Epsilon Aminocaproic Acid for the Prevention of Delayed Postoperative Bleeding in Retired Racing Greyhounds Undergoing Gonadectomy

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Objective: To evaluate the effects of epsilon aminocaproic acid (EACA) on the prevalence of postoperative bleeding in retired racing Greyhounds (RRG), and to assess its effects on selected thrombelastography (TEG) and fibrinolysis variables.
Study Design: Double-blinded, prospective, randomized study.
Methods: 100 RRG had elective ovariohysterectomy or orchiectomy and were administered EACA or placebo for 3 days after surgery. TEG variables were analyzed preoperatively and 24, 48, and 72 hours after surgery.
Results: Thirty percent (15/50) of RRG in the placebo group had delayed postoperative bleeding starting 36–48 hours after surgery compared with 10% (5/50) in the EACA group ($P = .012$). On the TEG variables, the slopes for R and K time were significantly different between treatment groups ($P < .05$); the R and K time decreased over time in the EACA group after surgery whereas they increased in the placebo group. The angle, maximal amplitude (MA), and G slopes were also significantly different between treatment groups ($P = .001$, .001, and .006, respectively). The angle, MA, and G increased postoperatively over time in the EACA group and decreased in the placebo group. All these changes are supportive of hypercoagulability associated with EACA administration.
Conclusion: Postoperative administration of EACA significantly decreased the prevalence of postoperative bleeding in RRG undergoing surgery by increasing the clot strength.

The popularity of retired racing Greyhounds (RRG) as pets has increased markedly in the United States (Gary Guccione, National Greyhound Association, personal communication). Currently, the number of Greyhounds that have retired from racing around the world exceeds those actively racing, and there are now over 130,000 RRG in North America. Most Greyhounds that complete racing careers are sexually intact and will be spayed or neutered at the time of adoption (some adoption groups make sure the pets are spayed/neutered before them being adopted); this represents as many as 15,000–20,000 surgeries a year.

Hemostatic complications associated with ovariohysterectomy (OHE) or orchiectomy in dogs can be classified as "surgical" (ie, attributed to faulty surgical technique and failure to control bleeding from the ovarian, uterine, or testicular vessels),[1] or "non-surgical" (ie, failure of hemostatic pathways).[2] The latter includes primary or secondary hemostatic defects; potential causes of failure of primary hemostasis include thrombocytopenia, platelet dysfunction, or von Willebrand's disease (vWD); causes of secondary hemostatic defects include hypofibrinogenemia, hypoprothrombinemia, hemophilia A or B, factor VII deficiency, or combined clotting factor deficiencies, such as those associated with disseminated intravascular coagulation (DIC) or rodenticide toxicity, among others.[2]

The hemostatic system is a complex sequence of events described as enzymatic reactions initiated by a traumatic or surgical injury;[2] these events result in the formation of thrombin, which is responsible for the conversion of fibrinogen into fibrin, thus resulting in the formation of a blood clot at the site of the injury.[2] The fibrinolytic process is in charge of terminating the coagulation phase once the clot is formed, followed by the elimination of fibrin deposits and reshaping of the thrombus, and the stabilization of the whole process while the endothelium is repaired.[2]

Recently, we demonstrated that 26% of the RRG develop delayed postoperative bleeding 36–48 hours after routine gonadectomy.[3] This prevalence is considerably higher than previously reported after OHE or orchiectomy in other dog breeds (ie, 0 to 2%).[4–7] With a prevalence of bleeding

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of 26%, as many as 3500–5000 RRG may be readmitted after surgery. The potential pathogenesis of this postoperative bleeding tendency was investigated by evaluating primary and secondary hemostasis preoperatively.[3] There were no significant differences between bleeders and nonbleeders for any of the following factors: platelet count (PLT), hematocrit (HCT), platelet function using the PFA-100[a], von Willebrand factor antigen (vWF:Ag), one-stage prothrombin time (OSPT), activated partial thromboplastin time (APTT), fibrinogen concentration (FIB), factor XIII (F-XIII), plasminogen (Plmg), and D-dimer.[3] Greyhounds with spontaneous bleeding had normal platelet counts for the breed, vWF, FIB, OSPT, and APTT at the time of postoperative hemorrhage, making common bleeding disorders such as thrombocytopenia, platelet dysfunction, and clotting factor or vWF deficiencies unlikely causes of the bleeding.[3] However, antiplasmin (AP) and antithrombin (AT) activities were significantly lower in dogs that bled than in those that did not (although they were within the reference interval).[3] These results and the delayed onset suggest that the postoperative bleeding in RRG may be because of abnormalities in clot maintenance or the fibrinolytic system or endothelial dysfunction, rather than primary or secondary hemostatic defects.[3]

Fibrinolytic inhibitors have proven to be effective in people and horses where complications are associated with enhanced fibrinolysis, but they have also been beneficial in patients with systemic bleeding because of other mechanisms.[8,9] Antifibrinolytic lysine analogs include epsilon aminocaproic acid (EACA) and tranexamic acid.[10,11] EACA prevents activation of plasminogen into plasmin on the fibrin surface,[12] by preventing the binding of plasminogen to C-terminal lysine residues on partially degraded fibrin, thus blocking the plasminogen binding site, which is essential for efficient plasmin formation.[13] EACA neutralizes bleeding states created experimentally in dogs by infusion of plasmin or a plasminogen activator.[11,13–17] EACA has a wide therapeutic index; no relevant adverse effects were reported in toxicologic studies in dogs, rabbits, and rats, with doses as high as 0.5 g/kg.[11,18,19]

Thromboelastography (TEG) is an *in vitro* technique that allows global evaluation of the blood coagulation process.[20] It is a novel device that evaluates the primary and secondary hemostasis, and the fibrinolytic pathway by assessment of the speed and strength of clot formation.[21] The TEG variables we used were (1) R time = latency time; from the time blood was placed in the TEG analyzer until the initial fibrin formation; (2) K time = a measure of the speed to reach a specific level of clot strength; (3) the alpha measures the rapidity of fibrin build-up and cross-linking (clot strengthening); (4) the maximal amplitude (MA) and G represent a direct function of the maximum dynamic properties of fibrin and platelet bonding via GPIIb/IIIa and represents the ultimate strength of the fibrin clot; and (5) the LY30 and LY60 measure the rate of amplitude reduction 30 and 60 minutes after MA.

We are unaware of any prospective studies evaluating the effect of EACA in spontaneously occurring fibrinolytic disorders or other hemostatic abnormalities in dogs. Our purpose was to evaluate the prevalence and severity of postoperative bleeding in RRG undergoing OHE or orchiectomy administered either EACA or placebo in a prospective, double-blinded, randomized study. Further, we evaluate the effects of EACA on selected TEG and fibrinolysis variables (ie, R, K, angle, MA, G, LY60, and routine coagulation tests). We hypothesized that EACA would significantly decrease the prevalence of postoperative bleeding in RRG and result in significant changes in selected TEG and fibrinolysis variables.

MATERIALS AND METHODS

One hundred RRG from a local adoption group (www.greyhoundadoptionofoh.org), were spayed or neutered as part of a 3rd and 4th year veterinary student operative practice curriculum over a 2-year period. Blood samples in all dogs were collected after signed consent from the director of the rescue group.

All dogs were evaluated preoperatively by physical examination; presurgical jugular venous samples (15 mL) collected through a 21-g butterfly catheter and Vacutainer (Franklin Lakes, NJ) in tubes with sodium EDTA (Sherwood, St. Louis, MO) for complete blood count (CBC), sodium citrate for hemostasis assays, and tubes without anticoagulant for biochemical profiles and rapid SNAP 4DX (IDEXX Laboratories, Westbrook, ME) test for some common vector borne diseases; a separate blood sample (6 mL) from the jugular vein was obtained with a 20-g needle and 6-mL plastic syringe for TEG analysis (TEG Haemoscope, Niles, IL). CBCs were performed using a LaserCyte (IDEXX Laboratories); biochemical profiles using a COBAS analyzer (ABX Diagnostics, Montpellier, France); and hemostasis panels OSPT (STA Neoplastine c15-PT), APTT (STA C.K. Prest-PTT), FIB (STA Fibrinogen 5), Antiplasmin (STA Stachrom Antiplasmin), D-dimer (STA Liatest D. Di), and Plasminogen (STA Stachrom Plasminogen) using an Stago compact analyzer (Diagnostica Stago Parsippany, NJ) and commercially available reagents.

Venous blood samples (6 mL) were obtained at 24, 48, and 72 hours after surgery from the external jugular vein using a 20-g needle and 6-mL plastic syringe; 4.5 mL of blood was immediately placed into a 3.2% buffered sodium citrate glass tube (Xanodyne pharmaceuticals, Inc, Newport, KY). Samples for TEG analysis were stored for 30–45 minutes at room temperature in a tube rack and analyzed within 30–45 minutes of collection. After TEG analysis, the residual blood samples were centrifuged (1380 g for 10 minutes) within 45 minutes of sampling, and plasma was stored at −80°C for ~15 months for other hemostasis assays (OSPT, APTT, FIB, plasminogen, and antiplasmin). CBCs were performed with 0.8mL of EDTA blood in a LaserCyte (IDEXX Laboratories), and duplicate packed cell volume (PCV) were run from the remaining blood.

Before surgery, all RRG were administered buprenorphine (0.05 mg/kg) and acepromazine (0.5 mg/total dose) intramuscularly (IM); in some dogs (those with pyoderma) a prophylactic dose of intravenous (IV) cefazolin sodium (22mg/kg IV) was administered. Anesthesia was induced with ketamine (5 mg/kg) and diazepam (0.25 mg/kg) IV, and maintained using isoflurane in oxygen. Breathing was supported with intermittent positive-pressure ventilation and lactated Ringer's solution (10 mL/kg/hour IV) was administered. Veterinary students and surgery residents performed the surgeries under the supervision of a board-certified surgeon. Dogs were monitored during surgery with pulse oximetry, respirometry, measurement of peripheral arterial pressure, and body temperature. After surgery, a single dose of carprofen (2.2mg/kg IM) was administered for analgesia. Dogs were monitored postoperatively until recovery, and then transferred to a boarding area. Daily physical examinations were preformed for 4 days.

Dogs were randomized (using a randomization table) to receive either EACA (500 mg orally every 8 hours for 5 days starting the night of surgery) or placebo, which consisted of lactose-containing capsules of identical volume and concentration. The senior investigator (GC), based on previous clinical experience, determined the dose of EACA used, which was extrapolated from that used in people.[22] Both EACA and placebo were packaged into gelatin capsules of identical size by the Veterinary Medical Center Pharmacy, and labeled as drug "A" and "B." Clinicians were blinded to the type of drug administered.

Although there is no standardized scale to evaluate the severity of bleeding in dogs, a system with scores ranging from 0 to 4, was adapted from the one proposed by Buchanan and Adix for children with idiopathic thrombocytopenic purpura and recently validated in Greyhounds (Table 1, Fig 1).[23,24] Bleeding scores were recorded once a day by the same person (LM), and surgical areas were photographed digitally. The final bleeding score assigned to each RRG corresponded with the highest score recorded during the postoperative period. Dogs were classified as nonbleeders if they had a bleeding score of 0 or 1 and as bleeders if they had a score of ≥2.

Statistical Analysis

The primary statistical analysis was to test if there is an association between the hematologic and hemostatic variables and the drug treatment (EACA versus placebo), whether or not the dogs bled after surgery, and the time (day 1 to 3). All 2-way interactions between treatment, bleeding, and time were included in the model and were kept in the model, if significant. Because of the longitudinal nature of the data, we used a random-effects (slope and intercept) linear regression model to test the main effects and interactions. Random-effects models take into account the variability within and between dogs to estimate the standard error used to test model coefficients. The model also adjusted for the baseline hematologic variables. This model allowed us to test main effects, interactions, and whether slopes (outcome per time) over treatment or bleeding were significantly different from each other and whether the individual slopes were significantly different from zero. Outcome differences across treatment and bleeding on day 3 were also tested using this model. A bleeding prediction model using baseline variables was developed using logistic regression. A Holm's procedure was used to adjust for the type 1 error as a result of performing multiple comparisons. All analyses were run using either Graph Pad Prism software (Prism version 4.0, GraphPad Software Inc, San Diego, CA) or Stata 11.1 (Stata Corporation, College Station, Texas). Variables that were included in the model were treatment, gender, weight, age, color, month of surgery, CBC including red blood cells (RBC), HCT, hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), reticulocytes, PLT, white blood cell count (WBC), neutrophil count (NEU), lymphocytes (LYM), monocytes (Mono), eosinophils (EOS), and TEG (R, K, angle, MA, G, LY30, LY60).

Table 1 Bleeding Score Modified From Buchanan and Addix

	Description
Score 0	Definitely no new bleeding
Score 1	Questionable new petechiae or bruising
Score 2	Definite new cutaneous and/or mucosal hemorrhagic lesions
Score 3	Moderate to severe cutaneous or mucosal bleeding without measurable decline in hematocrit (HCT)
Score 4	Severe external bleeding of sufficient magnitude to decrease HCT by ≥6% points

RESULTS

Signalment and Prevalence of Postoperative Bleeding

We evaluated 100 Greyhounds; the EACA group included 32 females (64%) and 18 males (36%), median age of 3 years (range, 2–4 years), and median weight of 28.5 kg (range 26.8–32 kg). The placebo group included 32 females (64%) and 18 males (36%), median age of 3 years (range, 2–5 years), and median weight of 27.8 kg (range, 26.6–31.7 kg). There were no significant differences in age, gender, and weight between the EACA and the placebo group.

None of the dogs had intraoperative or immediate postoperative bleeding; however, 15/50 RRGs (30%) in the placebo group had delayed postoperative bleeding 36–48 hours after surgery, compared with only 5/50 RRGs (10%) in the EACA group ($P = .012$)

In affected dogs, bleeding consisted of cutaneous bruising that extended from the area of the surgical incision toward the periphery (Fig 1). There was no bleeding from mucosal surfaces or in areas distant from the surgical site. None of the dogs required transfusion of blood components and the bleeding was self-limiting. In the bleeders, bruising was still present when the dogs were discharged 4

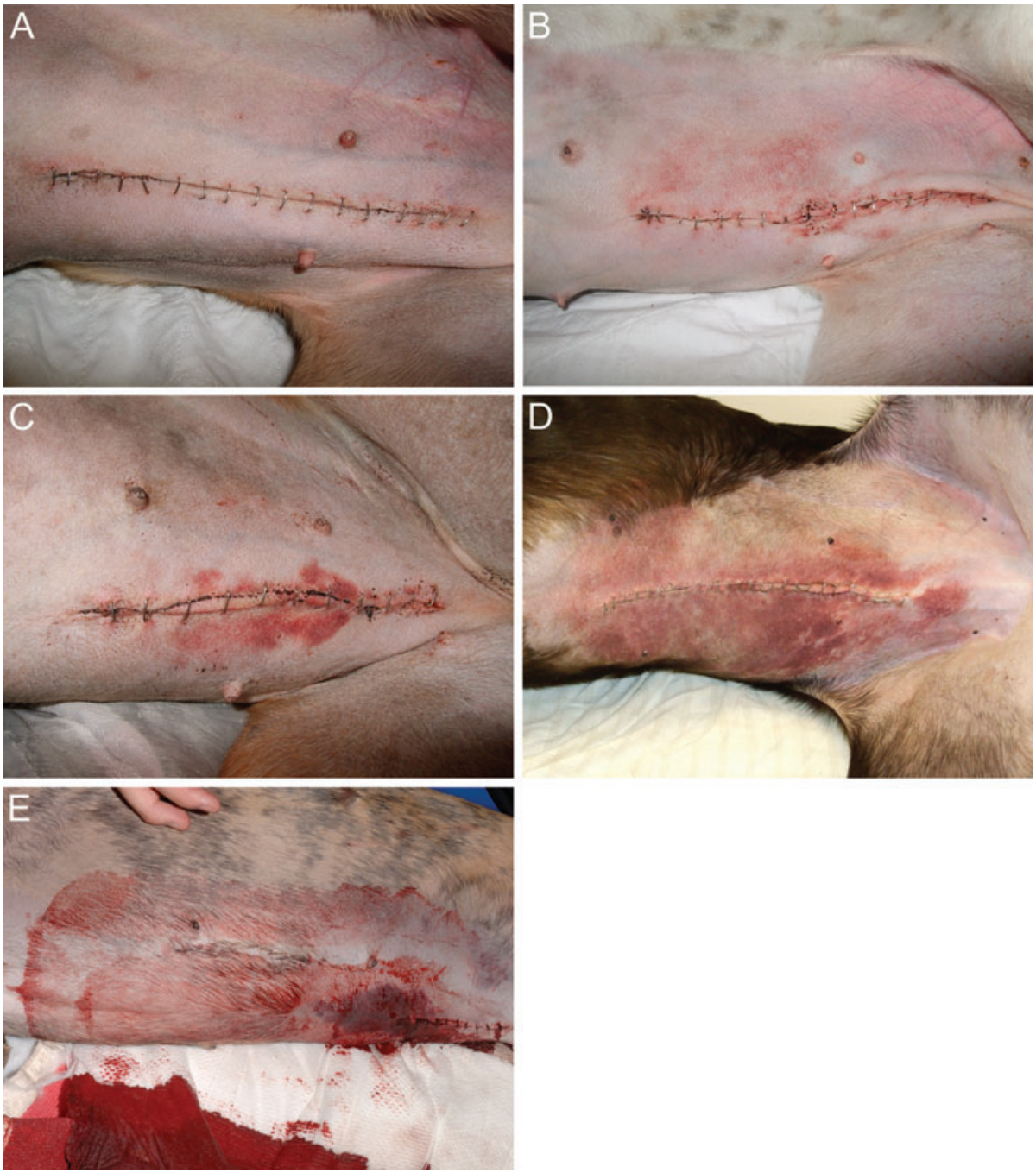

Figure 1 Surgical sites of Greyhounds after gonadectomy with bleeding scores from 0 to 4. (A) Bleeding score 0. (B) Bleeding score 1. (C) Bleeding score 2. (D) Bleeding score 3. (E) Bleeding score 4.

days after surgery. None of the RRG that received EACA had any adverse effect.

The estimated probability of bleeding based on the logistic regression model was 29.1% (95% CI: 17.4%–44.4%) with placebo and 7.4% (95% CI: 2.7%–18.8%) with EACA. The odds of bleeding increased 19% (OR = 1.18, $P = .050$) for every 1 kg increase in body weight, after adjustment for use of EACA. The odds of bleeding decreased 18% (OR = 0.82, $P = .058$) for every 0.1 unit increase in the baseline eosinophil count (EOS), after adjustment for use of EACA. Finally, use of EACA decreased the odds of bleeding by 79% (OR = 0.21, $P = .011$) after adjustment for baseline weight and EOS, assuming that the average dog weight was 29.4 kg and the average EOS was 0.8×10^9/L. None of

the other variables included in the logistic regression model were predictors nor had an association with the bleeding status.

Comparison of Preoperative Hemostatic and TEG Variables Between EACA Versus Placebo Groups and Between Bleeders Versus Nonbleeders.

Preoperatively, all variables were within reference intervals for the breed and there were no significant differences between EACA and placebo groups or between bleeders and nonbleeders for any of the following variables: RBC, HCT, Hb, MCV, MCH, MCHC, RDW, reticulocytes, PLT, WBC, NEU, LYM, MONO, EOS from the CBC. R, K, angle, MA, G, LY30, LY60 from the TEG and OSPT, APTT, fibrinogen, antiplasmin, D- dimers, and plasminogen. Changes in hematologic, hemostatic, and thrombelastography variables in RRG from the EACA and placebo group before and 24, 48, and 72 hours after gonadectomy are summarized in Table 2 and 3.

Comparison of Hematological and Hemostasis Test Slopes (Rate Change) Over 24, 48, and 72 Hours After Surgery Between EACA and Placebo

There were no significant postoperative differences between the slopes of EACA and placebo for any of the following hemostatic variables: RBC, HCT, Hb, MCV, MCH, MCHC, RDW, PLT, WBC, NEU, LYM, MONO, EOS from the complete blood count, and OSPT, APTT, D-dimers, fibrinogen, and antiplasmin.

The only hematologic variable that had significantly different slopes between EACA and placebo group was the reticulocyte count ($P = .028$), the placebo group had persistently higher reticulocyte counts compared with the EACA group after surgery (Fig 2). In both groups, the reticulocyte count increased over time after surgery. There was no significant difference between EACA and placebo group reticulocyte slopes at day 3. The only coagulation variable that had significantly different slopes between EACA and placebo group was the plasminogen ($P = .022$). The slope of the plasminogen concentration in the placebo group was lower 24 hours after surgery compared with the EACA group, in both groups the plasminogen increased over time after surgery (Fig 2); however the plasminogen concentration of the placebo group increased at a higher rate. There was no significant difference between EACA and placebo plasminogen slopes at day 3.

Comparison of TEG Slopes (Rate Change) Over 24, 48, and 72 Hours After Surgery Between EACA and Placebo

There were significant differences in the TEG slopes for R, K, angle, MA, G, and LY60 when compared at 24, 48, and 72 hours after surgery (Fig 3). The slopes for LY30 were not significantly different between EACA and placebo

Table 2 Selected Hematology, Hemostatic and Thrombelastography Variables in Dogs Receiving Epsilon Aminocaproic Acid (n = 50) Before and 24, 48, and 72 hours After Gonadectomy

	Preoperative			24 Hours			48 Hours			72 Hours		
EACA	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max
HCT (%)	51.1 ± 5.7	37.9	61.6	46.4 ± 6.7	28.1	59.5	45.9 ± 8.5	4	61.1	46.1 ± 6.6	27.1	59.6
HB (g/dL)	17.5 ± 1.3	14.8	20.6	15.9 ± 1.6	12.6	18.8	15.6 ± 1.6	10.6	18.3	15.7 ± 1.5	11.8	18.3
Reticulocytes (k/μL)	30.3 ± 8.8	16.1	51.6	25 ± 7.1	10.6	40.5	25.1 ± 7.3	9.7	48.5	26.6 ± 8.8	8.7	56.1
Platelets (k/μL)	203.3 ± 55.2	6	294	215.4 ± 98.2	55	712	202.7 ±65	41	336	219.1 ± 69	49	373
WBC (k/μL)	7 ± 1.8	3.6	10.4	9.9 ± 3.1	1.5	17.3	7.2 ± 2.1	3.1	15.4	6.3 ± 1.6	3	9.4
OSPT (sec)	7.2 ± 0.3	6.5	8	7.1 ± 0.3	6.5	8.3	6.9 ± 0.3	6.5	7.9	7 ± 0.3	6.5	7.8
APTT (sec)	11.5 ± 0.9	8.9	13.5	11.6 ± 1	10.2	14.1	11.6 ± 1.1	10.1	14.6	11.7 ± 1	10.4	15
D-DIMERS (μg/mL)	84.1 ± 34.7	0	150	134.3 ± 72.9	50	450	130.9 ± 78.5	40	420	135.8 ± 90.4	40	670
Fibrinogen (mg/dL)	159 ± 39.6	87	309	243.3 ± 70.3	99	405	238.8 ± 78.4	151	460	226 ± 57	133	373
Antiplasmin (%)	94.3 ± 13.9	63	121	121.1 ± 20.6	87	161	116 ± 16.6	89	161	114.1 ± 8.6	99	148
Plasminogen (%)	72.4 ± 14.6	38	102	70.7 ± 24.2	31	140	81.7 ± 16.3	53	141	87 ± 17	60	136
R time (min)	5 ± 1.8	2.4	9.8	4.3 ± 2.2	2.1	16.4	4.2 ± 1.2	1.5	8.2	4.1 ± 1.2	2	6.8
K time (min)	3.4 ± 1	1.4	6.4	2.5 ± 1.4	1.3	9.7	2.6 ± 1.6	1.3	10.1	2.2 ± 0.7	1	3.8
Angle (Degrees)	51.2 ± 7.5	36.8	68.2	59.2 ± 9.1	25.7	70.8	58 ± 10.4	24.5	72.1	61.3 ± 6.1	47.2	73.4
MA (mm)	49.4 ± 6.2	39	65.4	56 ± 6.5	42.4	66.5	56.4 ± 7.6	42.6	72.4	56.8 ± 6.6	42.9	70.2
G (d/sc)	4442.4 ± 2127.8	0	9469.8	6600 ± 1671.9	3674.4	9925.4	6558.5 ± 2542.2	0	13135.4	6842.6 ± 1934	3752.7	11753.9
LY30 (%)	0.5 ± 1.1	0	4.8	0.2 ± 0.6	0	3.2	0.6 ± 2.1	0	11.2	0.2 ± 0.4	0	2
Ly60 (%)	2.7 ± 3.1	−0.2	12.1	2.3 ± 2.3	−0.1	9.2	2.2 ± 2.8	0	13.2	2.2 ± 1.8	0	7

Table 3 Selected hematology, hemostatic and thrombelastography variables in dogs from a placebo group (n = 50) before and 24, 48, and 72 hours after gonadectomy

	Preoperative			24 Hours			48 Hours			72 Hours		
Placebo	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max
HCT (%)	52.4 ± 5.9	41.3	63	47.5 ± 9.9	4	62.4	48.5 ± 9	5	64.8	49.8 ± 7.9	18.8	64
HB (g/dL)	17.5 ± 1.2	15	19.7	16 ± 1.3	13.9	19.1	16.4 ± 1.4	11.9	19	16.8 ± 1.6	13.9	21.9
Reticulocytes (k/μL)	32.3 ± 7.3	17.5	49.5	27.8 ± 6.8	12.4	47	29.3 ± 11.1	14.2	65.9	28.9 ± 9.6	3	45.5
Platelets (k/μL)	222.9 ±55.1	95	374	202.9 ± 50.5	92	328	203.3 ± 55.9	51	344	215.6 ± 58.5	95	373
WBC (k/μL)	7.3 ± 2	4	12.2	10.4 ± 3	1	15.6	7.7 ± 1.6	4.9	12	7.1 ± 1.8	2.4	11.8
OSPT (sec)	7.4 ± 0.5	6.6	8.9	7.2 ± 0.4	6.6	8.9	6.9 ± 0.3	6.2	7.6	7 ± 0.4	6.4	8
APTT (sec)	11.6 ± 1	9.9	14.1	11.8 ± 1	10.5	14.7	11.7 ± 1.1	10.4	15.1	11.9 ± 1	9.5	15
D-DIMERS (μg/mL)	88.4 ± 50.2	0	290	122.9 ± 56.6	40	300	121.2 ± 82.4	0	530	134.8 ± 140.7	20	1030
Fibrinogen (mg/dL)	149.1 ± 38	74	244	240.9 ± 74.4	90	464	249.3 ± 69.1	146	526	227.4 ± 63.4	100	482
Antiplasmin (%)	95.2 ± 12.2	63	129	122.2 ± 21.1	81	165	117.3 ± 13.9	97	177	112.5 ± 8.1	93	129
Plasminogen (%)	73.2 ± 20.1	37	122	63.1 ± 17.5	40	112	85.3 ± 18.8	55	148	89.1 ± 16.6	57	147
R time (min)	5.2 ± 2	2.5	12.3	4.4 ± 1.9	1.8	12.4	4.7 ± 2.7	1.9	19.4	5.1 ± 2.8	2.4	18.2
K time (min)	3.6 ± 1.3	1.6	6.5	2.4 ± 0.9	1	6.2	2.7 ± 1.6	1.4	10.7	3.1 ± 1.9	1.2	10.8
Angle (Degrees)	50.1 ± 9.1	32	67.3	59.7 ± 7.8	35.8	74.5	57.9 ± 9.5	18.8	70.1	55.1 ± 11.5	21.7	72.2
MA (mm)	50 ± 5.5	36.8	64.2	57.6 ± 6.1	43.6	68.8	56.7 ± 5.7	44.4	66.2	54.8 ± 6.9	32.1	70.1
G (d/sc)	4712.7 ± 1842.8	0	8976.9	7026.1 ± 1781.8	3858.1	11000	6607.8 ± 1782.3	0	9790.8	6302.5 ± 1694.2	2363.1	11714.5
LY30 (%)	0.6 ± 1.9	0	10.5	0.2 ± 0.5	0	2.3	0.4 ± 1	0	3.8	0.3 ± 1.2	0	7.5
LY60 (%)	2.7 ± 4.4	−0.1	22	1.8 ± 1.9	−3.5	6.4	1.9 ± 2.6	−3.6	9.3	1.1 ± 2.3	−3.7	11.8

groups. The slopes for R and K time were significantly different between treatment groups ($P = .050$). The R and K time decreased over time in the EACA group, whereas they increased in the placebo group. The R time of the EACA group was 0.9 minutes shorter at day 3 compared to the placebo group ($P = .033$). The K time of the EACA group was 0.7 minutes shorter at day 3 compared to the placebo group ($P = .019$; Fig 3).

The angle, MA, and G slopes were significantly different between treatment groups ($P = .001$, .001, and .006). The angle, MA, and G increased postoperatively over time in the EACA group whereas they decreased in the placebo group. The angle of the EACA group was 4.7 degrees higher at day 3 compared with the placebo group ($P = .010$). Although not significantly different, the MA of the EACA group was 1.9 mm larger at day 3 and the G value was 424 d/sc higher at day 3 compared to the placebo group (Fig 3).

The LY60 slopes were significantly different between treatment groups ($P = .028$). In both groups, LY60 decreased over time after surgery, the EACA group was 0.7 units (%) higher than the placebo group at all time points. The slopes were parallel for treatment and placebo (Fig 3).

DISCUSSION

As previously reported,[3] none of the dogs included in the study experienced intraoperative or immediate postoperative bleeding, even though less experienced surgeons (students and surgery residents) performed the surgical procedures; however, 30% of the dogs in the placebo group had delayed postoperative bleeding 36–48 hours after surgery, compared to only 10% of the dogs in the EACA group ($P = .012$). These results demonstrate that postoperative administration of aminocaproic acid (EACA) significantly decreases the prevalence of delayed postoperative bleeding in RRG.

The dosage of EACA we used (500mg per dog) was not based on body weight; because the odds of bleeding increased 19% for every 1 kg increase in body weight, dosing EACA at 15mg/kg every 8 hour may be more appropriate, and it may decrease the prevalence of postoperative bleeding even further.

TEG variables used to quantify the velocity of clot growth and clot strength included R, K, angle, MA, and G. RRG administered EACA for the prevention of bleeding in spay/neuter surgeries had significant differences in the slopes of these variables 72 hours after surgery when compared with dogs not administered EACA. The significantly shorter slopes of R and K times 72 hours after surgery in the dogs administered EACA, suggest that the initial clot starts forming more rapidly, and the clot strength is reached faster in dogs administered EACA. Differences in the angle, G, and MA slopes 72 hours after surgery in the dogs administered EACA, suggest that EACA amplifies strengthening of the fibrin clot. The rate changes in the R, K, angle, G, and MA suggest that dogs administered EACA tend to become hypercoagulable, as occurs in people.[25,26]

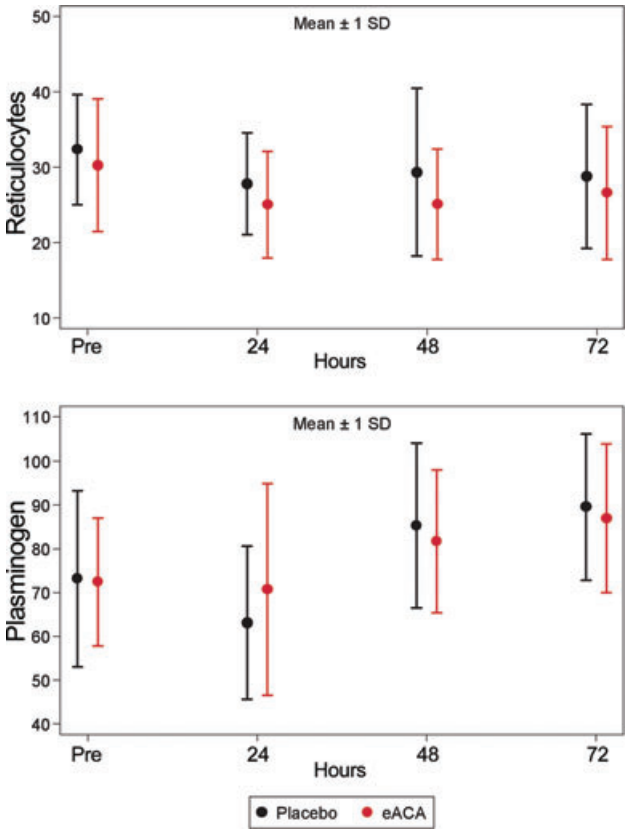

Figure 2 Mean (±SD) reticulocyte count and plasminogen concentration in the epsilon aminocaproic acid (n = 50) and placebo (n = 50) groups before and 24, 48, and 72 hours after gonadectomy.

Our results are similar to those of Hamada et al in 30 human patients undergoing upper abdominal surgery, that randomly received placebo or carbazochrome sodium sulfonate (CS) and tranexamic acid (TA), a drug similar to EACA.[27] Similarly, significant differences occurred between presurgical and postsurgical TEG variables in both groups, and all patients became hypercoagulable after surgery and no significant differences occurred in TEG variables between placebo and treatment groups. However, their single analysis was done only 2 hours after administration of TA, which limits the interpretation of the results, since a single TEG analysis does not necessarily represent the hemostatic status of the patient.

The trend toward hypercoagulability in the postoperative period has been described since 1977[28] and was supported using TEG analysis in 1987;[29] however, these studies were limited to a single TEG measurement and to a short postoperative period. Therefore, serial TEG evaluation was necessary to detect the postsurgical hypercoagulable changes over time in people. A more recent study[30] demonstrated a continuous increase in clot firmness 2–6 days after surgery. Similar to our study, OSPT and APTT did not reflect hypercoagulability after major surgery.[30] Our results corroborate the fact that surgery triggers the coagulation process and that dogs also develop a hypercoagulable state; they also support the fact that administration of EACA enhances the already established hypercoagulable state, therefore decreasing the prevalence of bleeding in RRG. It is unlikely that postoperative administration of nonsteroidal anti-inflammatory drugs (NSAIDs) contributed to changes in coagulation and TEG variables as has been previously reported.[31,32]

The proposed mechanism of hypercoagulability after surgery is associated with the local tissue trauma, release of tissue factor from damaged vessels, decreased blood flow, activation of inflammation, and compromised fibrinolysis.[28–30] Tuman et al, demonstrated an association between hypercoagulability (determined by TEG) and the risk of arterial and venous thrombotic events in people.[29] Interestingly, in our study the TEG values remained within the reference intervals for Greyhounds[21] and none of the dogs developed clinically detectable thromboembolic events.

The fact that postoperative bleeding in RRG is not associated with abnormalities in routine hemostasis and coagulation assays, that it is delayed, and that there appears to be a low antiplasmin activity in the breed, suggest that the cause of the bleeding is not likely attributable to a primary or secondary hemostatic defect. Enhanced fibrinolysis has been the proposed mechanism responsible for the delayed postoperative bleeding in RRG.[3,33] We have demonstrated that RRG that developed delayed postoperative bleeding had significantly lower activities of antiplasmin and antithrombin[3]; however, we have been unable to document increased fibrinolytic variables on TEG in our dogs.[34]

Surgical trauma, deficiencies of antiplasmin or plasminogen activator inhibitor type 1 (PAI 1), hyperactivity of fibrinolytic enzymes, and iatrogenic are among the causes of hyperfibrinolytic syndromes in people.[35] In general, enhanced fibrinolysis occurs when the balance between fibrinolytic activators and inhibitors is disturbed. In people, increased levels of fibrin degradation products (or D-dimer) and low fibrinogen concentration are used to establish a diagnosis of hyperfibrinolysis.[35] Although in our previous and current study we found no differences in D-dimer concentration between groups postoperatively,[3] preliminary data using TEG had suggested enhanced fibrinolysis as a possible mechanism for the bleeding.[3]

In 1961, Fichera et al using TEG demonstrated that EACA inhibits fibrinolysis in people.[26,36] In 2007, another study demonstrated the *in vitro* efficacy of EACA in people with severe hemophilia, showing that its administration normalized the TEG patterns in affected patients.[25] Interestingly, in our study we did not find evidence of enhanced fibrinolysis; LY60 decreased over time after surgery in both treatment groups.[30]

We (and others) have been unable to document the association between increased D-dimer concentration, fibrinogen/fibrin degradation products (FDPs), and increased fibrinolytic variables on TEG in dogs that has been reported in people.[20,34,37] Based on these and other data (not shown),

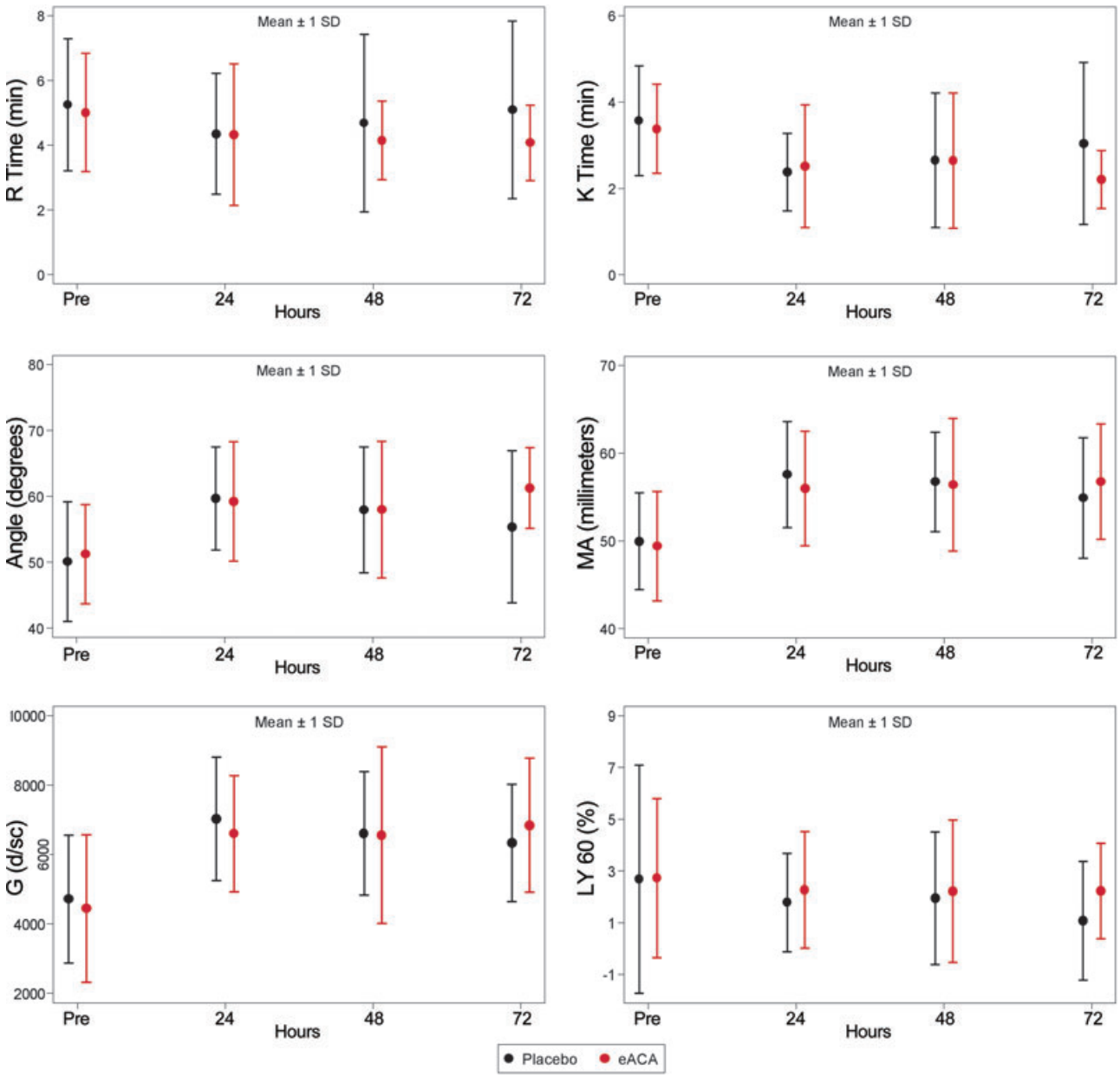

Figure 3 Mean (±SD) selected TEG variables (R, K, angle, MA, G and LY60) in the epsilon aminocaproic acid (n = 50) and placebo group (n = 50) before and 24, 48, and 72 hours after gonadectomy.

we propose that the TEG may not be a valuable tool to assess fibrinolysis in dogs.

Recently, a study using human plasma showed that derived TEG variables could be used more accurately to evaluate fibrinolysis and clot stability.[38] The rationale is that the TEG assessment of fibrinolysis has been based on determinations based on the MA of the clot, which is thought to be a subjective nonparametric measure.[38] Therefore Nielsen et al, used a methodology, referred to as the "elastic modulus," to measure the velocity of clot growth and disintegration based on an equation determined by changes in amplitude.[38] Further studies are needed in veterinary medicine to establish if elastic modulus evaluation will be a more effective method to assess fibrinolysis.

We concluded that administration of EACA significantly decreased the prevalence of delayed postoperative bleeding in RRG undergoing surgery by amplifying strengthening of the fibrin clot.

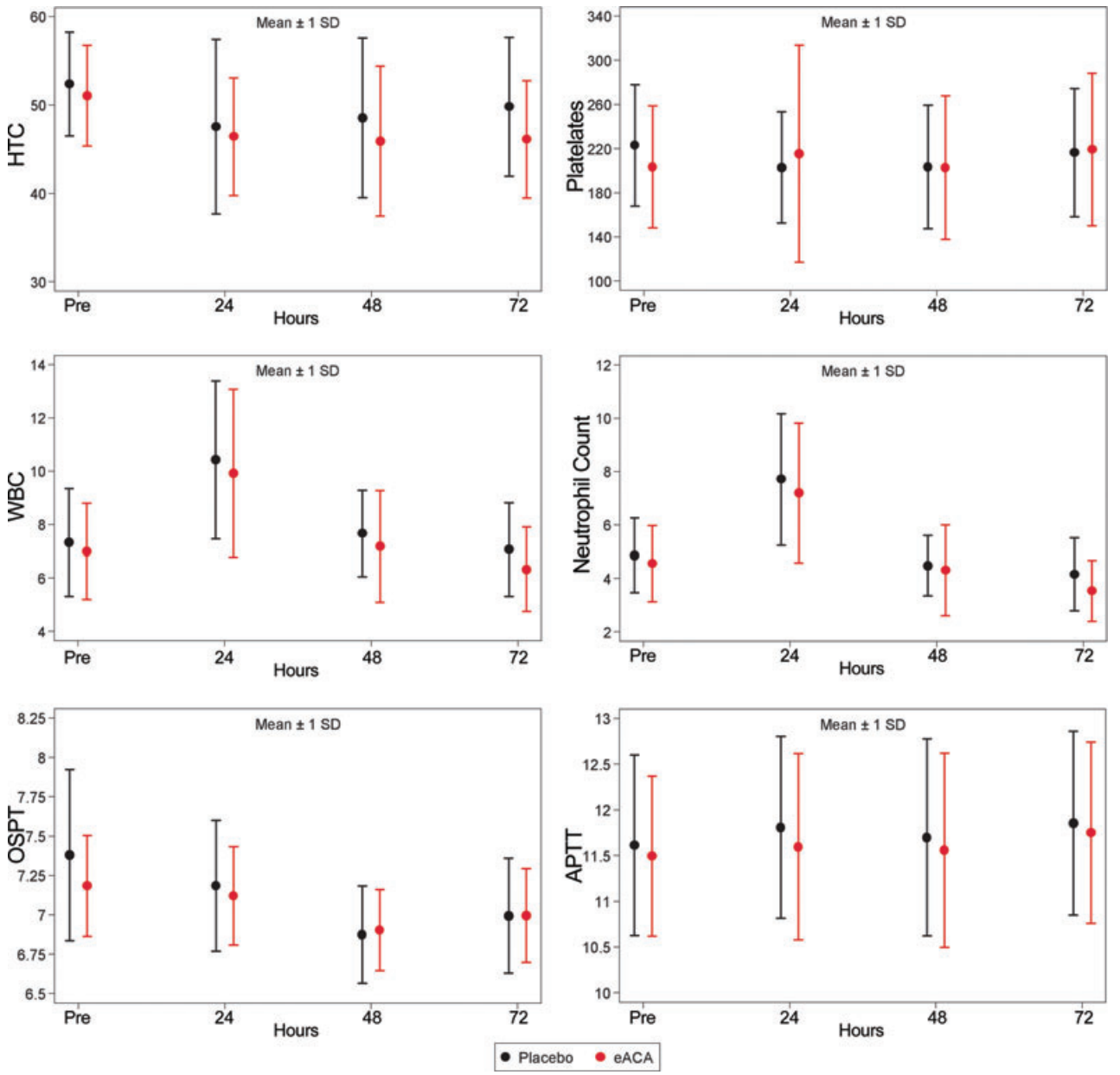

Figure 4 Mean (±SD) HCT, platelets, WBC, neutrophil count, OSPT, and APTT in the epsilon aminocaproic acid (n = 50) and placebo group (n = 50) before and 24, 48, and 72 hours after gonadectomy.

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