prepucial/vulva area was then prepared aseptically with 4% chlorhexidine gluconate solution using swab. Thereafter, the prepuce was retracted caudally exposing the *glanspenis* (in males), and vulva lips (in females) were parted to expose the urethral opening. Sterile lubricant (K-Y Lubricating jelly(R) - Johnson-Johnson Jult, Belgium) and 2% lidocaine hydrochloride solution (Labcalin(R) - LABORATE Phamaceutical, India) were then placed on a sterile gloved-hand. The sterile 10-Fr Foley catheter (improvised with scalp vein tube, in males) was lubricated with the K-Y jelly on the gloved hand. The 2%-lidocaine hydrochloride solution was allowed to fill the lumen of the catheter, and the tip of the catheter was inserted into the urethra and gradually advanced to the urinary bladder. The contrast medium (Urografin76%(R)- Berlimed S.A. Spain, reconstituted to 20% solution) administered at 5 ml/kg was then infused into the urinary bladder through the catheter (Plates XVIII and XIX). Lateral and ventro-dorsal radiographic views of the abdomino-pelvic region were taken by the x-ray machine (Muhammad *et al*., 2016b).



Plate XVIIIA: Urograffin solution, (5 ml/kg) being infused via 10 Fr Foley catheter (transurethrally) into urinary bladder of a bitch for evaluation of the bladder conformation post-cystoplasty by urography



Plate XVIIIB: Urograffin solution (5 ml/kg) being infused via improvised (35 cm long flexible transparent tubing of a scalp vein) catheter (transurethrally) into urinary bladder of a male dog for evaluation of the bladder conformation post-cystoplasty, by urography

3.2.8.5 Gross examination of the urinary bladder

Exploratory laparotomies on male and female subjects were performed at 108thweek post-surgery to check the reconstructed urinary bladders *in-situ*.

3.2.8.6 Histopathology

Tissue sections (biopsies)for histological preparation and examination of the composite colo-peritoneal-vesical site were harvested at weeks 14 and 108 post-surgery. The biopsies were fixed in 10 % buffered-neutral formalin. The composite colo-peritoneal-vesical tissue was then processed for histopathological assessment using the method outlined by Baker *etal*. (2002).

3.3Data Recording and Analysis

The data generated on vital parameters, urinalysis, haematology, plasma cytokines, serum electrolytes, urea, protein, glucose and enzymes were expressed as Mean ± standard error of the mean (Mean ± SEM) and summarised into tables and graphs. GraphPad Prism$^{(R)}$ [GraphPad Software (2007), version 5.01 for Windows, San Diego, California, USA] was used to analyse the data. Paired *t*-test was used to compare pre- and post-surgical means.Data on vital parameters, haematology and plasma cytokines were further subjected to the repeated measures analysis of variance (ANOVA) and Pearson's correlation analysis. Values of $P < 0.05$ were considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Pre-operative Findings and Results Post-treatment

4.1.1 Physical examination

Physical examination revealed that two of the dogs suffered from tick infestation and had enlargement of superficial lymph nodes. Isosporosis was diagnosed in two dogs and babesiosis in one dog. The dogs were all infected with *Taenia* species without clinical signs. The dogs were treated based on the afore-mentioned conditions and they all recovered fully (Table 4.1) prior to the commencement of the experiment.

Table 4.1: Pre-surgical parasitic screening outcome and management in experimental dogs

Dogs	***Physical examination findings***	***Parasitic screening results***	***Management instituted***
EC_1	Weakness, enlarged superficial lymphnodes, presence of ticks, pale ocular mucous membranes, pyrexia	*Babesia canis, Taenia spp Rhipicephalus sanguineus*	Diminazene aceturate (4 mg/kg IM), Praziquantel (10 mg/ kg PO), Ivermectin (0.4 mg/kg SC)
EC_2	Enlarged superficial lymphnodes and presence of ticks	*Rhipicephalus sanguineus Taenia spp*	Oxytetracycline (20 mg/kg), Ivermectin (0.4 mg/kg SC), Praziquantel (10 mg/ kg PO)
EC_3	Weakness and presence of proglottids in the faeces	*Isospora* oocyst *Taenia spp.*	Sulphadimidine, Praziquantel (10 mg/ kg PO)
EC_4	Proglottids and worms in the faeces, presence of fleas	*Taenia spp, Dipylidium caninum, Ctenocephalides canis*	Praziquantel (10 mg/ kg PO), Ivermectin (0.4 mg/kg SC).

Table 4.2: Pre-surgical (base-line) vital parameters of the experimental dogs

Dogs	*Days*	*Temperature (^oC)*	*Pulse rate (beats/min.)*	*Respiratory rate (cycles/min.)*
EC_1				
	7	39.1	102	28
	6	39.2	98	18
	5	38.8	100	22
	4	38.9	96	24
	3	39.0	98	20
	2	39.2	94	16
	1	38.5	986	18
	0	38.0	102	30
EC_2				
	7	38.5	120	28
	6	38.9	104	26
	5	39.1	98	22
	4	38.6	90	26
	3	38.7	96	20
	2	38.8	100	20
	1	38.6	96	18
	0	38.4	140	44
EC_3				
	7	38.8	94	30
	6	39.1	120	36
	5	38.7	106	26
	4	37.9	108	18
	3	38.3	104	20
	2	38.9	102	16
	1	38.3	98	18
	0	39.1	100	20
EC_4				
	7	38.1	100	18
	6	38.2	106	17
	5	38.9	102	18
	4	38.5	100	20
	3	38.0	98	18
	2	38.7	112	20
	1	38.6	105	17
	0	38.8	103	16

Table 4.3: Pre-surgical (base-line) Haemogram of the experimental dogs

Dogs	*Days*	*PCV (%)*	*Hb. (g/dl)*	*TWBC (10^9/L)*	*Neut. (10^9/L)*	*Lymp. (10^9/L)*	*Eosin. (10^9/L)*	*Mon. (10^9/L)*	*Baso. (10^9/L)*
EC_1									
	7	60	20.0	11.4	8.44	2.74	0.0	0.23	0.0
	4	55	18.3	9.6	6.91	2.69	0.0	0.00	0.0
	1	50	16.7	8.8	6.16	2.46	0.0	0.18	0.0
EC_2									
	7	50	16.7	8.4	5.54	2.86	0.0	0.00	0.0
	4	46	15.3	11.0	6.60	3.96	0.0	0.44	0.0
	1	45	15.0	10.6	7.42	2.86	0.0	0.32	0.0
EC_3									
	7	30	10.0	9.0	7.20	1.80	0.0	0.00	0.0
	4	43	14.3	8.0	4.00	4.00	0.0	0.00	0.0
	1	41	13.7	10.5	7.25	3.26	0.0	0.00	0.0
EC_4									
	7	39	13.0	14.0	9.24	4.20	0.0	0.56	0.0
	4	45	15.0	10.9	7.09	3.71	0.0	0.11	0.0
	1	45	15.0	6.0	3.72	2.28	0.0	0.00	0.0

EC = Enterocystoplasty; 1,2,3,4 = Dog identity; PCV = Packed cell volume; Hb = Haemoglobin concentration; TWBC = Total white blood cells; Neut. = Neutrophils; Lymp. = Lymphocytes; Mon. = Monocytes; Eosin. = Eosinophils; Baso. = Basophils

4.2 Clinical Evaluation

The dorsal aspect of the urinary bladder used as the recipient site (partially cystectomised) was adequate and adjacent to the descending colon (sero-muscular colonic pedicle flap) along with free peritoneal sheath in the form of composite colo-peritoneal pedicle tissue (as donor). The composite tissue retained its viability both pre- and post-transplantation despite excessive manipulation.

Generally, the pre-surgical values (taken within a week) of vital parameters (Table 4.2), urinalysis (Appendices VI-X), complete blood counts (Table 4.3), concentrations of serum electrolytes (Appendices XXIII-XXVI), including urea, glucose, albumin,and plasma cytokines (Appendices XIV-XVII), and activities of enzymes (Appendices XXIII-XXVI), obtained during acclimatisation following treatment and full recovery of the dogs treated were used as base-line data for each dog. The pre-surgical mean values were also considered the mean base-line references.

Haematuria (between 4 and 5 days) was observed in all the dogs post-surgery. Gross skin closure (apposition) of the laparotomy incisions occur between 5 and 15 days post-surgery. Post-surgical complications observed were straining in all dogs, incisional hernia (Plate XIXA) which is reducible (Plate XIXB) in EC_3 and constipation in EC_2. The complications (straining and constipation) were addressed positively by supportive intervention given to the dogs in form of drenching with liquid paraffin for 3-5 days twice daily and soapy enema once daily for 3 days. The EC_3 (with incisional hernia) responded well to surgical corrections (herniorrhaphy).

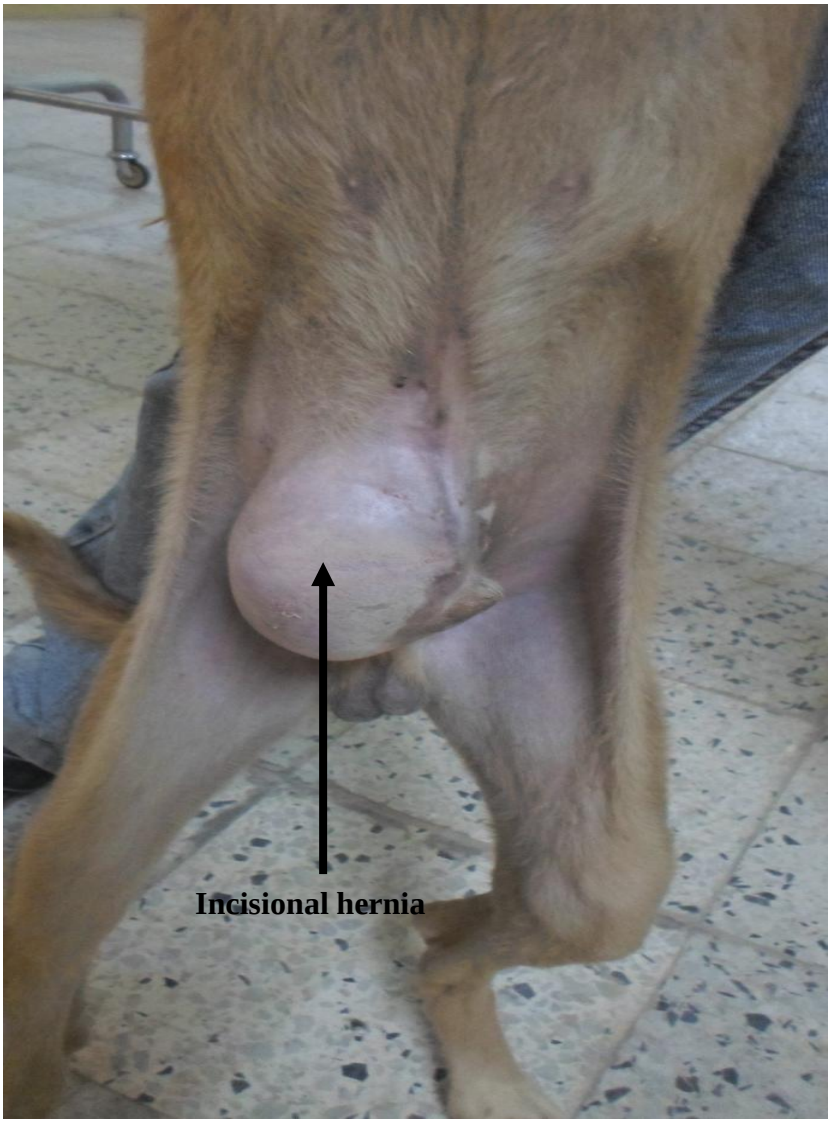


Plate XIXA:Incisional reducible hernia in EC_3 dog



Plate XIXB:Reduced incisional hernia in the EC_3 dog

4.2.1 Vital parameters

The values of vital parameters observed post-surgically in the dogs showed slight deviation within the first week, compared to their respective pre-surgical values. The base-line rectal temperature ranged between 38.50 ± 0.07 ^{0}C and 38.90 ± 0.11 ^{0}C, with the mean value (38.6 ± 0.16 ^{0}C) observed to be slightly deviated immediately after the surgery. The highest values recorded on days 1, 2, 4, and 6 were 39.23 ± 0.28 ^{0}C, 39.23 ± 0.34 ^{0}C, 38.78 ± 0.17 ^{0}C and 38.83 ± 0.12 ^{0}C, respectively, but the difference in the values was not significant. Thereafter, the rectal temperature values fluctuated between 38.25 ± 0.06 ^{0}C and 38.70 ± 0.04 ^{0}C (Figure 4.1).

Pre-surgical pulse rate ranged between 98.50 ± 3.77 beats/minute and 103.25 ± 1.59 beats/minute with the mean value of 105.04 ± 3.26 beats/minute, which fluctuated immediately post-surgery. The post-surgical fluctuations wereinsignificantly ($P > 0.05$)higher within the 5 days post-surgery (106.00 ± 7.12 - 130.00 ± 15.69 beats/minute).However, it subsequently dropped to the pre-surgical base-line ranges till the end of the study (Figure 4.1).

The mean pre-surgical respiratory rate was 21.36 ± 1.78 cycles/minute (ranging between 17.75 ± 0.25 cycles/minute and 26.00 ± 2.71 cycles/minute), but was later higher post-surgery on days 1, 2 and 3, with the values of 34.75 ± 3.86 cycles/minute, 36.00 ± 5.42 cycles/minute and 26.50 ± 4.72 cycles/minute, respectively. Although the difference in the values was not significant ($P > 0.05$), the respiratory rate later stabilized and fluctuated within the reference values (Figure 4.1).

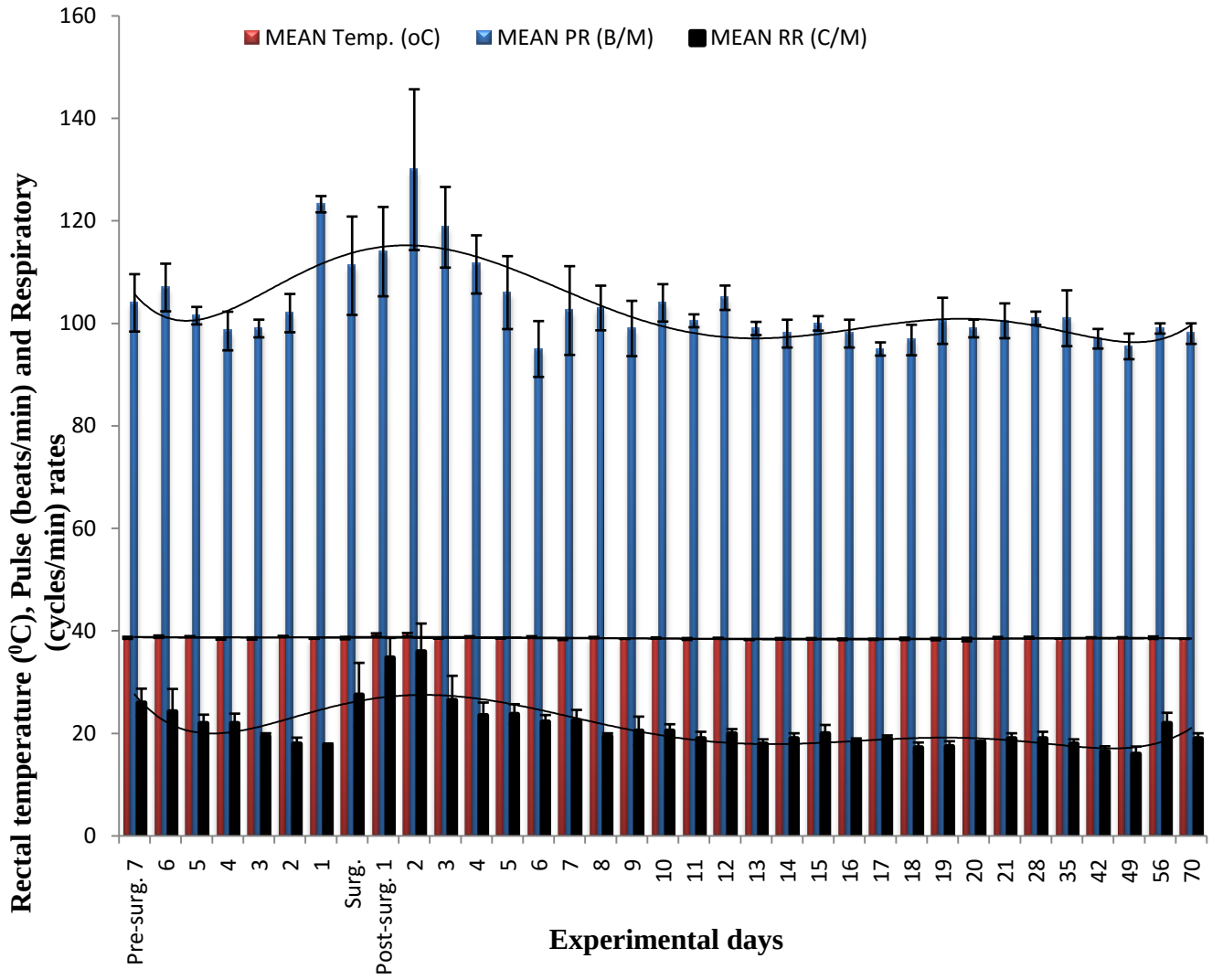


Figure 4.1: Fluctuations in vital parameters showing inflammatory response to colo-peritoneo-cystoplatic procedure in dogs

4.2.2 Urinalysis

The urine specific gravity (SG) ranged between 1.015 ± 0.002 and 1.021 ± 0.002 prior to the commencement of the surgery. The pre-surgical mean SG (1.018 ± 0.003) decreased post-surgically on days 7 (1.006 ± 0.002), 10 (1.005 ± 0.005) and 13 (1.010 ± 0.004), but later fluctuated between 1.008 ± 0.002 and 1.020 ± 0.004 as shown in Table 4.4up to termination of the sampling on day 42. The pre-surgical pH (6.81 ± 0.50) value in the dogsfluctuated post-surgically and was maintained within the range of 6.50 ± 0.15 and 7.50 ± 0.29 for 18 days. The urine pH later dropped to 5.75 ± 0.48, 6.25 ± 0.25, 6.00 ± 1.00 and 6.00 ± 0.00 on days 21, 28, 35 and 42, respectively, at the termination of the experiment (Table 4.4).

Protein was observed post-surgically in the urine of the dogs for the first 7 days, but gradually reduced and, subsequently, disappeared completely on day 21 till completion of the experiment. Glucose was also detected, but in the urine of one dog on day 7 post-surgery. Heamaturia observed was persistent within the first 16 days post-surgery, and it gradually reduced on days 18 and 21 post surgery. Thereafter, the bloody urine ceased completely up to the termination of the experiment. Leucocytes were initially observed 5 days pre-surgically in the urine of 50% of the dogs only. The leucocyturia was observed and persisted in 2 to 3 out of the dogs from day 1 to day 18 post-surgery, which later dropped to 25 % on day 21, and subsequently to 0% on day 28 post-surgery till termination of the experiment.

Ascorbic acid was detected in the urine of the dog on the first day post-surgery, which consistently fluctuated irregularly up to the end of the experiment (Table 4.4). Urobilinogen, bilirubin and nitrate were detected on 4, 5 and 10 days, respectively post-surgery in 2 of the dogs.

Table 4.4: Mean urinalysis results of the dogs pre- and post-colo-peritoneo-cystoplasty (proteinuria, haematuria and leucocyturia)

Parameters	S.G	P^H	Pro.	Sur.	Bld.	Leu.	K.B	Bil.	U.Bil.	A.A.	Nit.
NORM	1.015-1.045	(6-7)	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Days											
Pre-surgery											
7	1.016±0.004	7.00±0.41	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
5	1.021±0.002	7.25±0.48	- - - -	- - - -	- - - -	- - ++	- - - -	- - - -	- - - -	- - - -	- - - -
3	1.015±0.002	6.75±0.48	- - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
1	1.020±0.004	6.25±0.63	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
Post-surgery											
1	1.018±0.004	7.00±0.58	++++	- - - -	++++	++ - +	- - - -	- - ++	- - ++	+++ -	++++
4	1.019±0.002	6.75±0.25	++++	- - - -	++++	++ - +	- - - -	- - ++	- - ++	- - - -	- - ++
7	1.006±0.002	7.50±0.29	++++	- + - -	++++	- - ++	- - - -	- - + -	- - - -	+ - - -	+ - + -
10	1.005±0.005	6.50±0.65	- ++ -	- - - -	++++	+ - ++	- - - -	- - - -	- - - -	- - - -	+ - - +
13	1.010±0.004	6.75±0.25	+ - ++	- - - -	++++	+ - ++	- - - -	- - - -	- - - -	- - - -	- - - -
16	1.019±0.005	7.00±0.58	+ - ++	- - - -	++++	++++	- - - -	- - + -	- - - -	+ - - -	- - - -
18	1.014±0.004	6.50±0.65	+ - + -	- - - -	+ - + -	- - ++	- - - -	- - + -	- - - -	- - - -	- - - -
21	1.020±0.004	5.75±0.48	- - - -	- - - -	+ - - -	- - - +	- - - -	- - + -	- - - -	+ - - -	- - - -
28	1.018±0.003	6.25±0.25	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
35	1.008±0.002	6.00±1.00	+ -	- -	- -	- -	- - - -	- -	- -	+ -	- -
42	1.015±0.005	6.00±0.00	- -	- -	- -	- -	- - - -	- -	- -	+ -	- -

S.G = Specific gravity; p^H = urine acidity; Pro. = Protein; Sur. = Sugar; Bld. = Blood; Leu. = Leucocytes; K.B. = Ketone bodie; Bil. = Bilirubin; U.Bil. = Urobilinogen; A.A. = Ascobic acid; Nit. = Nitrate; NEG. = Negative; - = Absent in 1 dog; - - = Absent in 2 dogs; - - - = Absent in 3 dogs; - - - - = Absent in 4 dogs; + = Present in 1 dog; ++ = Present in 2 dogs; +++ = Present in 3 dogs; ++++ = Present in 4 dog

4.2.3 Haemogram

4.2.3.1 Packed cell volume (PCV)

Haematologically, the pre-surgical PCV was between 44.75 ± 6.52 % and 47.25 ± 2.66 % (mean= 45.84 ± 3.67 %).The PCVprogressively decreased below the pre-surgical base-line reference value to 39.25 ± 2.56 %, 35.25 ± 1.60 %, 33.50 ± 2.53 %, 36.50 ± 2.22 %, 38.00 ± 2.92 %, 35.75 ± 1.89 % and 35.25 ± 3.09 % on days 1, 4, 7, 10, 13, 16 and 18 respectively (Figure 4.2). Thereafter, the PCV improved and fluctuated between 40.00 ± 3.89 % and 45. 00 ± 1.00 % until the termination of the study, but the difference in the values was not significant ($P > 0.05$). The haemoglobin concentration followedthe same trend as the PCV (Figure 4.2).

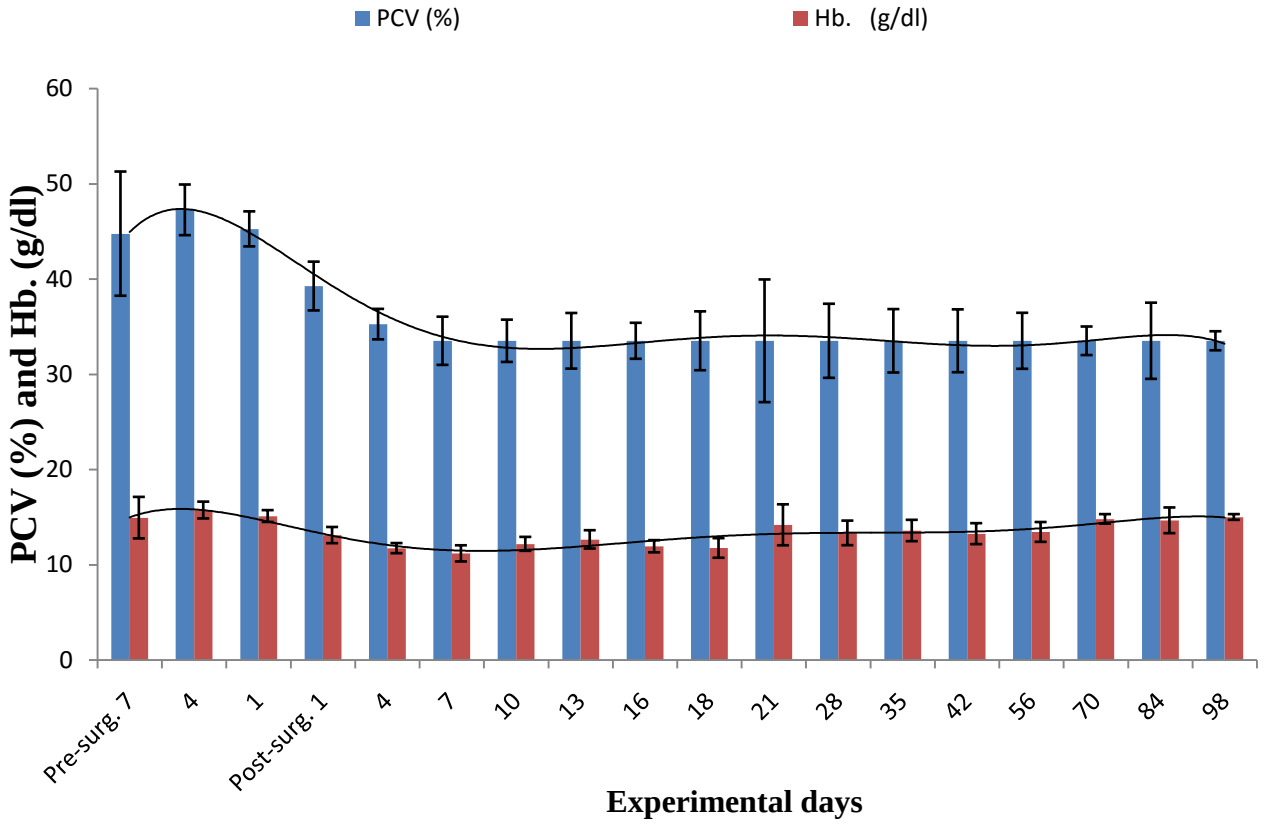


Figure 4.2: Packed cell volume (PCV) and haemoglobin concentration (Hb) of the dogs measured during pre- and post-surgical observations

4.2.3.2 Total leucocyte count (TLC)

Progressive increase in TLC above base-line values was observed after the surgery. The base-line mean TLC(9.85 ± 1.02 x 10^9/L) increased to 12.43 ± 2.06 x 10^9/L, 14.20 ± 2.77 x 10^9/L and 13.25 ± 2.60 x 10^9/L post-surgically on days 4, 7 and 10, respectively. On day 13 post-surgery, the TLC stabilised within the base-line range (8.98 ± 1.07 x 10^9/L-10.70 ± 1.28 x 10^9/L) and remained relatively unchanged,with the counts following between 7.37 ± 1.27 x 10^9/L and 10.83 ± 2.81 x 10^9/L. The changes observed in the TLC were due to fluctuations in neutrophil counts that increased from the pre-surgical mean value (6.63 ± 0.79 x 10^9/L) to the post-surgical values of 8.31 ± 1.51 x 10^9/L, 10.00 ± 2.39 x 10^9/L and 8.92 ± 1.69 x 10^9/L recorded on the 4^{th}, 7^{th} and 13^{th} days post-surgical respectively. The changes were later stabilised and maintained relatively within the base-line values. There was no significant difference ($P > 0.05$) between the pre-surgical and post-surgical values (Figure 4.3).

The pre-surgical lymphocyte counts ranged between 2.72 ± 0.22 x 10^9/L and 3.59 ± 0.31 x 10^9/L.The post-surgical counts recorded on days 1, 4, 7 and 10 were 3.60 ± 0.33 x 10^9/L, 4.42 ± 0.82 x 10^9/L, 3.64 ± 0.66 x 10^9/L and 4.01 ± 0.88 x 10^9/L, respectively. Thereafter, they fluctuated within the pre-surgical base-line counts, but thealteration was not significant ($P > 0.05$).

There was no evidence of eosinophils in circulation pre-surgically. However, presence of eosinophils was established immediately after, from day 1, which fluctuated between 0.02 ± 0.02 x 10^9/L and 0.41 ± 0.39 x 10^9/L, and was sustained for 5 weeks post-surgery. The difference in values was not significant ($P > 0.05$). Thereafter, the values returned to pre-surgical counts (Figure 4.4).

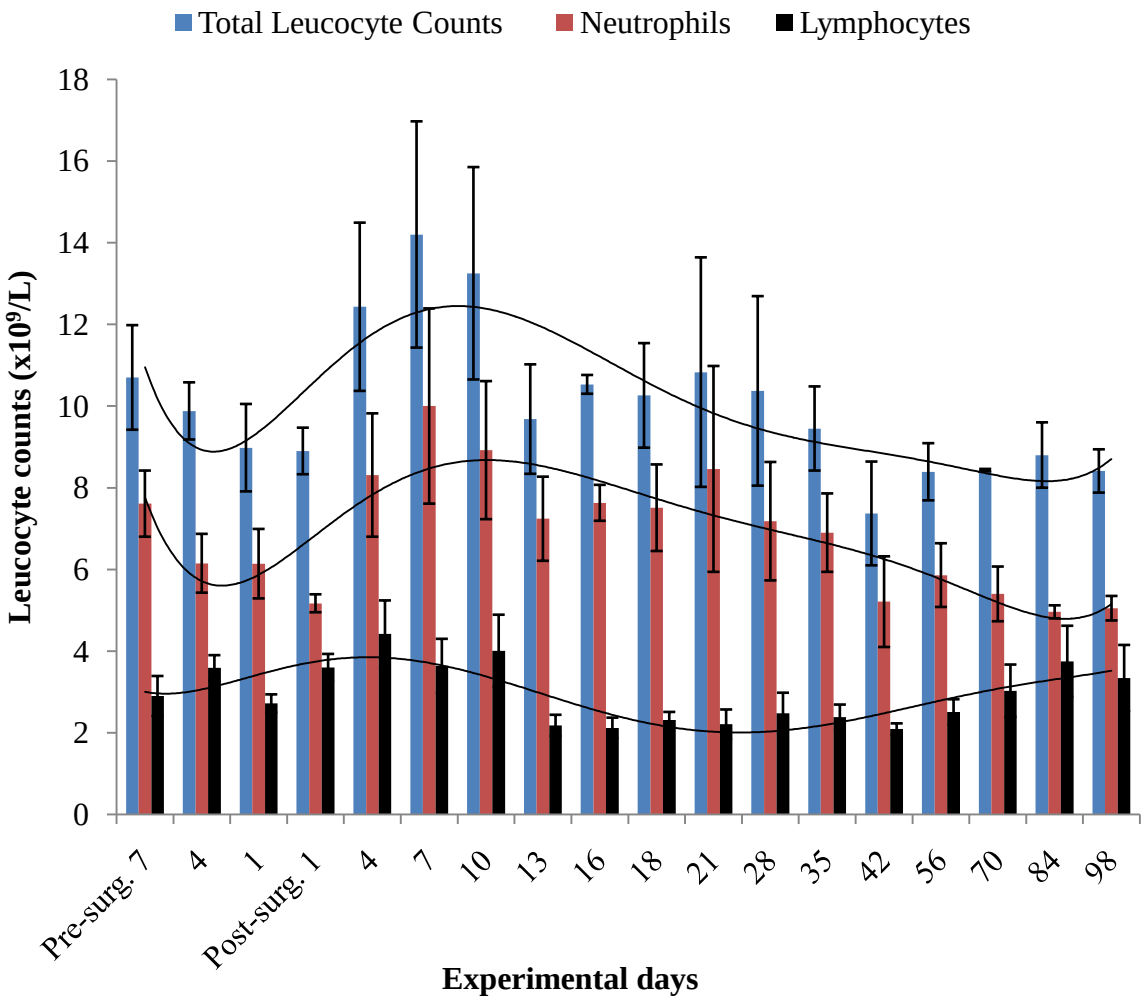


Figure 4.3: Mean total leucocyte, neutrophil and lymphocyte counts of the dogs

The mean monocyte count was 0.16 ± 0.10 x 10^9/L pre-surgically. This value was reduced post-surgically to 0.07 ± 0.02 x 10^9/L, 0.08 ± 0.04 x 10^9/L, 0.06 ± 0.03 x 10^9/L and 0.05 ± 0.04 x 10^9/L on days 1, 4, 10, and 13, respectively. However, on days 16, 18 and 28, there was an increase in the monocyte counts, to 0.21 ± 0.16 x 10^9/L, 0.42 ± 0.21 x 10^9/L and 0.46 ± 0.46 x 10^9/L, respectively; which was not significant ($P > 0.05$). There was relative decrease in the circulating monocyte count below the base-line on day 35 post-surgery (0.14 ± 0.14 x 10^9/L), maintained at the lower counts till termination of the study (Figure 4.4).

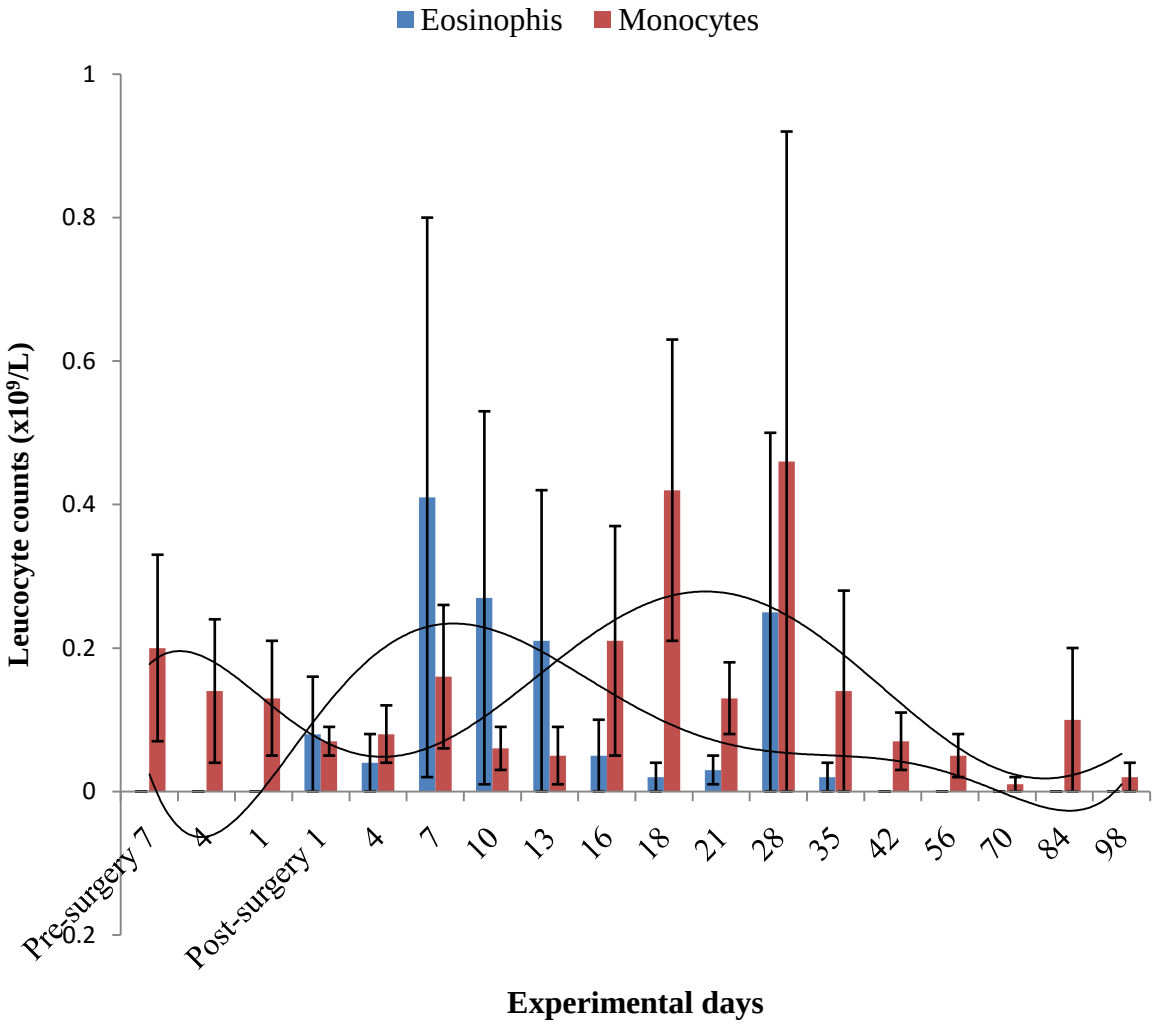


Figure 4.4: Mean total eosinophil and monocyte counts of the dogs

4.2.4 Plasma cytokine levels

The pre-surgical plasma mean concentrations of IL-6 and IL-10 were 7639.45 ± 146.81 pg/ml and 93.95 ± 17.18 pg/ml, respectively. The levels of IL-6 detected days 7, 4 and 1 pre-surgically were 7597.65 ± 204.48 pg/ml, 7639.23 ± 33.31 pg/ml and 7681.47 ± 202.63 pg/ml, respectively. The post-surgical plasma IL-6 concentration suddenly increased above the pre-surgical base-line values, which was maintained between 7916.62 ± 32.97 pg/ml and 7878.98 ± 71.47pg/ml, within the first 5 days post-surgery (Figure 4.5). It then dropped to 7657.38 ± 242.94 pg/ml on day 7 and subsequently undulated within the range of 7355.88 ± 190.57 pg/ml - 7923.52 ± 86.40 pg/ml up till the termination of the experiment. The differences between the pre-surgical and post-surgical IL-6 concentration was not significant.

The base-line mean plasma concentration of IL-10 was gradually elevated immediately post-surgery and attained 449.70 ± 125.79 pg/ml on day 9 post-surgery. The concentrations returned to pre-surgical levels and were maintained thereafter between 185.32 ± 8.80 pg/ml and 457.20 ± 95.78 pg/ml until termination of the experiment (Figure 4.6). There was no significant difference in the plasma concentration of IL-10.

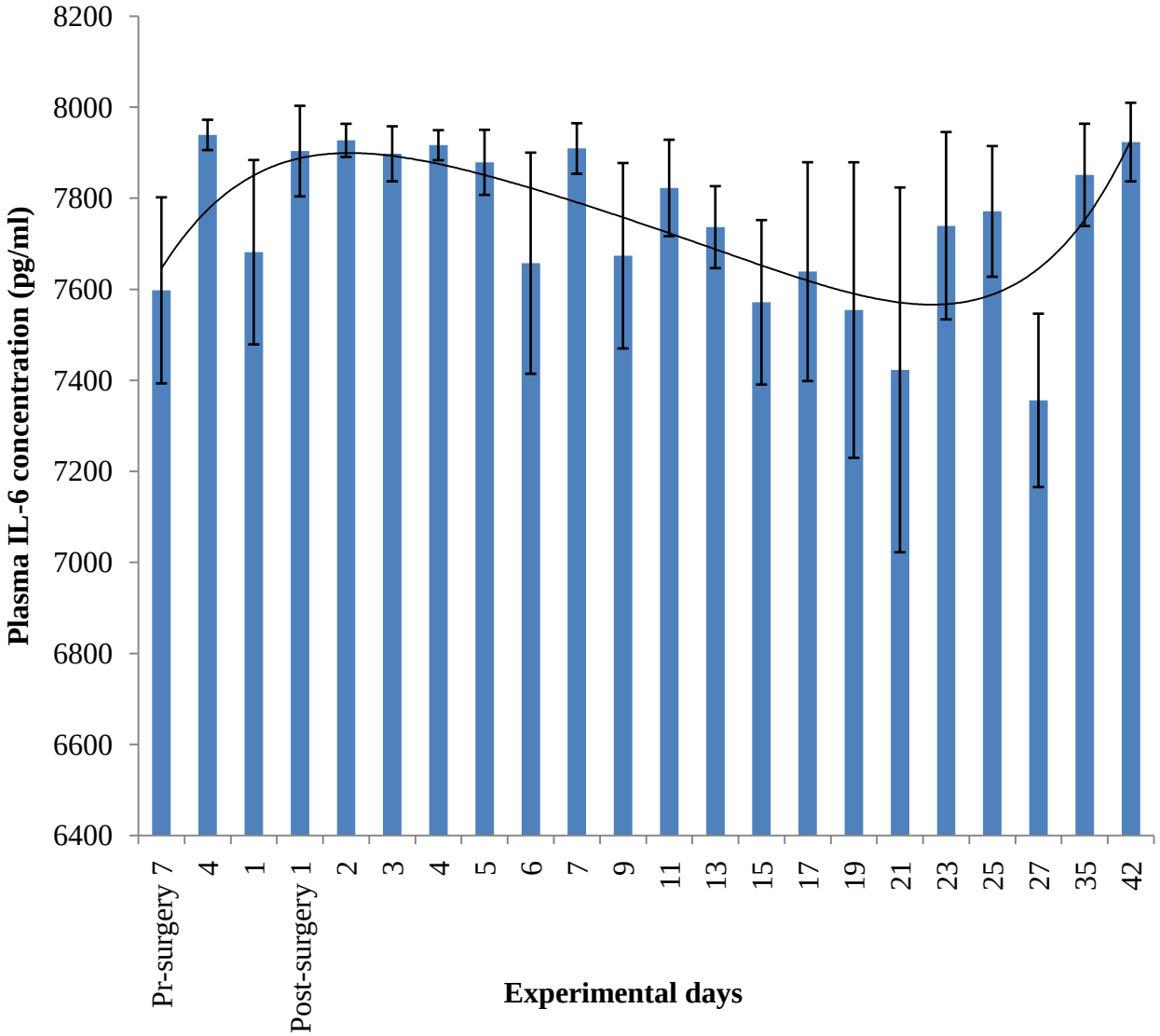


Figure 4.5: Mean pre- and post-surgical plasma canine interleukin 6 (IL-6) concentrations

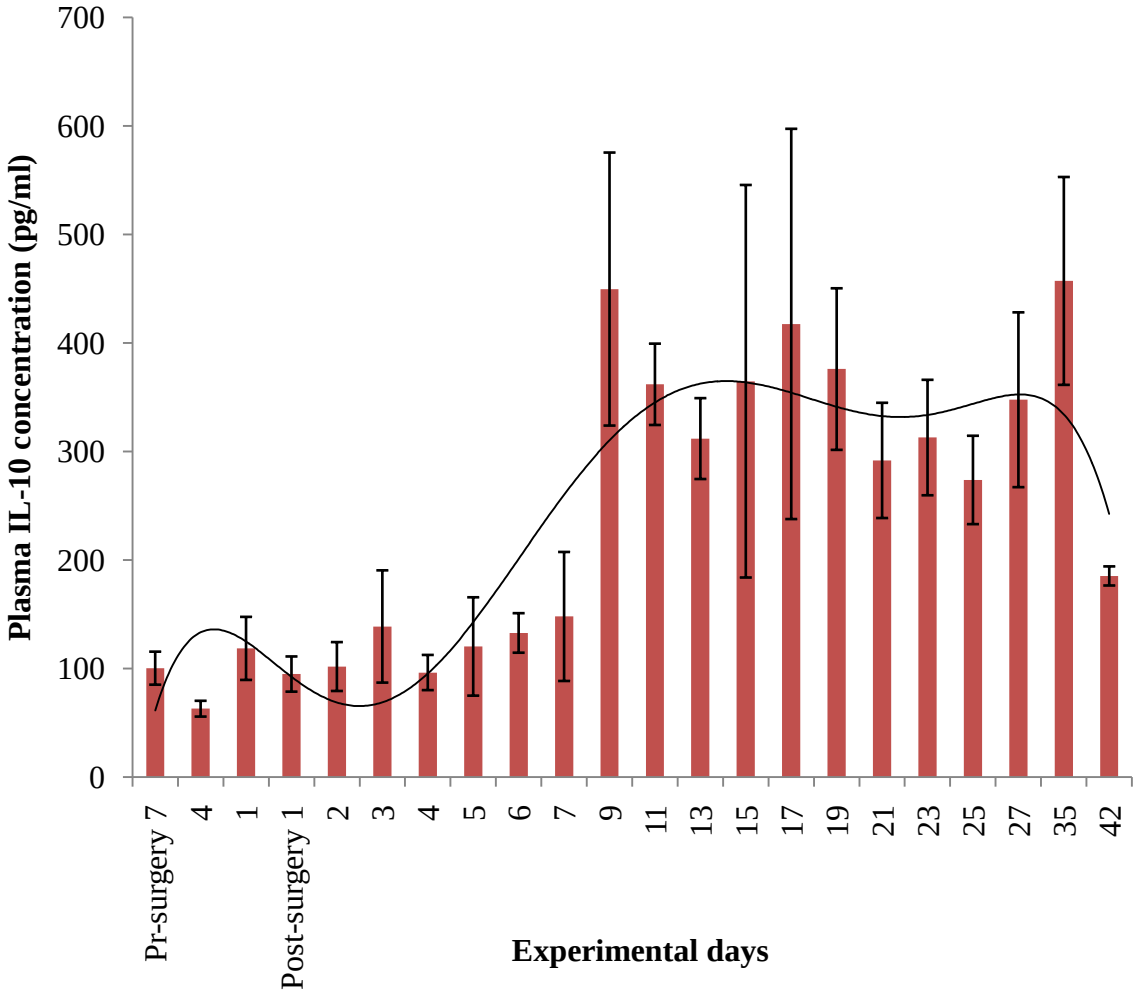


Figure 4.6: Mean pre- and post-surgical plasma canine interleukin 10 (IL-10) concentration

4.2.5 Relationship between concentrations of cytokines (IL-6 and IL-10 levels) and values of haematological and vital parameters

The associations between the cytokines (IL-6 and IL-10) with each other, and with the haematological and vital parameters were as follows:

There was an insignificant negative correlations between the IL-6 and IL-10 levels; IL-6 and packed cell volume, haemoglobin concentration, neutrophil count and rectal temperature values, except for monocyte count that was significant ($r = 0.57$; $P < 0.05$). Non-significant but positive correlations were also obtained between the IL-6 concentration and total leucocyte count, lymphocytes, eosinophil concentration, pulse and respiratory rates. However, very weak correlations were recorded between IL-6 concentration and the neutrophilcount ($r = 0.01$), rectal temperature ($r = 0.01$), eosinophil count ($r = 0.05$), respiratory rate and TLC (Figures 4.7 and 4.8).

Very weak correlation was also observed between IL-10 and TLC ($r = 0.02$) only, but non-significant negative correlations were recorded between the IL-10 and IL-6 levels. The IL-10 and packed cell volume, haemoglobin concentration, lymphocyte count, temperature and pulse rate were negatively correlated. Positive non-significant correlations were obtained between the IL-10 and total leucocyte, neutrophil, monocyte and eosinophil counts except for respiratory rate that was significant ($r = 0.559$; $P < 0.05$) (Figures 4.9 and 4.10).

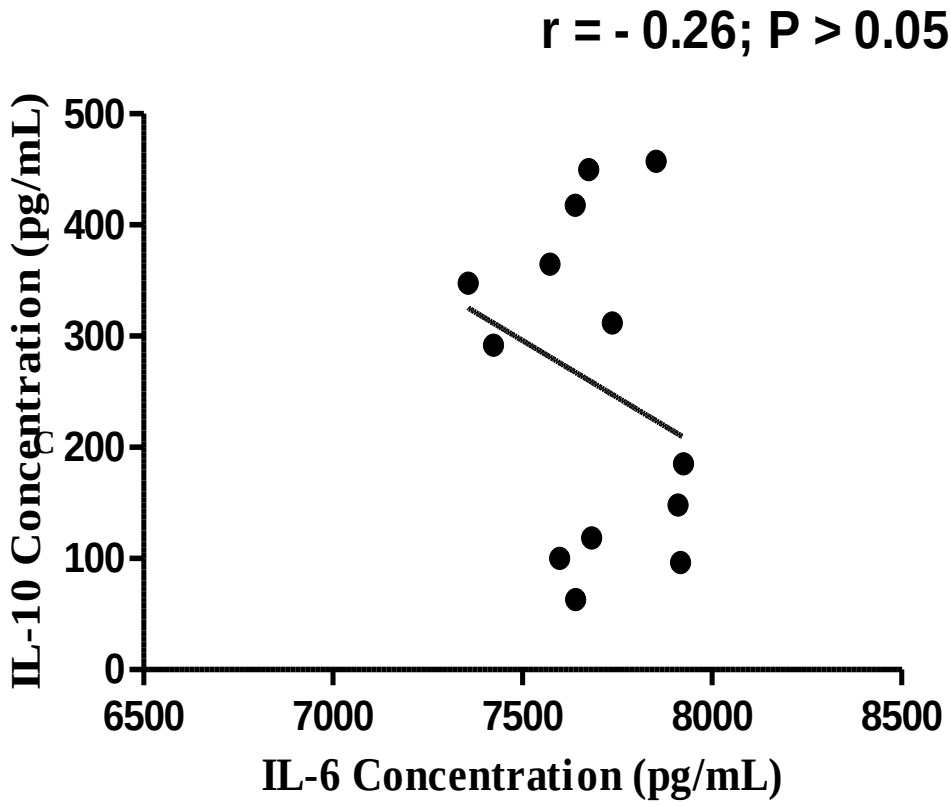


Figure 4.7A: Relationship between the levels of interleukin 6 (IL-6) and interleukin 10 (IL-10). r = Pearson's correlation coefficient.

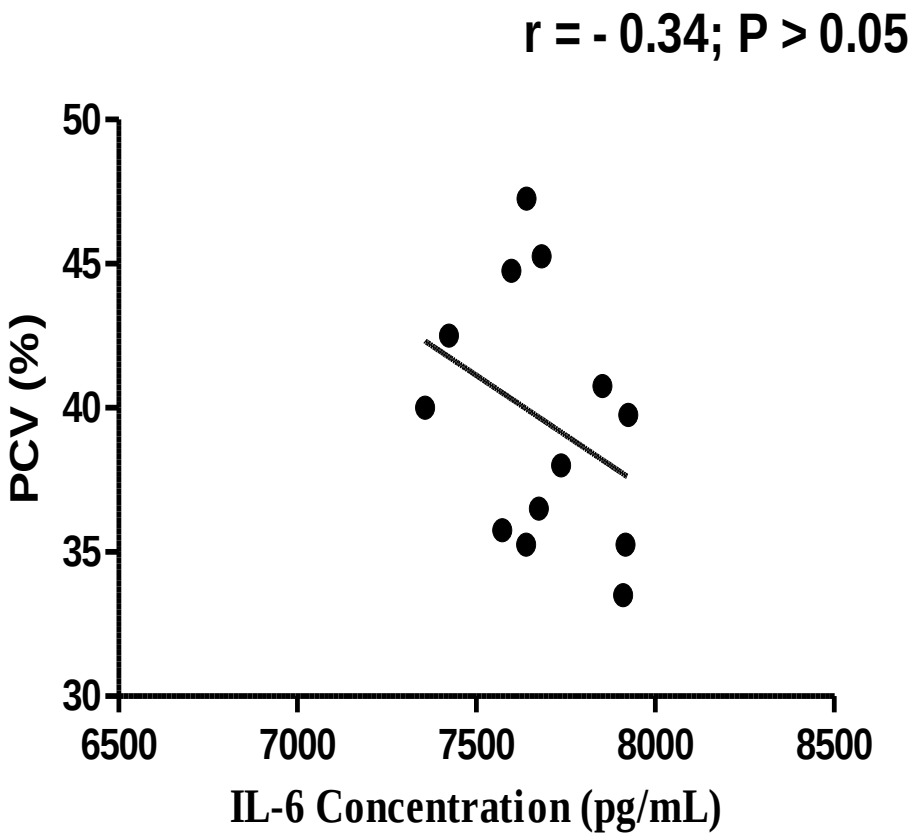


Figure 4.7B: Relationship between the levels of interleukin 6 (IL-6) andPacked cell volume (PCV).r = Pearson's correlation coefficient.

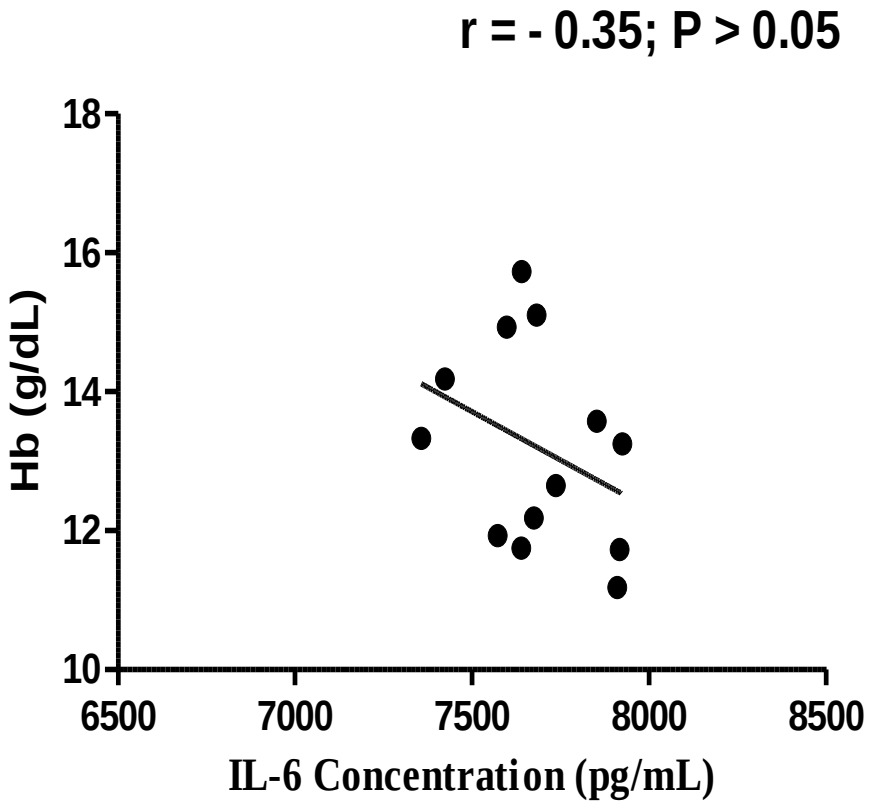


Figure 4.7C: Relationship between the levels of interleukin 6 (IL-6) and Haemoglobin (Hb). r = Pearson's correlation coefficient.

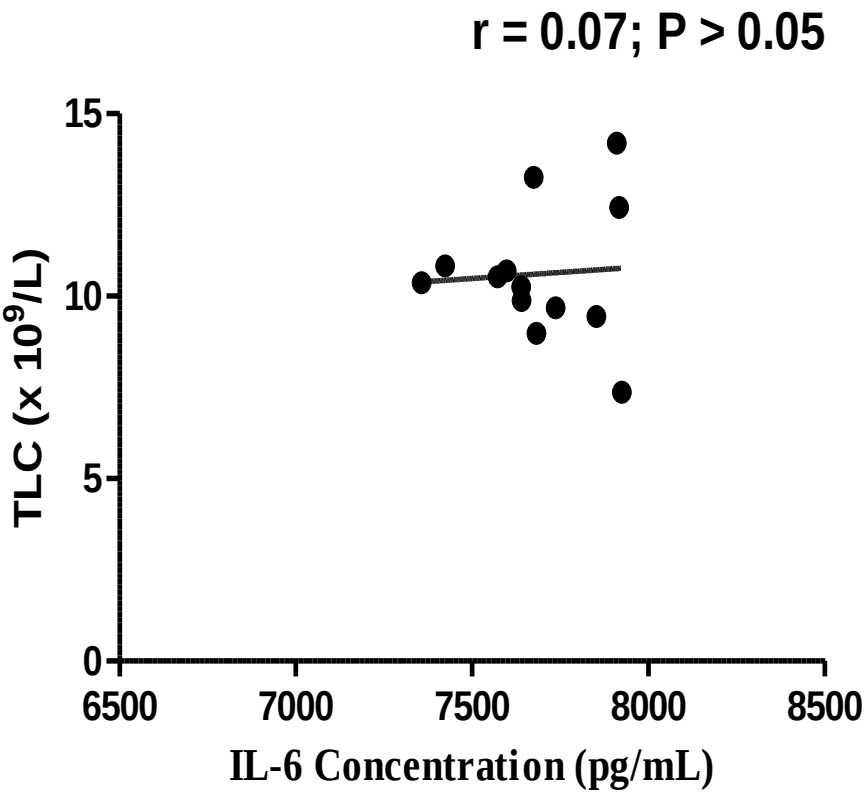


Figure 4.7D: Relationship between the levels of interleukin 6 (IL-6) and Total leucocyte counts (TLC) and IL-6. r = Pearson's correlation coefficient.

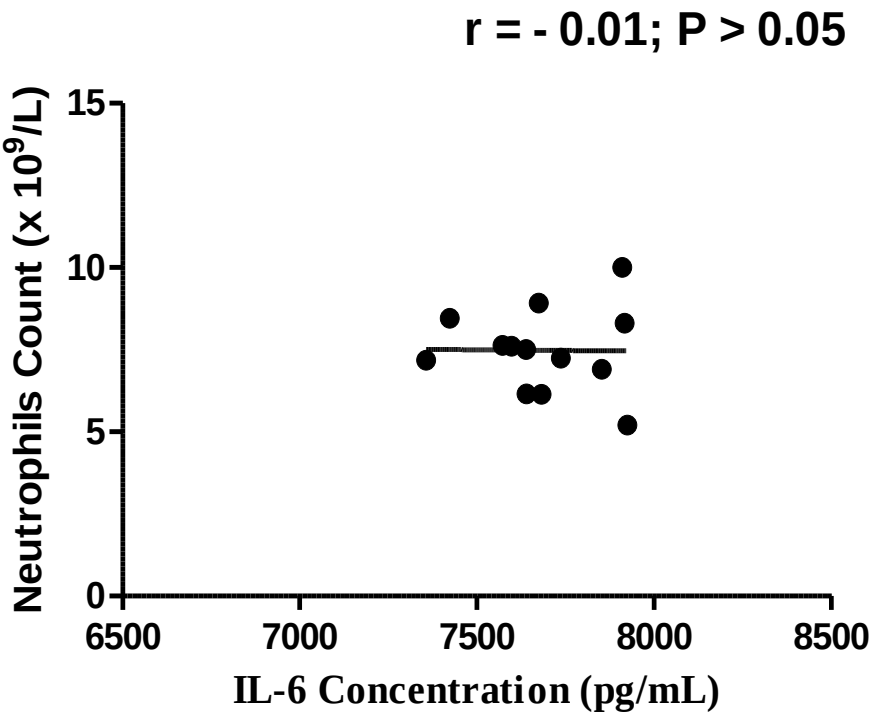


Figure 4.7E: Relationship between the levels of interleukin 6 (IL-6) and Neutrophils. r = Pearson's correlation coefficient.

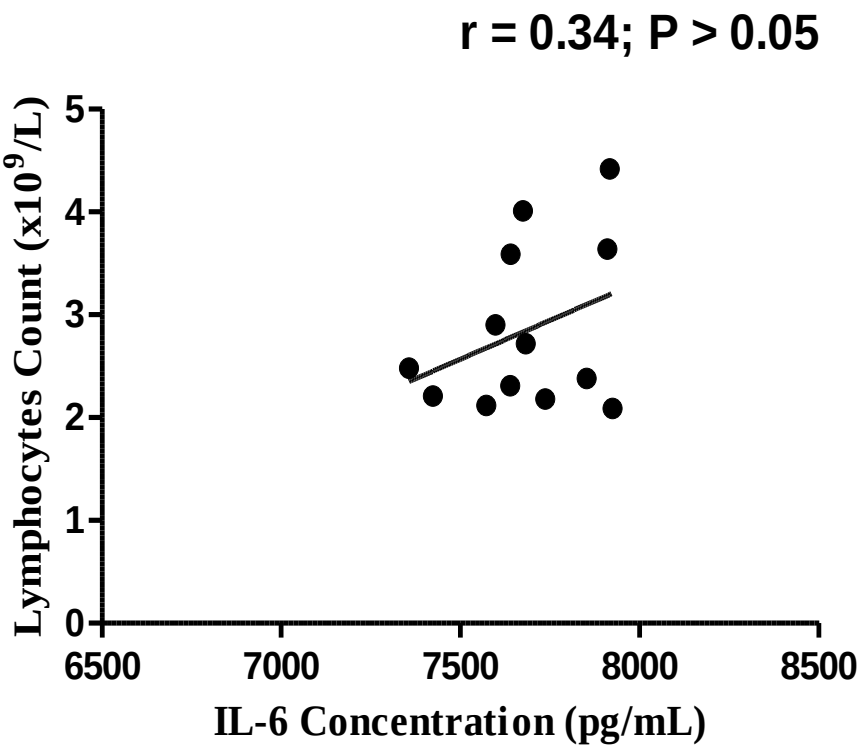


Figure 4.7F: Relationship between the levels of interleukin 6 (IL-6) and Lymphocytes. r = Pearson's correlation coefficient.

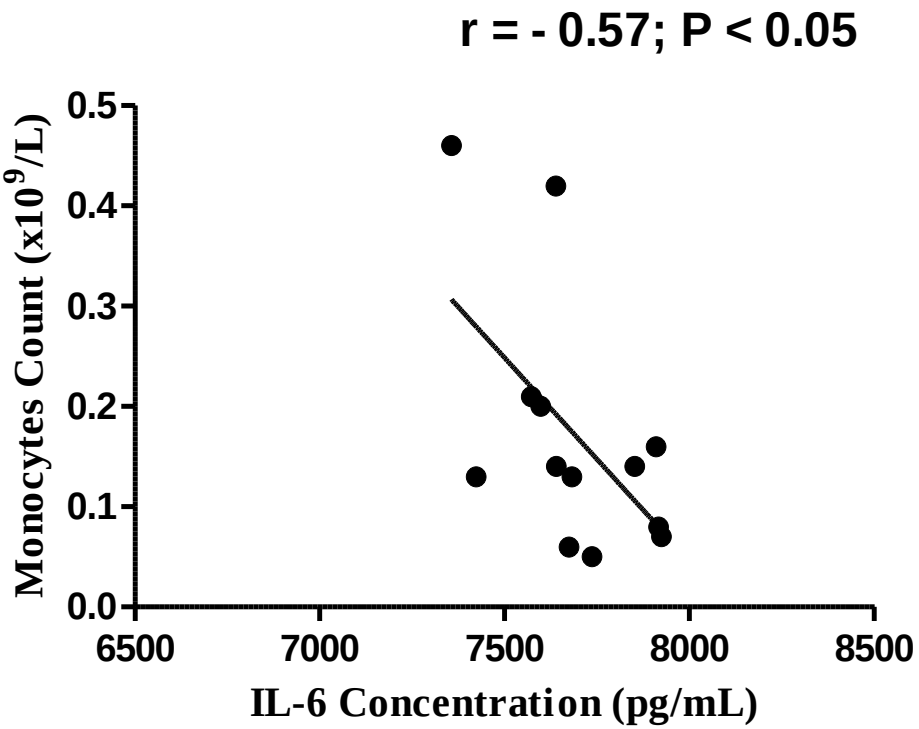


Figure 4.8A: Relationship between the levels of interleukin 6 (IL-6) and Monocytes. r = Pearson's correlation coefficient.

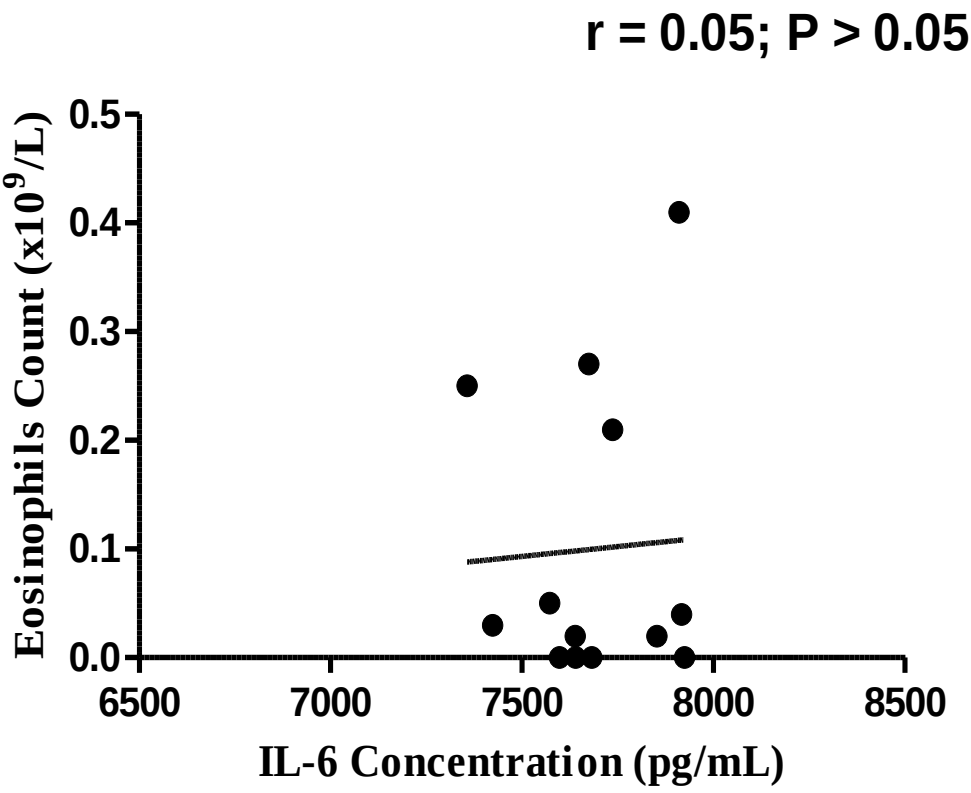


Figure 4.8B: Relationship between the levels of interleukin 6 (IL-6) and eosinophils. r = Pearson's correlation coefficient.

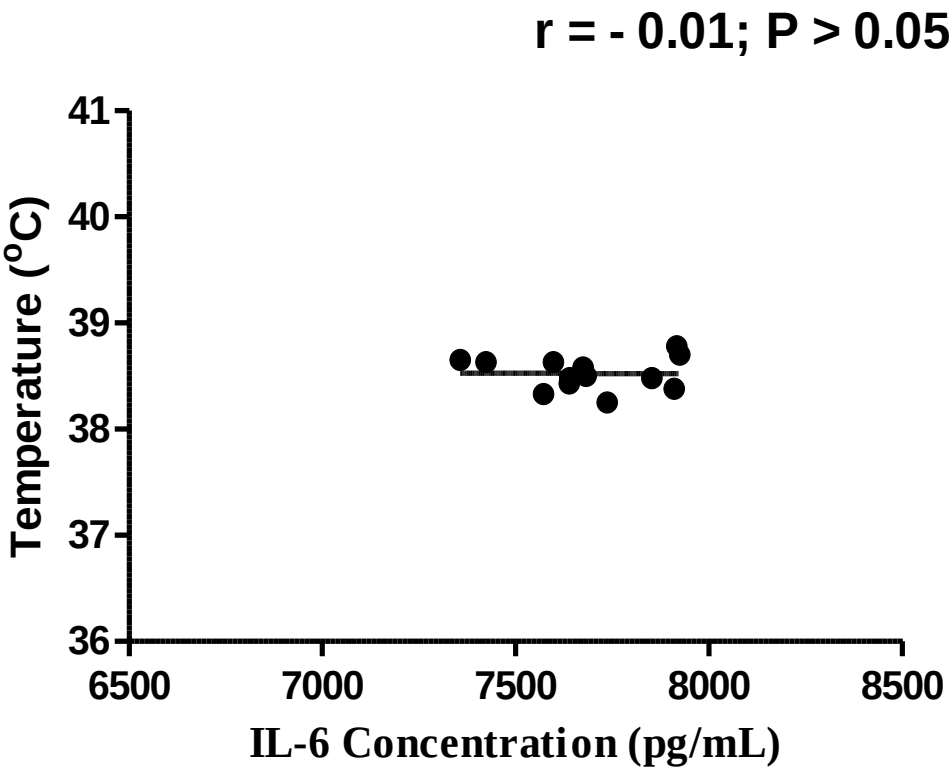


Figure 4.8C: Relationship between the levels of interleukin 6 (IL-6) and temperature. r = Pearson's correlation coefficient.

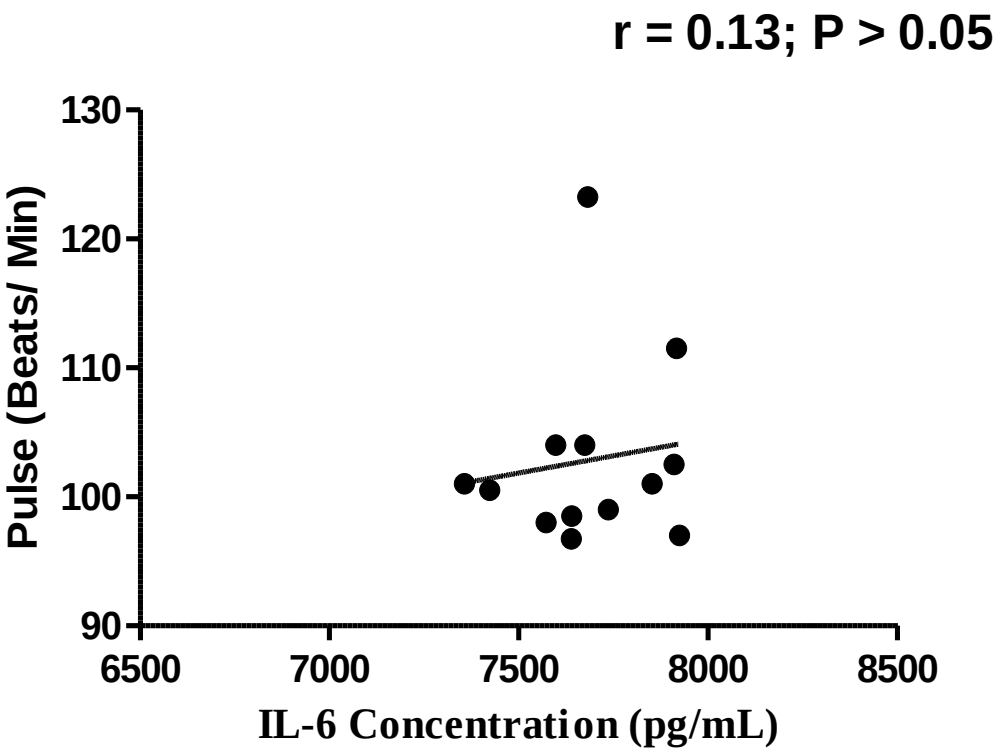


Figure 4.8D: Relationship between the levels of interleukin 6 (IL-6) and pulse rate. r = Pearson's correlation coefficient.

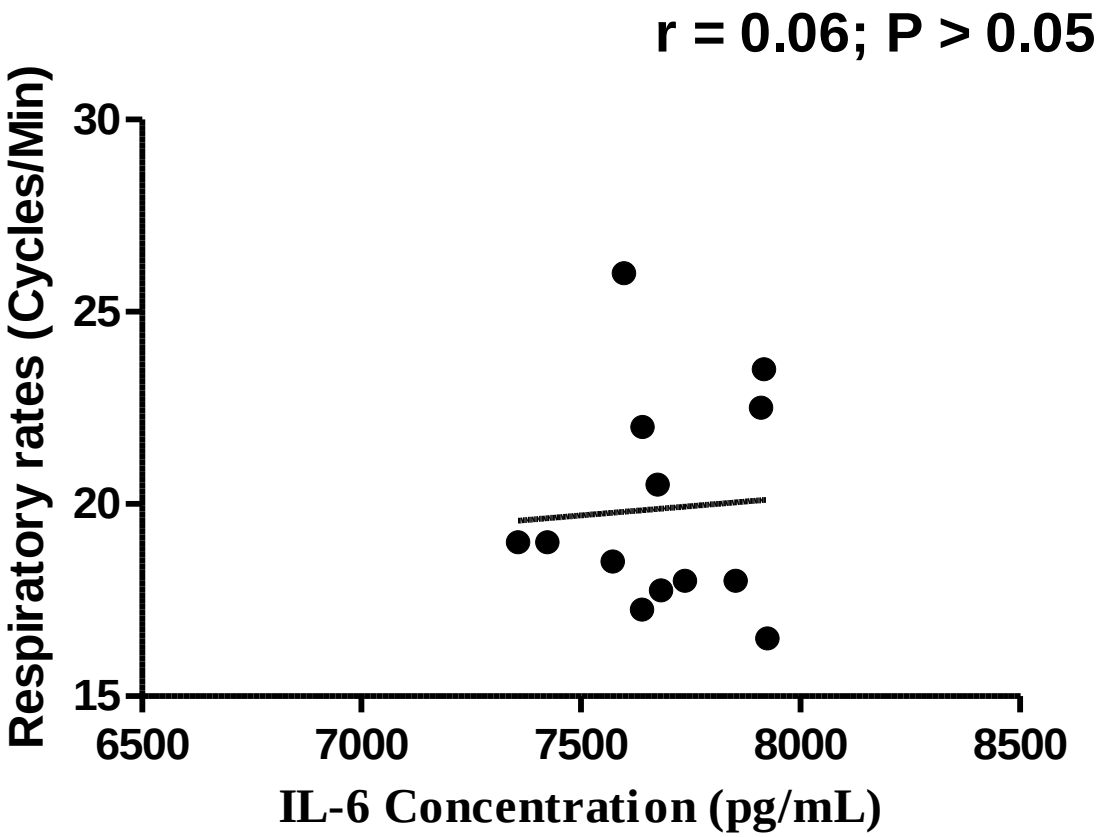


Figure 4.8E: Relationship between the levels of interleukin 6 (IL-6) and respiratory rate. r = Pearson's correlation coefficient.

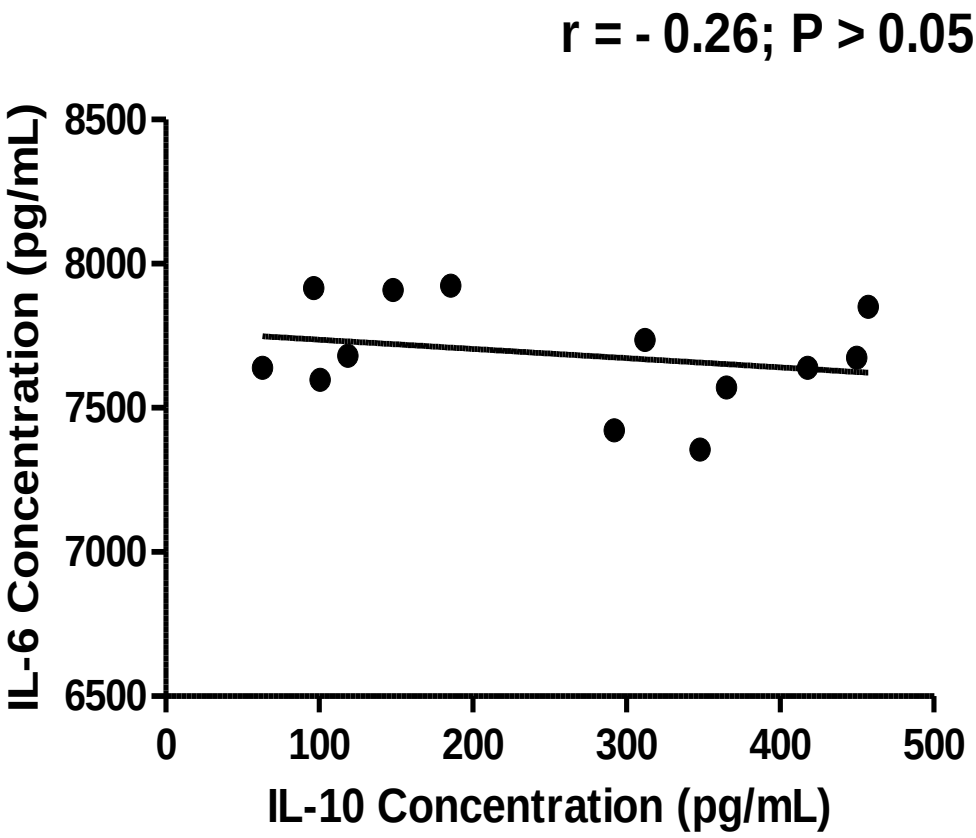


Figure 4.9A: Relationship between the levels of interleukin 10 (IL-10) and interleukin 6 (IL-6). r = Pearson's correlation coefficient.

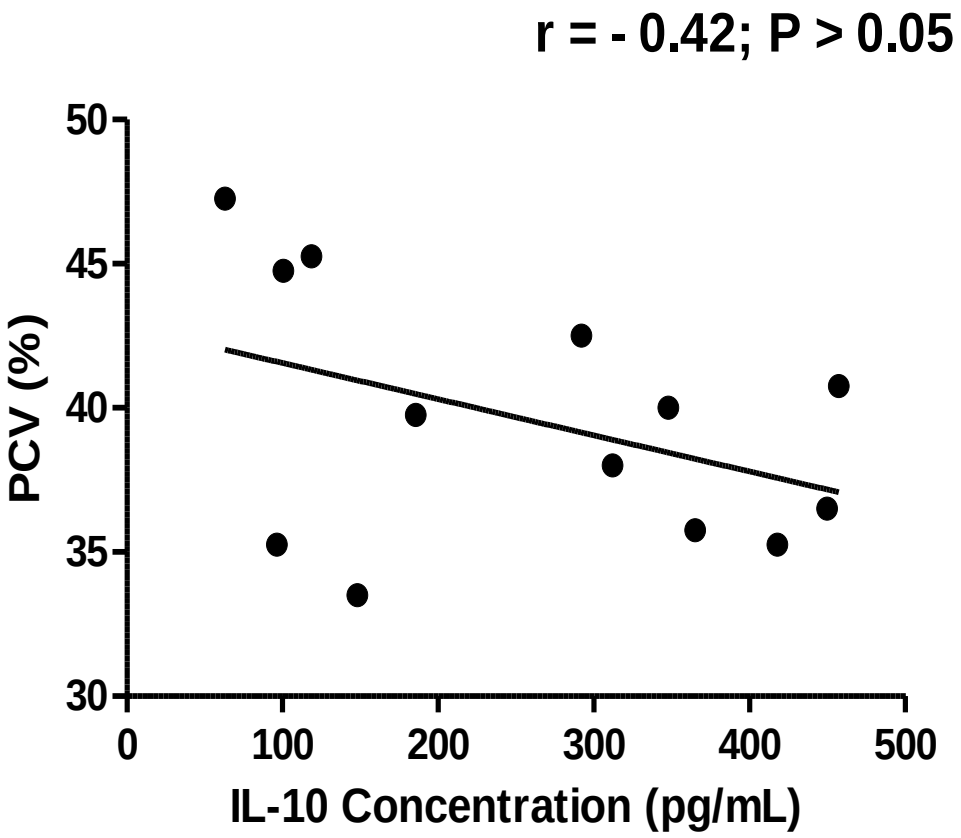


Figure 4.9B: Relationship between the levels of interleukin 10 (IL-10) and Packed cell volume (PCV). r = Pearson's correlation coefficient.

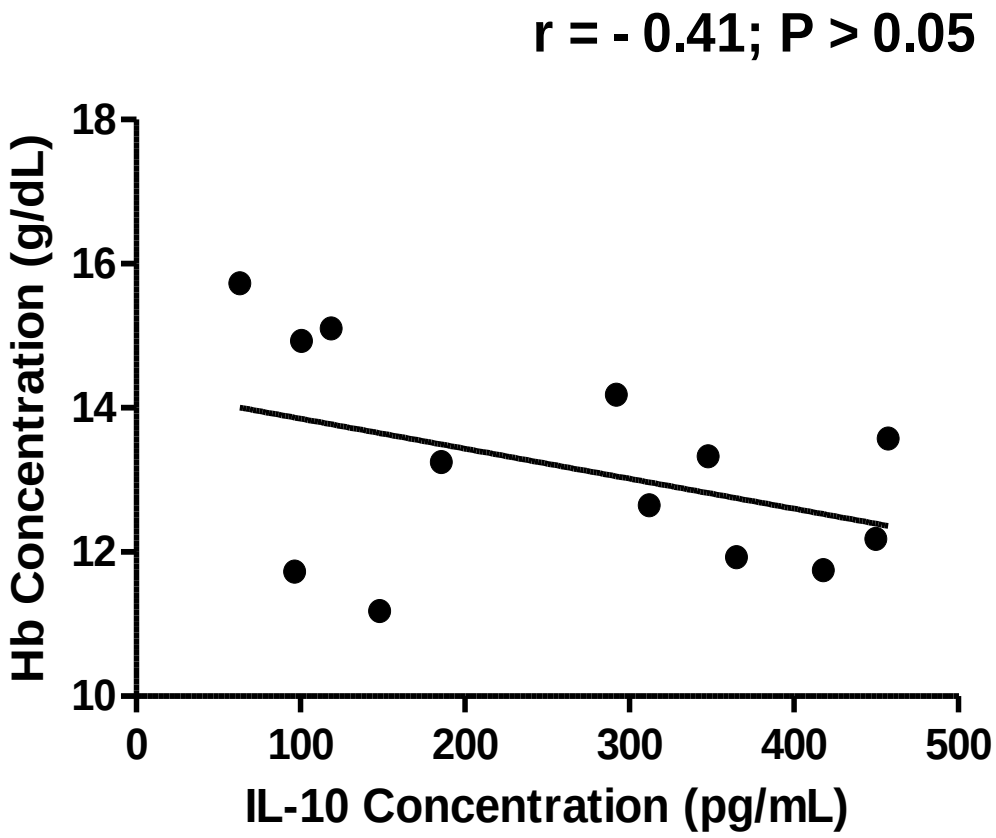


Figure 4.9C: Relationship between the levels of interleukin 10 (IL-10) Haemoglobin (Hb). r = Pearson's correlation coefficient.

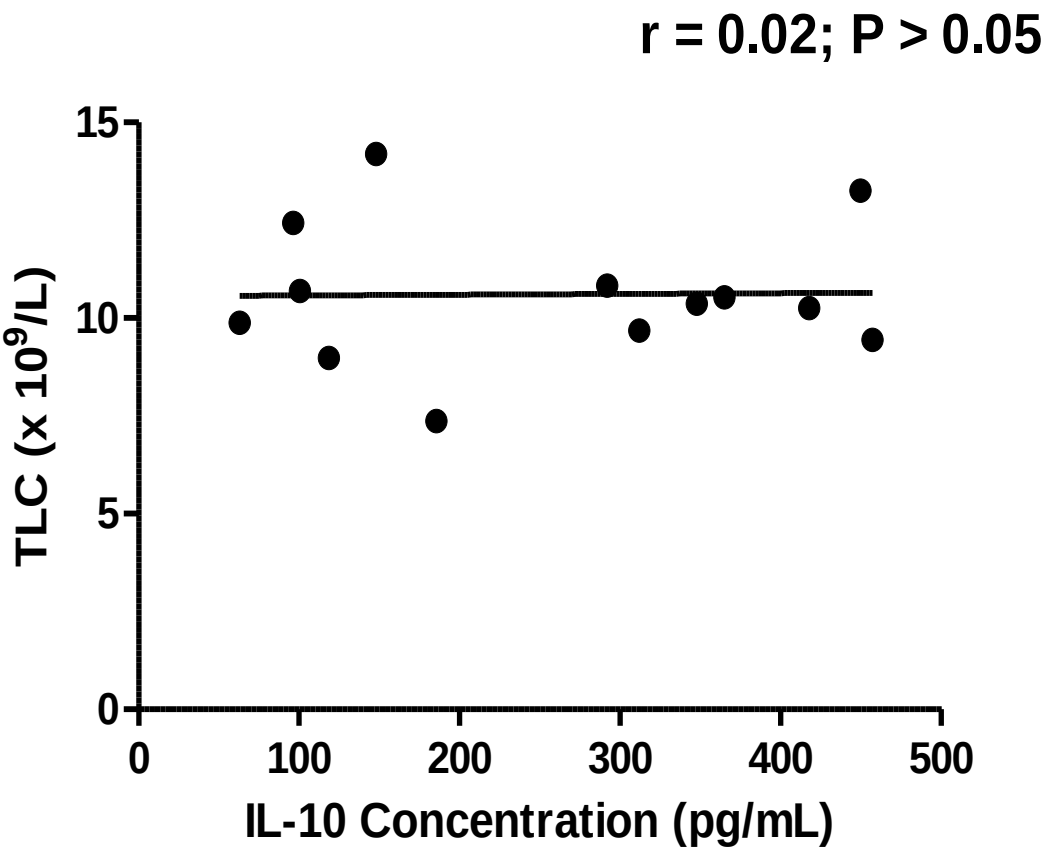


Figure 4.9D: Relationship between the levels of interleukin 10 (IL-10) and Total leucocyte counts (TLC). r = Pearson's correlation coefficient.

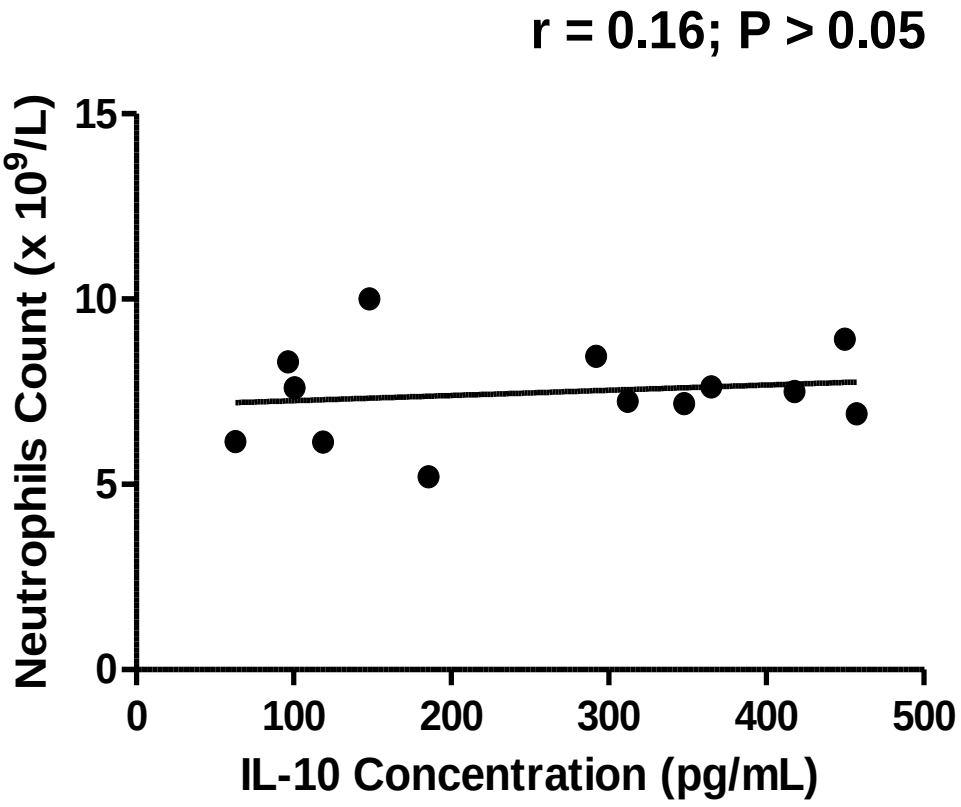


Figure 4.9E: Relationship between the levels of interleukin 10 (IL-10) and Neutrophils. r = Pearson's correlation coefficient.

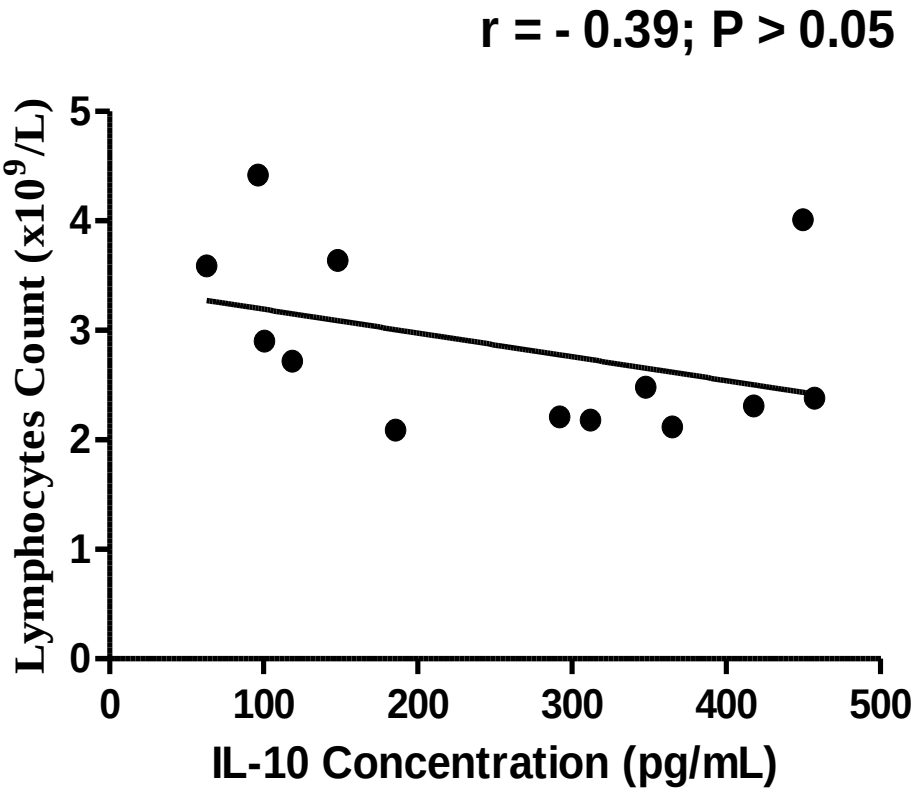


Figure 4.9F: Relationship between the levels of interleukin 10 (IL-10) and Lymphocytes. r = Pearson's correlation coefficient.

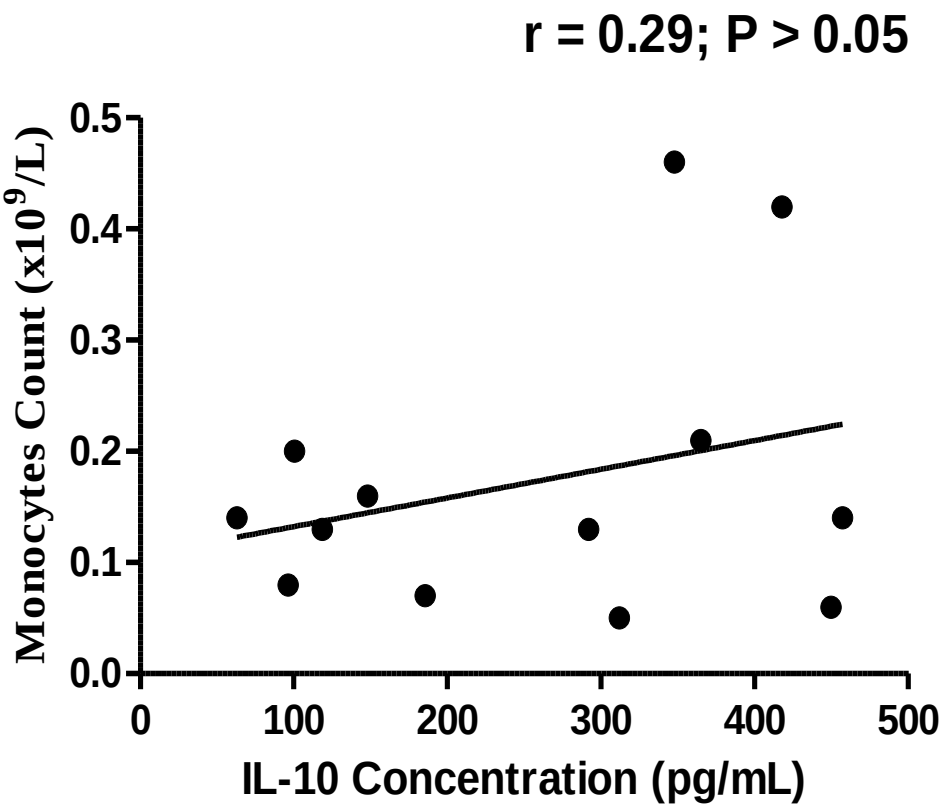


Figure 4.10A: Relationship between the levels of interleukin 10 (IL-10) and Monocytes. r = Pearson's correlation coefficient.

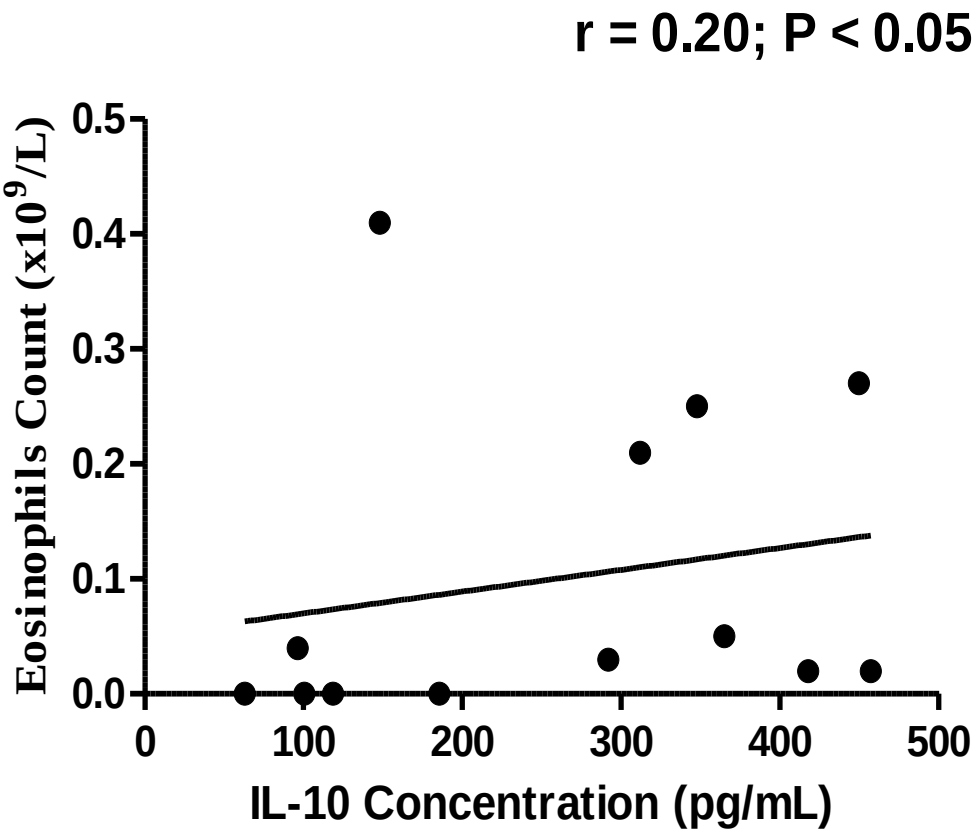


Figure 4.10B: Relationship between the levels of interleukin 10 (IL-10) and eosinophils).r = Pearson's correlation coefficient.

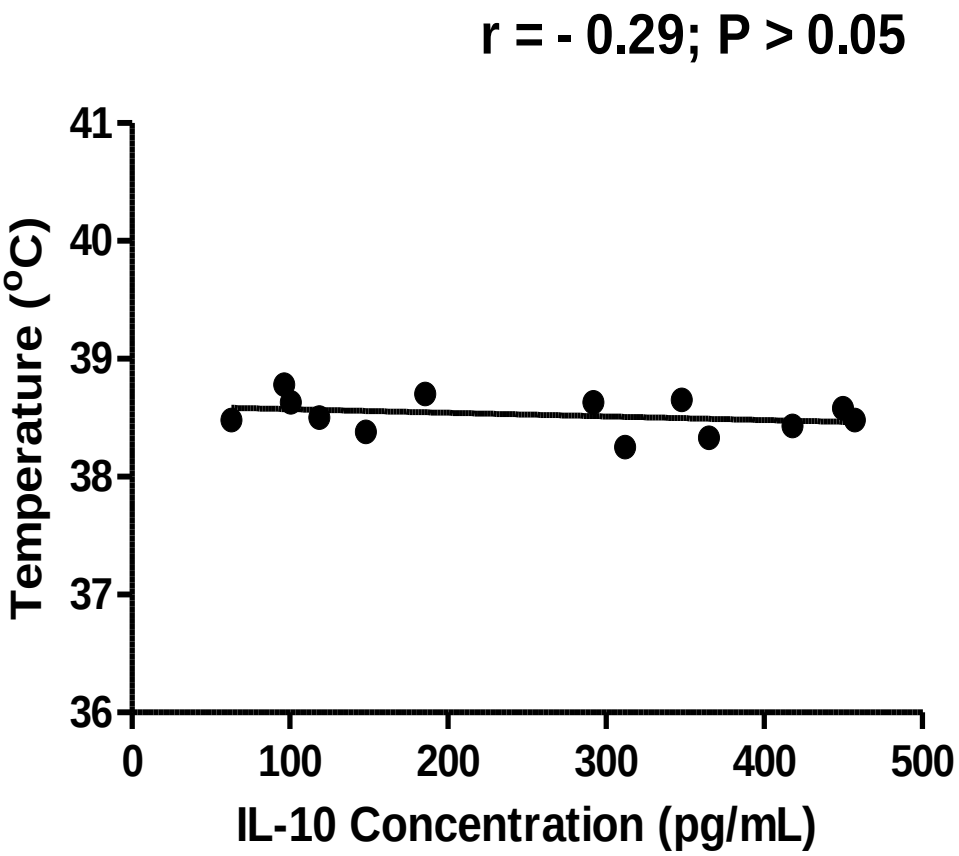


Figure 4.10C: Relationship between the levels of interleukin 10 (IL-10) and temperature.r = Pearson's correlation coefficient.

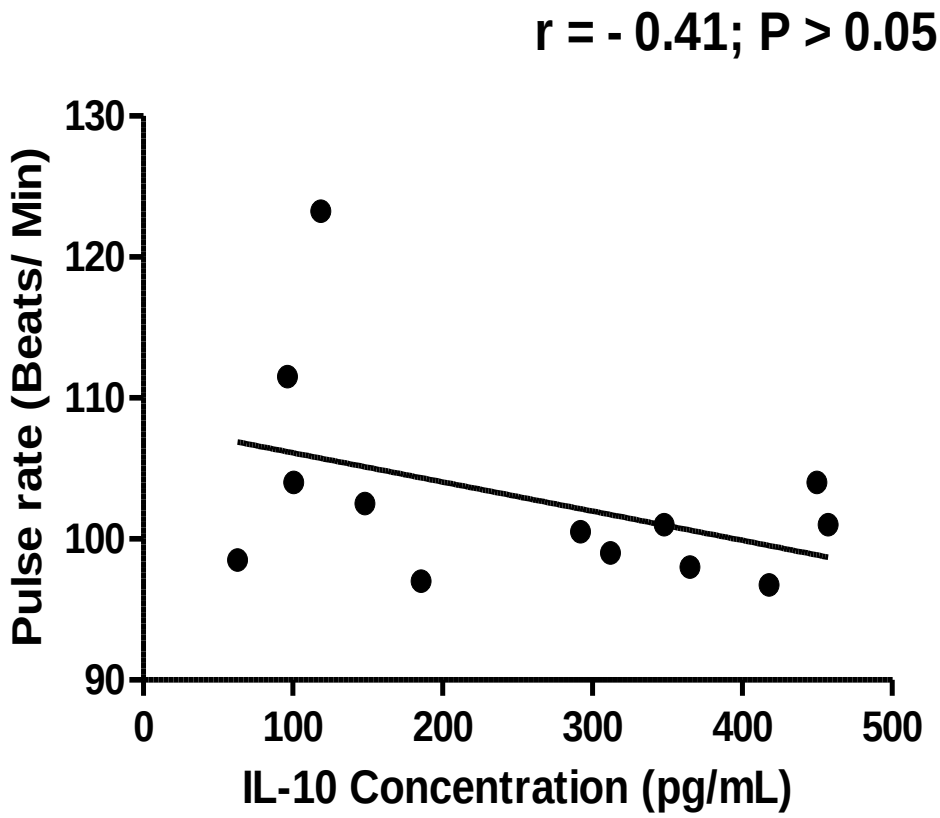


Figure 4.10D: Relationship between the levels of interleukin 10 (IL-10) and pulse rate. r = Pearson's correlation coefficient.

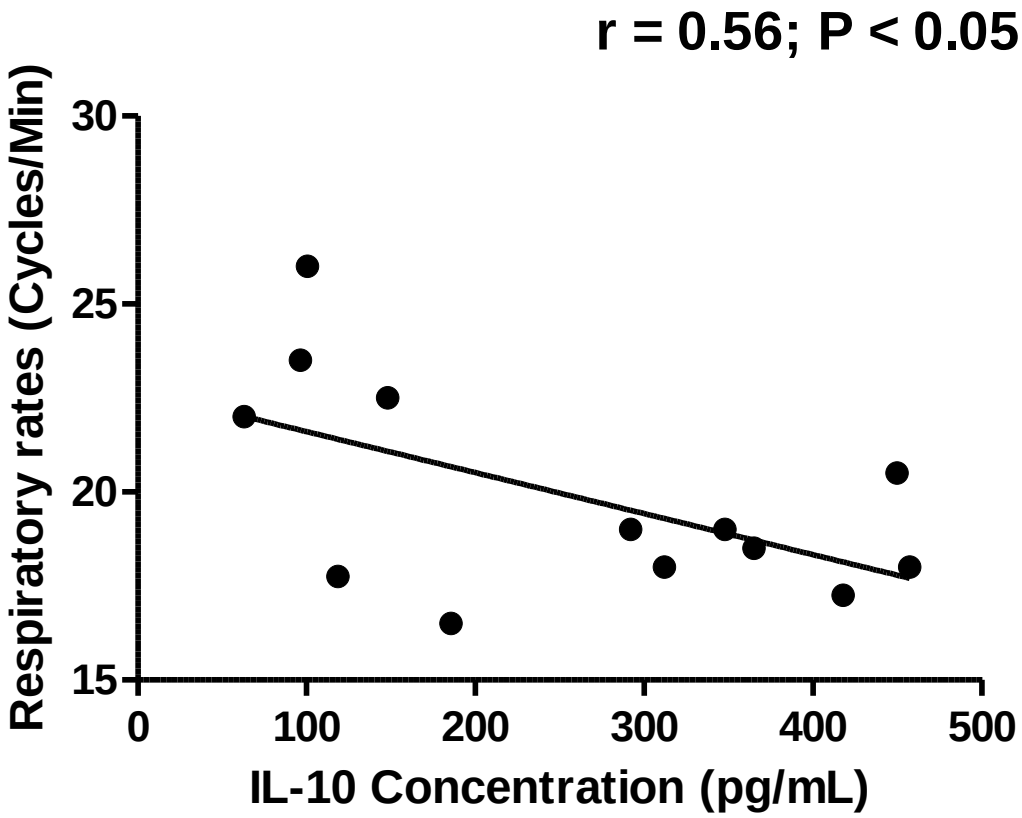


Figure 4.10E: Relationship between the levels of interleukin 10 (IL-10) and respiratory rate.r = Pearson's correlation coefficient.

4.2.6 Serum biochemistry

4.2.6.1 Serum electrolyte levels

There was slight change in the mean pre-surgical serum electrolyte concentrations following the colo-peritoneo-cystoplasty. The pre-surgical values of sodium ions (Na^+) measured on days 1, 4 and 7 were143.75 ± 1.75 mmol/L, 143.50 ± 3.52 mmol/L and 142.75 ± 2.25 mmol/L,respectively. The mean (143.33 ± 2.51 mmol/L) pre-surgical Na^+decreased to 136.00 ± 2.04 mmol/L a day post-surgery, which fluctuated from 143.00 ± 4.38 mmol/L on day 4 to 138.50 ± 4.09 mmol/L on day 7 post-surgery. Thereafter, on the 10^{th} day post-surgery, the [Na^+] was elevated to 142.25 ± 1.31 mmol/L and was subsequently maintained within the range of 138.75 ± 6.34 mmol/L and 145.00 ± 2.65 mmol/L to the end of the study (Appendix XXVII). There was no significant difference ($P > 0.05$) between the pre- and post- surgical serum Na^+concentration.

Pre-surgically, the mean serum potassium (K^+) measured was 5.14 ± 0.03 mmol/L, but not significantly changed post-surgery. The post-surgical concentration of the K^+ was maintained within pre-surgical range of 4.50 ± 0.21 mmol/L and 5.78 ± 0.21 mmol/L throughout the observation, except for passive decrease only on days 16 (3.85 ± 0.32 mmol/L) and 98 (3.20 ± 0.20 mmol/L) post-colo-peritone-ocystoplasty (Appendix XXVII). There was no significant difference in the values.

The concentrations of the serum chloride ion (Cl^-) were 40.00 ± 1.96 mmol/L, 43.25 ± 0.75 mmol/L and 35.25 ± 3.35 mmol/L on days 7, 4 and 1 before surgery, respectively and they serve as base-line values. Similarly, the Cl^-concentrations post-surgery did not differ significantly from the base-line values, which fluctuated between 34.00 ± 4.00 mmol/L and 42.67 ± 3.48 mmol/L throughout the period of observation (Appendix XXVII). There was no significant difference ($P > 0.05$) between base-line and post-surgical serumCl^- concentration.

Base-line serum bicarbonate (HCO_3^-) measured at 7, 4 and 1 days were respectively 20.75 ± 1.93 mmol/L, 20.75 ± 0.63 mmol/L and 22.50 ± 1.04 mmol/L. The post-operative concentration of HCO_3^- slightly deviated above the pre-surgical values on day 35 (23.75 ± 2.43 mmol/L), but did not differ significantly. Thereafter, the HCO_3^- concentrations were maintained within the base-line values to the end of the study (Appendix XXVII). There was no significant difference in between the pre-surgical and post-surgical serum bicarbonate concentrations.

The pattern displayed by the post-surgical serum Ca^{2+} concentration was different from the pre-surgical mean serum Ca^{2+} concentration (1.97 ± 0.29 mmol/L). The concentrations observed post-operatively increased above the base-line range (1.80 ± 0.30 mmol/L – 2.19 ± 0.06 mmol/L) to 2.27 ± 0.07 mmol/L and 2.42 ± 0.16 mmol/L on days 1 and 4, respectively, but it returned to the pre-surgical concentration(2.01 ± 0.29 mmol/L) on the 7^{th} day. It then fluctuated slightly above pre-surgical concentration on day 16 (2.35 ± 0.05 mmol/L) and 35 (2.33 ± 0.03 mmol/L). Furthermore, serum Ca^{2+} concentration slightly increased above the base-line on day 70 (2.32 ± 0.10 mmol/L), and was sustained to the end of the experiment (Figure 4.11). There was no significant difference in fluctuations between the base-line and post-surgical serum calcium ion (Ca^{2+})concentrations.

Similarly, the trend of fluctuations observed in the concentration of serum phosphate ions (PO_4^-) was that of increase, though at different pace, above the pre-surgical values. Initially, the measured pre-surgical concentrations of serum PO_4^- were 0.81 ± 0.28 mmol/L, 1.03 ± 0.05 mmol/L and 0.61 ± 0.17 mmol/L for days 7, 4 and 1, respectively. The increase above the base-line concentration was basically observed on days 7 (1.16 ± 0.06 mmol/L), 10 (1.18 ± 0.12 mmol/L), 13 (1.16 ± 0.20 mmol/L) and 70 (1.36 ± 0.24 mmol/L) post-operation (Figure 4.11), but the difference in the concentrations was not significant.

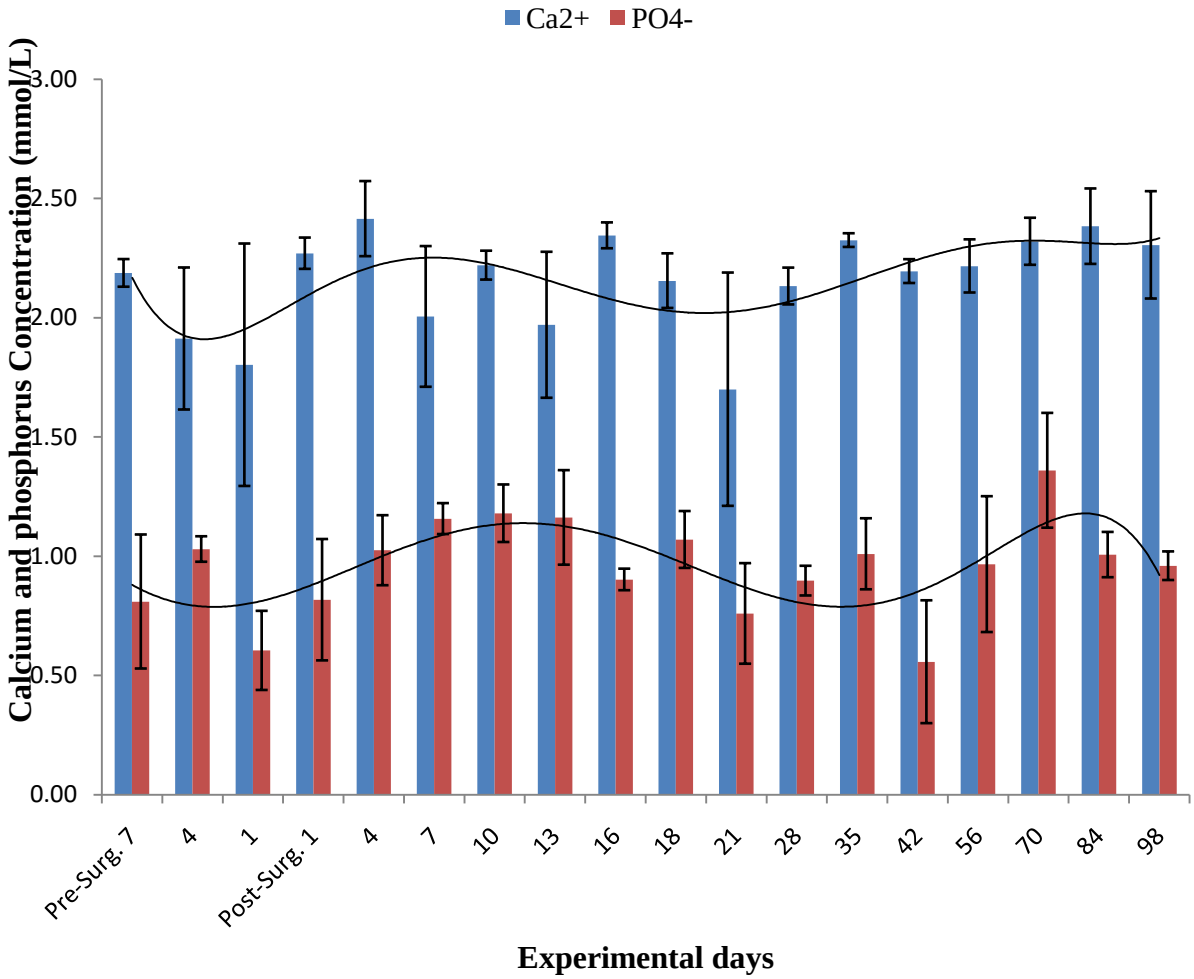


Figure 4.11: Pre- and post-surgical levelsin serum calcium [Ca^{2+}] and phosphorus [PO_4^-]concentrations

4.2.6.2 Serum urea, glucose and albumin concentrations

Serum urea concentrations recorded as base-line on days 7, 4 and 1 before the colo-periotoneo-cystoplasty were 5.38 ± 1.03 mmol/L, 5.13 ± 0.69 mmol/L and 5.13 ± 0.43 mmol/L respectively. The concentrations increased immediately after the surgery on days 1 (5.63 ± 1.84 mmol/L), 4(5.75 ± 1.38 mmol/L), 7 (5.88 ± 0.63 mmol/L) and 10 (8.00 ± 1.17 mmol/L). The concentration subsequently returned to the pre-surgical level on day 13 (5.38 ± 0.97 mmol/L) was sustained relativelyto day 21 post-surgery. The values thereafter, deceased and fluctuated between the base-line concentrations (Figure 4.12). The ($P < 0.05$) phosphate concentration pre-surgery was significantly lower than that post-surery**.**

The pre-surgical serum glucose concentration, measured between 3.05 ± 0.39 mmol/L and 3.75 ± 0.55 mmol/L, increased immediately after surgery and fluctuated between 3.88 ± 0.91 mmol/L and 5.40 ± 0.56 mmol/L throughout the study. An exception to the fluctuation occurred on days 10 and 28 whenthe concentrationsrecorded were 3.60 ± 0.52 mmol/L and 3.60 ± 0.50 mmol/L, respectively (Figure 4.12). There was no significant difference ($P < 0.05$) between the pre- and post- surgical concentrations.

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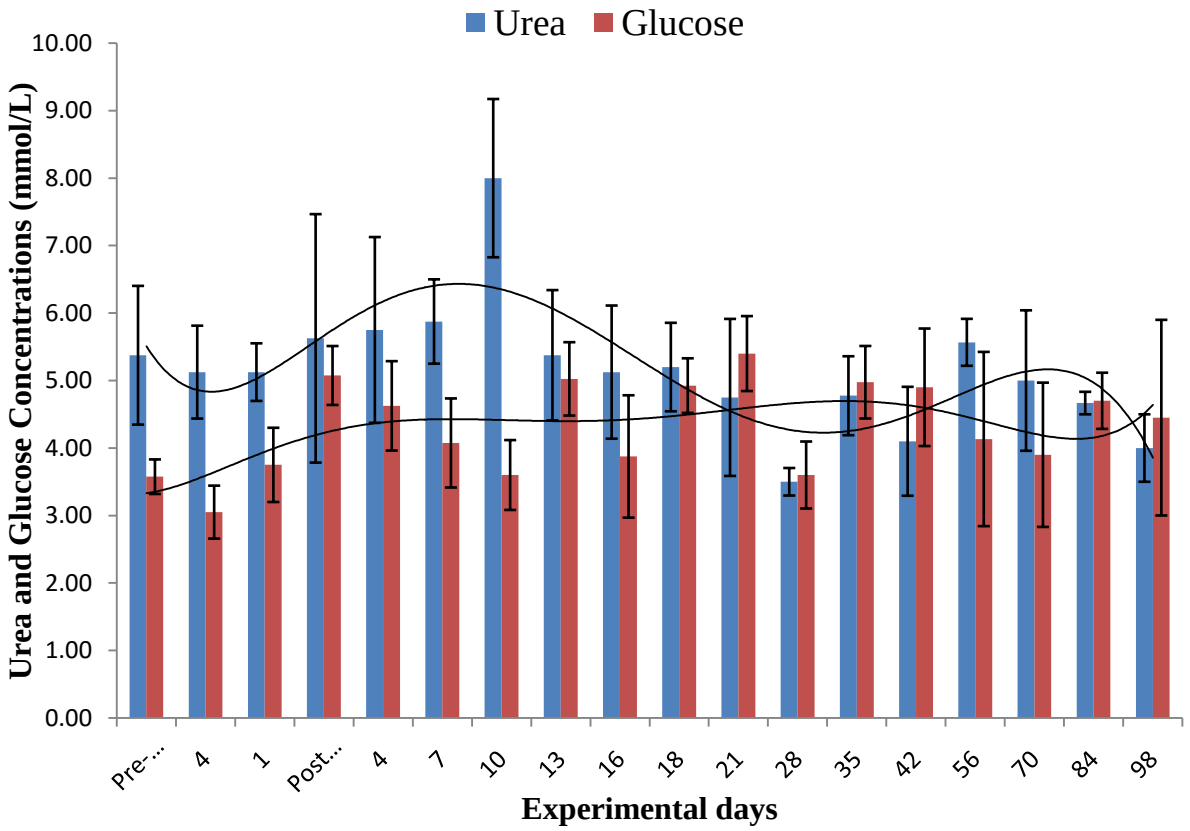


Figure 4.12: Pre- and post-surgical serum urea and glucose concentrations in colo-peritoneo-cystoplastic dogs

Total serum protein (TP) at the base-line level was between 104.75 ± 4.96 mmol/L to 108.50 ± 3.33 mmol/L. Post-surgically, the concentration transiently fluctuated below and above the base-line values with no unique pattern, between the reference base-line and the slightly changed values of 95.25 ± 8.01 mmol/L and 112.00 ± 5.29 mmol/L (Appendix XXVII). The changes were not significant ($P > 0.05$). However, the pattern in serum albumin concentration was completely different from that of the TP, though not significant ($P > 0.05$). The serum concentration of the albumin fell below the base-line range (73.00 ± 3.11 mmol/L – 80.75 ± 1.55 mmol/L) post-surgically, and within the first 16 days between 63.25 ± 3.15 mmol/L and 72.00 ± 4.71 mmol/L (Appendix XXVII). The concentration subsequently stabilised within the reference values up to the completion of the study, except for days 35 (68.75 ± 1.25 mmol/L) and 56 (68.67 ± 3.48 mmol/L).

4.2.6.3 Serum enzyme activities

The pre-surgical mean serum alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) activities were 10.67 ± 2.93 mmol/L, 17.17 ± 5.16 mmol/L and 52.17 ± 7.41 mmol/L, respectively. The post-surgical ALT activity slightly decrease below the base-line activity on days 4 (6.50 ± 1.32 mmol/L) and 7 (7.00 ± 2.55 mmol/L), and subsequently rose to 18.50 ± 5.91 mmol/L on day 10, but returned to 10.00 ± 2.92 mmol/L and 13.25 ± 5.82 mmol/L (within base-line limits). The ALT activity on day 18 increased above the base-line level and fluctuated between 14.33 ± 6.33 mmol/L and 20.25 ± 2.02 mmol/L, until the activity returned within the base-line value at day 84 (13.67 ± 1.76 mmol/L) (Figure 4.13). There was no significant difference ($P > 0.05$) between the pre-surgical and post-surgical activities.

The alteration in serum AST activity was lower than that of the base-line activity on day 1 post-surgery (9.00 ± 2.04 mmol/L), but subsequently increased to 14.75 ± 1.65 mmol/L on

day 4. Thereafter, the activity fluctuated between 9.00 ± 2.04 mmol/L and 19.67 ± 1.45 mmol/L throughout the study period (Figure 4.13), althuough the difference in the activities was not significant.

The serum ALP activity was elevated above the base-line range on days 1 (76.75 ± 2.90 mmol/L), 4 (62.25 ± 9.01 mmol/L) and 70 (83.00 ± 8.00 mmol/L) post-surgery (Appendix XXVII). Again the difference in the activities was not significant.

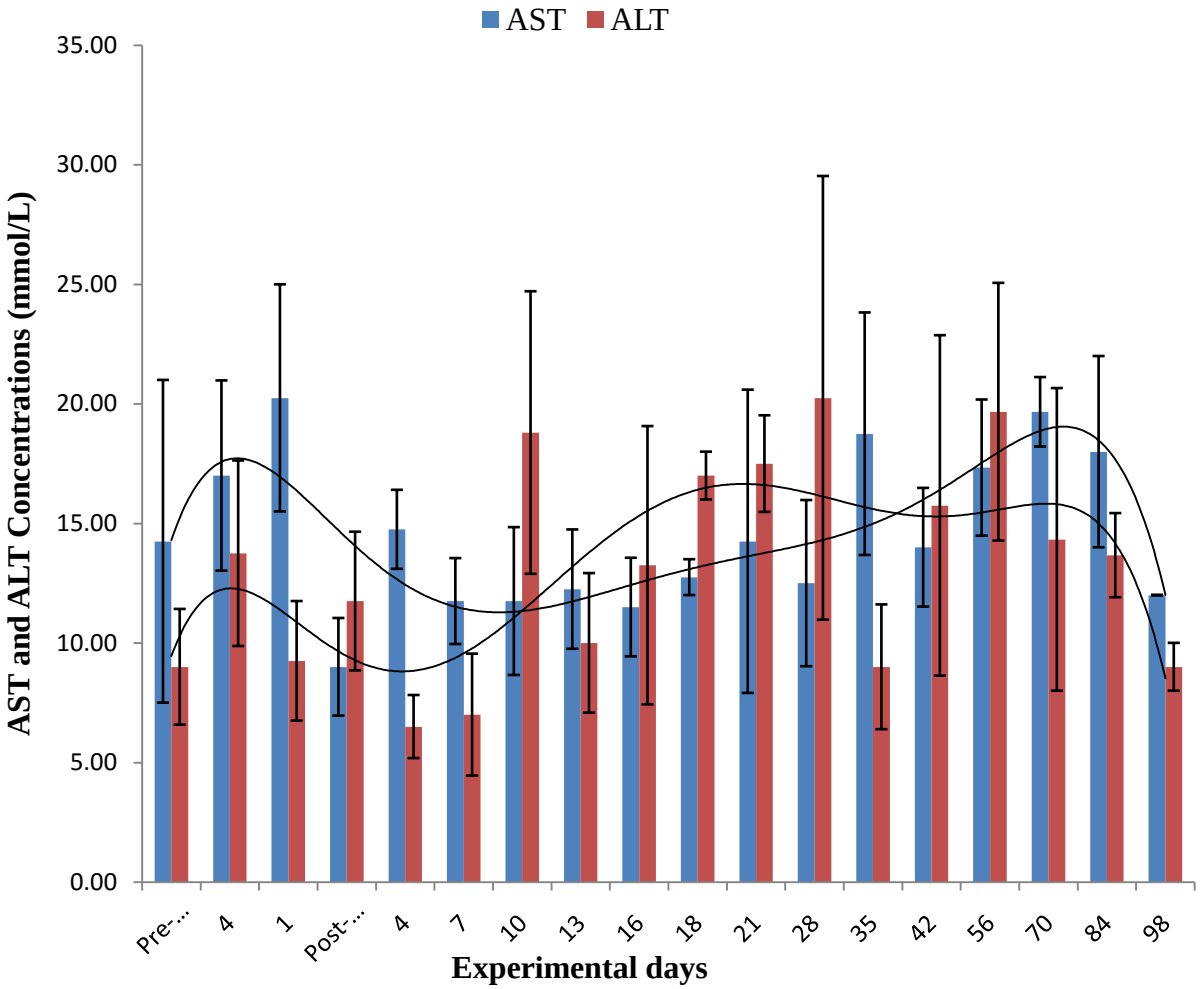


Figure 4.13: Pre- and post-colo-peritoneo-cystoplastic activities of serum alanine transaminase (ALT) and aspartate transaminase (AST)

4.2.7 Diagnostic imaging

4.2.7.1 Cystosonography

Ultrasonographic assessments performed on day 8 post-surgery showed anechogenic appearance of the bladder content, with thickened bladder wall at the point of patch (dorsum) compared to the ventrum (Plates XXA and XXB). Similarly, the dorsal bladder wall (point of patch) presented amarked decrease in thickness at day 21 post surgery (Plate XXI)**.**

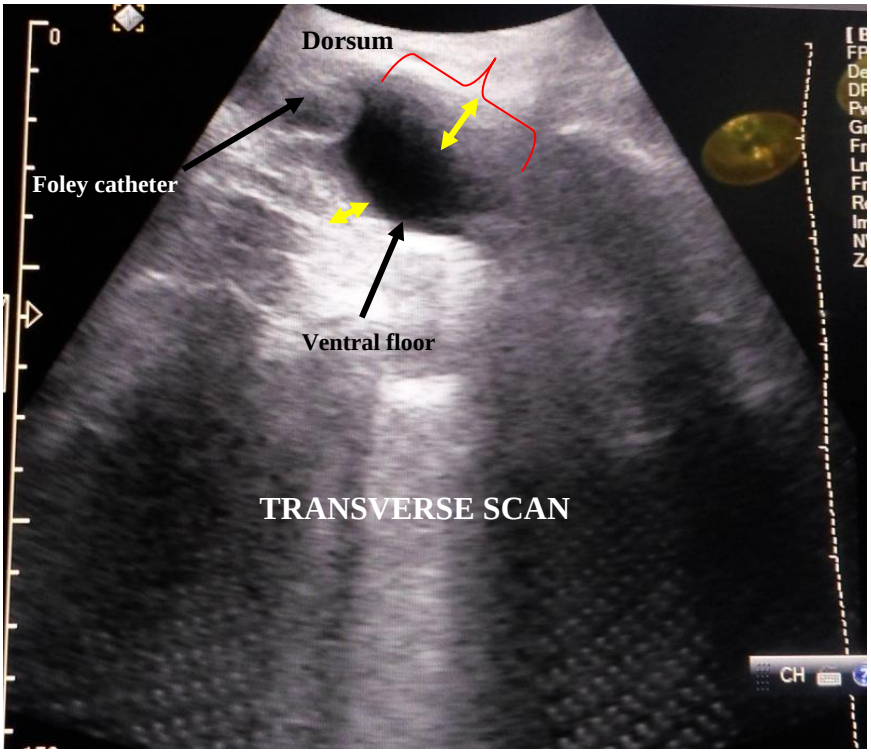


Plate XXA: Sonographic appearance of the urinary bladder at day 8 post-surgery showing anechogenic appearance of the bladder content in male. Note the thickened bladder wall at the dorsum (point of composite colo-peritoneal patch) relative to the ventral (native) wall of the bladder

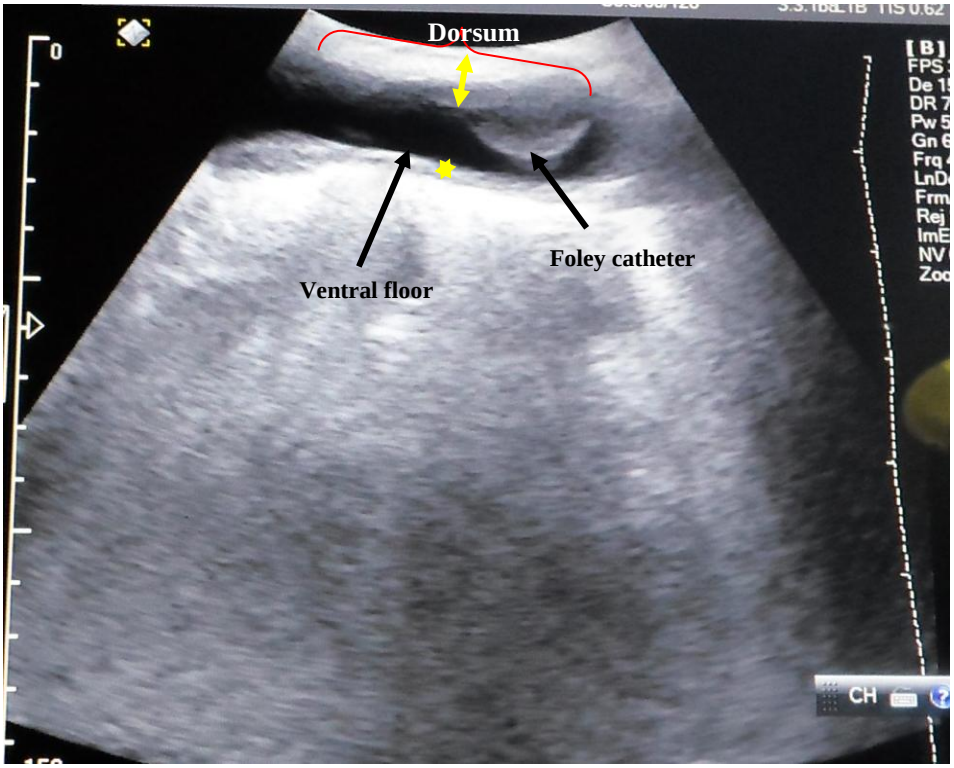


Plate XXB: Sonographic appearance of the urinary bladder at day 8 post-surgery showing anechogenic appearance of the bladder content in female. Note the thickened bladder wall at the dorsum (point of composite colo-peritoneal patch) relative to the ventral (native) wall of the bladder

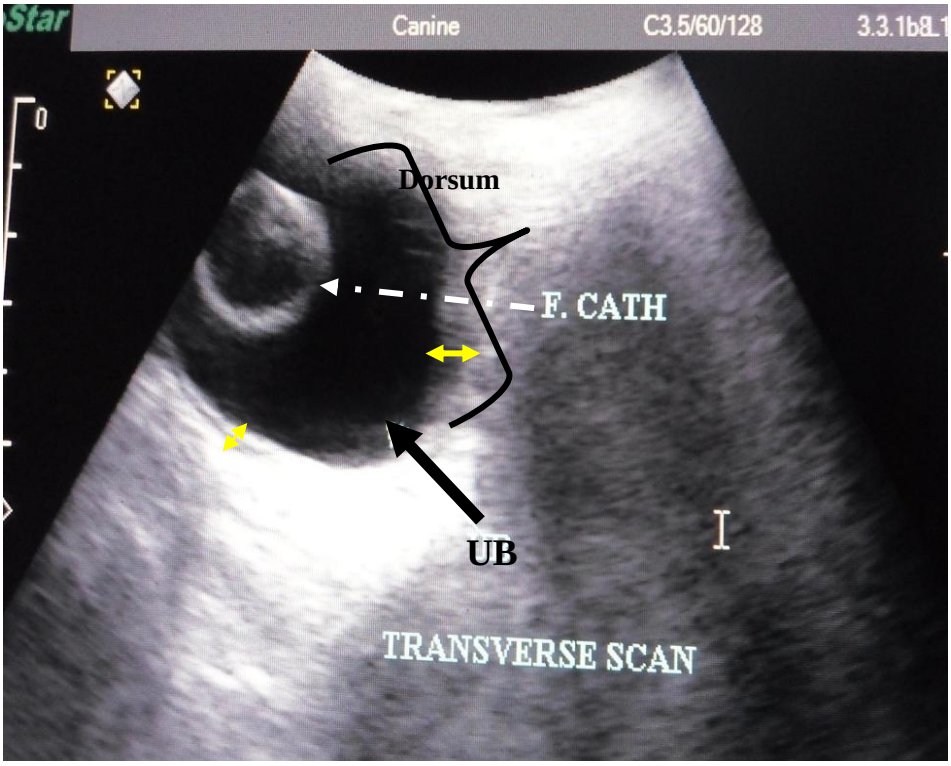


Plate XXI: Sonograph of the urinary bladder showing marked decrease in the thickness of the dorsal bladder wall (composite coloperitoneal tissue) at day 21 post-surgery

4.2.7.2 Cystography

The contrast radiographic evaluation of the reconstructed urinary bladder (with composite colo-peritoneal tissue) at different periods of the study revealed high opacification only within the urinary bladder in both the ventro-dorsal and lateral views, indicating distinctive urinary bladder silhouettes. There was gradual transition of the urinary bladder architecture at the point of patch. It appeared with clear delineation and minimal blurring at week 2 post-surgery (Plates XXIIA and XXIIB) that gradually disappeared following subsequent evaluations at weeks 10,14 and 108 post-operatively (Plates XXIIIA and XXIIIB, XXIVA and XXIVB and XXVA and XXVB respectively). There was evidence of bulging or diverticulum at the reconstructed site of the urinary bladder (dorsum) in one of the females (EC_2) at108 post-operatively (Plate XXVB).

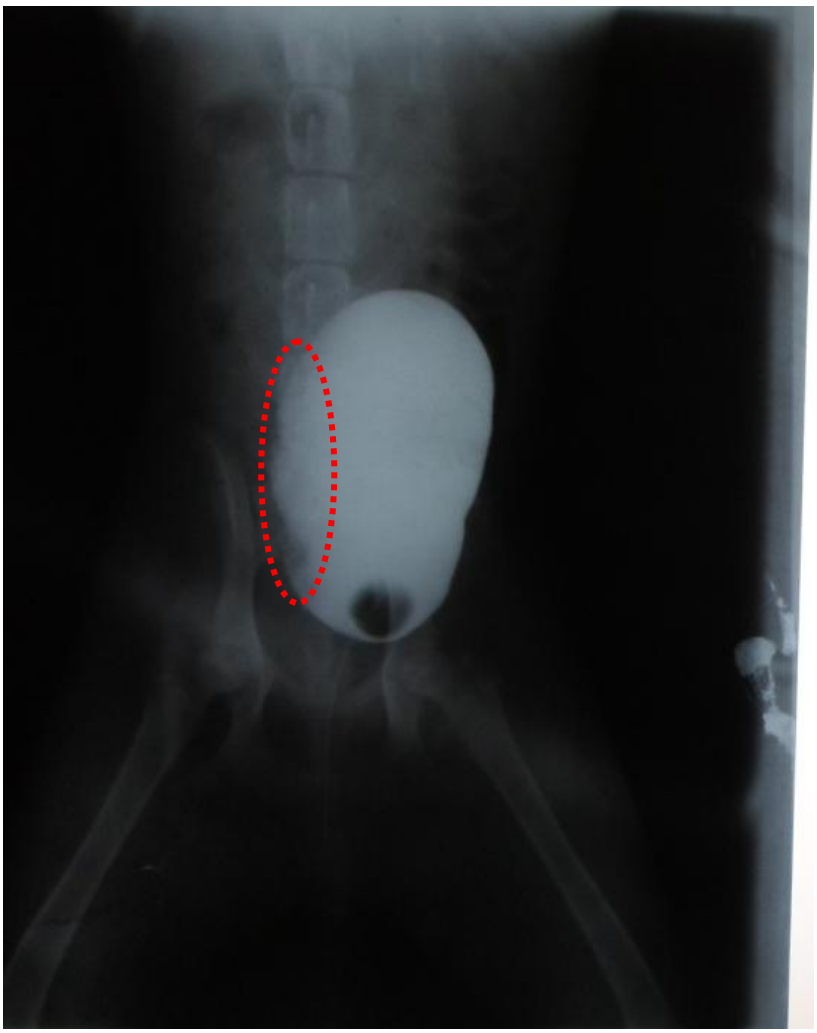


Plate XXIIA: Ventro-dorsal cystograph two weeks after colo-peritoneo-cystoplasty. Note the clear delineation of the urinary bladder architecture and irregularity at the point of patch (circled) in a dog.

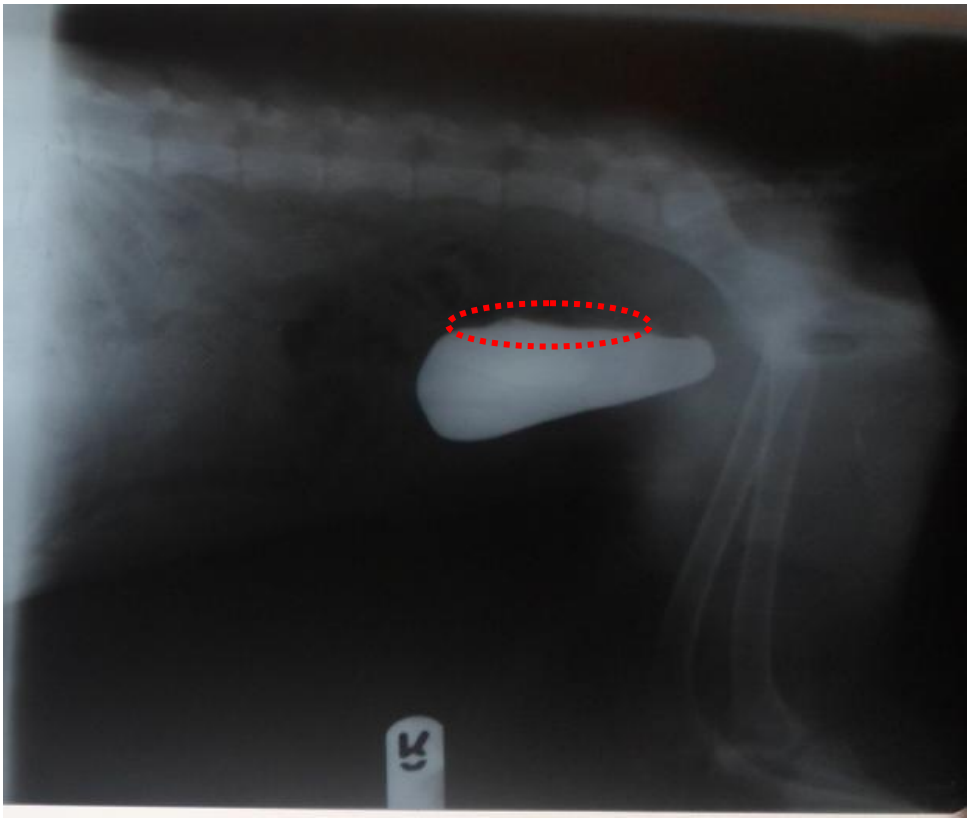


Plate XXIIB: lateral cystograph two weeks after colo-peritoneo-cystoplasty. Note the clear delineation of the urinary bladder architecture and irregularity at the point of patch (circled) in a dog.

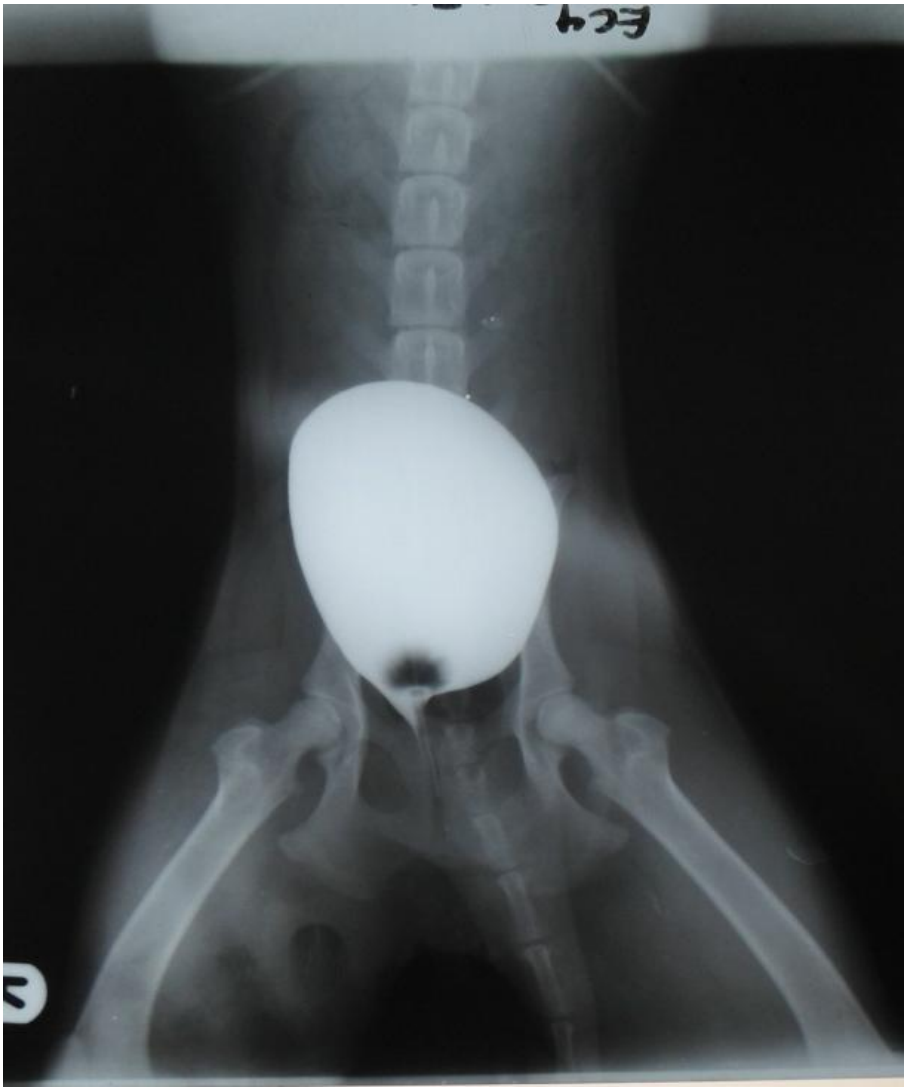


Plate XXIIIA: Ventro-dorsal contrast cystograph of the reconstructed urinary bladder 10 weeks post-cystoplasty. Note the clear delineation of the urinary bladder architecture in a dog

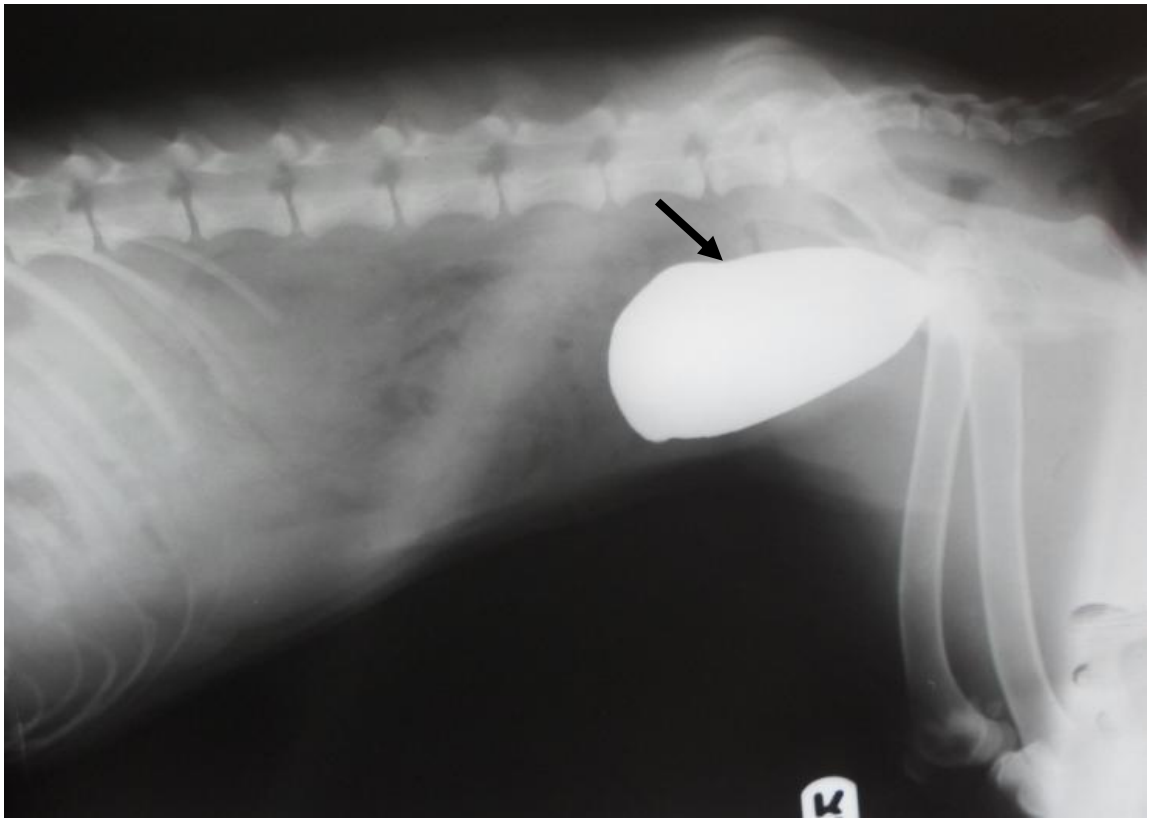


Plate XXIIIB: Lateral contrast cystograph of the reconstructed urinary bladder 10 weeks post-cystoplasty. Note the clear delineation of the urinary bladder architecture and slight dent at the point of patch (arrowed) in a dog



Plate XXIVA: Ventro-dorsal contrast cystograph of the reconstructed urinary bladder 14 weeks post-colo-peritoneo-cystoplasty. Note the clear delineation of the urnary bladder architecture and uniform regularity in a dog.

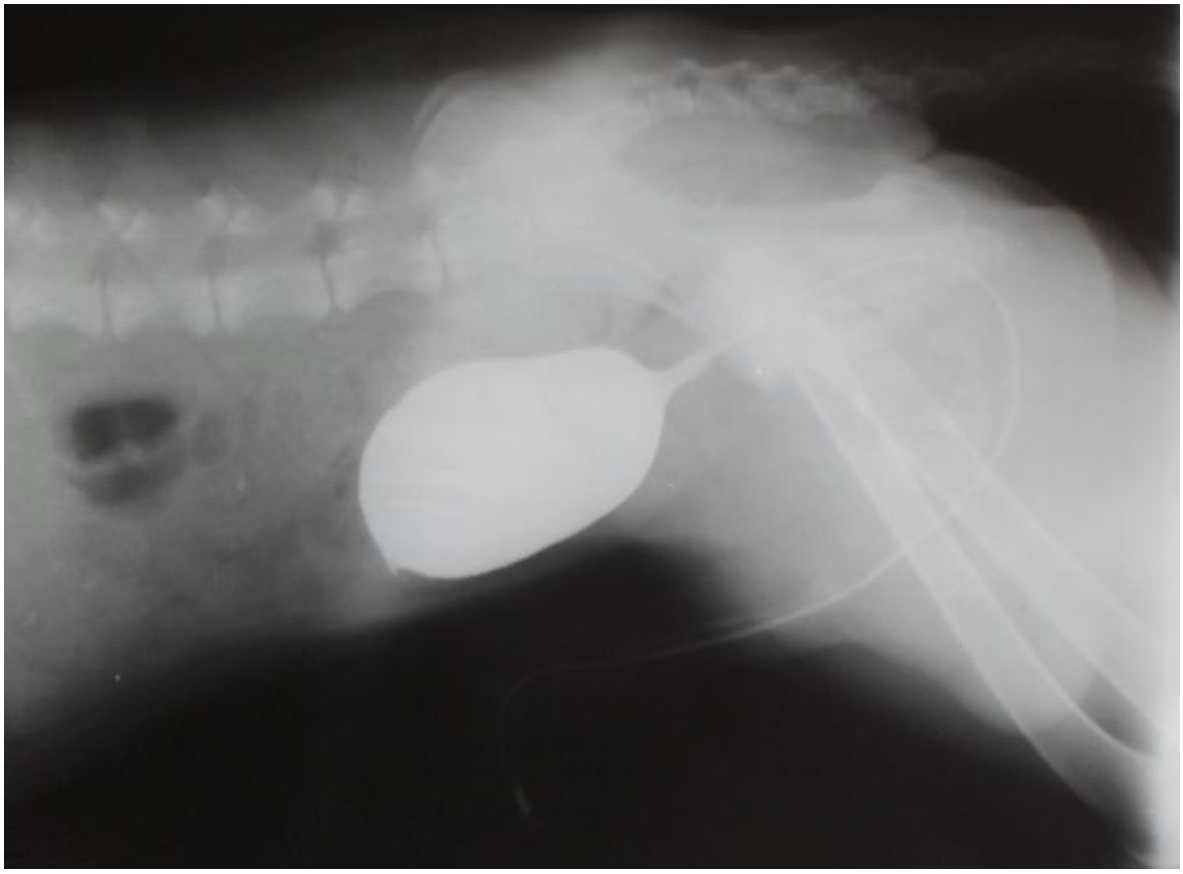


Plate XXIVB: Lateral contrast cystograph of the reconstructed urinary bladder 14 weeks post-colo-peritoneo-cystoplasty. Note the clear delineation of the urnary bladder architecture and uniform regularity in a dog.

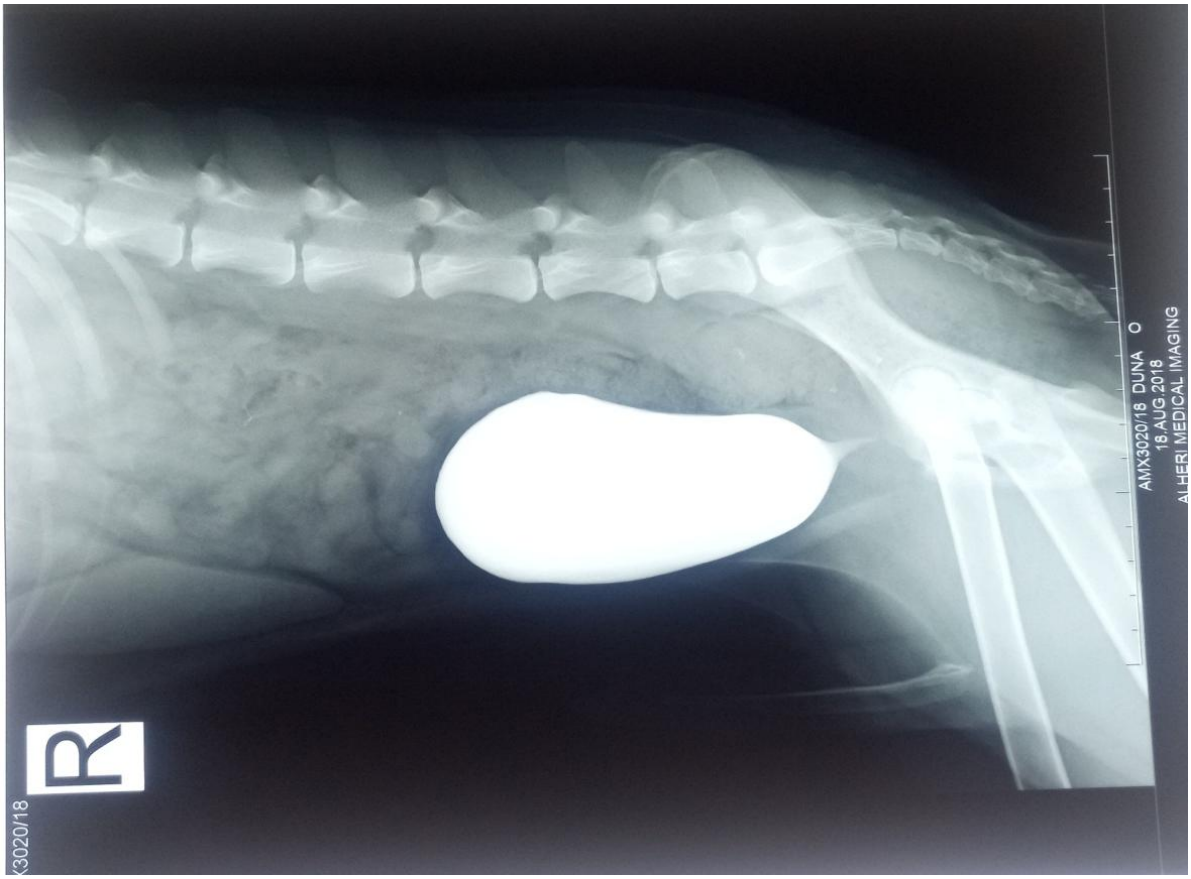


Plate XXVA: Lateral contrast cystograph of the reconstructed urinary bladder 108th week post-colo-peritoneo-cystoplasty in a male dog. Note the clear delineation of the urnary bladder architecture.

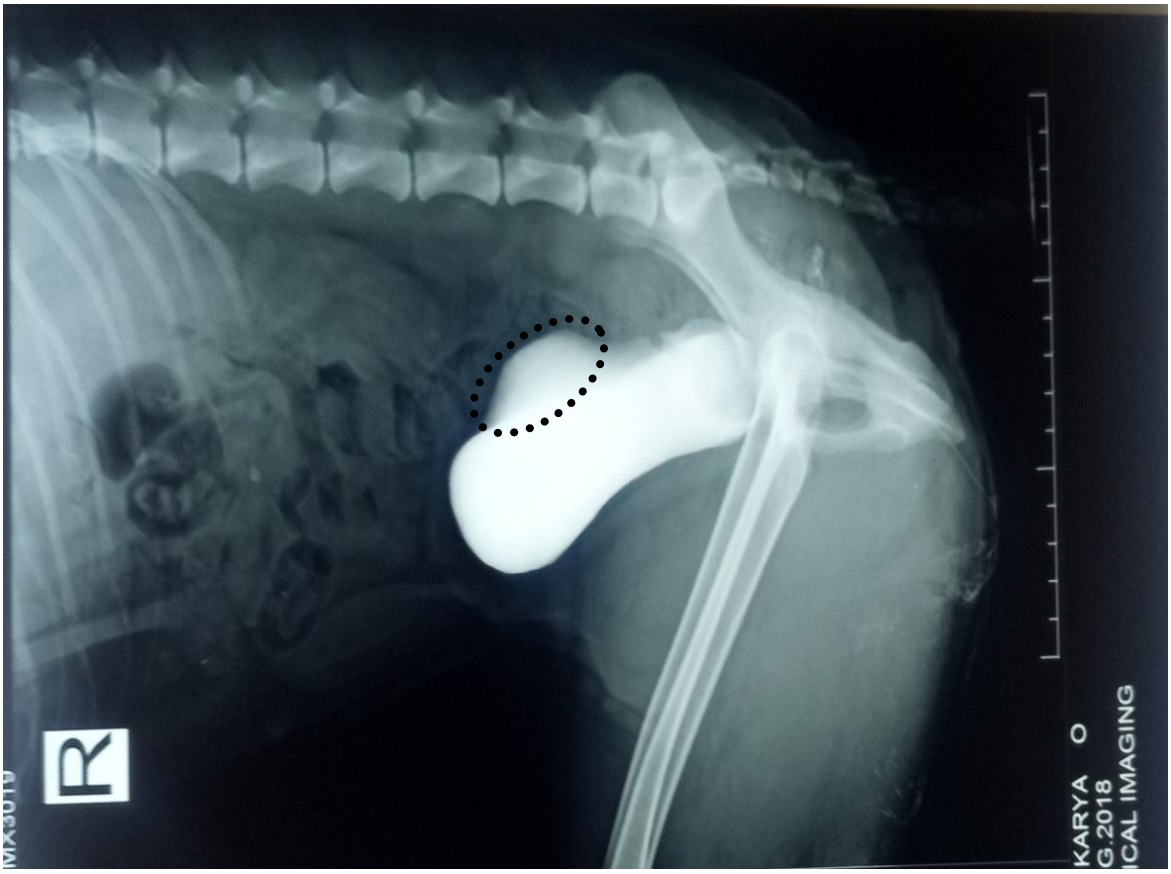


Plate XXVB: Lateral contrast cystograph of the reconstructed urinary bladder 108th week post-colo-peritoneo-cystoplasty in a female dog. Note the clear delineation of the urnary bladder architecture withdiverticulum (circled).

4.2.8 Gross evaluation

Gross evaluation following exploratory surgeries (laparotomies)on the 108th week post- colo-peritoneo-cystoplasty revealed omental adhesions on the dorsum of the urinary bladder in both male and female dogs (Plates XXVIA,XXVIB and XXVIIA), and urinary bladder diverticulum on the dorsum in a female subject (Plate XXVIIB).

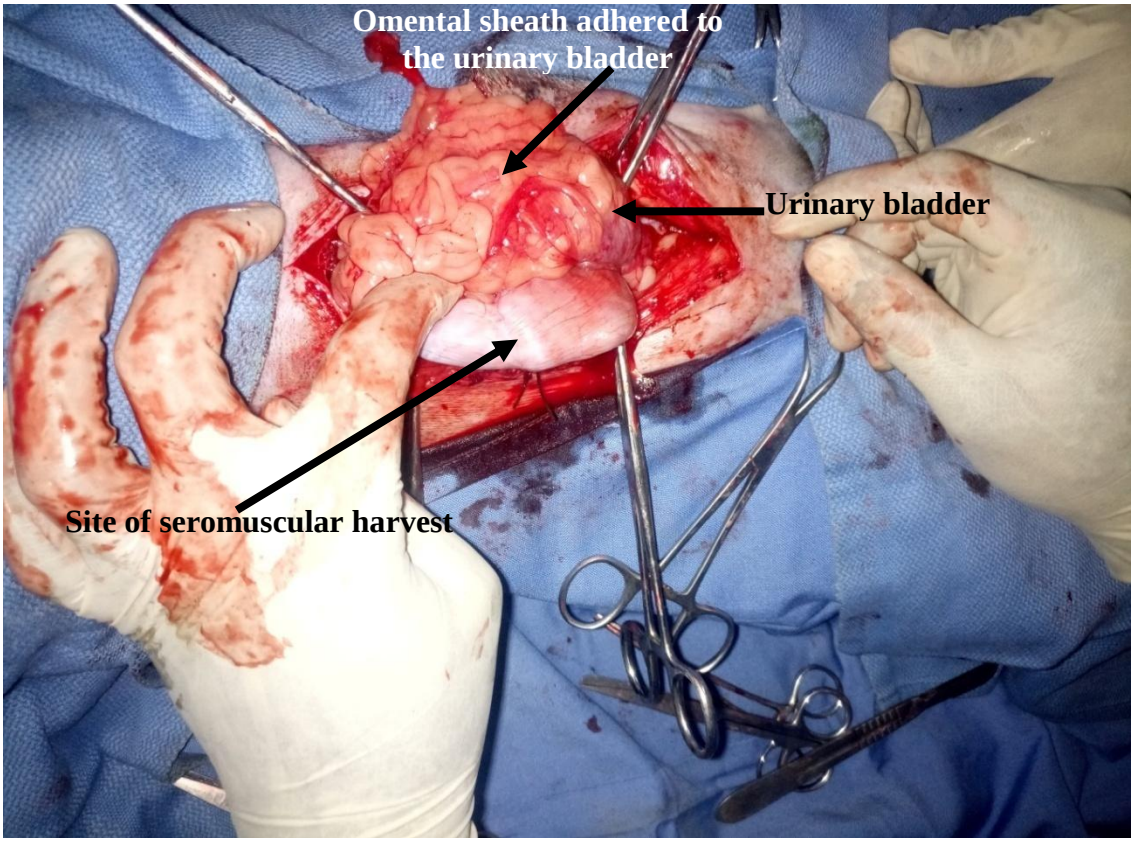


Plate XXVIA: Gross appearance of the urinary bladder 108th week post-colo-peritoneo-cystoplasty in male dog showing omental adhesion on the urnary bladder

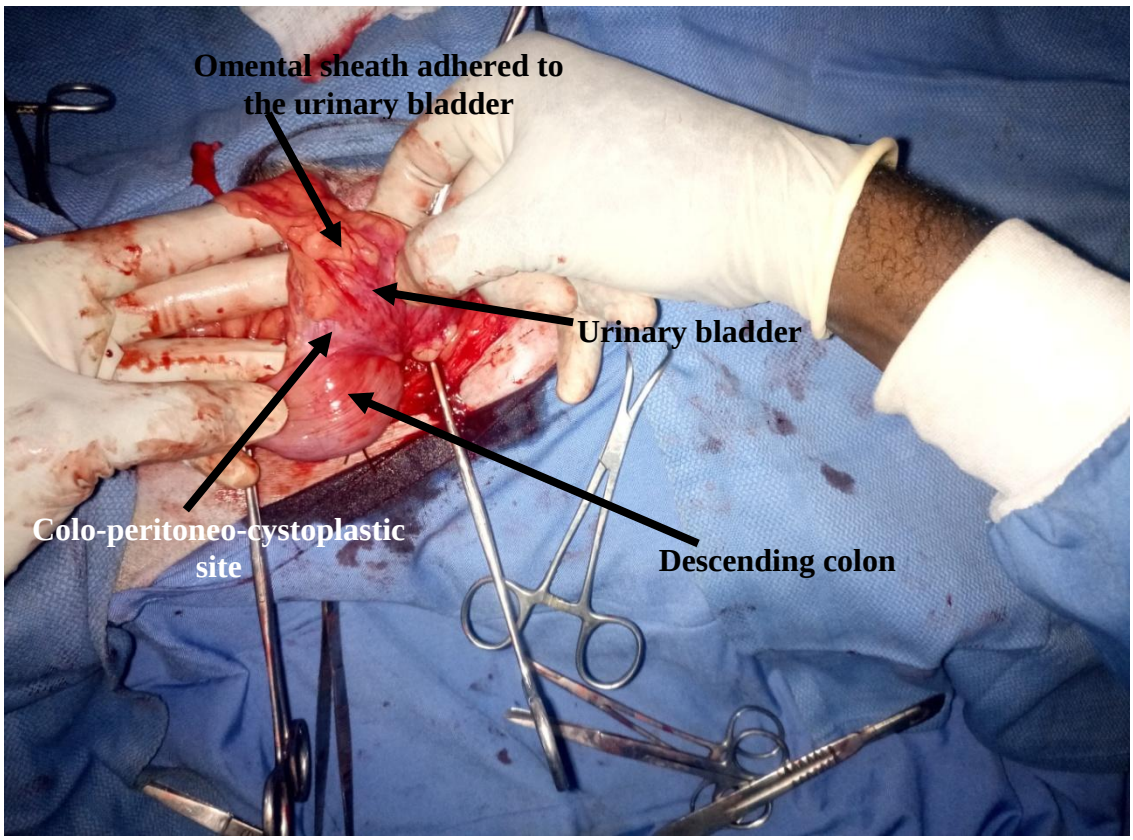


Plate XXVIB: Detachement of the adhered omentum from the urinary bladder 108th week post-colo-peritoneo-cystoplasty in male dog.

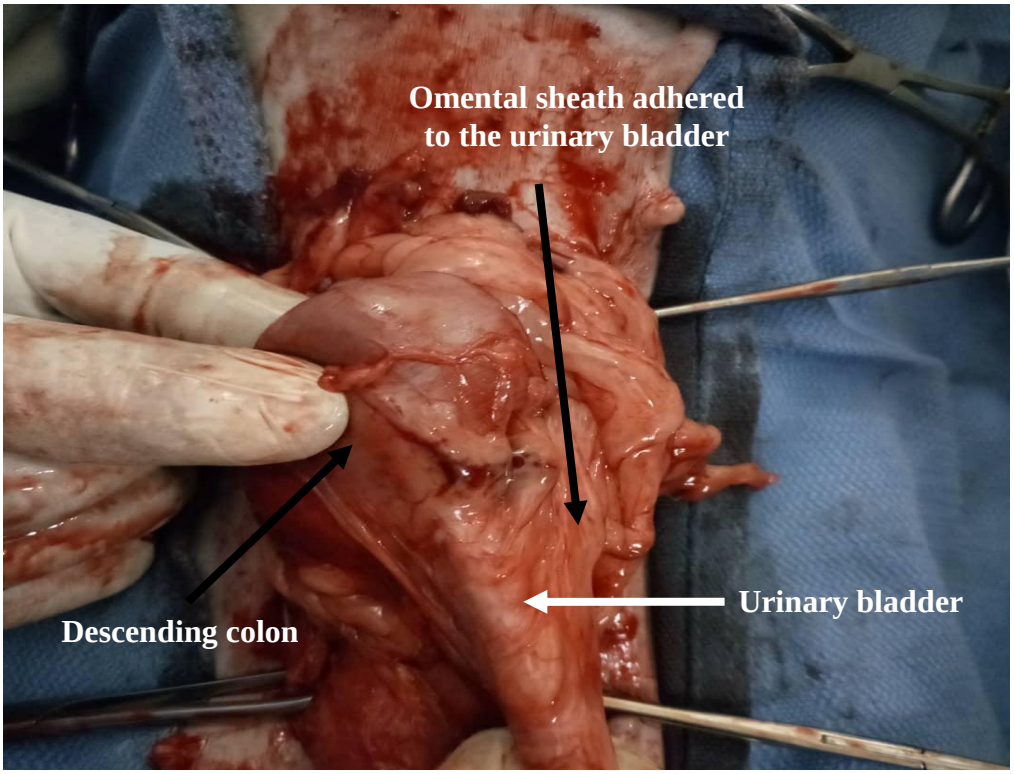


Plate XXVIIA: Gross appearance of the urinary bladder 108th week post-colo-peritoneo-cystoplasty in female dog showing omental adhesion on the urnary bladder.

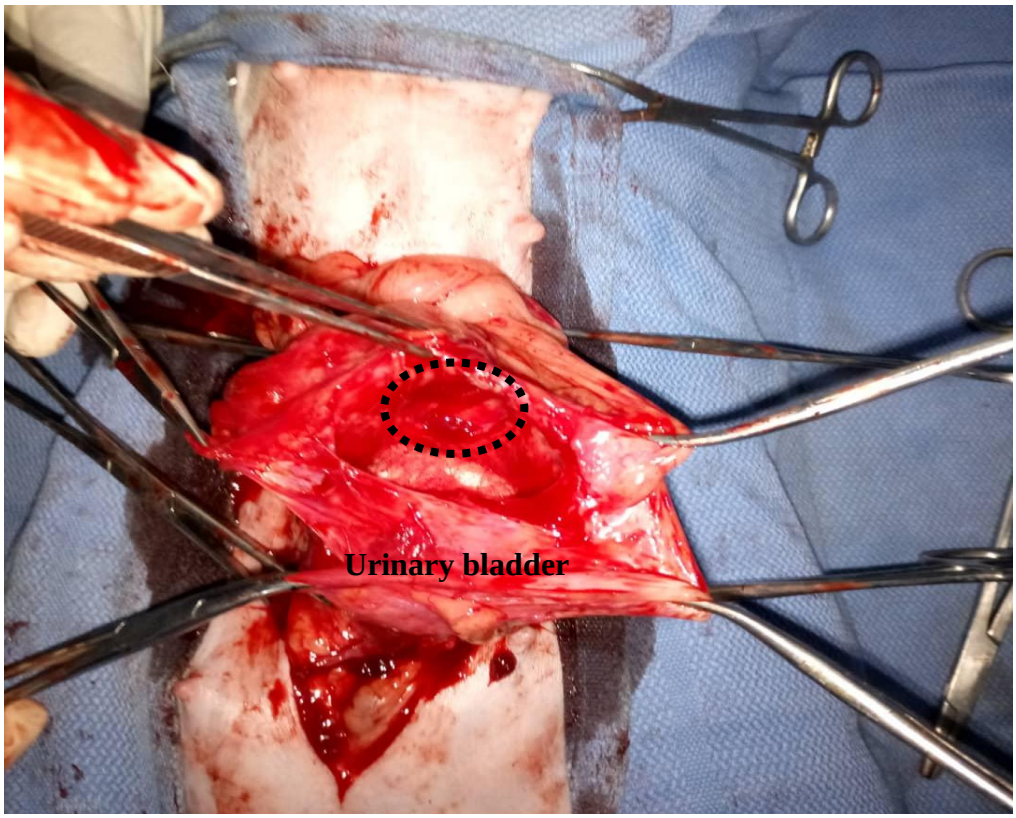


Plate XXVIIB: Cystotomised urinary bladder 108th week post-colo-peritoneo-cystoplasty in a female dog showing diverticulum (circled) on the dorsum.

B

4.2.9Histopathology

The histological section of the colo-peritoneo-cystoplastic tissue at 14th and 108th weeks post-surgery has no clear line of demarcation between the mucosae of the urinary bladder and composite coloperitoneal tissue at the site of anastomosis. There was evidence of squamous and columnar cellson the composite tissue (Plate XXVIII). Multiple blood vessels (vascular buds) with mononuclear cellular infiltration at submucosae of the colo-peritoneo-cystoplastic junction and the composite colo-peritoneal segment were observed at the108th week post-surgery (Plate XXIX).

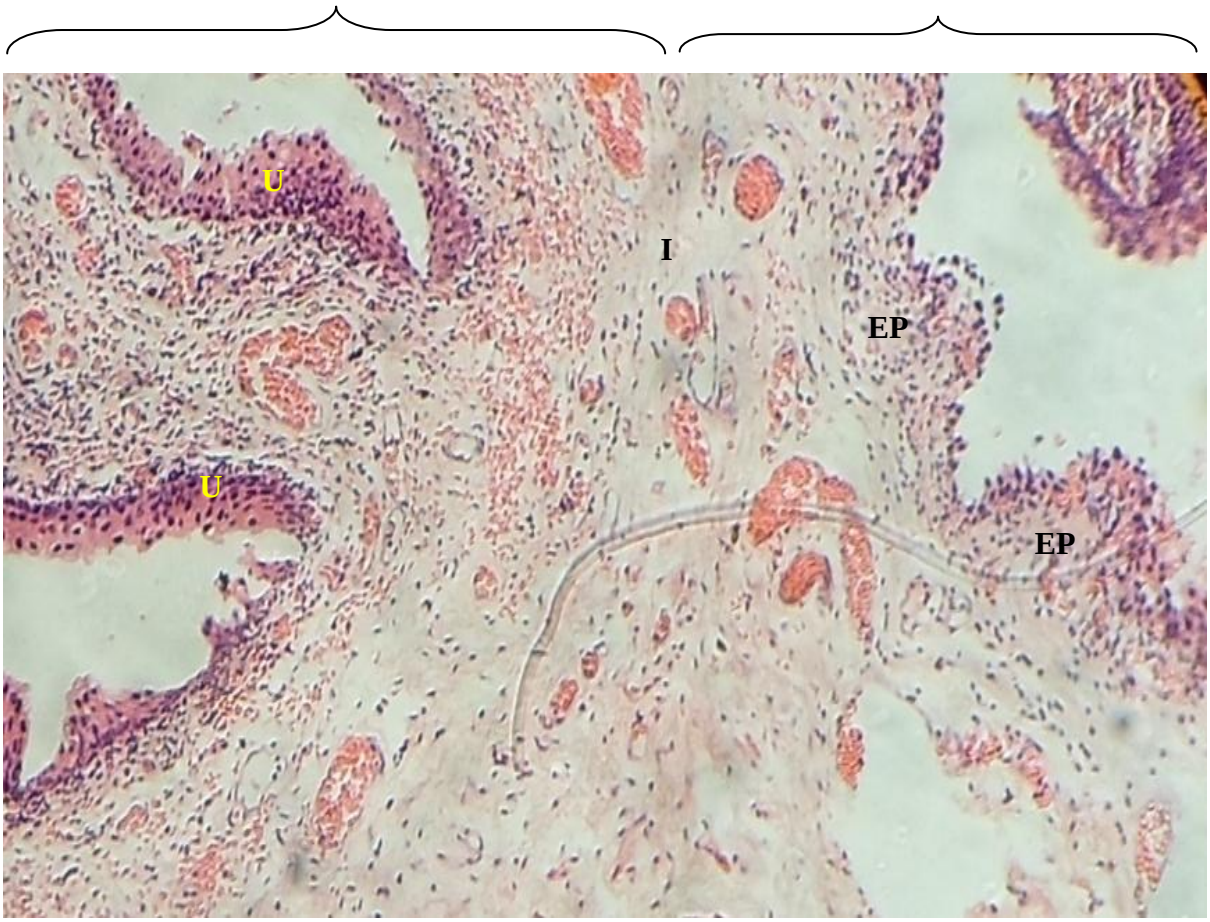


Plate XXIX : Photomicrograph of colo-peritoneo-cystoplastic tissue section showing urothelium (U), epithelium of peritoneal sheath (EP) and evidence of integration (I) at 14th week (H and E×1000)

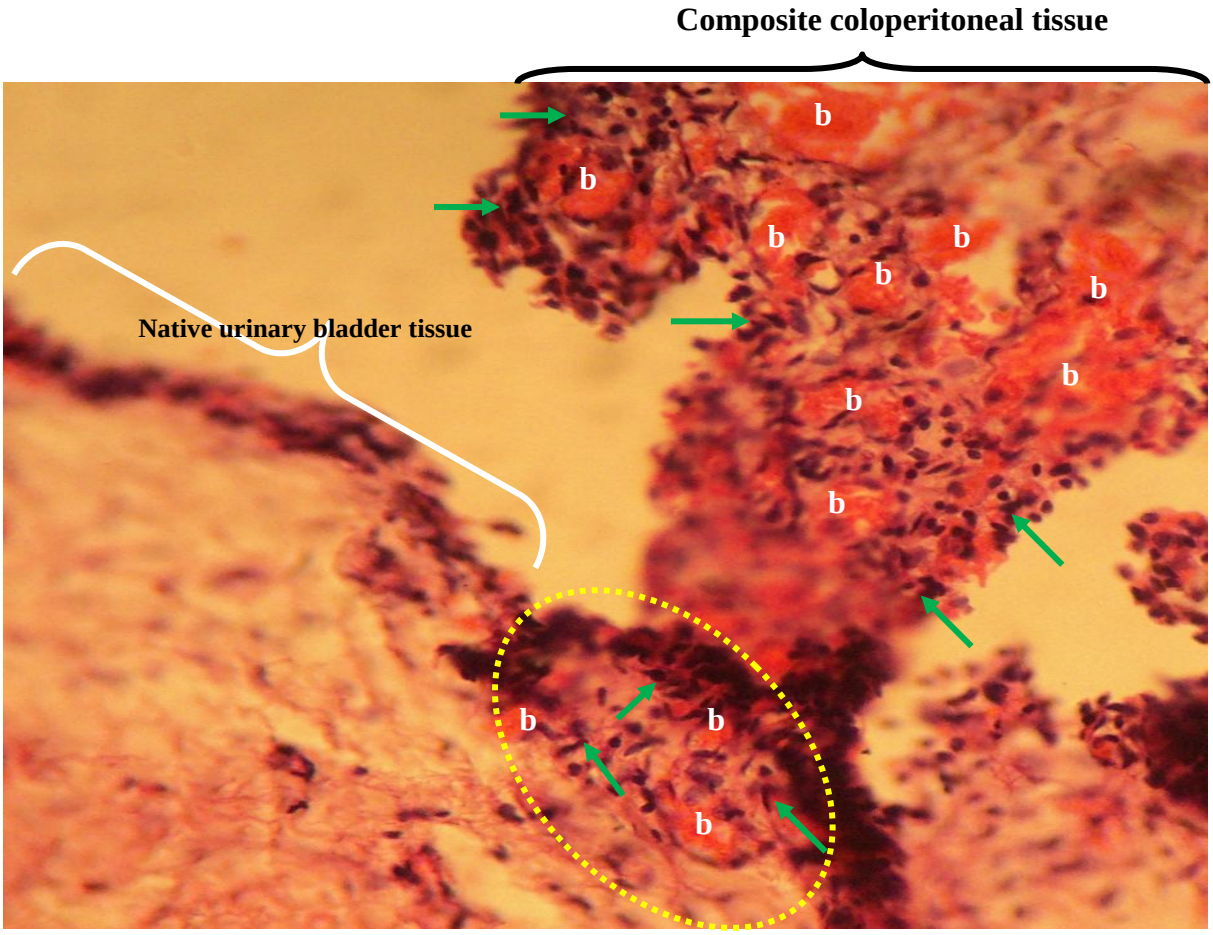


Plate XXX: Photomicrograph of colo-peritoneo-cystoplastic tissue section showing multiple neovascularisation (b) with mononuclear cellular infiltration (arrows)at submucosae of the colo-peritoneo-cystoplastic junction (circled) and the composite colo-peritoneal segmentat 108thweek (H and E × 400)

CHAPTER FI

5.0 DISCUSSION

Suitable guide for selecting the NIDs that were used in this study was based on the results of pre-surgical evaluations of physical findings, vital parameters, haematological and some biochemical parameters. Tick infestation, isosporosis, babesiosis and taeniasis observed prior to the commencement of the study were not unusual due to the endemicity of the parasites causing such conditions in the environment (Ogbaje *etal*., 2015).However, regular acaricide spray, appropriate anticoccidial, babesiocidal and antihelminthic therapies instituted based on the confirmatory diagnoses and prompt kennel hygiene were employed as control measures and in providing optimally conducive environment for the experimental dogs used in the study.

The afore-mentioned, evaluated parameters, urinalysis, ultrasonographic, radiographic, histological and cytokine evaluations were used to monitor and assess the pre-surgical (base-line) and post-surgical responses of the NIDs.The responses of the dogs were used to determine the quality of the autologous composite colo-peritoneal pedicle grafting technique, employed in the repair of experimentally-created urinary bladder defects in dogs. It was deduced that the defect created on the urinary bladder mimicked conditions resulting in loss (due to disease or surgery) of significant portion of the bladder, which necessitated surgical repair by grafting technique.

This research was significant in evaluating relatively medium-term responses by NIDs following autologous composite colo-peritoneo-cystoplastic procedure. The decision on success of the technique by reconstruction was based on the concept of ‘composite’ cystoplasty, involving transplantation of histologically different tissues in order to facilitate the restoration of function and structural reliability.The cystoplasty was the culmination of

progress in two disparate surgical disciplines - reconstructive surgery and transplantation surgery. The surgery consideredthe status of the optimum functional kidneys,theconcentrating defect, and the intestinal segment and location of the parietal peritoneal sheath to be selected. It also determined the amount of the composite colo-peritoneal tissue (formed) required to achieve adequate compliance, configuration of the segment on the native bladder, and assessment of the patient for the colo-peritoneo-cystoplastic procedure, considered a quality-of-life intervention. The surgical technique was averagely completed within 3 hours 30 minutes, which was consistent with the team of surgeons' experiences and understanding.

The dorsum of the urinary bladder used as the site for partial cystectomy was appropriate because the bladder is adjacent to the descending colon (donor) which can be easily mobilised or manipulated around the urinary bladder. The composite colo-peritoneal tissue was able to retain its architecture and viability both pre- and post-transplantation; despite excessive manipulation, which suggested possible gross compatibility.

Post-surgically, the dogs were calm but inactive within the first four days, which was attributable to the intramuscular administration of an opioid pain reliever, pentazocine. The laparotomy incisional wounds on the skin grossly apposed within 5 to 7 days in three of the dogs, while that of one 4thdog lasted for fifteen days, which could be due in part to excessive force on the abdominal incision, while straining and the behaviour of the dog towards the incision site that led to incisional hernia (Gower *etal*., 2009). However, this finding (incisional hernia) has not been reported by researchers following similar surgical procedures, from the available literature. The omental adhesions on the urinary bladder observed occurred during inflammatory phase of urinary bladder wound healing probably due to tissue anoxia and serosal injury on the bladder and the grafted composite colo-peritoneal tissue (Desch and

Wagner, 1986). The omentum plays a role in guarding against post-operative massive adhesion of the urinary bladder at the repaired site to adjacent tissues/oragans (Alkattam, 2014), due to its role in fibrinolytic inhibition(Ayman *etal*., 2003). The bladder diverticulum observed in this study was due to the surgical trauma and probably transient urine outflow obstruction post-surgery as reported by Klauschner *etal*. (1983) and Scheepens and L'Eplatter (2005). However, the diverticulum and the omental adhesions did not interfere with the functionality of the urinary bladder in this study.

Haematuria observed in this study was part of the anticipated early post-surgical complications of the procedure, which agreed with an earlier report by Awasum (2010) and Muhammad (2013) that surgical trauma to the urinary bladder resulted in extravasation of blood into the bladder lumen to initiate coagulation cascade, which maylead to clot formation and reduce further blood loss. Haematuria observed in this study was minimal and naturally resolved, and was similar to the haematuria-dysuria syndrome reported following gastrocystoplasty by Nguyen *et al*. (1993). However, blood transfusion is sometimes required in some patients (≤ 3%) undergoing gastrocystoplastic procedure (Greenwell *et al*., 2001), which was not the case in this study.

Among post-surgical complications recorded were straining, constipation and incisional hernia, possibly due to the colo-colic intussusceptions (Valiei and Beheshti, 2011; Kim *etal*., 2012), where the intussuscepting de-seromuscularised donor colonic segment (intussusceptum) caused partial or transient stenosis of the colon. The stenosis leads to transient interference of normal intestinal peristalsis, discomfort and persistent straining that resulted to undue pressure on the sutured inner laparatomy incision, and finally incisional hernia that ensued. The straining and constipation were addressed appropriately by enhancing smooth intestinal evacuation through supportive interventions in form of drenching with

liquid paraffin for 3-5 days twice daily and soapy enema once daily for 3 days (Muhammad *etal*., 2014b). The incisional hernia was corrected by herniorrhaphy as recommended by Henrotay *etal*.(2010)in order to avert further complications that may follow the hernia.

The differences observed in the vital parameters post-surgery could be ascribed to the inflammatory processes that occurred, which normally followsurgical trauma inflicted on dogs. The result agreed with the finding of by Greenwell *et al*. (2001) and Muhammad (2013), who recorded ≤ 6.4% of common and early surgical complications related to enterocystoplasty. The slight insignificant increase in the mean rectal temperature coincided with inflammatory response to the surgical trauma (Muhammad, 2013). This could be explained on the basis of lack of sedative effect, muscle contraction and opened abdominal cavity established contribute to lower body temperature prior to recovery from anaesthesia. The immediate post-surgical sharp increase in pulse rate might be due, in part, to post-surgical response to pain by the dogs following the invasive nature of the procedure, despite the administration of pentazocine for pain alleviation. The pulse rate recorded signifies decreased pain sensation. The fluctuation in the respiratory rate coincided with the response to surgical pain and followed similar pattern as that of the pulse rate(Muhammad, 2013).

Among the major factors that determine urine composition, the material(s) added to the glomerular filtrate (post-renal), as it flows through the urinary bladder and urethra is also essential. The slight decrease in specific gravity observed within the first 2 weeks and the maintenance of urine pH around the pre-surgical value within 18 days post-surgery were apparently, due to increased fluid intake through both oral and intravenous administrations of physiological saline solution leading to production of diluted urine with relative normal urine pH and decreased SG (Albasan *etal*., 2003). Upon withdrawal of the intravenous fluid and complete return to feeding pre-surgical meal *per os*, the urine pH decreased (Margi, 1995),

but thereafter stabilised within the base-line. Traces of protein in the urine were evidence of haemorrhage and inflammatory responses, caused by irritation of the surgical site in contact with urine, surgical trauma, leading to gross and occult haemorrhages, and inflammatory exudation of plasma protein (secretory protenuria). The persistent haematuria observed could be due to entrance of erythrocytes into the urinary bladder as a result of haemorrhages caused by the surgical trauma on the bladder, inflammatory process and the corrosive nature of the urine on the anastomotic site (Schumann and Schweitzer, 1996). Leucocyturia is an indicator of inflammatory response by the urinary tract following surgical trauma (Vail *etal*., 1986). Ascorbic acid detected is essential in stimulating body immunity due to its anti-inflammatory effect as observed by Sorice *etal*.(2014). Urobilinogen observed in the urine was of no diagnostic importance as some drugs can react the same way. Bilirubin, which is usually detected in urine of healthy dogs, may be attributed to the proteinuria in form of conjugated bilirubin that may subsequently hydrolyse into unconjugated bilirubin (Osborne and Stevens, 1999), and nitrate may be due to urine contamination post-collection because the urine samples collected during that period were not promptly evaluated.

The erythrocyte count showed initial progressive decrease in the mean PCV which was accompanied with corresponding reduced Hb concentration. The changes observed could be attributed to the transient intra- and post-surgical haemorrhage following enterocystoplastic procedures as earlier reported by Greenwell *et al*. (2001) and Muhammad (2013).

The post-surgical increase in total leucocyte counts due to neutrophilia observed may be attributed to inflammatory response by the body following surgical intervention (Hassan *etal*., 2006). The neutophilia was gradual and attained its peak 7 days post-surgery as a result of activation of compliment cascade that led to migration of the marginated and circulating neutrophils by diapedesis. Thus, the stimulated neutrophilia occurred due to excessive

production and release of more neutrophils from bone marrow into the peripheral systemic circulation (Hassan *etal*., 2006). The increased lymphocytic count above the base-line values recorded within 10 days post-surgery was a clear indication of transient migration of the cells into the injured tissues following other inflammatory cells, during the proliferative phase. The lymphocytosis recorded in the present study attained its peak at day 4, which disagreed with that obtained atday 7 following skin injuries (Fishel *etal*., 1987; Park and Barbul, 2004). This early lymphocytosis in the peripheral circulation might be due to the invasive nature of the surgery. It indicated significant role of lymphocytes, playing an inhibitory role in wound healing, possibly enhancing the down-regulation of the excessive inflammatory and proliferative states of the healing wound (Barbul, 1988; Park and Barbul, 2004; Martin and Leibovich, 2005).

The increase in monocyte count observed between day 16-28 post-surgery suggested that the cells were attracted and on transit to the injured site at later phase of the inflammation.At this phase, monocytes underwent a phenotypic change to become tissue macrophages and function as phagocytes (Martin and Leibovich, 2005; Tsirogianni *etal*., 2006). Due to the invasive nature and extent of tissue injury, monocytes persistently observed in peripheral circulation for 5 weeks, but subsequently disappeared completely from circulation, implying decrease in their activity.

The post-surgical increase in mean plasma IL-6 concentration above the base-line values, maintained within the first 5 days could be attributed to the systemic manifestation of the acute phase inflammatory response following the invasive surgical trauma. The response may lead to the production of pro-inflammatory cytokines (IL-6) from the local sites of the injury into the systemic circulation, which was proportional to the extent of injury (Lin *etal*., 2000; Mizuno *etal*., 2015). The circulating IL-6 subsequently induced activation and recruitment of

phagocytes (including neutrophils and monocytes) reported by Warren *etal*. (2009), John *etal*. (2013) and Avazi (2017). This response was similar to that earlier reported by Mizuno *etal*. (2015) following cardiopulmonary bypass surgery in dogs, that demonstrating IL-6 induces differentiation of lymphocytes into plasma cells and stimulation of neutrophil-mediated reperfusion injury. In the present study, increased lymphocyte (day 4) and neutrophil (days 4 and 7) counts, observed post-surgery corresponds to the peak concentration of IL-6 (within the 1st week).

The elevation of concentration of pro-inflammatory and anti-inflammatory (IL-10), were observed following invasive surgical procedures such as cardiopulmonary bypass in humans (Steinberg *etal*. 1993; Wan *etal*., 1996; Mizuno *etal*., 2015). The gradual elevation of plasma IL-10 concentrationpost-surgery was similar to the findings of Opal *etal.* (1998) and Moore *etal*. (2001), who reported that IL-10 is synthesised later than other pro-inflammatory cytokines following injuries, in lymphocytes and monocytes, suggesting its regulatory role in the later phases of inflammatory response. The IL-10 may increase concentration of reduce neutrophil adhesion to activated endothelial cells. However, the trend in the increase in the concentration of IL-10 in this study disagreed with the report of Mizuno *etal*. (2015) that IL-10 level increase earlier than that of IL-6 following cardiopulmonary bypass. Thus, the late expression of IL-10 was to inhibit macrophages and lymphocyte synthesis of pro-inflammatory cytokines (Sikka *etal*., 2012).

The negative insignificant association observed between the concentrations of IL-6 and IL-10 suggested the existing dynamic equilibrium between the pro-inflammatory and anti-inflammatory cytokines (McBride *etal*., 1996). The association also showed that the cytokines exerted regulatory effect on the haematological and vital parameters (Francisco *etal*., 1999). The negative significant association observed between the pro-inflammatory (IL-

6) and monocyte count, and the non-significant association between the anti-inflammatory (IL-10) and lymphocyte countshowed the important relationship between these leucocyte types and the different cytokines in wound healing (Mehdi and Maryam, 2014). This relationship, therefore, suggests the stability and improvement in wound healing under the regulation of IL-6 and IL-10 (Strecker-McGraw *etal*., 2007).

Since the composite colo-peritoneal tissue was to serve as a means of repairing the urinary bladderdefect in this study, it could be expected that the values of the serum electrolytes may significantly alter immediately following its (composite colo-peritoneal tissue) transplantation. The alteration may be due to the inherent properties of each of the two dissimilar tissues (peritoneal sheath and colonic segment) in fluid and electrolyte exchange, occuring between the systemic circulation and the urine stored in the urinary bladder. Thus, this alteration recorded in this study was due to a transient decrease in mean values of sodium ion (Na^+) concentration within the first week post-surgery. The decrease could be attributable to the increased fluid intake through enteral and parenteral routes, which may dilute the Na^+ level in the blood, leading to relative decrease in its concentration (Steven and Michael, 2008; Esievo, 2017). The decrease could also be due to diffusion of Na^+ from capillaries across the peritoneal sheath as a result of peritoneal inflammation, which increases the peritoneal solute transport rate and sieving coefficient of the peritoneal membrane (Wang and Lindholm, 2003).The decrease may also occur due to thecontact between the urine in the bladder and peritoneal tissue (as an 'alien urothelium'), similar to the explanation given by Ciftci*etal*.(1999) and Vilos *etal*. (2001) in patients with intra-peritoneal bladder perforations.

The result of this study was further supported by the decreased fluid intake and decreased inflammatory process that caused stability in the level of Na^+. The concentrations of the other serum electrolytes (K^+, Cl^-, PO_4^- and HCO_3^-) did not differ from the pre-surgical values

throughout the study period. This finding clearly suggested the absence of uroperitoneum due to urine leakage (as an anticipated post-surgical complication) contrast to the earlier report on post-surgical findings by Muhammad (2013) and reported significant high level potassium (Vilos *etal*., 2001). The finding of this study on the concentrations of serum electrolytes differed considerably from the previous reports by Lockhart *et al*. (1994), Racioppi *et al*. (1999) and Muhammad (2013) who recorded an increase in values of the serum electrolytes (Na^+, K^+, Cl^-, PO_4^- and HCO_3^-), urea and creatinine, and acid-base changes following interposition of the intestinal tissues in the urologic tissue, indicating metabolic disturbances.

The initial post-surgical increase in [Ca^{2+}] could be due to its resorption from the urine in the bladder by the peritoneal surface of the composite tissue and a decrease in serum albumin (transporter of Ca^{2+}) concentration induced by IL-6 on hepatocytes. The result was similar to the finding of Steven and Michael (2008) in ruptured urinary bladder in horses resulting in hypercalcaemia. This is because the peritoneum rapidly absorbsCa^{2+} via the infrequent "large pores," which are permeable to protein (Blackburn and Stantom, 2014). Similarly, the relative short-term increase in serum [urea] and [PO_4^-] obtained in this study were anticipated and normal because urea is a freely diffusible molecule to most membranes (including peritoneum).It is passively reabsorbed into systemic circulation, thereby causing transient increase in serum urea concentration (Steven and Michael, 2008). This finding was deduced from the physiological properties of the peritoneum, which enhance thecapillary-peritoneal flow through one of the three pores; the frequent "small pores," which allow passage of water and small solutes like urea, and thus elevate the concentration of serum urea (Blackburn and Stantom, 2014). Similarly, the increase in PO_4^- is a normal finding following muscular injury (for example, due to surgery in this case), its leakage from intracellular to extracellular space, and its aborption from urine through the composite tissue into the systemic circulation (Steven and Michael, 2008).

Post-surgical concentration of serum total protein (TP) showed its fluctuations, with no unique pattern, between the reference base-line values and the slight changes, suggestive of inflammatory condition,which enhance increase in production of some serum proteins at different time interval following stimulation of the hepatocytes by IL-6 (Petersen *etal*., 2004; Ceron *etal*., 2005). However, the pattern in serum albumin concentration was completely different from that of the TP, with distinct pattern where decreased serum concentration of the albumin fell below the base-line post-surgical values within the first 16 days. The decrease in serum albumin concentration was partly due to haemodilution (through enteral and parenteral routes) and the action of IL-6 on the hepatocytes (Steven and Michael, 2008), affecting transportation of some cations (such as Ca^{2+} and Mg^{2+}). Thus, caused the increase in serum cation concentration. Furthermore, the decrease in serum albumin concentration occurred during the inflammatory process,because the permeability of theperitoneal membrane increases, which in turn enhance the opening of the infrequent “large pores,” that is permeable to protein. Therefore, protein loss through the urine caused hypoalbuminaemia (Wang and Lindholm, 2003; Rippe and Davies, 2011; Blackburn and Stantom, 2014).

The increase in serum glucose concentration, though within reference values, could be attributed in part to the initial intravenous administration of dextrose post-surgery, surgical stress and increase in cellular activities during healing and repair processes. The surgical stress and increased cellular activities during healing process may cause release of anabolic steroid,insulin and other hormones, which cause production and mobilisation of glucose from non-carbohydrate source (Kramer and Hoffmann, 1997;Sorice *etal*., 2014).

Serum enzymes usually originate from cells (exogenous to serum) with recognised function in blood.Their presence in tissues does not necessarily cause damages to the tissue, but will increase the activities ofthe serum enzyme, and may not be diagnostic in nature, but provides

some clue. Increased serum ALT and AST (released by skeletal myocytes) activities are usually caused by severe muscular trauma (Valentine *etal*., 1990). However, the decrease observed in AST activity is of no diagnostic value because of its short half-life of less than 24 hours (Moss and Henderson, 1999). The ALPactivity was relatively maintained throughout the study period, except the sporadic non-relevant increases which are of no significant value (Kramer and Hoffmann, 1997).

Early cystosonographic appearance of the urinary bladder with thickened bladder wall at the point of patch is one of the most common findings, similar to the reportin cases of acute bladder cystitis and benign lesions of the urinary bladder (Takiguchi and Inaba 2005; Sidhu *etal*., 2010). The evaluation was also essential following enterocystoplasty for evidence of anastomotic leaks as suggested by Sidhu *etal*. (2010) that sonographic evaluation of the urinary bladder post-bladder augmentation and replacement procedures often reveals unexpected findings, resulting from incorporation of bowel into the urinary bladder wall. This observation was the basis of the later evaluation performed on the 3rd week after the colo-peritoneo-cystoplastythat presented marked decrease in the thickness of the dorsal bladder wall as evidence of subsided post-surgical inflammatory response.

The cystogram revealed an intact and engorged urinary bladder at day 21 post-surgery with irregularity at the point of patch. The subsequent slight dent observed at the point of patch (10th week), final uniformity and regular smooth topography (14th week) indicated near perfectly, preserved urinary bladder, devoid of leaks/abnormality.It was also evidence of adaptation between the neocystic (composite colo-peritoneal portion) and the transitional tissues of the urinary bladder. The finding was in agreement with that of Muhammad *etal*. (2016b)and Probst *et al.* (2000), who reported uniform and smooth urinary bladder topography in dogs following colocystoplasty.The cystograms were also similar to the

illustration recorded by Muhammad *etal*. (2014a), who employed contrast cystography (using 20 % solution of Urografin) to evaluate the urinary bladder following ileocystoplasty.

The evidence of cells with squamous and columnar attributes (such as tall, aligned cells with basally located nuclei) on the composite colo-peritoneal tissue (used in colo-peritoneo-cystoplastic technique) was clear indication of graft take histologically. This was particularly so that demarcation was observed between the bladder and the composite tissue at the site of anastomosis.

Furthermore, free peritoneal graft was implanted within the urinary tract (urethroplasty in male rabbits) and yielded positive result. Three weeks after the surgery (peritoneal grafting), the grafted peritoneal tissue was transformed with uniformly lined normal urothelium, presented good graft survival (Nanni *etal*., 2001). In this study neovascularity develops within the subepithelial layer suggests positive engraftment of the free graft peritoneal graft with subsequent transformation of the mesothelium into urothelium, apparently due to the in-growth of transitional urothelium along the scaffolding provided by the graft. This result suggested that the peritoneal mesothelium underwent a metaplastic process, caused by contact with the urine (Shaul *etal*., 1996).Mononuclear cellular infiltration observed was similar to what was reported by Nanni *etal*. (2001) as chronic granulomatous inflammation.

Turner*etal*. (2011) documented that augmented segments were completely lined by confluent urothelium, with minimal inflammatory changeshistologically, and healthy smooth muscle with no evidence of fibrosis. However, clean plane of separation at the level of the lamina propria (between urinary bladder and colonic segment) was recorded in contrast to the present finding in this study, thus preventing any colonic mucosal-cell regeneration from the basal crypt compartment, signifying effective barrier function. The finding of this studydid not agree with the report of Frasier *etal*. (2003), who observed re-growth of the colonic

epithelium following composite colocystoplasty with cultured urothelial cells in pigs. It also disagreed with the findings of Gitlin *etal*. (1999), who recorded the overgrowth of a hyperplastic transitional epithelium post-enterocystoplasty at the entero-vesical segment, suggesting transformation of the urothelial cells into intestinal cells.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

Four NIDs were used to study the efficiency of autologous composite colo-peritoneal pedicle grafts in the repair of experimental urinary bladder defects in dogs. The efficiency study was based onthe following anatomical and physiological evaluations:

i. Demonstration of the suitability of the seromuscular pedicle flap and peritoneal free flap as composite graft by:
   a. harvesting 3 cm x 5 cm sero-muscular colonic pedicle flap.
   b. harvesting 3 cm x 5 cm autologous free parietal peritoneal flap.
   c. formation of composite colo-peritoneal pedicle tissue, where muscular surface of the sero-muscular pedicle flap was underlined with the parietal (muscular) surface of the pre-harvested peritoneal flap and sutured.
   d. partial cystectomy of the urinary bladder, in which predetermined 2 cm x 4 cm dimension of a full thickness rectangular flap was excised from the dorsum.
   e. The finely 'tailored' composite colo-peritoneal pedicle flap, grafted on the defective site of the urinary bladder by colo-cystoplasty technique.

ii. Imaging evaluation of the urinary bladder architecture post-repair with the composite coloperitoneal grafts through:
   a. Cystosonographic examination on day 8 post-surgery, which revealed thickened bladder wall at the point of patch (dorsum) and later showed marked decrease in thickness at 21 days post-surgery.
   b. Contrast cystographic evaluation at 2^{nd}, 10^{th}, 14^{th} and 108^{th}weeks post-surgery, which indicated distinctive urinary bladder silhouettes with gradual transition of the UB

architecture at the point of patch. It also appeared with clear delineation and minimal blurring at week 2 that gradually disappeared following subsequent evaluations at weeks 10 and 14 post-operatively. However, there was evidence of UB diverticulum in one of the subjects on the 108^{th} week popst-surgery.

iii. Comparingpre- and post-surgical urinalyses. Urinalysis test strip was used for the evalution.

a. Protein was observed post-surgically in the urine of the dogs for the first 7 days.

b. Glucose was also detected in the urine of one dog on day 7 post-surgery.

c. Haematuria was observed within the first 16 days post-surgery.

d. Leucocyturia was obtained and persisted in 3 dogs from day 1 to day 18 post-surgery.

iv. Demonstration of haematological alteration following colo-peritoneo-cystoplasty:

a. The pre-surgical PCV (45.84 ± 3.67 %) decreased post-operatively to 39.25 ± 2.56 %, 35.25 ± 1.60 %, 33.50 ± 2.53 %, 36.50 ± 2.22 %, 38.00 ± 2.92 %, 35.75 ± 1.89 % and 35.25 ± 3.09 % on days 1, 4, 7, 10, 13, 16 and 18, respectively.

b. The base-line TLC observed was 9.85 ± 1.02 x 10^9/L, which increased to 12.43 ± 2.06 x 10^9/L, 14.20 ± 2.77 x 10^9/L and 13.25 ± 2.60 x 10^9/L post-surgically on days 4, 7 and 10 respectively. The TLC stabilised and fluctuated relatively between 7.37 ± 1.27 x 10^9/L and 10.83 ± 2.81 x 10^9/L.

c. The fluctuation observed in the TLC was due to increase in neutrophil counts from the pre-surgical mean of 6.63 ± 0.79 x 10^9/L to the post-surgical values of 8.31 ± 1.51 x 10^9/L, 10.00 ± 2.39 x 10^9/L and 8.92 ± 1.69 x 10^9/L on the 4^{th}, 7^{th} and 13^{th} days respectively, and was relatively maintained within the base-line values.

d. The base-line lymphocyte counts ranged between 2.72 ± 0.22 x 10^9/L and 3.59 ± 0.31 x 10^9/L. The post-surgical counts observed on days 1, 4, 7 and 10 were 3.60 ± 0.33 x 10^9/L, 4.42 ± 0.82 x 10^9/L, 3.64 ± 0.66 x 10^9/L and 4.01 ± 0.88 x 10^9/L, respectively.

e. Eosinophil counts were not detected in circulation pre-surgically, but were observed from day 1 after surgery, and the counts were between 0.02 ± 0.02 x 10^9/L and 0.41 ± 0.39 x 10^9/L for 5 weeks post-surgery.

f. The monocyte count was 0.16 ± 0.10 x 10^9/L pre-surgically. The count fluctuated post-surgically as 0.07 ± 0.02 x 10^9/L, 0.08 ± 0.04 x 10^9/L, 0.06 ± 0.03 x 10^9/L and 0.05 ± 0.04 x 10^9/L on days 1, 4, 10, and 13, respectively.

v. Comparative evaluation of plasma cytokines (IL-6 and IL-10) post-surgery:

a. The pre-surgical plasma value of IL-6 mean concentration was detected at 7639.45 ± 146.81 pg/ml. The plasma IL-6 concentration was measured between 7916.62 ± 32.97 pg/ml and 7878.98 ± 71.47pg/ml within the first 5 days following surgery.

b. The base-line mean plasma level of IL-10 detected was 93.95 ± 17.18. The plasma level of IL-10 fluctuated between 138.76 ± 51.72 pg/mland 449.70 ± 125.79 pg/ml for 9 days post-surgery.

vi. Correlation of plasma levels of IL-6 and IL-10 with each other, and with haematological and vital parameters were also evaluated.

a. There were negative correlation between the IL-6 and IL-10 levels; IL-6 and PCV, haemoglobin concentration, neutrophil count and rectal temperature ($r = 0.57$; $P < 0.05$). Weak correlations were observed between IL-6 and neutrophil count ($r = 0.01$), rectal temperature ($r = 0.01$), eosinophil count ($r = 0.05$), respiratory rate ($r = 0.06$) and TLC ($r = 0.07$).

b. Weak correlation was observed between IL-10 and TLC ($r = 0.02$).Negative correlations were recorded between the IL-10 and IL-6 levels; IL-10 and PCV, haemoglobin concentration, lymphocytes, temperature and pulse rate. Positive insignificant correlation was obtained between the IL-10 and TLC, neutrophils,

monocytes and eosinophils except for respiratory rate that was significant ($r = 0.559$; $P < 0.05$).

vii. Evaluation of serum biochemical changes following autologous colo-peritoneo-cystoplasty:

a. Pre-surgical Na^{+}concentration measured on days 7, 4 and 1 were 142.75 ± 2.25 mmol/L, 143.50 ± 3.52 mmol/L and 143.75 ± 1.75 mmol/L, respectively. The post-surgical serum concentration recorded were 136.00 ± 2.04 mmol/L, 143.00 ± 4.38 mmol/L, 138.50 ± 4.09 mmol/L and 142.25 ± 1.31 mmol/L on day 1, 4, 7 and 10respectively.

b. The pre-surgical serum Ca^{2+} concentration was 1.97 ± 0.29 mmol/L. The concentrations observed post-operatively ranged from 1.80 ± 0.30 mmol/L to 2.01 ± 0.29 mmol/L within 7 days. On day 16 and 35 the concentrations were 2.35 ± 0.05 mmol/L and 2.33 ± 0.03 mmol/L, respectively.

c. Serum urea base-line concentrations on day 7, 4 and 1 before the colo-periotoneo-cystoplasty were 5.38 ± 1.03 mmol/L, 5.13 ± 0.69 mmol/L and 5.13 ± 0.43 mmol/L respectively. The value increased post-operatively, to 8.00 ± 1.17 mmol/L on day 10.

d. The pre-surgical serum aspartate transaminase (AST) activity was 17.17 ± 5.16 mmol/L,and after surgery, the concentrations decreased significantly ($P< 0.05$) to 9.00 ± 2.04 mmol/L, on day 1. Thereafter, the activity fluctuated between 9.00 ± 2.04 mmol/L and 19.67 ± 1.45 mmol/L.

viii. Histological evaluation of the composite tissue after healing showed presence squamous and columnar cells, on the composite tissue. Also mononuclear cellular infiltration at the submucosae of the colo-peritoneo-cystoplastic junction and the composite colo-peritoneal segment were observed after long term evaluation.

6.2 Conclusions

The study concluded that:

1. Successful grafting of autologous composite colo-peritoneal tissue in the reconstruction of defectiveurinary bladder in dogs by means of colo-peritoneo-cystoplasty is possible through:

i. harvesting and transplanting autologous peritoneal sheath onto harvested vascularised, seromuscular colonic pedicle segment to form “a pediculated composite colo-peritoneal tissue”;

ii. grafting of the pediculated autologous composite colo-peritoneal tissue onto the partially cystectomised urinary bladder.

2. Post-surgical gross physical and clinicalcomplications associated with the colo-peritoneo-cystoplasty werehaematuria (4/4), straining (4/4) incisional hernia (1/4) and constipation (1/4). Long term evaluation revealed omental adhesion (2/4) to the UB and UB diverticulum (1/4) at the point of patch.
3. The base-line vital parameters were briefly affected post-surgery due to the surgical trauma inflicted.
4. Haematuria, proteinuria and leucocyturia (pyuria) were the commonest early findings of urinalysis post colo-peritoneo-cystoplasty.
5. Generally, changes observed post-surgery in the haemogram were:

i. Transient decrease in the PCV.

ii. Transient increased total leucocyte count due to increased neutrophil and lymphocyte counts.

iii. Sudden appearance of eosinophils in the peripheral circulation occurred only post-surgery (lasted for 5 weeks), and late increased monocyte count was observed between day 16-28 post-surgery.

6. Regulatory interplay of plasma IL-6 (pro-inflammatory) and IL-10 (anti-inflammatory) cytokines in response to the surgery, in which IL-6 level rose immediately post-surgery and was sustained for few days before the IL-10 increased to regulate the activity of the pro-inflammatory cytokines.
7. There were established associations between IL-6 and IL-10,and relationship between the cytokines and the haematological and vital parameters.
8. Metabolic changes observed were minimal and transient biochemical alterations in concentration of serum electrolytes, occurring only during healing process.
9. Cystosonographic appearance of the urinary bladder with thickened bladder wall at the point of graft (early) that later decreased in the thickness as an e evidence of advanced healing.
10. Cystographs revealed engorged and well preserved urinary bladder architecture with no evidence of leaks, indicative of advanced healing and adaptation between composite colo-peritoneal portion and the urinary bladder.
11. Histological assessment of the grafted site showed:

i. No clear demarcation between submucosae of urinary bladder and composite colo-peritoneal tissue at the site of anastomosis, suggesting tissue integration and further supportedabsence of the bladder leakages.Demonstration squamous and columnar cells, on the composite tissue.

ii. Vascular buds with mononuclear cellular infiltration at submucosae of the colo-peritoneo-cystoplastic junction and the composite colo-peritoneal segment were observed.

6.3 Recommendations

It was recommended that:

1. The technique should be the preferred operative procedure for managing extensive urinary bladder defects in dogs (and possibly humans) because it has the potential to alleviate complications due to metabolic disorders, associated with conventional enterocystoplasty.The technique has obvious time advantage in emergency situations over tissue culture.
2. Other diagnostic imaging modalities should be used for gross evaluation of the technique apart from ultrasonographic scan and radiology.
3. Further studies involving urinalysis should include microscopic examination of urine sediment.
4. Further study should be conducted on the histology of the composite colo-peritoneal tissue using immunohistochemistry apart from normal H & E staining technique.
5. Long-term study should be conducted on the clinico-pathological parameters post colo-peritoneo-cystoplasty in dogs.
6. Evaluation of IL-6 and IL-10 levels should not be restricted to plasma, but should include urine and other body fluids, following colo-peritoneo-cystoplasty.
7. Investigation into other pro-inflammatory and anti-inflammatory cytokines post-colo-peritoneo-cystoplasty should be performed.

6.4 Limitations

1. The study used only four NIDs, aged between 0.75-1 years,as the subjects. The use of more subjects of varying age groups (that is paediatric, adult and geriatric subjects) would have provided a more representative result.
2. Health status of the experimental dogs was evaluated and ascertained through the assessment of vital parameters, basic haematology, urinalysis and serology. Gross evaluations of the organs involved through imaging would have provided additional information on the animals.
3. Interleukin 6 (IL-6) and IL-10 were the only cytokines evaluated, and from plasma alone. Other cytokines such as Interleukin 1 (IL-1), Interleukin 8 (IL-8), tumour necrosis factor and vascular endothelial growth factor, could also have been evaluated to expand this component of the study.
4. The urinary bladderdefect experimentally induced mimicked conditions resulting in loss (due to disease or surgery) of significant portion of the bladder. However, in clinical/natural case, the nature of the defect may not be the same with the experimental created defect.
5. The diagnostic imaging modalitiesused for gross evaluation of the technique were ultrasonography and radiography. Other imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) may play important role in accurate gross evaluation of the urinary bladder, which were absent the study area.
6. The histologicaltissue was only processed and stainedby normal H & E technique, and viewed under light microscopy.Immunohistochemical method would be more informative, if it was also done on the composite tissue.

7. The study was considered to be medium-termhistologicalevaluation of the composite colo-peritoneal tissue. Long-term evaluation will be useful in ascertaining the level of transformation of the composite tissue.

6.5 Contributions to Knowledge

To the best of our knowledge this is the first report on:

1. The use of free harvested peritoneal sheath with harvested vascularised, seromuscular colonic segment to form “a composite colo-peritoneal tissue”.
2. The transformation of the peritoneal mesothelium into urothelium after its transplantation in the urinary bladder.
3. The outcome of the study has the potential to address the common complications due to metabolic disorders, associated with conventional enterocystoplasty.

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APPENDICES

APPENDIX I: Consumables and surgical materials used in the study

S/No.	Description of item	Quantity
I.	**Consumables**	
1.	Detergent powder and table soap	10 kg
2.	Chlorhexidine gluconate B.P (0.3% w/v- Purit®)	12 litres
3.	Sodium hypochlorite (0.5% w/v- Jik(R))	80 litres
4.	Surgical gloves (sizes 7.5 and 8)	2 packets
5.	Pack of disposable hand (latex) gloves (100 pieces)	2 packs
6.	Razor blades	1 packet
7.	Sterile disposable syringes (20 ml)	1 packet
8.	Sterile disposable syringes (10 ml)	1 packet
9.	Sterile disposable syringes (5 ml)	4 packets
10.	Sterile disposable syringes (2 ml)	2 packet
11.	Elizabethan collars (improvised)	12 pieces
12.	Dog chains and collars	12 pieces
13.	Gauze	4 rolls
14.	Gauze bandage	40 rolls
15.	Cotton wool	12 bundles
16.	Adhesive plaster (large size; 10 cm x 5 m)	3 packets
17.	5 % Povidone iodine solution	1litre
18.	Povidone iodine ointment (20 g)	12 bottles
19.	Foley catheters (sizes 10 and 24 Fr)	32 pieces
20.	Intravenous cannula	20 pieces
II.	**Surgical materials**	
21.	Standard general surgical pack	2 packs
22.	Surgical gowns	2 sets
23.	Surgical drapes	2 sets
24.	Surgical blades (No. 15 and 23)	16 pieces
III.	**Suture materials**	
25.	Polygalactin 910 size 4-0, Vicryl(R)	2 packets
26.	Polygalactin 910 size 2-0, Vicryl(R)	3 packets
27.	Polygalactin 910 size 1, Vicryl(R)	1 packet
28.	Chromic catgut size 2-0	2 packets
29.	Nylon with needle size 2	1 packet
IV.	**Antibiotics**	
30.	Metronidazole infusion (0.5 % -100 ml)	40 bottles
31.	Ciprofloxacin syrup (60 ml)	4 bottles
32.	Oxytetracycline Long-Acting(R) injection (100 ml)	1 bottle
33.	Sulphadimidine injection (100 ml)	1 bottle
V.	**Anaesthetics, premedications and other related items**	
34.	Thiopentone sodium injection (1 g vial)	3 bottles
35.	Chlorpromazine hydrochloride injection (ampoules)	28 ampoules
S/No.	**Description of item**	**Quantity**
36.	Atropine sulphate injection (ampoules)	20 ampoules
37.	Diazepam injection (ampoules)	10 ampoules
38.	Ketamine hydrochloride (Ketamine®)	7 bottles
39.	Pentazocine injection (30 mg/ml)	10 ampouls
40.	Halothane solution (B.P 100% $^{V}/_{V}$ -250 ml)	1 bottle

41. Lignocaine hydrochloride with adrenaline (B.P 2% $^{W}/_{V}$- 50 ml)	1 bottle
42. Endotracheal tubes (sizes 7.5)	4 pieces
43. Scrub suit	2 sets
44. Surgical caps and masks	2 packets
VI. Other drugs and items	
45. Dextrose solution infusion 50% (100 ml)	30 sachets
46. Dextrose- saline solution infusion (500 ml)	60 sachets
47. Normal physiologic saline solution 0.9% (500 ml)	50 sachets
48. Infusion sets (macro- drip sets)	150 sets
49. Scalp vein infusion set (21 G)	150 pieces
50. Paracetamol injection (2 ml ampoules)	12 bottles
51. Multivitamins injection (ml)	1 bottle
52. Diazinon solution (Diazintol$^{(R)}$)	10 litres
53. Amitraz solution (Amitik$^{(R)}$)	10 litres
54. Pyrethrin soap (Ectocide$^{(R)}$)	2 kg
55. Ivermectin injection (1 % w/v)	50 ml
56. Ivermectin (Mectizan$^{(R)}$) (3 mg/tablets)	200 tablets
57. Pyrantelpamoate (125 mg/tablet)	24 tablets
58. Praziquantel (600 mg/tablet)	12 tablets
59. Diminazene aceturate (2.05 g/sachet)	4 sachets
60. K-Y$^{(R)}$ Lubricating Jelly (42 g/tube)	2 tubes
61. Urografin76%$^{(R)}$ (sodium amidotrizoate + meglumine amidotrizoate + Iodine) (20 ml/ampoule)	36 ampoules
VII. Laboratory and imaging materials	
62. Radiographic films (35 x 43 cm)	20 pieces
63. Giemsa stain (powder)	10 mg
64. Methanol solution	1 litre
65. Ethanol solution	1 litre
66. Glass slides (packet)	1 litre
67. Microhaematocrit capillary tubes	200 pieces
68. Urine test kits, Combi 11$^{(R)}$ and Combi 9$^{(R)}$ (packet)	2 packets
69. 10 % buffered formalin	1 litre
70. Haematoxylin and eosin powder	50 g
71. ELISA Cytokine Kits	2 kits

APPENDIX II: List of food, feeding and drinking items, and other related objects used during the study

S/No.	Description of item	Quantity
1.	Maize, Beans, Salt, Blood meal, Bones/beef and Palm oil (kg per day)	100 kg
2.	Kennel attendants (Cook and Cleaner)	2 persons
3.	Feeding and drinking bowls for the dogs	16 sets
4.	Water buckets	2 pieces
5.	Water reservoirs (150-litre capacity)	2 pieces
6.	Sweeping brooms	4 bundles

APPENDIX III: Pre- and post-surgery vital parameters of EC_1 dog that underwent colo-peritoneocystoplasty on the dorsum of the urinary bladder.

Parameters / Days	Temperature (^oC) (38.5-39.4)	Pulse rate (beats/min.) (65-90)	Respiratory rate (cycles/min.) (15-30)
Pre-surgical			
7	39.1	102	28
6	39.2	98	18
5	38.8	100	22
4	38.9	96	24
3	39.0	98	20
2	39.2	94	16
1	38.5	98	18
Surgical	38.0	102	30
Post-surgical			
1	38.8	104	38
2	39.0	109	40
3	38.4	116	20
4	39.1	120	22
5	38.4	114	21
6	39.1	94	20
7	38.1	112	22
8	38.8	110	20
9	38.3	106	26
10	38.4	112	24
11	38.1	104	20
12	38.3	110	18
13	38.3	102	16
14	38.8	100	18
15	38.3	102	20
16	38.2	100	18
17	38.5	98	20
18	38.2	101	17
19	38.3	108	16
20	37.9	100	18
21	39.0	106	20
28	39.1	104	16
35	38.5	98	20
42	38.8	100	18
49	38.8	96	14
56	38.9	98	20
70	38.5	100	18

EC = Enterocystoplasty; 1 = Dog identity

APPENDIX IV: Pre - and post-surgery vital parameters of EC_2 Bitch that underwent colo-peritoneocystoplasty on the dorsum of the urinary bladder.

Parameters / Days	Temperature (^oC) (38.5-39.4)	Pulse rate (beats/min.) (65-90)	Respiratory rate (cycles/min.) (15-30)
Pre-surgical			
7	38.5	120	28
6	38.9	104	26
5	39.1	98	22
4	38.6	90	26
3	38.7	96	20
2	38.8	100	20
1	38.6	96	18
Surgical	38.4	140	44
Post-surgical			
1	40.0	140	44
2	39.1	168	44
3	38.4	140	40
4	38.6	110	30
5	38.5	118	26
6	38.8	102	24
7	38.0	122	28
8	38.8	110	20
9	38.5	105	24
10	38.8	108	20
11	38.1	100	22
12	38.5	100	20
13	38.2	96	18
14	38.5	90	18
15	38.8	102	16
16	38.2	100	18
17	38.3	96	20
18	38.0	100	16
19	37.9	108	18
20	37.6	102	19
21	38.8	106	20
28	38.8	102	18
35	38.5	116	16
42	38.7	100	18
49	38.8	102	20
56	38.4	100	24
70	38.4	96	20

EC = Enterocystoplasty; 2 = Dog identity

APPENDIX V: Pre- and post-surgery vital parameters of EC_3 dog that underwent colo-peritoneocystoplasty on the dorsum of the urinary bladder.

Parameters	Temperature (^oC) (38.5-39.4)	Pulse rate (beats/min.) (65-90)	Respiratory rate (cycles/min.) (15-30)
Days			
Pre-surgical			
7	38.8	94	30
6	39.1	120	36
5	38.7	106	26
4	37.9	108	18
3	38.3	104	20
2	38.9	102	16
1	38.3	98	18
Surgical	39.1	100	20
Post-surgical			
1	39.3	108	27
2	40.2	143	40
3	38.6	117	26
4	39.0	120	24
5	38.5	106	20
6	38.9	104	25
7	38.6	86	18
8	38.8	100	20
9	38.6	102	18
10	38.3	96	20
11	38.5	100	18
12	38.8	102	22
13	38.1	98	20
14	38.3	102	18
15	38.1	100	24
16	38.8	102	20
17	38.5	94	18
18	39.1	88	16
19	39.0	96	20
20	39.1	100	18
21	38.4	98	16
28	38.2	98	20
35	38.4	100	18
42	38.7	96	16
49	38.6	90	14

EC = Enterocystoplasty; 3 = Dog identity

APPENDIX VI: Pre- and post-surgery vital parameters of EC_4 bitch that underwent colo-peritoneocystoplasty on the dorsum of the urinary bladder.

Parameters	Temperature (^oC) (38.5-39.4)	Pulse rate (beats/min.) (65-90)	Respiratory rate (cycles/min.) (15-30)
Days			
Pre-surgical			
7	38.1	100	18
6	38.2	106	17
5	38.9	102	18
4	38.5	100	20
3	38.0	98	18
2	38.7	112	20
1	38.6	105	17
Surgical	38.8	103	16
Post-surgical			
1	38.8	104	30
2	38.6	100	20
3	38.8	102	20
4	38.4	96	18
5	38.9	86	28
6	38.5	80	20
7	38.8	90	22
8	38.3	92	18
9	38.4	83	14
10	38.8	100	18
11	38.9	98	16
12	38.6	108	20
13	38.4	100	18
14	38.1	100	22
15	38.5	96	20
16	38.1	90	18
17	38.0	92	18
18	38.4	98	20
19	38.3	90	16
20	38.6	94	18
21	38.3	92	20
28	38.5	100	22
35	38.5	90	18
42	38.6	92	14
49	38.5	94	16

EC = Enterocystoplasty; 4 = Dog identity

APPENDIX VII: Pre- and post-surgery vital parameters means and standard error of mean (SEM) of four dogs that underwent colo-peritoneocystoplasty on the dorsum of their urinary bladder

Parameters	Temperature (^oC)	Pulse rate (beats/min.)	Respiratory rate (cycles/min.)

Day			
Pre-surgical			
7	38.63±0.21	104.00±5.60	26.00±2.71
6	38.85±0.23	107.00±4.65	24.25±4.40
5	38.88±0.09	101.50±1.71	22.00±1.63
4	38.48±0.21	98.50±3.77	22.00±1.83
3	38.50±0.22	99.00±1.73	19.50±0.50
2	38.90±0.11	102.0±3.74	18.00±1.15
1	38.50±0.07	103.25±1.59	17.75±0.25
Surgical	38.58±0.24	111.25±9.60	27.50±6.24
Post-surgical			
1	39.23±0.28	114.00±8.72	34.75±3.86
2	39.23±0.34	130.00±15.69	36.00±5.42
3	38.55±0.10	118.75±7.87	26.50±4.72
4	38.78±0.17	111.50±5.68	23.50±2.50
5	38.58±0.11	106.00±7.12	23.75±1.93
6	38.83±0.12	95.00±5.45	22.25±1.31
7	38.38±0.19	102.50±8.66	22.50±2.06
8	38.68±0.13	103.00±4.36	19.50±0.50
9	38.45±0.06	99.00±5.40	20.50±2.75
10	38.58±0.13	104.00±3.65	20.50±1.26
11	38.40±0.19	100.50±1.26	19.00±1.29
12	38.55±0.10	105.00±2.38	20.00±0.82
13	38.25±0.06	99.00±1.29	18.00±0.82
14	38.43±0.15	98.00±2.71	19.00±1.00
15	38.43±0.15	100.00±1.41	20.00±1.63
16	38.33±0.16	98.00±2.71	18.50±0.50
17	38.33±0.12	95.00±1.29	19.00±0.58
18	38.43±0.24	96.75±2.98	17.25±0.95

19	38.38±0.23	100.50±4.50	17.50±0.95
20	38.30±0.34	99.00±1.73	18.25±0.25
21	38.63±0.17	100.50±3.40	19.00±1.00
28	38.65±0.19	101.00±1.29	19.00±1.29
35	38.48±0.02	101.00±5.45	18.00±0.82
42	38.70±0.04	97.00±1.91	16.50±0.96
49	38.68±0.07	95.50±2.50	16.00±1.41
56	38.65±0.25	99.00±1.00	22.00±2.00
70	38.45±0.05	98.00±2.00	19.00±1.00

APPENDIX VIII: Pre- and post-surgery urinalysis results of EC_1 dog that underwent colo-peritoneocystoplasty on the dorsum of the urinary bladder.

Parameters	S.G	P^H	Pro.	Sur.	Bld.	Leu.	K.B	Bil.	U.Bil.	A.A.	Nit
Reference Days	**1.015-1.045**	**(6-7)**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**
Pre-surgical											
7	1.010	6.0	-	-	-	-	-	-	-	-	-
5	1.015	8.0	-	-	-	-	-	-	-	-	-
3	1.010	6.0	-	-	-	-	-	-	-	-	-
1	1.010	6.0	-	-	-	-	-	-	-	-	-
Post-surgical											
1	1.005	8.0	+++	-	+++	+++	-	-	-	+++	+
4	1.015	7.0	+++	-	+++	+++	-	-	-	-	-
7	1.005	8.0	+	-	++	-	-	-	-	+	+
10	1.000	5.0	-	-	+++	+	-	-	-	-	+
13	1.005	7.0	+	-	+	++	-	-	-	-	-
16	1.010	8.0	+	-	+	++	-	-	-	+	-
18	1.010	8.0	+++	-	+	-	-	-	-	-	-
21	1.020	5.0	-	-	+	-	-	-	-	++	-
28	1.015	6.0	-	-	-	-	-	-	-	-	-
35	1.010	7.0	+	-	-	-	-	-	-	++	-
42	1.010	6.0	-	-	-	-	-	-	-	+	-

EC = Enterocystoplasty; 1 = Dog identity; S.G = Specific gravity; p^H = urine acidity; Pro. = Protein; Sur. = Sugar; Bld. = Blood; Leu. = Leucocytes; K.B. = Ketone bodie; Bil. = Bilirubin; U.Bil. = Urobilinogen; A.A. = Ascobic acid; Nit. = Nitrate; NEG. = Negative; - = Absent; + = Small; ++ = Moderate; +++ = Large

APPENDIX IX: Pre- and post-surgery urinalysis results of EC_2 bitch that underwent colo-peritoneocystoplasty on the dorsum of the urinary bladder

Parameters	S.G	P^H	Pro.	Sur.	Bld.	Leu.	K.B	Bil.	U.Bil	A.A	Nit
Reference Days	**1.015-1.045**	**(6-7)**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**
Pre-surgical											
7	1.025	7.0	-	-	-	-	-	-	-	-	-
5	1.025	6.0	-	-	-	-	-	-	-	-	-
3	1.020	8.0	-	-	-	-	-	-	-	-	-
1	1.020	8.0	-	-	-	-	-	-	-	-	-
Post-surgical											
1	1.020	6.0	+++	-	++	+	-	-	-	+++	+
4	1.015	7.0	+++	-	++	+	-	-	-	-	-
7	1.000	8.0	++	+++	+	-	-	-	-	-	-
10	1.000	6.0	+	-	+	-	-	-	-	-	-
13	1.005	7.0	-	-	+	-	-	-	-	-	-
16	1.010	8.0	-	-	+	+	-	-	-	-	-
18	1.015	6.0	-	-	-	-	-	-	-	-	-
21	1.010	7.0	-	-	-	-	-	-	-	-	-
28	1.010	6.0	-	-	-	-	-	-	-	-	-
35	1.005	5.0	-	-	-	-	-	-	-	-	-
42	1.020	6.0	-	-	-	-	-	-	-	-	-

EC = Enterocystoplasty; 2 = Dog identity; S.G = Specific gravity; p^H = urine acidity; Pro. = Protein; Sur. = Sugar; Bld. = Blood; Leu. = Leucocytes; K.B. = Ketone bodie; Bil. = Bilirubin; U.Bil. = Urobilinogen; A.A. = Ascobic acid; Nit. = Nitrate; NEG. = Negative; - = Absent; + = Small; ++ = Moderate; +++ = Large

APPENDIX X: Pre- and post-surgery urinalysis results of EC_3 dog that underwent colo-peritoneocystoplasty on the dorsum of the urinary bladder.

Parameters	S.G	P^H	Pro.	Sur.	Bld.	Leu.	K.B	Bil.	U.Bil.	A.A.	Nit
Reference Days	1.015-1.045	(6-7)	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Pre-surgical											
7	1.010	8.0	-	-	-	-	-	-	-	+	-
5	1.020	7.0	-	-	-	++	-	-	-	-	-
3	1.015	6.0	-	-	-	-	-	-	-	-	-
1	1.020	5.0	-	-	-	-	-	-	-	-	-
Post-surgical											
1	1.025	6.0	+	-	+++	-	-	+	+	+	+
4	1.025	6.0	+	-	+++	-	-	+	+	-	+
7	1.010	7.0	+	-	+++	+	-	+	-	-	+
10	1.020	7.0	+	-	+++	+	-	-	-	-	-
13	1.020	6.0	+	-	+	+	-	-	-	-	-
16	1.030	6.0	+	-	++	+	-	+	-	-	-
18	1.005	7.0	+	-	++	+	-	+	-	-	-
21	1.025	5.0	-	-	-	-	-	+	-	-	-
28	1.020	6.0	-	-	-	-	-	-	-	-	-

EC = Enterocystoplasty; 3 = Dog identity; S.G = Specific gravity; p^H = urine acidity; Pro. = Protein; Sur. = Sugar; Bld. = Blood; Leu. = Leucocytes; K.B. = Ketone bodie; Bil. = Bilirubin; U.Bil. = Urobilinogen; A.A. = Ascobic acid; Nit. = Nitrate; NEG. = Negative; - = Absent; + = Small; ++ = Moderate; +++ = Large

APPENDIX XI: Pre- and post-surgery urinalysis results of EC_4 bitch that underwent colo-peritoneocystoplasty on the dorsum of the urinary bladder.

Parameters	S.G	P^H	Pro.	Sur.	Bld.	Leu.	K.B	Bil.	U.Bil.	A.A.	Nit
Reference Days	**1.015-1.045**	**(6-7)**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**
Pre-surgical											
7	1.020	7.0	-	-	-	-	-	-	-	-	-
5	1.025	8.0	-	-	-	++	-	-	-	-	-
3	1.015	7.0	-	-	-	-	-	-	-	-	-
1	1.030	6.0	-	-	-	-	-	-	-	-	-
Post-surgical											
1	1.020	8.0	+	-	+++	+	-	+	+	-	+
4	1.020	7.0	+	-	+++	+	-	+	+	-	+
7	1.010	7.0	+	-	+++	+	-	-	-	-	-
10	1.000	8.0	-	-	+++	+	-	-	-	-	+
13	1.010	7.0	+	-	+++	+	-	-	-	-	-
16	1.025	6.0	+	-	+	+	-	-	-	-	-
18	1.025	5.0	-	-	-	+	-	-	-	-	-
21	1.025	6.0	-	-	-	+	-	-	-	-	-
28	1.025	7.0	-	-	-	-	-	-	-	-	-

EC = Enterocystoplasty; 4 = Dog identity; S.G = Specific gravity; p^H = urine acidity; Pro. = Protein; Sur. = Sugar; Bld. = Blood; Leu. = Leucocytes; K.B. = Ketone bodie; Bil. = Bilirubin; U.Bil. = Urobilinogen; A.A. = Ascobic acid; Nit. = Nitrate; NEG. = Negative; - = Absent; + = Small; ++ = Moderate; +++ = Large

APPENDIX XII: Pre - and post-surgery haematology results of EC_1 dog that underwent colo-peritoneocystoplasty on the dorsum of the urinary bladder

Parameters Days	PCV (%) (37-55)	Hb. (g/dl) 12.3-18.3	TWBC (10^9/L) 6-17.0	Neut. (10^9/L) 5-13.50	Lymp. (10^9/L) 1-4.80	Mon. (10^9/L) 0.15-1.35	Eosin. (10^9/L) 0.1-1.25	Baso. (10^9/L) RARE	Tpp. (g/dl) 5.7-7.7
Pre-surgical									
7	60	20.0	11.4	8.44	2.74	0.23	0.00	0.00	7.8
4	55	18.3	9.6	6.91	2.69	0.00	0.00	0.00	6.8
1	50	16.7	8.8	6.16	2.46	0.18	0.00	0.00	6.4
Post-surgical									
1	32	10.7	10.4	5.82	4.16	0.10	0.31	0.00	5.4
4	33	11.0	16.4	11.32	6.40	0.16	0.16	0.00	6.2
7	31	10.3	14.1	9.87	4.23	0.00	0.00	0.00	6
10	35	11.7	12.4	8.95	3.42	0.01	0.02	0.00	6.8
13	32	10.7	11.8	8.89	2.87	0.04	0.01	0.00	7
16	33	11.0	11.0	8.80	1.54	0.66	0.00	0.00	6.6
18	39	13.0	10.1	7.07	2.42	0.61	0.00	0.00	7.2
21	56	18.7	6.4	4.61	1.60	0.13	0.06	0.00	7.8
28	45	15.0	6.4	5.06	1.34	0.00	0.00	0.00	8.4
35	42	14.0	7.8	6.11	1.69	0.00	0.00	0.00	7.2
42	37	12.3	5.9	4.13	1.77	0.00	0.00	0.00	5.8
56	40	13.3	7.2	4.68	2.52	0.00	0.00	0.00	6.5
70	43	14.3	8.4	4.73	3.67	0.00	0.00	0.00	6.8
84	40	13.3	9.6	4.80	4.61	0.19	0.00	0.00	7
98	44	14.7	8.93	4.75	4.15	0.03	0.00	0.00	7.5

EC = Enterocystoplasty; 1 = Dog identity; PCV = Packed cell volume; Hb = Haemoglobin concentration; TWBC = Total white blood cells; Neut. = Neutrophils; Lymp. = Lymphocytes; Mon. = Monocytes; Eosin. = Eosinophils; Baso. = Basophils; Tpp. = Total plasma protein.

APPENDIX XIII: Pre- and post-surgery haematology results of EC_2 bitch that underwent colo-peritoneocystoplasty on the dorsum of the urinary bladder

Parameters	PCV (%) (37-55)	Hb. (g/dl) 12.3-18.3	TWBC (10^9/L) 6-17.0	Neut. (10^9/L) 5-13.50	Lymp. (10^9/L) 1-4.80	Mon. (10^9/L) 0.15-1.35	Eosin. (10^9/L) 0.1-1.25	Baso. (10^9/L) RARE	Tpp. (g/dl) 5.7-7.7

Days									
Pre-surgical									
7	50	16.7	8.4	5.54	2.86	0.00	0.00	0.00	7.0
4	46	15.3	11.0	6.60	3.96	0.44	0.00	0.00	7.2
1	45	15.0	10.6	7.42	2.86	0.32	0.00	0.00	7.0
Post-surgical									
1	40	13.3	8.0	5.04	2.88	0.08	0.00	0.00	6.0
4	34	11.3	11.0	6.60	4.4	0.00	0.00	0.00	6.4
7	32	10.7	19.6	15.48	2.35	0.20	1.57	0.00	6.5
10	35	11.7	16.3	10.87	4.23	0.15	1.05	0.00	7.4
13	34	11.3	11.5	8.55	1.97	0.15	0.83	0.00	7.0
16	36	12.0	10.0	7.80	1.86	0.16	0.18	0.00	6.5
18	42	14.0	8.4	5.54	2.6	0.17	0.08	0.00	6.0
21	51	17.0	6.4	4.61	1.6	0.13	0.06	0.00	7.8
28	40	13.3	7.1	4.83	2.27	0.00	0.00	0.00	8.0
35	45	15.0	8.9	6.15	2.75	0.00	0.00	0.00	7.0
42	43	14.3	6.6	4.55	1.98	0.07	0.00	0.00	6.0
56	42	14.0	7.5	5.46	2.08	0.00	0.00	0.00	7.0
70	46	15.3	8.5	6.06	2.39	0.01	0.00	0.00	7.4
84	48	16.0	8.0	5.12	2.88	0.00	0.00	0.00	6.8
98	46	15.3	7.9	5.35	2.53	0.00	0.00	0.00	8.0

EC = Enterocystoplasty; 2 = Dog identity; PCV = Packed cell volume; Hb = Haemoglobin concentration; TWBC = Total white blood cells; Neut. = Neutrophils; Lymp. = Lymphocytes; Mon. = Monocytes; Eosin. = Eosinophils; Baso. = Basophils; Tpp. = Total plasma protein

APPENDIX XIV: Pre- and post-surgery haematology results of EC_3 dog that underwent colo-peritoneocystoplasty on the dorsum of the urinary bladder

Parameters Days	PCV (%) (37-55)	Hb. (g/dl) 12.3-18.3	TWBC (10^9/L) 6-17.0	Neut. (10^9/L) 5-13.50	Lymp. (10^9/L) 1-4.80	Mon. (10^9/L) 0.15-1.35	Eosin. (10^9/L) 0.1-1.25	Baso. (10^9/L) RARE	Tpp. (g/dl) 5.7-7.7
Pre-surgical									
7	30	10.0	9.0	7.20	1.80	0.00	0.00	0.00	5.4
4	43	14.3	8.0	4.00	4.00	0.00	0.00	0.00	6.5
1	41	13.7	10.5	7.25	3.26	0.00	0.00	0.00	7.6
Post-surgical									
1	44	14.7	9.2	5.00	4.14	0.09	0.00	0.00	7.4
4	34	11.3	15.0	10.35	4.50	0.15	0.00	0.00	6.4
7	30	10.0	16.5	10.83	5.20	0.42	0.05	0.00	6.0
10	33	11.0	18.0	11.70	6.30	0.00	0.00	0.00	5.6
13	43	14.3	9.4	7.14	2.26	0.00	0.00	0.00	6.8
16	33	11.0	10.8	7.13	2.59	0.00	0.00	0.00	6.0
18	30	10.0	13.9	10.53	2.48	0.91	0.00	0.00	7.0
21	31	10.3	18.1	15.20	2.66	0.24	0.00	0.00	5.0
28	29	9.7	16.5	11.05	2.58	1.83	0.99	0.00	7.5
35	31	10.3	12.5	9.76	2.05	0.56	0.08	0.00	6.0
42	32	10.7	11.0	8.51	2.45	0.02	0.00	0.00	5.5
49	32	10.7	10.3	8.14	2.06	0.10	0.00	0.00	4.2

EC = Enterocystoplasty; 3 = Dog identity; PCV = Packed cell volume; Hb = Haemoglobin concentration; TWBC = Total white blood cells; Neut. = Neutrophils; Lymp. = Lymphocytes; Mon. = Monocytes; Eosin. = Eosinophils; Baso. = Basophils; Tpp. = Total plasma protein

APPENDIX XV: Pre- and post-surgery haematology results of EC_4 bitch that underwent colo-peritoneocystoplasty on the dorsum of the urinary bladder

Parameters Days	PCV (%) (37-55)	Hb. (g/dl) 12.3-18.3	TWBC (10^9/L) 6-17.0	Neut. (10^9/L) 5-13.50	Lymp. (10^9/L) 1-4.80	Mon. (10^9/L) 0.15-1.35	Eosin. (10^9/L) 0.1-1.25	Baso. (10^9/L) RARE	Tpp. (g/dl) 5.7-7.7
Pre-surgical									
7	39	13.0	14.0	9.24	4.20	0.56	0.00	0.00	6.0
4	45	15.0	10.9	7.09	3.71	0.11	0.00	0.00	7.5
1	45	15.0	6.0	3.72	2.28	0.00	0.00	0.00	8.8
Post-surgical									
1	41	13.7	8.0	4.80	3.20	0.00	0.00	0.00	7.0
4	40	13.3	7.3	4.97	2.37	0.00	0.00	0.00	6.5
7	41	13.7	6.6	3.83	2.77	0.00	0.00	0.00	5.0
10	43	14.3	6.3	4.16	2.08	0.06	0.00	0.00	7.8
13	43	14.3	6.0	4.38	1.62	0.00	0.00	0.00	6.0
16	41	13.7	10.3	6.80	2.47	0.00	0.00	0.00	6.5
18	30	10.0	8.6	6.88	1.72	0.00	0.00	0.00	7.0
21	32	10.7	12.4	9.42	2.98	0.00	0.00	0.00	7.0
28	46	15.3	11.5	7.78	3.74	0.00	0.00	0.00	6.5
35	45	15.0	8.7	5.60	3.05	0.00	0.00	0.00	8.0
42	47	15.7	6.0	3.66	2.16	0.18	0.00	0.00	7.2
49	46	15.7	8.5	5.14	3.38	0.10	0.00	0.00	7.0

EC = Enterocystoplasty; 4 = Dog identity; PCV = Packed cell volume; Hb = Haemoglobin concentration; TWBC = Total white blood cells; Neut. = Neutrophils; Lymp. = Lymphocytes; Mon. = Monocytes; Eosin. = Eosinophils; Baso. = Basophils; Tpp. = Total plasma protein

APPENDIX XVI: Pre- and post-surgery interleukins 6 and 10 results of EC_1 dog that underwent colo-peritoneocystoplasty on the dorsum of the urinary bladder

	Interleukin 6		Interleukin 10	
Days	**Absorbance**	**Concentration (pg/ml)**	**Absorbance**	**Concentration (pg/ml)**
Pre-surgical				
7	1.044	7819.36	0.208	124.96
4	1.012	7663.68	0.141	64.89
1	1.424	6313.63	0.236	153.18
Post-surgical				
1	1.134	8011.06	0.154	75.67
2	1.138	8012.91	0.250	167.92
3	1.224	7899.48	0.152	73.99
4	1.147	8014.86	0.201	118.18
5	1.138	8012.91	0.239	156.31
6	1.025	7744.83	0.250	167.92
7	1.055	7857.38	0.134	59.27
9	1.055	7857.38	0.310	235.46
11	1.007	7664.24	0.376	316.86
13	1.010	7678.33	0.408	358.57
15	0.918	7136.70	0.246	163.67
17	1.157	8013.39	0.332	261.84
19	1.153	8014.44	0.510	498.77
21	1.200	7961.72	0.330	259.41
23	1.651	8003.23	0.430	387.96
25	1.151	8014.74	0.248	165.79
27	0.937	7266.01	0.458	426.10
35	1.197	7967.78	0.546	550.13
42	1.291	7837.12	0.258	176.53

EC = Enterocystoplasty; 1 = Dog identity

APPENDIX XVII: Pre- and post-surgery interleukins 6 and 10 results of EC_2 bitch that underwent colo-peritoneocystoplasty on the dorsum of the urinary bladder

	Interleukin 6		Interleukin 10	
Days	**Absorbance**	**Concentration (pg/ml)**	**Absorbance**	**Concentration (pg/ml)**
Pre-surgical				
7	0.781	7861.44	0.200	117.22
4	1.103	7672.87	0.162	82.52
1	1.199	7963.78	0.138	62.47
Post-surgical				
1	1.132	8009.91	0.144	67.34
2	1.200	7961.72	0.170	89.54
3	1.200	7961.72	0.123	50.70
4	1.060	7873.39	0.142	65.71
5	2.577	7933.15	0.115	44.68
6	1.114	7992.91	0.237	154.22
7	1.172	8003.84	0.219	135.84
9	1.081	7931.68	0.716	796.22
11	1.134	8011.06	0.480	456.59
13	1.171	8004.75	0.392	337.55
15	0.975	7498.24	0.296	219.10
17	0.891	6939.02	0.256	174.36
19	3.297	6636.19	0.308	233.10
21	1.430	6233.36	0.474	448.23
23	1.100	7971.59	0.432	390.65
25	0.441	7680.42	0.398	345.40
27	1.329	7319.25	0.204	121.07
35	1.210	7938.78	0.620	657.24
42	1.132	8009.91	0.274	194.12

EC = Enterocystoplasty; 2 = Dog identity

APPENDIX XVIII: Pre- and post-surgery interleukins 6 and 10 results of EC_3 dog that underwent colo-peritoneocystoplasty on the dorsum of the urinary bladder

	Interleukin 6		Interleukin 10	
Days	**Absorbance**	**Concentration (pg/ml)**	**Absorbance**	**Concentration (pg/ml)**
Pre-surgical				
7	1.019	7719.03	0.131	56.90
4	1.012	7606.25	0.129	55.34
1	1.089	7950.00	0.263	181.97
Post-surgical				
1	0.995	7605.32	0.223	139.87
2	1.061	7876.49	0.162	82.52
3	1.122	8001.93	0.233	150.08
4	1.066	7891.53	0.149	71.48
5	1.010	7678.33	0.12	48.43
6	0.892	6946.62	0.166	86.01
7	1.033	7777.56	0.156	77.37
9	1.050	7840.57	0.49	470.58
11	1.120	7999.9	0.354	289.00
13	1.001	7635.29	0.402	350.65
15	1.133	8010.51	0.252	170.06
17	1.060	7873.39	0.348	281.51
19	0.985	7553.14	0.332	261.84
21	1.009	7673.66	0.32	247.35
23	2.820	7854.15	0.246	163.67
25	1.108	7984.63	0.964	326.98
27	1.061	7876.49	0.504	490.28
35	1.188	7983.71	0.444	406.93

EC = Enterocystoplasty; 3 = Dog identity

APPENDIX XIX Pre- and post-surgery interleukins 6 and 10 results of EC_4 bitch that underwent colo-peritoneocystoplasty on the dorsum of the urinary bladder

	Interleukin 6		Interleukin 10	
Days	**Absorbance**	**Concentration (pg/ml)**	**Absorbance**	**Concentration (pg/ml)**
Pre-surgical				
7	1.672	6900.78	0.184	102.19
4	1.516	7677.12	0.121	49.18
1	1.072	7908.48	0.155	76.52
Post-surgical				
1	1.185	7988.28	0.178	96.71
2	3.121	7858.01	0.144	67.34
3	1.021	7727.75	0.347	280.27
4	1.228	7886.69	0.213	129.87
5	1.066	7891.53	0.307	231.93
6	0.690	7945.18	0.206	123.01
7	1.119	7998.82	0.378	319.43
9	0.908	7065.34	0.360	296.53
11	0.997	7615.42	0.428	385.26
13	2.753	7627.80	0.280	200.84
15	1.002	7640.19	0.794	906.12
17	1.266	7729.54	0.828	952.46
19	1.140	8013.61	0.518	510.12
21	1.045	7822.98	0.290	212.20
23	0.917	7129.67	0.370	309.20
25	0.959	7404.91	0.328	256.98
27	0.894	6961.75	0.404	353.28
35	1.478	7515.11	0.292	214.50

EC = Enterocystoplasty; 4 = Dog identity

APPENDIX XX: Pre- and post- surgery means (and standard error of the means) cytokine (interleukins 6 and 10) levels of four dogs that underwent colo-peritoneocystoplasty

Days	Mean ± SEM IL-6 (pg/ml)	Mean ± SEM IL-10 (pg/ml)
Pre-surgical		
7	7597.65 ± 204.48	100.32 ±15.22
4	7639.23 ± 33.31	62.98 ± 7.27
1	7681.47 ± 202.63	118.54 ± 29.06
Post-surgical		
1	7903.64 ± 99.58	94.90 ± 16.21
2	7927.28 ± 36.40	101.83 ± 22.51
3	7897.72 ± 60.50	138.76 ± 51.72
4	7916.62 ± 32.97	96.31 ± 16.22
5	7878.98 ± 71.47	120.33 ± 45.32
6	7657.38 ± 242.94	132.79 ± 18.21
7	7909.40 ± 55.53	147.98 ± 59.44
9	7673.74 ± 203.77	449.70 ± 125.79
11	7822.66 ± 106.05	361.93 ± 37.48
13	7736.54 ± 90.09	311.90 ± 37.27
15	7571.41 ± 180.71	364.74 ± 180.89
17	7638.83 ± 240.37	417.55 ± 179.82
19	7554.35 ± 324.76	375.96 ± 74.45
21	7422.93 ± 400.86	291.80 ± 53.10
23	7739.66 ± 205.84	312.87 ± 53.20
25	7771.17 ± 143.55	273.79 ± 40.73
27	7355.88 ± 190.57	347.69 ± 80.55
35	7851.34 ± 112.46	457.20 ± 95.78
42	7923.52 ± 86.40	185.32 ± 8.80

IL-6 = Interleukin 6; IL-10 = Interleukin 10; SEM = Standard error of the mean

APPENDIX XXI: Relationship between IL-6 and IL-10 with hematological and vital parameters in EC_1 dog and EC_2 bitch that underwent colo-peritoneocystoplasty

	IL-10	PCV	HB	TLC	NEUT	LYMP	MONO	EOSIN	TEMP	PULSE	RESP
EC1 IL-6											
Pearson r	-0.192	0.091	0.092	0.177	0.009	0.469	-0.292	0.364	0.141	-0.079	0.410
P value (two-tailed)	0.511	0.756	0.756	0.545	0.976	0.091	0.312	0.201	0.631	0.790	0.145
Summary	Ns	ns	ns	Ns	ns	ns	ns	Ns	Ns	ns	ns
R square	0.037	0.008	0.008	0.031	8.07E-05	0.220	0.085	0.132	0.020	0.006	0.168
EC1 IL-10	**IL-6**										
Pearson r	-0.192	0.006	0.008	-0.447	-0.312	-0.514	-0.139	-0.326	-0.061	-0.133	-0.503
P value (two-tailed)	0.511	0.984	0.979	0.109	0.278	0.060	0.636	0.255	0.837	0.650	0.067
Summary	Ns	ns	ns	Ns	ns	ns	ns	ns	Ns	ns	ns
R square	0.037	3.41E-05	5.89E-05	0.200	0.097	0.264	0.019	0.107	0.004	0.018	0.253
EC2 IL-6	**IL-10**										
Pearson r	-0.118	-0.389	-0.390	0.430	0.361	0.435	0.088	0.246	-0.024	0.202	0.288
P value (two-tailed)	0.687	0.169	0.168	0.125	0.205	0.120	0.764	0.396	0.934	0.489	0.319
Summary	Ns	ns	ns	Ns	ns	Ns	ns	ns	Ns	ns	ns
R square	0.014	0.152	0.152	0.185	0.130	0.190	0.008	0.061	0.001	0.041	0.083
EC2 IL-10	**IL-6**										
Pearson r	-0.118	-0.025	-0.020	0.190	0.176	0.051	-0.179	0.312	-0.202	-0.041	-0.341
P value (two-tailed)	0.687	0.931	0.945	0.516	0.548	0.862	0.540	0.277	0.488	0.888	0.232
Summary	Ns	ns	ns	Ns	ns	Ns	ns	ns	Ns	ns	ns
R square	0.014	0.001	0.000	0.036	0.031	0.003	0.032	0.097	0.041	0.002	0.116

EC = Enterocystoplasty; 1, 2 = dogs identity; IL-6 = Interleukin 6; IL-10 = Interleukin 10; PCV = Packed cell volume; HB = Haemoglobin concentration; TLC = Total leucocyte count; NEUT = Neutrophils; LYMPH = Lymphocytes; MONO = Monocytes; EOSIN = Eosinophils; TEMP = Temperature; PULSE = Pulse rates; RESP = Respiratory rates. r = Pearson's correlation coefficient; R^2 = Coefficient of Determinant; P values are levels of significance where (* = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$), ns = non significant.

APPENDIX XXII: Relationship between IL-6 and IL-10 with hematological and vital parameters in EC_3 dog and EC_4 bitch that underwent colo-peritoneocystoplasty

EC3 IL-6	**IL-10**	**PCV**	**HB**	**TLC**	**NEUT**	**LYMP**	**MONO**	**EOSIN**	**TEMP**	**PULSE**	**RESP**
Pearson r	0.046	-0.181	-0.182	-0.026	-0.105	-0.001	0.147	0.117	-0.479	0.160	-0.280
P value (two-tailed)	0.882	0.554	0.553	0.933	0.732	0.998	0.632	0.704	0.098	0.602	0.354
Summary	Ns	ns	ns	ns	ns	Ns	ns	ns	Ns	ns	ns
R square	0.002	0.033	0.033	0.001	0.011	8.02E-07	0.0216	0.014	0.230	0.026	0.079
EC3 IL-10	**IL-6**										
Pearson r	0.046	-0.249	-0.247	0.438	0.438	-0.094	0.524	0.511	-0.274	-0.170	-0.438
P value (two-tailed)	0.882	0.413	0.415	0.135	0.135	0.761	0.066	0.074	0.365	0.579	0.135
Summary	Ns	ns	ns	ns	ns	Ns	ns	ns	ns	ns	ns
R square	0.002	0.062	0.061	0.192	0.191	0.009	0.275	0.261	0.075	0.029	0.192
EC4 IL-6	**IL-10**										
Pearson r	0.082	-0.357	-0.351	-0.194	-0.154	-0.200	-0.430	-	0.288	-0.055	0.475
P value (two-tailed)	0.790	0.231	0.240	0.526	0.617	0.514	0.143	-	0.340	0.857	0.101
Summary	Ns	ns	ns	ns	ns	Ns	ns	-	ns	ns	ns
R square	0.007	0.128	0.123	0.038	0.024	0.040	0.185	-	0.083	0.003	0.226
EC4 IL-10	**IL-6**										
Pearson r	0.082	-0.462	-0.461	0.038	0.147	-0.416	-0.254	-	-0.344	-0.331	-0.104
P value (two-tailed)	0.790	0.112	0.113	0.903	0.631	0.157	0.403	-	0.250	0.269	0.735
Summary	Ns	ns	ns	ns	ns	Ns	ns	-	ns	ns	ns
R square	0.007	0.213	0.213	0.001	0.022	0.173	0.064	-	0.118	0.110	0.011

EC = Enterocystoplasty; 3, 4 = dogs identity; IL-6 = Interleukin 6; IL-10 = Interleukin 10; PCV = Packed cell volume; HB = Haemoglobin concentration; TLC = Total leucocyte count; NEUT = Neutrophils; LYMPH = Lymphocytes; MONO = Monocytes; EOSIN = Eosinophils; TEMP = Temperature; PULSE = Pulse rates; RESP = Respiratory rates. r = Pearson's correlation coefficient; R^2 = Coefficient of Determinant; P values are levels of significance where (* = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$), ns = non significant.

APPENDIX XXIII: Relationship between means IL-6 and IL-10 with mean hematological and vital parameters in the four dogs that underwent colo-peritoneocystoplasty

MEAN IL-6	IL-10	PCV	HB	TLC	NEUT	LYMP	MONO	EOSIN	TEMP	PULSE	RESP
Pearson r	-0.256	-0.345	-0.347	0.066	-0.011	0.343	-0.569	0.048	-0.009	0.133	0.062
P value (two-tailed)	0.399	0.249	0.246	0.829	0.970	0.251	0.042	0.876	0.976	0.666	0.841
Summary	Ns	ns	Ns	ns	ns	Ns	*	ns	ns	ns	ns
R square	0.066	0.119	0.120	0.004	0.000	0.118	0.324	0.002	8.97E-05	0.018	0.004
MEAN IL-10	**IL-6**										
Pearson r	-0.256	-0.416	-0.414	0.015	0.161	-0.395	0.291	0.201	-0.287	-0.412	-0.559
P value (two-tailed)	0.399	0.158	0.160	0.960	0.600	0.182	0.336	0.510	0.342	0.162	0.047
Summary	Ns	ns	Ns	ns	ns	Ns	ns	ns	ns	ns	*
R square	0.066	0.173	0.171	0.000	0.026	0.156	0.084	0.040	0.082	0.170	0.313

IL-6 = Interleukin 6; IL-10 = Interleukin 10; PCV = Packed cell volume; HB = Haemoglobin concentration; TLC = Total leucocyte count; NEUT = Neutrophils; LYMPH = Lymphocytes; MONO = Monocytes; EOSIN = Eosinophils; TEMP = Temperature; PULSE = Pulse rates; RESP = Respiratory rates.

r = Pearson's correlation coefficient; R^2 = Coefficient of Determinant; P values are levels of significance where (* = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$), ns = non significant.

APPENDIX XXIV: Pre- and post-surgery haematology means and standard error of the means (SEM) results of four dogs that underwent colo-peritoneocystoplasty

Parameters / Days	PCV (%)	HB(g/dl)	TWBC(10^9/L)	NEUT(10^9/L)	LYMP(10^9/L)	EOSIN(10^9/L)	MON(10^9/L)	BASO(10^9/L)	TPP(g/dl)
Pre-surgical									
7	44.75±6.52	14.93±2.18	10.70±1.28	7.61±0.81	2.90±0.49	0.00±0.00	0.20±0.13	0.00±0.00	6.55±0.53
4	47.25±2.66	15.73±0.88	9.88±0.70	6.15±0.72	3.59±0.31	0.00±0.00	0.14±0.10	0.00±0.00	7.00±0.22
1	45.25±1.84	15.10±0.62	8.98±1.07	6.14±0.85	2.72±0.22	0.00±0.00	0.13±0.08	0.00±0.00	7.45±0.51
Post-surgical									
1	39.25±2.56	13.10±0.85	8.90±0.57	5.17±0.22	3.60±0.33	0.08±0.08	0.07±0.02	0.00±0.00	6.45±0.46
4	35.25±1.60	11.73±0.53	12.43±2.06	8.31±1.51	4.42±0.82	0.04±0.04	0.08±0.04	0.00±0.00	6.38±0.06
7	33.50±2.53	11.18±0.85	14.20±2.77	10.00±2.39	3.64±0.66	0.41±0.39	0.16±0.10	0.00±0.00	5.88±0.31
10	36.50±2.22	12.18±0.73	13.25±2.60	8.92±1.69	4.01±0.88	0.27±0.26	0.06±0.03	0.00±0.00	6.90±0.48
13	38.00±2.92	12.65±0.96	9.68±1.34	7.24±1.03	2.18±0.26	0.21±0.21	0.05±0.04	0.00±0.00	6.70±0.24
16	35.75±1.89	11.93±0.64	10.53±0.23	7.63±0.44	2.12±0.25	0.05±0.05	0.21±0.16	0.00±0.00	6.40±0.14
18	35.25±3.09	11.75±1.03	10.26±1.28	7.51±1.06	2.31±0.20	0.02±0.02	0.42±0.21	0.00±0.00	6.80±0.27
21	42.50±6.44	14.18±2.15	10.83±2.81	8.46±2.52	2.21±0.36	0.03±0.02	0.13±0.05	0.00±0.00	6.90±0.66
28	40.00±3.89	13.33±1.29	10.37±2.32	7.18±1.45	2.48±0.50	0.25±0.25	0.46±0.46	0.00±0.00	7.60±0.41
35	40.75±3.33	13.58±1.12	9.45±1.03	6.9±0.96	2.38±0.31	0.02±0.02	0.14±0.14	0.00±0.00	7.05±0.41

42	39.75±3.30	13.25±1.10	7.37±1.27	5.21±1.11	2.09±0.14	0.00±0.00	0.07±0.04	0.00±0.00	6.13±0.37
56	40.00±2.94	13.43±1.04	8.39±0.70	5.86±0.78	2.51±0.31	0.00±0.00	0.05±0.03	0.00±0.00	6.18±0.67
70	44.50±1.50	14.80±0.50	8.43±0.03	5.40±0.67	3.03±0.64	0.00±0.00	0.01±0.01	0.00±0.00	7.10±0.30
84	44.00±4.00	14.65±1.35	8.80±0.80	4.96±0.16	3.75±0.87	0.00±0.00	0.10±0.10	0.00±0.00	6.90±0.10
98	45.00±1.00	15.00±0.30	8.41±0.53	5.05±0.30	3.34±0.81	0.00±0.00	0.02±0.02	0.00±0.00	7.75±0.25

EC = Enterocystoplasty; 1 = Dog identity; PCV = Packed cell volume; HB = Haemoglobin concentration; TWBC = Total white blood cells; NEUT. = Neutrophils; LYMP. = Lymphocytes; MON. = Monocytes; EOSIN. = Eosinophils; BASO. = Basophils; TPP. = Total plasma protein

APPENDIX XXV: Pre- and post-surgery serum chemistry results of EC1 dog that underwent colo-peritoneocystoplasty on the dorsum of the urinary bladder

Parameters DAYS	Urea	Na^+	K^+	Cl^-	HCO_3^-	Ca^{2+}	PO_3^-	U/A	TPP	Alb	Glu	Cholest	AST	ALT	ALP
Pre-Surgical															
7	8	148	5.9	109	23	2.26	0.78	80	82	44	4.1	3.8	3	13	37
4	6	142	4.1	100	19	2.22	0.93	90	81	45	4.1	4	6	24	54
1	5	145	5.4	103	22	0.28	0.14	70	79	42	4.7	4.2	25	6	34
Post-Surgical															
1	3.5	130	5.2	90	22	2.2	0.9	90	70	41	4.3	5	11	4	60
4	7	144	3.8	107	22	2.8	0.63	60	72	35	3.3	5.3	14	7	87
7	7.5	142	6.7	69	21	1.14	1.07	100	73	38	3.2	3.3	11	2	57
10	7	140	4.6	101	24	2.3	0.88	80	60	37	2.6	5.3	5	26	47
13	7.5	146	4.7	110	23	2.58	1.54	80	66	43	3.5	3.7	19	4	30
16	6	130	4	106	22	2.47	0.8	80	76	45	4.4	4.5	6	30	95
18	6.9	136	3.1	108	21	2	1.09	70	83	29	4	6.1	12	14	78
21	4	146	5	106	23	2.16	0.89	80	75	33	6.1	4	30	23	40
28	3	143	4.9	118	23	2.18	0.8	75	70	30	3.2	3	16	10	98
35	4.1	138	4.1	100	19	2.28	1.02	70	72	49	3.8	2.3	11	4	39

42	5.9	143	5.3	107	20	2.14	0.15	80	85	44	5.6	4.4	12	6	41
56	6.2	140	6	104	26	2.37	0.43	40	75	44	4.6	5.9	15	12	49
70	7	149	4.7	110	17	2.14	1.03	60	79	39	4.6	6.9	20	23	91
84	5	140	5.8	106	23	2.14	1.07	70	76	42	4.1	3.4	14	11	33
98	4.5	141	3	96	20	2.08	1.02	100	69	30	5.9	2.1	12	8	61

Na^+ = Sodium ion; K^+ = Potassium ion; Cl^- = Chloride ion; HCO_3^- = Bicarbonate ion; Ca^{2+} = Calcium ion; PO_3^- = Phosphate ion; U/A = Uric acid; TPP = Total plasma protein; Alb = Albumin; Glu = Glucose; Cholest = Cholesterol; AST = Aspartate transaminase; ALT = Alanine transaminase; ALP = Alkaline phosphatase

APPENDIX XXVI: Pre- and post-surgery serum chemistry results of EC2 bitch that underwent colo-peritoneocystoplasty on the dorsum of the urinary bladder

Parameters DAYS	Urea	Na^+	K^+	Cl^-	HCO_3^-	Ca^{2+}	PO_3^-	U/A	TPP	Alb	Glu	Cholest	AST	ALT	AP
Pre-Surgical															
7	4	139	5.4	99	22	2.02	0.89	70	72	42	3.9	4.3	15	10	57
4	4.5	136	5	104	21	2.04	0.99	40	85	44	3	5.6	18	15	71
1	5.5	142	4.3	118	20	2.33	0.6	60	71	40	2.3	5	31	16	50
Post-Surgical															
1	3	138	5.8	105	21	2.14	1.07	70	85	20	4.4	3.7	10	18	95
4	9	155	5	113	22	2.12	1.06	60	83	30	5.5	4.5	15	10	57
7	6	139	5.3	110	23	2.12	1.06	90	63	30	4.4	3.6	15	14	38
10	6.5	146	5.5	106	23	2.04	1.2	80	68	35	3.7	4.1	17	14	61
13	4	133	3.8	93	22	1.12	1.41	40	66	43	5.9	4	12	15	83
16	3.5	141	3.4	103	30	2.22	1.02	60	63	29	2.7	3.4	16	12	58
18	5.5	147	5.5	109	24	2.38	1.4	60	83	41	4.5	2.8	15	18	34
21	7.5	141	6.1	75	21	1.88	0.88	40	81	41	5.5	3.5	19	14	64
28	4	138	4.4	100	20	2	1	70	72	49	2.4	2.5	19	10	60
35	6	153	4	117	25	2.28	1.4	87	68	39	6.3	2.5	19	14	44

42	2.5	136	3.7	101	24	2.18	0.08	70	70	34	6	3	17	18	30
56	5.5	153	6.3	122	20	2	1.4	94	63	34	1.7	3	23	17	24
70	4.5	140	4.3	102	23	2.48	0.88	60	78	35	1.8	4	22	18	91
84	4.5	136	2.9	104	16	2.68	0.82	60	72	49	5.5	6	22	13	60
98	3.5	139	3.4	96	22	2.53	0.9	50	72	38	3	4.8	12	10	57

Na^+ = Sodium ion; K^+ = Potassium ion; Cl^- = Chloride ion; HCO_3^- = Bicarbonate ion; Ca^{2+} = Calcium ion; PO_3^- = Phosphate ion; U/A = Uric acid; TPP = Total plasma protein; Alb = Albumin; Glu = Glucose; Cholest = Cholesterol; AST = Aspartate transaminase; ALT = Alanine transaminase; ALP = Alkaline phosphatase

APPENDIX XXVII: Pre- and post-surgery serum chemistry results of EC3 dog that underwent colo-peritoneocystoplasty on the dorsum of the urinary bladder

Parameters DAYS	Urea	Na^+	K^+	Cl^-	HCO_3^-	Ca^{2+}	PO_3^-	U/A	TPP	Alb	Glu	Cholest	AST	ATT	AP
Pre-Surgical															
7	6	145	5.5	112	23	2.2	0.1	30	70	39	3.3	3.7	6	11	47
4	3.5	143	4.7	106	21	2.35	1.02	100	79	42	2.9	4	25	6	37
1	6	140	4.5	104	25	2.36	0.88	80	83	30	4.5	4	15	5	34
Post-Surgical															
1	11	139	4.6	101	22	2.3	0.08	40	69	37	6.1	5.5	3	13	74
4	4	136	3.9	103	20	2.2	1.07	30	60	44	6	4.2	11	4	44
7	4.5	146	5.3	110	20	2.36	1.16	60	80	30	2.9	6.2	14	7	80
10	7	142	5.5	107	23	2.26	1.47	100	69	33	3.1	3.8	8	4	71
13	6.5	143	5.3	108	24	2.1	0.66	60	82	46	5	4	7	15	31
16	3.5	134	3.3	100	20	2.3	0.9	90	74	55	6.2	2.1	12	7	39
18	3.9	122	2.9	96	22	2.32	0.9	90	72	42	5.5	6.8	12	18	47
21	5.5	147	5.4	110	22	2.48	1.12	40	73	29	3.8	6	5	15	37
28	3.5	149	4.7	117	23	2.02	1.01	30	74	44	4.2	4.9	12	48	41
35	3.5	146	5.3	103	30	2.34	0.94	80	66	43	4.5	4.5	12	5	50

42	5	144	5.1	113	22	2.34	1.07	102	65	30	2.3	2.3	8	4	34

Na^+ = Sodium ion; K^+ = Potassium ion; Cl^- = Chloride ion; HCO_3^- = Bicarbonate ion; Ca^{2+} = Calcium ion; PO_3^- = Phosphate ion; U/A = Uric acid; TPP = Total plasma protein; Alb = Albumin; Glu = Glucose; Cholest = Cholesterol; AST = Aspartate transaminase; ALT = Alanine transaminase; ALP = Alkaline phosphatase

APPENDIX XXVIII: Pre- and post-surgery serum chemistry results of EC4 bitch that underwent colo-peritoneocystoplasty on the dorsum of the urinary bladder

Parameters	Urea	Na^+	K^+	Cl^-	HCO_3^-	Ca^{2+}	PO_3^-	U/A	TPP	Alb	Glu	Cholest	AST	ALT	AP
DAYS															
Pre-Surgical															
7	3.5	139	6.3	114	15	2.27	1.47	70	68	35	3	3.9	11	2	57
4	6.5	153	4.2	113	22	1.04	1.18	26	78	42	2.2	6	33	10	84
1	4	148	6.4	94	23	2.24	0.8	60	63	29	3.5	4	19	10	64
Post-Surgical															
1	5	137	4.3	98	22	2.44	1.22	30	79	45	5.5	2.3	10	12	78
4	3	137	4.4	109	21	2.54	1.34	30	73	40	3.7	3	12	5	61
7	5.5	127	3.7	94	20	2.4	1.34	30	66	43	5.8	3.6	19	5	30
10	11.5	141	4.6	103	24	2.28	1.17	40	56	38	5	4.6	7	30	51
13	3.5	142	6	104	23	2.08	1.04	53	70	24	5.7	6	17	6	41
16	7.5	153	4.7	116	22	2.39	0.89	80	66	41	2.2	3.3	11	4	50
18	4.5	150	6.3	111	21	1.92	0.89	90	66	42	5.7	3.4	12	18	37
21	2	132	5.2	90	27	0.28	0.15	20	72	38	6.2	3	12	18	44
28	3.5	140	3.8	108	23	2.33	0.78	40	82	44	4.6	3.8	3	13	37
35	5.5	129	9.2	107	21	2.4	0.68	40	69	38	5.3	4	3	13	74

42	3	143	4.7	73	23	2.12	0.93	90	68	34	5.7	5.3	33	35	54
56	5	140	5.4	110	22	2.28	1.07	106	68	39	6.1	2.2	19	30	68
70	3.5	146	5.3	110	20	2.34	1.33	60	70	43	5.3	2.9	14	2	67
84	4.5	142	4.4	105	20	2.33	1.13	60	81	37	4.5	2.3	17	17	51

Na^+ = Sodium ion; K^+ = Potassium ion; Cl^- = Chloride ion; HCO_3^- = Bicarbonate ion; Ca^{2+} = Calcium ion; PO_3^- = Phosphate ion; U/A = Uric acid; TPP = Total plasma protein; Alb = Albumin; Glu = Glucose; Cholest = Cholesterol; AST = Aspartate transaminase; ALT = Alanine transaminase; ALP = Alkaline phosphatase

APPENDIX XXIX: Pre- and post-surgery serum means and standard error of the means (SEM) results of four dogs that underwent colo-peritoneocystoplasty

Parameters	Urea	Na	K	Cl	HCO_3	Ca	PO_3	U/A	TPP	Alb	Glu	Chol	AST	ALT	ALP
Days															
Pre-surgical															
7	5.38±1.03	142.75±2.25	5.78±0.21	40.00±1.96	20.75±1.93	2.19±0.06	0.81±0.28	62.50±11.09	73.00±3.11	108.50±3.33	3.58±0.26	3.93±0.13	14.25±6.75	9.00±2.42	49.50±4.79
4	5.13±0.69	143.50±3.52	4.50±0.21	43.25±0.75	20.75±0.63	1.91±0.30	1.03±0.05	64.00±18.24	80.75±1.55	105.75±2.72	3.05±0.39	4.90±0.53	17.00±3.98	13.75±3.88	61.50±10.22
1	5.13±0.43	143.75±1.75	5.15±0.48	35.25±3.35	22.50±1.04	1.80±0.51	0.61±0.17	67.50±4.79	74.00±4.43	104.75±4.96	3.75±0.55	4.30±0.24	20.25±4.75	9.25±2.50	45.50±7.23
Post-surgical															
1	5.63±1.84	136.00±2.04	4.98±0.33	35.75±5.50	21.75±0.25	2.27±0.07	0.82±0.25	57.50±13.77	75.75±3.82	98.50±3.18	5.08±0.44	4.13±0.72	9.00±2.04	11.75±2.90	76.75±2.90
4	5.75±1.38	143.00±4.38	4.28±0.28	37.25±3.04	21.25±0.48	2.42±0.16	1.03±0.15	45.00±8.66	72.00±4.71	108.00±2.08	4.63±0.66	4.25±0.48	14.75±1.65	6.50±1.32	62.25±9.01
7	5.88±0.63	138.50±4.09	5.25±0.61	35.25±3.20	21.00±0.71	2.01±0.29	1.16±0.06	70.00±15.81	70.50±3.80	95.75±9.68	4.08±0.66	4.18±0.68	11.75±1.80	7.00±2.55	51.25±11.13
10	8.00±1.17	142.25±1.31	5.05±0.26	35.75±1.11	23.50±0.29	2.22±0.06	1.18±0.12	75.00±12.58	63.25±3.15	104.25±1.38	3.60±0.52	4.45±0.33	11.75±3.09	18.50±5.91	57.50±5.38
13	5.38±0.97	141.00±2.80	4.95±0.47	39.00±5.50	23.00±0.41	1.97±0.31	1.16±0.20	58.25±8.35	71.00±3.79	103.75±3.79	5.03±0.54	4.43±0.53	12.25±2.50	10.00±2.92	46.25±12.50
16	5.13±0.99	139.50±5.04	3.85±0.32	42.50±5.38	23.50±2.22	2.35±0.05	0.90±0.05	77.50±6.29	69.75±3.12	106.25±3.47	3.88±0.91	3.33±0.49	11.50±2.06	13.25±5.82	60.50±12.14
18	5.20±0.66	138.75±6.34	4.45±0.85	38.50±3.18	22.00±0.71	2.16±0.11	1.07±0.12	77.50±7.50	76.00±4.22	106.00±3.39	4.93±0.40	4.78±0.99	12.75±0.75	17.00±1.00	49.00±10.06
21	4.75±1.16	141.50±3.43	5.43±0.24	35.25±2.66	23.25±1.31	1.70±0.49	0.76±0.21	45.00±12.58	75.25±2.02	95.25±8.01	5.40±0.56	4.13±0.66	14.25±6.34	17.50±2.02	46.25±6.09
28	3.50±0.20	142.50±2.40	4.45±0.24	41.75±4.09	22.25±0.75	2.13±0.08	0.90±0.06	53.75±11.06	74.50±2.63	110.75±4.23	3.60±0.50	3.55±0.52	12.50±3.48	20.25±9.28	59.00±13.93
35	4.78±0.59	141.50±5.17	5.65±1.22	42.25±2.50	23.75±2.43	2.33±0.03	1.01±0.15	69.25±10.36	68.75±1.25	106.75±3.71	4.98±0.54	3.33±0.55	18.75±5.07	9.00±2.61	51.75±7.75

42	4.10±0.81	141.50±1.85	4.70±0.36	35.50±2.99	22.25±0.85	2.20±0.05	0.56±0.26	85.50±6.85	72.00±4.45	98.50±8.89	4.90±0.87	3.75±0.68	14.00±2.48	15.75±7.12	39.75±5.27
56	5.57±0.35	144.33±4.33	5.90±0.26	39.00±2.89	22.67±1.75	2.22±0.11	0.97±0.28	80.00±20.30	68.67±3.48	112.00±5.29	4.13±1.29	3.70±1.12	17.33±2.85	19.67±5.39	47.00±12.74
70	5.00±1.04	145.00±2.65	4.77±0.29	39.00±2.31	20.00±1.73	2.32±0.10	1.03±0.24	60.00±0.00	75.67±2.85	107.33±2.67	3.90±1.07	4.60±1.19	19.67±1.45	14.33±6.33	83.00±8.00
84	4.67±0.17	139.33±1.76	4.37±0.84	42.67±3.48	19.67±2.03	2.38±0.16	1.01±0.09	63.33±3.33	76.33±2.60	105.00±0.58	4.70±0.42	3.90±1.10	18.00±4.00	13.67±1.76	48.00±7.94
98	4.00±0.50	140.00±1.00	3.20±0.20	34.00±4.00	21.00±1.00	2.31±0.23	0.96±0.06	75.00±25.00	70.50±1.50	96.00±0.00	4.45±1.45	3.45±1.35	12.00±0.00	9.00±1.00	59.00±2.00

Na^+ = Sodium ion; K^+ = Potassium ion; Cl^- = Chloride ion; HCO_3^- = Bicarbonate ion; Ca^{2+} = Calcium ion; PO_3^- = Phosphate ion; U/A = Uric acid; TPP = Total plasma protein; Alb = Albumin; Glu = Glucose; Cholest = Cholesterol; AST = Aspartate transaminase; ALT = Alanine transaminase; ALP = Alkaline phosphatase.Prof