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Dynamics of Hemostatic Parameters in some Ungulates

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Summary

Most of the ungulates are now on the verge of extinct due to heavy hunting and unfair management system either in the natural reservoir or zoo. Heamostatis plays an important role in animal survival during injury or other hemostatic disease. Information on the most of the ungulates are scarce. In this studiy, we have determined different hemostatic parameters and compared them with other ungulates. These information are important for the wildlife management to give a speedy response to these animals in case of any vital injury or diseases.

Abstract

Context and aims: Normal coagulation parameters are vital for evaluating and understanding clinical manifestation of hemorrhagic diseases.

Methods: Therefore, the objective of this study was to investigate the interspecies and intersex differences in certain coagulation parameters in three gazelle species namely Idmi (n=16), Reem (n=36), Erlangeri gazelles (n=22) and she-camel (n=20).

Results: The obtained results showed prothrombin time (PT, 13.13 ± 0.22), activated partial prothrombin time (APPT, 23.69 ± 0.20), Factor VIII:C (3.25 ± 0.08), IX (1.23 ± 0.03) and X (1.21 ± 0.02) were significantly (P<0.05) lower in Reem compared to Idmi and Erlangeri irrespective of gender. Similarly, PT (18.46 ± 0.77), APPT (35.08 ± 1.95) and Factor IX (1.78 ± 0.07) were significantly (P<0.05) higher in Idmi compared to the other ungulates irrespective of gender. Factor VIII: C (13.10 ± 0.24) and X (1.29 ± 0.05) were significantly (P<0.05) higher in camel compared to the other ungulates. However, Fibrinogen (3.67 ± 0.03) was significantly (P<0.05) higher in Erlangeri. Gender-wise, PT, APPT, Factor VII and IX were higher (P<0.05) in Idmi male. Significantly (P<0.05) higher Factor VIII: C was found in male camel. Fibrinogen was significantly (P<0.05) lower in human male compared to Idmi, camel, Erlangeri, Ibdex, Oryx and Reem. Similarly, PT and APPT were significantly (P<0.05) higher in female Idmi while Factor VII was significantly (P<0.05) lower in all ungulates compared to human female.

Conclusion and implications: These information provides some basic information of coagulation time for these ungulates which may be important for veterinarian and wildlife managers.

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Introduction

Hemostasis is a protective dynamic mechanism involved in protecting the body against blood loss through bleeding by sealing the site of injury in the vascular system [1,2]. Diagnosis of diseases and health problems require reliable source of references [3]. Normal coagulation parameters are vital for evaluating and understanding clinical manifestations of hemorrhagic diseases such as hemorrhagic septicemia, malignant catarrhal fever, blue tongue and mucosal diseases in animals [4,5] in addition to other causes of hemorrhages including trauma [6], anticoagulant poisoning or clotting factors deficiency due to chronic liver disorders and wasting diseases [7]. In addition, during infection, animals are prone to disturbed hemodynamic profile [8].

Numerous studies have been carried out to determine coagulation parameters in domestic animals [9,10], however, studies on coagulation parameters in wild animals are scarce. Only a few studies are found in Erlangeri, Speke's, mountain and sand gazelles, Nubian Ibex, Oryx, fallow deer and red deer [11,12]. Hussain et al. [12] revealed that mountain gazelle and Nubian ibex have higher plasma fibrinogen concentration and factor VIII: C. Similarly, Siroka et al. [11] found that prothrombin time was significantly higher in fallow deer. Similar to camel, Arabian oryx have shorter APPT and higher Factor VIII [12]. Studies in camel have reported shorter prothrombin, higher fibrinogen and factor VIII: C [13].

Most of these animals can hardly be found in the jungle; therefore, they are mostly confined to the captivity centers to avoid their extinction. In addition, their numbers are very few and it is hard to get desired number of samples for determination of hematological profile. Few studies have documented different coagulation factor in these animals, however, no study is available on the comparison of these factors in these ungulates. The objective of the present study was to compare some coagulation parameters in three species of gazelles, and their comparison with other desert ungulates, camels and humans.

Materials and methods

A total of 74 apparently healthy gazelles, comprising of 36 Reem (18 males, 18 females), 22 Erlangeri (11 males, 11 females) and 16 Idmi (6 males, 10 females) gazelles were investigated, along with 17 Nubian Ibex (7 males, 10 females) and 16 Oryx (6 males, 10 females). All of these animals were born and raised in captivity at King Khalid Wildlife Research Center in Thumamah near Riyadh Saudi Arabia (24° 41′ N, 46° 42′ E). They were fed dried alfalfa and commercial concentrate (16% protein) with free access to water. All of them were healthy adults and none of the females was pregnant at the time of sampling. Blood samples from 20 naturally grazing she- camels were also included. For comparison, whole citrated blood samples from 10 healthy male human volunteers (20-23 yr. old) were also included, being kindly provided by King Khalid Hospital.

Blood samples were collected from the animals by jugular venipuncture into vacutainer tubes containing 3.2% trisodium citrate (0.11 M; Becton Dickinson) to give a citrate to blood ratio of 1:9 for the determination of coagulation parameters. Plasma was separated within five minutes from the whole citrated blood by centrifugation at 3,500g for 15 min. at 4°C, transferred into plastic tubes, and frozen at - 40°C.

The concentration of fibrinogen, Prothrombin Time (PT), Activated Partial Thromboplastin Time (APPT) and FVII, factor VIII:C, factor IX, factor X, and factor XI were measured in paired citrated plasma using an automated STA compact coagulation Analyzer (Diagnostic Stago, Roche, Basile, Switzerland) and commercial reagents, as described previously by Hussein et al. [12]. The PT was determined by adding 0.2 ml rabbit thromboplastin reagent (Simplastin; Organon Tekinka Corp., Durham, North Carolina, USA) to 0.1 ml citrated plasma, whereas APPT was measured by adding 0.1 ml actin-activated cephaloplastin reagent (Organon Tekinka) to 0.1 ml of plasma and mixed with 0.1 ml of $Ca\ Cl_2$ solution. Plasma samples for determination of both PT and APPT were incubated at 37^0 C for 3 min before adding reagents. Control values for PT and APPT were based on human citrated plasma reference plasma (Baxter Diagnostics, Deerfield, Illinois, USA). Fibrinogen concentration was assayed using commercial STA kit (Diagnostic, Stago, Roche). The PT and APPT fibrinogen concentration were measured in clot-detection system of STA analyzer.

The coagulation factors were determined using substrates of human plasma deficient in either factor VII, VIII: C, IX, or X (Organon Tekinka), and canine plasma deficient in factor XI (Dade Actin, Baxter Diagnostics). Activity of these factors in test samples was determined by adding the corresponding factor-deficient substrate to the sample, followed by addition of Simplastin reagent for factors VII, X and cephaloplastin reagent for the remaining factors (VIII: C, IX, XI). The mixture was incubated at 37°C for 3 min, and the clotting time was recorded. All the values in the control were within the normal acceptable range.

Because there is no reliable test for animals clotting factors, standard curves were prepared using humans reference plasma, and the clotting times were converted into units per liter of biological activity and compared with human plasma having been assigned as 1.0 U/l coagulation factor.

Statistical analysis

Data was statistically analyzed using one way Analysis of Variance (ANOVA) with the help of statistical software (Statistica version 10). Means were separated using Tukey test. P value less than 0.05 was considered to be statistically significant. Data was shown as mean and Standard Error (SE).

Results

The results of comparison between the three gazelles are shown in Table 1, while Table 2 and 3 show the comparison between the three species by gender. There was highly significant difference (P<.0001) between the overall PT and APPT of Idmi and the other two gazelle species. It was significantly longer in Idmi than both gazelles and also than humans and camels. The same trend was followed in males and females. No significant difference was recorded between the mean values of Factors VII, X, XI and fibrinogen concentration in the three gazelles; however, highly significant differences (<0.005) were noted between the means of the three gazelles in factors VIII: C and IX, the two factors being higher in Idmi gazelle than the other gazelles. In addition, a significant difference in factor X was noted between Idmi and the other two species of gazelles, while in males, differences were recorded between Idmi and Erlangeri gazelle and in females between Reem and other gazelle species.

Table 4 shows a comparison between the three species of gazelles, Nubian Ibex and Oryx with references to humans and camels, while Table 5 and 6 show the comparison between their genders.

The results revealed significant difference between the studied animal species versus humans in all parameters measured except factors VII and XI. The Idmi gazelle recorded longer PT and APPT times than other species and humans (18.6, and 35.08 respectively), while camel and Nubian Ibex recorded shorter values of PT (11.10 ± 0.10). Factor VII was almost similar in all animal species, but lower than humans (1.55 ± 0.10), while factor VIII: C differed in all species, with highest value in camels (13.1 ± 0.24) and the lowest in Reem gazelle (3.25 ± 0.08), but all were higher than human (1.30 ±.06). Significant difference between Idmi and all species including humans were recorded in factor IX. Humans had lower values of factor X, and higher values of factors XI and VII than all animal species under study.

The PT in females showed similar trend with higher values for Idmi (15.81 ± 0.84) and lower for camel and Ibex (11.1 ± .06, 11.07 ± 0.13 respectively), also APPT followed the same pattren with higher values in Idmi (32.67 ± 2.23), and lower values in camel and Ibex (19.1 ± 0.03, 21.67 ± 0.08 respectively). The same trend was followed by Factors VII, and VIII: C. Only Idmi gazelle showed higher values of factor IX, while humans showed higher values of Factor XI. Factor X was nearly similar in all animal species and human, while fibrinogen concentration was comparable in all animal species studied, but lowers in humans.

Males and females showed comparable results for PT and APPT, while factors VII, and VIII: C followed the same trend like the overall and females; factors IX, X, XI, were nearly similar in all animals and humans investigated except that the fibrinogen concentration was lower in humans than other species.

Inter-sex difference appeared only and clearly in Idmi gazelle, where the PT and APPT were higher in the males than females, while Factor VIII: C was higher in the females. The remaining factors tended to be similar in both sexes.

Table 1: Comparison between hemostatic parameters in Idmi, Erlangeri and Reem gazelles,
Mean values within a row having different superscripts differ significantly (P< 0.05).

Parameters	Idmi [16]	Erlangeri [22]	Reem [36]
PT (S)	18.46 ± 0.77^a	13.58 ± 0.23^b	13.13 ± 0.22^b
APPT (S)	35.08 ± 1.95^a	23.11± 0.17^b	23.69 ± 0.20^b
VII (IU)	1.24 ± 0.01	1.21± 0.02	1.19 ± 0.05
VIII:C (IU)	5.33±0.45^a	4.98 ± 0.10^a	3.25 ± 0.08^b
IX (IU)	1.78 ± 0.07^a	1.21 ± 0.02^b	1.23±0.03^b
X (IU)	1.25 ± 0.01^a	1.20 ± 0.02^b	1.21 ± 0.02^b
XI (IU)	1.58 ± 0.03	1.58 ± 0.02	1.54 ± 0.05
Fibrinogen (g/l)	3.37 ± 0.28	3.63 ± 0.04	3.67 ± 0.09

Table 2: Comparison between hemostatic parameters in Idmi, Erlangeri and Reem gazelles: Males,
Mean values bearing different superscripts in a row differ significantly (P<0.05).

Parameters	Idmi [6]	Erlangeri [11]	Reem [16]
PT. (S)	21.11 ± 0.96^a	13.96 ± 0.36^b	13.10 ± 0.36^b
APPT. (S)	35.08 ± 1.95^a	23.06 ± 0.30^b	23.82 ± 0.29^b
VII (IU)	1.24 ± 0.01^a	1.23 ± 0.03^a	1.18 ± 0.04^a
VIII: C (IU)	3.99 ± 0.56^a	4.94±0.10^a	3.25 ± 0.06^b
IX (IU)	1.85 ± 0.07^a	1.21 ± 0.02^b	1.23 ± 0.02^b
X. IU (IU)	1.25 ± 0.10^a	1.20 ± 0.03^a	1.21 ± 0.03^b
XI. IU (IU)	1.58 ± 0.01^a	1.58 ± 0.02^a	1.54 ± 0.06^b

Table 3: Comparison between hemostatic parameters in Idmi, Erlangeri and Reem gazelles: Females,
Mean values within a row having different superscripts differ significantly (P< 0.05)

Parameters	Idmi [10]	Erlangeri [11]	Reem [16]
PT. (S)	15.68 ± 0.84^a	13.19 ± 0.24^b	13.14 ± 0.31^b
APPT. (S)	32.49 ± 3.15^a	23.16 ± 0.19^b	23.61 ± 0.29^b
VII (IU)	1.24 ± 0.01	1.20 ± 0.03	1.20 ± 0.07
VIII:C (IU)	6.75 ± 0.56^a	5.03 ± 0.14^b	3.21 ± 0.13^c
IX (IU)	1.78 ± 0.11^a	1.25 ± 0.02^b	1.23± 0.05^b
X (IU)	1.25 ± 0.01^a	1.18 ± 0.03^b	1.22 ± 0.02^{ab}
XI (IU)	1.57 ± 0.03	1.62 ± 0.02	1.56 ± 0.08
Fibrinogen (g/l)	3.17 ± 0.23^b	3.71±0.05^a	3.63 ± 0.12^a

Table 4: Comparison between hemostatic parameters in some ungulates and human,
Mean values within a row having different superscripts differ significantly (P<0.05).

Parameters	Idmi [16]	Camel [20]	Erlangeri [22]	Human [10]	Ibex [17]	Oryx [16]	Reem [36]
PT (S)	18.46 ± 0.77[a]	11.10 ± 0.06[c]	13.58 ± 0.23[b]	12.30 ± 0.06[bc]	11.12 ± 0.08[c]	12.94 ± 0.07[b]	13.13 ± 0.22[b]
APPT (S)	35.08± 1.95[a]	19.10 ± 0.03[d]	23.11±0.17[cd]	27.60±0.80[b]	20.61 ± 0.11[cd]	21.70 ± 0.06[cd]	23.69 ± 0.20[bc]
VII (IU)	1.24 ± 0.01[b]	1.12±0.43[c]	1.21 ± 0.02[bc]	1.55 ± 0.10[a]	1.12 ± 0.03[c]	1.22 ± 0.05[bc]	1.19± 0.05[bc]
VIII:C (IU)	5.33 ± 0.45[d]	13.10 ± 0.24[a]	4.98 ± 0.10[d]	1.30 ± 0.06[f]	7.45 ± 0.06[c]	8.52±0.13[b]	3.25 ± 0.08[e]
IX (IU)	1.78 ± 0.07[a]	1.29 ± 0.030[b]	1.21 ± 0.02[b]	1.26 ± 0.04[b]	1.25 ± 0.03[b]	1.32 ± 0.01[b]	1.23±0.03[b]
X (IU)	1.25 ± 0.01[ab]	1.29 ± 0.05[a]	1.20±0.02[c]	1.19± 0.40[c]	1.25 ± 0.02[abc]	1.3±0. 07[a]	1.21 ± 0.02[bc]
XI (IU)	1.58 ± 0.03[bc]	1.62 ± 0.05[b]	1.58 ± 0.02[bc]	1.75± 0.08[a]	1.50 ± 0.02[c]	1.60±0.01[bc]	1.54 ± 0.05[bc]
Fibrinogen (g/l)	3.27 ± 0.18[b]	3.44 ± 0.43[ab]	3.67 ± 0.03[a]	2.10 ± 0.06[c]	3.60 ± 0.08[ab]	3.60 ± 0.01[ab]	3.59 ± 0.08[ab]

Table 5: Comparison between hemostatic parameters in some ungulates, and human: Males,
Mean values within a row having different superscripts differ significantly (P<0.05).

Parameters	Idmi [6]	Camel [20]	Erlangeri [11]	Human [10]	Ibex [7]	Oryx [6]	Reem [18]
PT (S)	21.11 ± 0.96[a]	11.10 ± 0.06[c]	13.96 ± 0.36 [b]	12.30 ± 0.06[bc]	11.07 ± 0.13[c]	12.83 ± 0.07[bc]	13.10 ± 0.36[b]
APPT (S)	37.67 ± 2.23[a]	19.1 0 ± 0.03[d]	23.06 ± 0.30[cd]	27.6 0 ± 0.08[b]	20.95 ± 0.20[cd]	21.68 ± 0.09[cd]	23.82 ± 0.29[bc]
VII IU (IU)	1.23 ± 0. 05[b]	1.12 ± 0.01[b]	1.23 ± 0.03[b]	1.55 ± 0.10 [a]	1.13 ± 0.02 [b]	1.22 ± 0.01[b]	1.18 ± 0.04[b]
VIII:C IU (IU)	3.99 ± 0.56[c]	13.10 ± 0.24[a]	4.94±0.15[cd]	1.3 ± 0.06[e]	7.43 ± 0.07[b]	8.42 ± 0.2[b]	3.30 ± 0.06[d]
IX IU (IU)	1.85 ± 0.08[a]	1.29 ± 0.03[b]	1.18 ± 0.02[b]	1.26 ± 0.04[b]	1.24 ± 0.05[b]	1.32 ± 0.02[b]	1.24 ± 0.02[b]
X IU (IU)	1.23 ± 0.10[a]	1.29 ± 0.03[a]	1.22 ± 0.03[a]	1.19 ± 0.04[a]	1.20 ± 0.02[a]	1.27 ± 0.01[a]	1.18 ± 0.03[b]
XI IU (IU)	1.63 ± 0.01[ab]	1.62 ± 0.05[ab]	1.55 ± 0.02[ab]	1.75 ± 0.08[a]	1.51 ± 0.05[b]	1.62 ± 0.02[ab]	1.52 ± 0.06[b]
Fibrinogen (g/l)	3.17 ± 0.28[b]	3.44 ± 0.04[ab]	3.63 ± 0.04[a]	2.10 ± 0.06[c]	3.61 ± 0.14[a]	3.57 ± 0.02[a]	3.53 ± 0.09[a]

Table 6: Comparison between hemostatic parameters in some ungulates and human: Females.
Mean values within a row having different superscripts differ significantly (P<0.05).

Parameters	Idmi [10]	Camel [20]	Erlangeri [11]	Human [10]	Ibex [10]	Oryx [10]	Reem [18]
PT (S)	15.68 ± 0.84[a]	11.10 ± 0.06[c]	13.19 ± 0.24[b]	12.30 ± 0.06[bc]	11.1 6 ± 0.10[c]	13.01 ± 0.1[b]	13.14 ± 0.31[b]
APPT (S)	32.49 ± 3.15[a]	19.10 ± 0.03[c]	23.16 ± 0.19[bc]	27.60 ± 0.08[ab]	20.63 ± 0.14[c]	21.67 ± 0.08[c]	23.55 ± 0.27[bc]
VII (IU)	1.24 ± 0.01[b]	1.12 ± 0.04[b]	1.20 ± 0.03[b]	1.55 ± 0.10[a]	1.11 ± 0.04[b]	1.23 ± 0.01[b]	1.19 ± 0.07[b]
V111:C (IU)	6.75 ± 0.56[c]	13.1 ± 0.24[a]	5.03 ± 0.14[d]	1.30 ± 0.06[f]	7.47 ± 0.09[c]	8.59 ± 0.17[b]	3.22 ± 0.12[e]
1X (IU)	1.70 ± 0.11[a]	1.29 ± 0.03[b]	1.25 ± 0.02[b]	1.26 ± 0.04[b]	1.26 ± 0.04[b]	1.3 2± 0.01[b]	1.23 ± 0.05[b]
X (IU)	1.25 ± 0.01[abc]	1.29 ± 0.03[a]	1.18 ± 0.03[c]	1.19 ± 0.04[c]	1.28 ± 0.02[ab]	1.28 ± 0.01[ab]	1.21 ± 0.02[bc]
X1 (IU)	1.57 ± 0.03[b]	1.62 ± 0.05[ab]	1.62 ± 0.02[ab]	1.75 ± 0.08[a]	1.49 ± 0.02[b]	1.59 ± 0.01[b]	1.56 ± 0.08[b]
Fibrinogen (g/l)	3.26 ± 0.23[b]	3.71 ± 0.05[ab]	3.71 ± 0.05[a]	2.1 ± 0.06[c]	3.57 ± 0.09[ab]	3.6 ± 0.01[a]	3.63 ± 0.12[a]

Discussion

Idmi gazelle exhibited significantly longer PT and APPT times than Erlangeri and Reem gazelle. These results were similar and almost equal to those reported by Hussein et al. [12,13] and AL-Jumaah and Hussein [14]. However, these were higher compared to those of other ungulates [14]. These differences could be attributed to handling stress, hematocrit resulting in artificial prolongation, or other factors such as citrate concentration, sampling interval, assay performance and lack of specificity to rabbit thromboplastin usually used in the test [10]. The average APPT value for Idmi was within the normal ranges for cattle [15], but was lower than that of horses [16].

All gazelle species studied showed higher factor VIII: C values compared to humans, and the highest value was recorded in Idmi. The herein recorded factor VIII: C was similar to that reported by Hussein et al. [13]. The levels of other coagulation factors reported in the present study for gazelle species were broadly similar to those reported for Reem and Idmi gazelles and Arabian Oryx [12]. Idmi gazelle exhibited higher value of factor IX compared to other ungulates, camel and humans. Similar finding was reported by Hussein et al. [12]. Ungulates in general showed higher value of factor VIII: C compared to humans. This is a peculiarity of ungulates since higher values of factor VIII: C has also been reported in other species such as cattle, pigs and cats [17]. However, it remains to be determined whether this increase in factor VIII: C reflects an actual circulatory increase or a thrombin activated one.

The female and male groups followed the same overall pattern of gazelle species, showing significantly higher PT and APPT values in both sexes of Idmi compared to both sexes of Reem and Erlangeri gazelles. Gender data revealed significant differences in PT and APTT between the females of different species following the same trend of the overall data. The effect of gender has been reported by Lemini et al. [18] who obtained significant differences in PT, APPT and fibrinogen in Wister rats. In addition, these authors also recorded intra- and inter-specific differences in PT, APPT, TT and fibrinogen values. Schewertz and Penckofer reported that gender plays an important role in hemostatic changes due to influence of the steroid sex hormone. Siroka et al. [11] recorded significant difference in APPT between males and females and hypothesized that the higher clotting times in males compared to females represent a physiological disadvantage of this gender to deal with injuries (due to frequent fighting during heat period). Idmi exhibited longer PT and APTT values, while Ibex and camels showed shorter values in PT compared to other animals. Camel, Erlangeri, Oryx, Reem and Ibex had similar APTT values, while Reem, oryx and Erlangeri had PT values similar to humans. No significant differences in factors VII, IX, X and XI were observed between female ungulates and camels. Humans showed higher values of factor VII and XI, while Idmi showed higher factor IX values. There were significant differences between all ungulate species in factor VIII: C, with the highest value being recorded for camels and the lowest for humans. Among wild ungulates the highest value was recorded for Oryx and the lowest for Reem. The fibrinogen concentration followed the same trend of overall data, with similar values in males and females in all animal species including camels. However, the lowest fibrinogen value was recorded in humans. Males exhibited the same trend as females concerning PT, APTT, factors VIII: C, VII, IX, X, XI, and fibrinogen concentration. Inter-sex differences in PT, APTT, factor VIII: C and fibrinogen concentration values were observed only in Idmi gazelles [19].

In conclusion, normal values for coagulation variable vary considerably between species, and also within the species and with the laboratory method indicating that the comparison between species must be done when the test is performed by the same method and in the same laboratory. Our data could constitute a baseline for coagulation parameters in wild animals.

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