Hemostatic response to surgical neutering via ovariectomy and ovariohysterectomy in dogs

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Objective—To investigate the hemostatic response to surgery and compare the response for ovariohysterectomy with that for ovariectomy and to evaluate the usefulness of thromboelastography on plasma samples.

Animals—42 female dogs.

Procedures—Dogs were assigned to undergo ovariohysterectomy or ovariectomy. Blood samples were collected immediately before and 1, 6, and 24 hours after surgery and stored at −80°C for subsequent analysis. Plasma samples were subjected to thromboelastography after thawing. In addition, coagulation variables were measured, including concentrations of von Willebrand factor antigen, fibrinogen, antithrombin, and protein C; activity of factor VIII; activated partial thromboplastin time; prothrombin time; and thrombin time. The fibrinolytic response was assessed via concentrations of D-dimer, plasminogen, and α2-antiplasmin (plasmin inhibitor).

Results—Substantial hemostatic and fibrinolytic activation was evident after surgery in both groups, as characterized by significantly increased global clot strength and an overall hypercoagulable state at 4 hours after surgery in addition to decreases in von Willebrand factor antigen and factor VIII concentrations and shortened prothrombin and thrombin times. The dogs also typically had activation of the fibrinolytic system, as evidenced by increased postoperative concentrations of D-dimer, plasminogen, and plasmin inhibitor. Differences between the 2 groups could not be detected for any variables.

Conclusions and Clinical Relevance—Elective surgery with limited tissue trauma induced hemostatic activation in dogs, which led to hypercoagulability after surgery. A difference between the ovariohysterectomy and ovariectomy groups was not detected. Thromboelastography can be used on plasma samples and may be useful for evaluating patterns over time. (Am J Vet Res 2012;73:1469–1476)

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Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>aPTT</td>
<td>Activated partial thromboplastin time</td>
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<tr>
<td>AT</td>
<td>Antithrombin</td>
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<td>MA</td>
<td>Maximum amplitude</td>
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<td>PT</td>
<td>Prothrombin time</td>
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<td>TT</td>
<td>Thrombin time</td>
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conducted to compare ovariectomy and ovariohysterectomy, and the authors are not aware of any studies conducted to compare the hemostatic part of the surgical stress response or short-term postoperative morbidity between the 2 procedures.

In humans, the effects of surgery on the hemostatic response and the development of an increased tendency for clot formation (ie, hypercoagulability) are known. Surgery and immobilization are considered some of the risk factors for the development of hypercoagulability and postoperative thromboses. The same activation of the hemostatic response has also been reported in horses, as indicated by decreased concentrations of AT and increased concentrations of D-dimer. Furthermore, decreases in TT, fibrinogen concentration, and AT con-
coagulation tests, and fibrinolysis tests. The secondary objective of the study reported here was to evaluate the use of plasma thromboelastography for measuring hemostatic trends over time.

### Materials and Methods

#### Animals

Forty-two client-owned healthy sexually intact bitches admitted to the Department of Clinical Sciences of Companion Animals for elective neutering between June 2006 and June 2007 were prospectively enrolled in a controlled randomized clinical trial. Of these, 12 were mixed-breed dogs and 30 were purebred dogs. Consent was obtained from the owners, and the study was approved by the Ethics and Research Committee of the Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, University of Utrecht, The Netherlands. Variables, including blood loss, surgical time, surgical wound characteristics, pain scores, and wound assessment scores, for these dogs have been reported.

Dogs underwent a thorough clinical examination to ensure they were healthy. Only dogs assigned to American Society of Anesthesiologists category 1 (clinically normal animals) were eligible for participation in the study. An inclusion requirement was that the last estrus of each dog had ended at least 6 weeks prior to surgery. Body condition score (1 = emaciated and 5 = obese) was recorded for each dog at the time of admission. The dogs were numbered consecutively at time of admission; dogs were assigned to 2 treatment groups (ovarioectomy or ovariohysterectomy) by use of a randomization procedure. Forty-two cards were made, 21 stating ovariohysterectomy and 21 stating ovariectomy. These were put in envelopes; the envelopes were mixed, then numbered from 1 to 42. The envelope corresponding to the number of a dog was opened after induction of anesthesia in that dog, and the indicated procedure (ovarioectomy or ovariohysterectomy) was performed.

#### Anesthesia, surgery, and analgesia

A catheter was inserted in a cephalic vein of each dog. Dogs were premedicated with medetomidine (1 mg/m², IV) and carprofen (4 mg/kg, IV), and anesthesia was induced with propofol (1 to 2 mg/kg, IV, to effect). Dogs then were intubated, and anesthesia was maintained by administration of isoflurane in oxygen and air. Intermittent positive-pressure ventilation was used to ensure normocapnia, and the tidal volume was regulated to ensure end-tidal Pco₂ remained within reference limits (4.5 to 5 kPa). All dogs were administered lactated Ringer’s solution at a maintenance rate (10 mL/kg/h, IV) throughout the course of anesthesia and surgery.

Intraoperative monitoring consisted of ECG, capnography, measurement of body temperature, and measurement of oxygen and vapor concentrations. In surgeries that lasted >1 hour, dogs received an additional dose of medetomidine that corresponded to half of the original medetomidine dose. After surgery, dogs were administered atipamezole (2.5 µg/m² IM).

All surgeries were performed by 1 experienced surgeon (MEP) with the help of an assistant. Standardized surgical protocols were used; both ovariectomy and ovariohysterectomy were performed as open surgical procedures, such as endoscopic cholecystectomy and subtotal thyroidectomy, but some variables changed significantly, although the changes were less pronounced, after less invasive procedures, such as endoscopic cholecystectomy and subtotal thyroid resection. In another study, AT concentration was the most sensitive marker for the degree of surgical trauma in humans.

Studies of surgery on dogs have involved the use of assays that assess only isolated parts or phases of the hemostatic process, and it has been difficult to evaluate the entire coagulant state. Thromboelastography performed on whole blood allows for global assessment of hemostatic function from initiation of clotting through amplification and propagation of clot formation to fibrinolysis. Although the equipment for thromboelastography is highly specialized and not readily available to all clinicians, it may be a valuable addition for the diagnosis of hemostatic disorders and assessment of hypercoagulability. Thromboelastography most commonly involves the use of whole blood but may be applied to frozen-thawed plasma samples. This allows for analysis of the samples in a single assay and has the added benefit of eliminating between-assay analytic variation.

The primary objective of the study reported here was to investigate the effects of elective surgery on the hemostatic response in dogs, including whether hypercoagulability is induced, and to compare the magnitude of this response in dogs in which surgical trauma was believed to be minimal (ovariectomy) with that in dogs in which the trauma was believed to be greater (ovariohysterectomy). Our hypothesis was that ovariectomy would have a less pronounced effect on the hemostatic response, compared with the effect for ovariohysterectomy, as measured via plasma thromboelastography,
procedures. For the ovariectomy group dogs, the ovaries were removed through a smaller incision than the incision used for the ovariohysterectomy group dogs. In addition, the uterus was removed from each ovariohysterectomy group dog.

All dogs were hospitalized for 24 to 32 hours after surgery. Administration of buprenorphine (10 µg/kg, SC, q 6 h for 24 hours) was initiated approximately 40 minutes before atipamezole administration. A rescue analgesia protocol consisted of administration of a higher dose of buprenorphine (20 µg/kg, SC) to dogs with pain scores > 15 on a modified version of the short form of the Glasgow Composite Measure Pain Scale. Treatment at home consisted of carprofen (2 mg/kg, PO, q 12 h) for an additional 2 days after discharge.

Collection of blood samples—Immediately after anesthesia was induced, a catheter was inserted in a jugular vein of each dog and secured in place. Immediately before the skin incision (time 0), before closure of the abdominal incision (1 hour), and 6 hours after surgery, blood samples were collected via this catheter. The catheter was removed from the jugular vein immediately after the sample was collected at 6 hours after surgery. A blood sample was obtained 24 hours after surgery via direct puncture of the contralateral jugular vein. For all samples, 11 mL of blood was collected in 1 serum tube followed by 2 tubes containing 3.2% sodium citrate. For samples collected via the jugular vein catheter, the first 5 mL of blood was discarded, and 11 mL of blood was then collected. In addition, immediately before the skin was incised, 10 mL of blood was collected into tubes containing heparin and EDTA. These samples were used for biochemical and hematologic analysis to confirm each dog’s health before enrollment in the study.

All serum and citrate tubes were immediately placed on ice; the citrate tubes were centrifuged within 5 minutes and the serum tubes were centrifuged within 1 hour after collection. Immediately after centrifugation (1,006 × g for 10 minutes at 4°C), serum and plasma were separated and stored at ~80°C until analysis. Frozen serum and citrated plasma samples were transported on dry ice to the Central Laboratory, Department of Small Animal Clinical Sciences, Faculty of Life Sciences, University of Copenhagen, Denmark, for analysis. Samples were analyzed in January 2008. The laboratory analysis was performed in a double-blind manner, and investigators were not apprised of the results until after statistical analysis of the data was completed. Only the surgeon knew the procedure that was performed on each dog.

Plasma samples were thawed in a water bath at 37°C for 5 minutes and centrifuged at 4,000 × g for 3 minutes. The supernatant was harvested and then analyzed. Variables analyzed in plasma and serum samples were alkaline phosphatase activity; BUN, serum creatinine, bile acids, total plasma calcium, phosphorus, sodium, and potassium concentrations; Hct; and total leukocyte and platelet counts.

Thromboelastography—Thromboelastography was performed on citrated plasma samples with diluted recombinant human tissue factor as an activator. The analysis was performed on a computerized thromboelastograph in accordance with methods described elsewhere.

Interpretation of thromboelastography results—Three thromboelastography variables were analyzed: R (reaction time), α angle, and MA. A measure of the overall coagulant state, G (global clot strength), was then calculated by use of the following equation: G = 5,000 × MA/(100 − MA). The R (in seconds) was determined as the distance in millimeters from the start of the tracing to the point at which a preset fibrin clot is achieved. The α angle represents the speed at which fibrin forms and cross-links (ie, clot formation). The MA is a measure of the strength of the fibrin clot.

Coagulation tests—All coagulation tests on citrated plasma samples were performed with an automated coagulation analyzer. Variables measured were aPTT, PT, and TT; activity of factor VIII; and fibrinogen, von Willebrand factor antigen, AT, and protein C concentrations.

Fibrinolysis tests—Concentrations of plasminogen and plasmin inhibitor were measured with the automated coagulation analyzer. In addition, concentrations of D-dimer were measured via an immunometric flow-through principle.

Statistical analysis—All statistical analyses were performed with a statistical software package. Variables were evaluated for a normal distribution and to determine correlations. Logarithmic transformation was performed when data did not have a normal distribution. When logarithmic transformation did not result in a normal distribution, a Box-Cox transformation was performed. Observations within each dog over time were not independent of each other. Therefore, linear mixed regression models, including a random effect for dogs, were used to detect differences between the treatment groups and between time points for each of the outcome variables. In an attempt to control for multiple comparisons, values of P < 0.01 were considered significant. The distribution of the residuals was assessed via a normal quantile plot.

The variable D-dimer concentration was not normally distributed and was logarithmically transformed before inclusion in the linear mixed model. However, transformations failed to result in a normal distribution for the variables plasminogen concentration, AT, and aPTT; thus, median values were used in graphs and a Box-Cox transformation was used in the linear mixed model for these 3 variables.

Results

Two dogs were excluded from the study (one was receiving phenobarbital because of epilepsy and the other had unexpected complications during surgery that increased the duration of the procedure but were not associated with the procedure per se). Thus, results from 40 dogs (20 dogs/group) were included in the statistical analysis. Mean ± SEM age of participating dogs was 2.8 ± 3.0 years in the ovariohysterectomy
group and 1.9 ± 1.2 years in the ovariecomy group. Mean weight of participating dogs was 26 ± 6.0 kg in the ovariohysterectomy group and 24.4 ± 7.3 kg in the ovariecomy group. The groups were comparable with regard to age, body weight, body condition score, and duration of surgery. Results of biochemical and hematologic analyses of samples obtained before surgery were within reference limits established by the laboratory. Values did not differ significantly between the groups. None of the dogs had pain scores > 15; thus, rescue analgesia was not provided to any of the dogs.

The intracluster correlation coefficients for the linear mixed models were consistently high (from ρ = 0.19 for R to ρ = 0.83 for plasminogen concentration), which indicated that values for the same dog were similar over time and that most of the variation in the data was between dogs. None of the variables analyzed differed between groups at any of the time points, although significant differences from values at time 0 were detected at several time points in both groups.

**Plasma thromboelastography**—The R (P = 0.005) and MA (P < 0.001) increased significantly at 24 hours after surgery (Figure 1). There was also a small but significant (P = 0.005) decrease in MA at 1 hour. The G decreased significantly (P < 0.01) at 1 hour but increased significantly (P < 0.001) at 24 hours after surgery (Figure 2). The G and MA values were above the reference limits established for plasma thromboelastography in dogs.17

**Coagulation variables**—Several variables differed significantly from the values at time 0. Concentration of von Willebrand factor antigen decreased significantly (P < 0.001) at 1 and 6 hours after surgery (Figure 3). Factor VIII concentration decreased significantly at 6 (P < 0.001) and 24 (P = 0.007) hours after surgery (Figure 4). The PT decreased significantly (P < 0.001) at 24 hours after surgery (Figure 5). The TT decreased significantly at 6 (P = 0.002) and 24 (P < 0.001) hours after surgery. Fibrinogen concentration increased sig-
significantly ($P < 0.001$) at 24 hours after surgery (Figure 6). The AT concentration increased significantly ($P < 0.001$) at 6 and 24 hours after surgery. For aPTT and protein C concentration, we did not detect significant differences over time. There was a significant negative correlation ($\rho = -0.85$) between fibrinogen concentration and TT.

**Fibrinolysis variables**—Concentration of D-dimer increased significantly ($P < 0.001$) at 6 hours after surgery (Figure 7). Plasminogen concentration increased significantly ($P < 0.001$) at 6 and 24 hours after surgery (Figure 8). Plasmin inhibitor concentration decreased significantly ($P = 0.001$) at 1 hour and increased significantly ($P < 0.001$) at 24 hours after surgery (Figure 9).

**Discussion**

Postoperative hypercoagulability was detected after both ovariectomy and ovariohysterectomy in dogs of the present study and was characterized by significant increases above the reference limits for plasma MA and G values at 24 hours after surgery, combined with a decreased von Willebrand factor antigen concentration, PT, TT, and factor VIII concentration. However, the hypothesis that there would be a difference between dogs undergoing ovariectomy and those undergoing ovariohysterectomy was not supported. The detection of hypercoagulability after surgery is in agreement with results of previous studies in humans. However, 2 studies conducted to evaluate PT, aPTT, AT concentration, platelet counts, hemograms, fibrin-degradable products, plasminogen concentration, plasmin inhibitor concentration, and tissue plasminogen activator concentration failed to find hypercoagulability in dogs after ovariohysterectomy. The detection of hypercoagulability in the study reported here may be explained by the use of plasma thromboelastography and a more extensive evaluation of coagulation variables.

The plasma thromboelastography profile changed over time. At 1 hour after the skin was incised, a tendency for a decrease in coagulation (ie, hypocoagulability) was observed in the plasma thromboelastography tracing as a decrease in MA, probably as a result of consumption of coagulation factors at that time point during surgery. In contrast, a significant increase in MA was observed at 24 hours after surgery, which indicated a tendency for hypercoagulability at that time point. Thromboelastography of canine plasma has been evaluated, but to the authors’ knowledge, no studies have directly compared plasma thromboelastography with whole blood thromboelastography. Thus, it would be difficult to draw conclusions regarding the overall hemostatic capability of a dog from a single plasma thromboelastography assessment. Findings in the present study indicated that plasma thromboelastography can be successfully used to evaluate hemostatic patterns over time.
The procoagulant state as detected via thromboelastography was confirmed because the fibrinogen concentration increased significantly over time, which is characteristic of the role of fibrinogen as an acute-phase protein. There was a significant increase at 24 hours after surgery. Presumably, this was attributable to increased production of fibrinogen by the liver that was induced by local inflammation at the surgery site, which obscured the decrease we expected to see as a result of consumption. Fibrinogen is considered an independent marker of hypercoagulability. The fact that factor VIII and von Willebrand factor antigen concentrations decreased soon after surgery before subsequently increasing toward reference limits suggested increased consumption.

The AT concentration increased unexpectedly at 6 and 24 hours after surgery, but median values were within the reference limits at all time points. Investigators in 1 study found that the AT concentration in pigs decreased 3 hours after surgery, which was followed by a gradual increase at 6 hours after surgery. Therefore, the decrease in AT concentration reported in other studies may have happened between 1 and 6 hours after surgery in the present study and thus may have been missed. The aPTT and protein C concentration did not change significantly in the present study.

Fibrinolysis was activated, as indicated by the significant increases in D-dimer, plasminogen, and plasmin inhibitor concentrations during the postoperative period. D-dimer is a specific marker of fibrinolysis (specifically the breakdown of cross-linked fibrin), and concentrations of D-dimer are a sensitive negative predictor of thrombosis in humans and dogs. The peak at 6 hours after surgery in the present study may have reflected increased fibrinolytic activity starting at approximately that time.

Studies in humans and horses have revealed that plasminogen concentration in plasma is decreased after surgery. Such results contrast with findings of the present study. A study in dogs revealed an increase in plasminogen concentration between 24 and 48 hours after surgery, which was thought to reflect increased de novo synthesis by the liver. Plasminogen activation increases as an acute fibrinolytic response to inflammation. In humans, this activation typically is counteracted by a delayed but sustained increase in plasminogen activator inhibitor type-1 concentration that results in an inhibition of fibrinolysis, which is commonly seen in humans after surgery and is thought to lead to an increased risk of thrombosis. Dogs have long been suspected to have increased fibrinolytic activity, compared with that in humans, because of remarkably fast lysis of thrombi in the pulmonary vasculature. This has been attributed to high activity of urokinase-type plasminogen activator and its production and secretion by pulmonary arterial endothelial cells, which may further explain the findings for plasminogen concentration in the present study.

Plasmin inhibitor is the main systemic inhibitor of fibrinolysis and controls the terminal step in fibrinolysis. In the present study, dogs had a significant decrease in plasma inhibitor concentrations at 1 hour, which may have been attributable to consumption when plasmin-antiplasmin complexes formed from plasmin released from the clots initiated by surgery. The significant increase at 24 hours after surgery was probably attributable to plasmin inhibitor’s role as an acute-phase protein, and the finding is in concordance with results of studies in humans and dogs. In humans, hemostatic activation is less marked when minimally invasive surgical techniques, such as laparoscopic procedures, are used. Similarly, orthopedic surgery in humans results in a more pronounced effect on coagulation. The latter has also been observed in dogs and provides evidence for an effect on hemostasis proportional to the degree of surgical insult in this species. The authors are not aware of any studies that have been conducted to assess hemostasis in dogs following use of minimally invasive techniques, but investigators in 1 study found that cortisol and glucose concentrations were higher after ovariohysterectomy via an open abdomen approach, compared with concentrations after laparoscopic ovariohysterectomy. Dogs undergoing ovariohysterectomy via an open abdomen approach also consistently needed more pain medication than did dogs undergoing laparoscopic ovariohysterectomy. On the basis of these results and the increasing evidence of a bidirectional link between catecholamine release and coagulation, it would be reasonable to assume that the hemostatic response would have the same differences in dogs. However, because ovariohysterectomy in the present study was not performed laparoscopically, the 2 procedures might have been too similar for subtle differences in hemostatic activation to be detected.

It cannot be excluded that the choice of anesthetic and analgesic agents influenced results of the present study to some degree. Carprofen can inhibit platelet aggregation and may have masked any potential postoperative complications attributable to altered hemostasis in the dogs of the present study. However, carprofen is the most widely used NSAID for perioperative analgesia in dogs; because the dogs were also subjected to behavioral evaluation, this protocol was chosen to mimic the clinical situation as closely as possible. The inclusion criteria allowed bitches to participate in the trial only if their last estrus had ended at least 6 weeks prior to surgery; however, the exact dates of estrus were not recorded. Thus, the bitches were likely at different stages of the estrous cycle, which may have influenced the results because progesterone concentrations can affect hemostasis. Nevertheless, in a clinical situation, bitches are commonly spayed at different phases of the estrous cycle; thus, the study reported here was considered representative of clinical situations.

The dogs were only monitored for 24 hours after surgery. Future studies should be conducted to evaluate the long-term hemostatic effects of elective surgery, including whether there is thrombosis, and if so, whether thrombosis results in important complications. Also, studies conducted to compare plasma thromboelastography with whole blood thromboelastography may shed more light on the potential use of thromboelastography on plasma samples.

In the study reported here, elective surgery (ie, ovariohysterectomy and ovariohysterectomy) induced a hyper-
static response in dogs that led to hypercoagulability.


References


33. ACL 9000, Instrumentation Laboratory, Bedford, Mass.

34. TEG 5000 hemostasis analyzer, Haemoscope Corp, Niles, Ill.

35. TEG 5000 hemostasis analyzer, Haemoscope Corp, Niles, Ill.


40. Prisco D, De Gaulo AR, Carla R, et al. Videolaparoscopic cho-
44. Hickford FH, Barr SC, Erb HN. Effect of carprofen on hemo-