Effects of Induced Haemorrhage on Thermoregulation, Blood Constituents and Serum Biochemical Parameters in Pregnant and Non-Pregnant Rabbits (Oryctolagus cuniculus)

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Authors’ contributions
This work was carried out in collaboration among all authors. Authors KAOS and AMA designed the study and managed analysis. All authors wrote the first draft of the manuscript. Authors KAOS and AOA performed the statistical analysis and managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

Background and Objectives: Haemorrhage is a leading cause of mortality and morbidity of mother and foetus. Pregnancy is associated with changes which may influence responses to bleeding. This study was designed to examine the influence of controlled haemorrhage and compare thermoregulation and haematological and biochemical parameters in pregnant and nonpregnant rabbits.

Materials and Methods: Twelve pregnant and nonpregnant rabbits, 6 in each group, were used in the studies. Both groups of animals were subjected to 20% haemorrhage of total blood volume on gestation day 21. The rectal temperature (Tr), respiratory rate (RR) and heart rate (HR) were monitored for 2 days following bleeding. Blood samples were collected at 24 hrs before induction of

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bleeding and then after bleeding at 30 min, 24 hrs and 48 hrs. The samples were used for measurements of haematological parameters; coagulation profile, arterial blood gases and serum electrolytes.

**Results:** The general trend indicates lower Tr values in pregnant rabbits at 30 min post-haemorrhage. The values of RR and HR were significantly (P ≤ 0.01) higher in pregnant and nonpregnant animals at 24 hrs post-haemorrhage. The pregnant rabbits showed significant (P<0.05) decrease in platelets count at 24 hrs post-haemorrhage compared to the nonpregnant values. In pregnant rabbits, haemorrhage was associated with significant increase in PT. The activated partial thromboplastin time (APTT) was significantly (Ps ≤ 0.01) prolonged at 24 hrs post-haemorrhage in pregnant and nonpregnant rabbits. The partial pressure of arterial oxygen (PaO₂) in pregnant and nonpregnant rabbits was significantly (Ps ≤ 0.01) increased at 24hrs and 48 hrs post-haemorrhage. The partial pressure of arterial carbon dioxide (PaCO₂) decreased significantly (Ps ≤ 0.01) at 30 min post-haemorrhage in pregnant rabbits. Haemorrhage in rabbits was associated with significant decreases in plasma osmolality in pregnant rabbits.

**Conclusion:** Pregnancy induces modifications in some physiological responses to haemorrhage. The information generated could be used in monitoring maternal health during pregnancy and risks of changes associated with haemorrhage in mammals.

**Keywords:** Rabbit; pregnancy; haemorrhage; thermoregulation; blood constituents.

1. INTRODUCTION

Haemorrhage is generally defined as blood escaping from the circulatory system [1]. It constitutes the leading cause of morbidity and mortality in surgery and trauma patients [2].

Haemorrhage has an impact on various body systems including thermoregulation. Previous studies in rabbits indicated that external thermal support reduced the mortality in rabbits exposed to haemorrhagic shock by reducing the metabolic effect of shock [3]. However, studies in goats reported that there was initial gradual increase in rectal temperature following haemorrhage and the normal value of rectal temperature was recovered after 5 hrs [4]. On the other hand, other reports showed that bleeding in rats caused a drop in body core temperature that was proportional to the bleeding volume [5].

Severe blood loss can alter body homeostasis by reducing blood volume and can lead to cardiovascular collapse, hypovolemic shock and death [6]. Tachycardia was reported after 20% bleeding in adult rabbits [7]. Likewise, studies in goats reported that the heart rate and respiration rate increased gradually in animals exposed to 20% haemorrhage [8]. Previous animal studies reported bradycardia phase of haemorrhage followed by rapid increase of heart rate as blood 44% of total blood loss. This was associated with reduction in mean arterial pressure in pigs [9]. Similar responses were observed in human patients exposed to haemorrhagic shock [10].

Acute blood loss involves a progressive increase in heart rate and peripheral vasoconstriction to maintain arterial pressure, and decomposition phase followed hypotension, bradycardia [11] and hypovolaemia in humans [12], and rats [13]. The arterial and venous blood bicarbonate level decreased in haemorrhage even when the pH and blood pressure maintained normal values [14]. However, failure of early management may lead to cellular hypoxia and tissues death [15].

Haemorrhage may induce alterations in the blood profile. Erythropoietin appears in circulation in response to blood loss to stimulate erythropoiesis in the bone marrow [16]. However, red blood corpuscles, PCV, Hb concentration and leukocyte counts were significantly decreased following 20% bleeding in goats [8]. In goats exposed to 15-30% haemorrhage, there was also a significant decrease in PCV, Hb concentration, total leukocytes and lymphocyte ratio [4]. Post-haemorrhage neutrophilic leukocytosis was reported and it was related to the shift of neutrophils from marginal pool and bone marrow reserve to the circulation [17].

Haemorrhage influences the concentrations of total plasma proteins and albumin and others blood metabolites. The changes were related to haemodilution induced by shifting of interstitial fluids [4]. Postoperative reductions of total proteins and albumin concentrations were reported in humans [18]. Decline in serum Ca level after haemorrhage was reported in sheep subjected to 20% bleeding [19]. However, Ware et al. [20] noted that Ca level was not
changed after haemorrhage in rats subjected to 20% bleeding.

Haemorrhage is a leading cause of mortality and morbidity of mother and fetus [21]. Previous studies reported that 50% of the women are presented to delivery in emergency room with vaginal bleeding and maternal haemorrhage was reported in 15% - 25% of all pregnancies [22, 23]. Pregnancy is associated with physiological changes which may impair the haemodynamic responses to bleeding; in late gestation the plasma volume and red cell mass increase by up to 40% [24]. Other studies indicated that the compensatory haemodilution initiates a drop in haemoglobin, haematocrit and RBCs, but maintains mean corpuscular haemoglobin concentration (MCHC) [25]. Moreover, gestation causes irresponsiveness to angiotensin II[26] and other vasoconstrictor agents [27,28]. This resistance to vasoconstrictors may influence the response to bleeding by inhibiting shunting of blood towards the brain and heart [29,30].

Previous studies reported haemodynamic changes and maternal outcome during haemorrhage in woman [31], rat [32] and rabbits [7]. But there is dearth of information in literature regarding changes in blood constituents and arterial blood gases during haemorrhage. Therefore, this experiment used a rabbit model, which controlled for both the timing and volume of haemorrhage in order to compare thermoregulation, haematological and biochemical parameters in pregnant and nonpregnant rabbits.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Twelve healthy, sexually mature pregnant and nonpregnant rabbits (Oryctolagus cuniculus), 6 in each group, were used in the studies. The animals were kept in an animal house at the Department of Physiology. The rabbits were obtained from the local market. The animals were aged 9-12 months with an average body weight of 1.50± 0.30kg. Female rabbits were isolated for one month individually in cages to ascertain their reproductive status and animals were judged to be in oestrus were housed together with sexually mature males in cages for mating on 1:1 basis. The day when copulation was confirmed was regarded as day 0 of gestation (GD0). Pregnancy was confirmed by abdominal palpation of the foetus at days 14- 16 of pregnancy [33]. During the studies, the rabbits were offered fresh Lucerne (Medicago sativa) and crushed sorghum grains and were given fresh tap water ad libitum.

2.2 Experimental Procedure

The complete randomized design was used to evaluate the effect of haemorrhage in the domestic rabbit. Pregnant and nonpregnant rabbits, 6 in each group. Both groups of animals were subjected to 20% haemorrhage of total blood volume on gestation day 21. 20% haemorrhage was calculated according to body weight of the experimental animals. Blood was withdrawn from the jugular vein over 20 min by syringes. The initial baseline physiological data including rectal temperature (Tr), respiratory rate (RR) and heart rate (HR) were monitored for 2 days following bleeding in order to determine the physiological responses to haemorrhage. Blood samples were collected from marginal vein and auricular artery of right ear at 24 hrs before induction of bleeding and then after bleeding at 30 min, 24hrs and 48 hrs. The samples were used for measurements of haematological parameters, coagulation profile arterial blood gases and serum electrolytes.

2.3 Rectal Temperature (Tr)

The Tr of animals was measured by a certified digital clinical thermometer (Hartman – United Kingdom). The thermometer was inserted into the rectum for a depth of approximately 4 cm for 2 min. The values were obtained with an accuracy of ±0.1℃.

2.4 Respiratory Rate (RR)

The RR of rabbits was measured visually by counting the flank movements for 1 min. using a stopwatch. The values were taken with the animals sitting quietly.

2.5 Heart Rate (HR)

The HR was measured by auscultation using a stethoscope on the left ventral chest wall, performed twice for one minute.

2.6 Collection of Blood Samples

The area of collection was shaved and scrubbed by a disinfectant (70% ethanol) before the marginal ear vein was punctured. Then 5 ml of blood was collected using plastic disposable syringes. Immediately after collection, 2ml of
blood was transferred to capped test tube containing di-sodium ethylene diamine tetra-acetate (Na$_2$ EDTA) as anticoagulant for measurements of haematological parameters. The rest of the blood was allowed to stay for 2 hrs at room temperature and then centrifuged at 3000 rpm for 15 min (Hettich-Zentrifugen-German) and haemolysis-free serum samples were pipetted into clean vials and immediately frozen at -20ºC for subsequent analysis. In addition, 2 ml of blood collected in heparinized tubes were centrifuged at 300 rmp for 15 min, and plasma samples obtained were used for determination of prothrombin time (PT) and activated partial thromboplastin (APTT) and osmolality.

2.7 Erythrocytic and Leukocytes Parameters

The standard methods described by Jain [34] were used for the determination of the parameters of erythrocytic series, erythrocytic count, packed cell volume (PCV), haemoglobin concentration (Hb) and total leukocyte count (TLC).

2.8 Blood Coagulation Parameters

2.8.1 Platelet count

The platelet count was performed microscopically (Olympus, Japan) under low power (X10 objective) using a haemocytometer according to the method described by Mary and Gretchen [35].

2.8.2 Prothrombin time (PT)

The prothrombin time (PT) was determined by the reagent (DiaPlastin, United Kingdom) using coagulometer bio- bas (Spinreact S.A. Spain) using the method described by Biggs and McFarlane [36].

2.8.3 Activated partial thromboplastin time (APTT)

The APTT was determined by the reagent Fortress Diagnostics APTT (United Kingdom) using analyzer bio- bas (Spinreact S.A. Spain) by the method described by Biggs [37].

2.9 Serum Electrolytes

The serum concentration of Na and K were determined by the flame photometry technique described by Wootton [38]. The serum Ca concentration was measured by the cresolphthalein method described by Robertson and Marshall [39] using a kit (Spinreact, Spain).

2.10 Arterial Blood Gas Analysis

Arterial blood gas analysis was performed using GEM primer 3000 analyzer (Instrumental Laboratory, Italy). The sample was collected from the auricular artery of rabbits by heparinized blood syringe (1.0 ml). The sample collected in plastic syringe was run within 10 min as described by Picandet et al. [40]. Then the sample was aspirated by ABG analyzer and the partial pressures of oxygen (PaO$_2$), carbon dioxide (PaCO$_2$) and bicarbonate (HCO$_3$) concentrations were measured directly by the analyzer.

2.11 Plasma Osmolality

The plasma osmolality was determined by a cryoscopic digital osmometer (Osmomat 030, Gonotec- Germany). The osmolality depends on the concentration of all osmotically active parts dissolved in the solvent. Since the freezing point depression is directly proportional to the dissolved parts; the osmomat 030 directly measures the osmolality.

2.12 Statistical Analysis

The data obtained from the studies were subjected to standard methods of statistical analysis using the Statistical Package of Science and Social (SPSS) version 16.0. The experiments were performed according to the complete randomized designs (CRD). Analysis of variance (ANOVA) test was used to evaluate the effect of haemorrhage on thermoregulation, haematological and biochemical parameters in pregnant and non-pregnant rabbits. The means values were compared for significance at P≤0.05 and the group results were presented as mean± SD.

3. RESULTS

3.1 Rectal Temperature (Tr), Respiratory Rate (RR) and Heart Rate (HR)

The results indicate that the body ore temperature (Tr) values were not significantly affected by haemorrhage in rabbits. However, the general trend indicates lower Tr values in
pregnant rabbits at 30 min post-haemorrhage. The respiratory rate (RR) values, were significantly (P<0.05) increased in pregnant and nonpregnant rabbits at 30 min and 24hrs post-haemorrhage compared to the control values. Furthermore, HR values were significantly (P<0.05) increased at 30 min and 24hrs post-haemorrhage in pregnant and nonpregnant rabbits compared to the respective control values (Table 1). There was increase in RR of rabbits in response to bleeding.

3.2 Erythrocytic and Leukocytes Parameters

The results indicate that PCV values, erythrocyte count and Hb concentration were significantly (P<0.05) decreased at 30 min and 24 hrs post-haemorrhage in pregnant and non-pregnant rabbits compared to respective control values (Table 2).

In current study, the TLC values were significantly (P<0.05) increased at 30 min and 48 hrs post-haemorrhage in the nonpregnant group of rabbits (Fig. 1).

3.3 Platelet Count

The platelet counts were significantly (P<0.05) decreased in pregnant and nonpregnant at 30 min, 24 and 48 hrs post-haemorrhage compared to the control values (Fig. 2). The pregnant rabbits showed significant (P<0.05) decrease in platelets count at 24 hrs post-haemorrhage compared to the nonpregnant values.

3.4 Prothrombin Time and Activated Partial Thromboplastin Time

Haemorrhage was associated with fluctuations in prothrombin time (PT) in nonpregnant rabbits, while significantly (P<0.05) increased PT in pregnant rabbits (Fig. 3). APTT was significantly (P<0.05) prolonged in pregnant and nonpregnant rabbits at 24hrs post-haemorrhage (Fig. 4).

3.5 Arterial Blood Gas Analysis

The current results showed a significant (P<0.05) increase in partial pressure of arterial oxygen (PaO$_2$) in pregnant and nonpregnant rabbits at 24 hrs and 48 hrs post-haemorrhage (Fig. 5). However, higher PaO$_2$ were observed in pregnant rabbits at 48 hrs post-haemorrhage.

The PaCO$_2$ was significantly (P<0.05) decreased at 30 min post-haemorrhage in pregnant rabbits, while nonpregnant rabbits had higher PaCO$_2$ at 30 min post-haemorrhage (Fig. 6).

The results indicated that the blood bicarbonate (HCO$_3^-$) level was significantly (P<0.05) increased in pregnant and nonpregnant rabbits at 24hrs post-haemorrhage. The general trend indicates a slight increase in HCO$_3^-$ level in pregnant rabbits (Fig. 7).

![Fig. 1. Effect of 20% haemorrhage on Total Leukocyte Count (TLC) in nonpregnant and pregnant rabbits](image)
3.6 Serum Electrolytes

The results showed that serum Na level was significantly increased in pregnant and nonpregnant rabbits at 30 min and 48 hrs post-haemorrhage (Fig.8). However, there was a mild increase in Na level in pregnant rabbits.

The serum K level was significantly (P<0.05) decreased at 30 min, 24hrs and 48 hrs post-haemorrhage in pregnant and non-pregnant rabbits (Fig. 9).

The serum Ca level showed fluctuations in nonpregnant rabbits, while a slight increase was detected in pregnant rabbits post-haemorrhage (Fig. 10).

3.7 Plasma Osmolality

Haemorrhage in rabbits was associated with significant decreases in plasma osmolality in pregnant rabbits, while the nonpregnant group maintained normal osmolality at 30 min, 24 hrs and 48 hrs post-haemorrhage (Fig. 11).

4. DISCUSSION

4.1 Thermoregulation and Heart rate

The decrease in Tr in pregnant rabbits may have resulted from hormonal changes during pregnancy. Oestrogen increases in late gestation and could exert its effect on thermoregulation by locally mediated peripheral effect by nitric oxide, such as relaxation of vascular smooth muscles and inhibition of vasoconstrictor tone [41,42]. This can lead to vasodilatation and enhance heat loss by convection. A decline in body temperature with the advance of pregnancy was previously reported in rabbits [43]. Hypothermia and sweating were reported as adverse effect of blood donation in humans [44,45].
Table 1. Effect of 20% haemorrhage on rectal temperature (Tr), respiratory rate (RR) and heart rate (HR) in non-pregnant and pregnant rabbits

<table>
<thead>
<tr>
<th>Parameter</th>
<th>24 hrs pre-haemorrhage</th>
<th>Post-haemorrhage</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
<td>24 hrs</td>
<td>48 hrs</td>
</tr>
<tr>
<td></td>
<td>Non-pregnant</td>
<td>Pregnant</td>
<td>Non-pregnant</td>
</tr>
<tr>
<td>Tr (°C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>38.60±0.42a</td>
<td>38.53±0.32a</td>
<td>38.64±0.26a</td>
</tr>
<tr>
<td>RR (breaths/min)</td>
<td>51.86±2.61d</td>
<td>45.28±2.50g</td>
<td>56.86±3.48d</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>227.14±17.99c</td>
<td>185.71±12.72g</td>
<td>224.28±15.12c</td>
</tr>
</tbody>
</table>

*Significant at P≤0.05; **Highly significant at P≤0.01; n.s Notsignificant at P > 0.05

Table 2. Effect of 20% haemorrhage on blood constituents in pregnant and nonpregnant rabbits

<table>
<thead>
<tr>
<th>Parameter</th>
<th>24 hrs pre-haemorrhage</th>
<th>Post-haemorrhage</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
<td>24 hrs</td>
<td>48 hrs</td>
</tr>
<tr>
<td></td>
<td>Non-pregnant</td>
<td>Pregnant</td>
<td>Non-pregnant</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>38.28±2.218a</td>
<td>37.57±3.21b</td>
<td>32.28±3.35d</td>
</tr>
<tr>
<td>Erythrocytes (x10⁶/μL)</td>
<td>5.59±0.38a</td>
<td>5.28±0.36a</td>
<td>4.62±0.48b</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>10.56±0.58a</td>
<td>10.68±0.31a</td>
<td>8.40±0.67h</td>
</tr>
</tbody>
</table>
Fig. 4. Effect of 20% of haemorrhage on activated partial thromboplastin time (APTT) in nonpregnant and pregnant rabbits.

Fig. 5. Effect of 20% of haemorrhage on partial pressure of arterial oxygen (PaO₂) in nonpregnant and pregnant rabbits.

Fig. 6. Effect of 20% haemorrhage on arterial blood partial pressure of carbon dioxide (PaCO₂) in nonpregnant and pregnant rabbits.
Fig. 7. Effect of 20% haemorrhage on bicarbonate (HCO₃⁻) level of arterial blood in nonpregnant and pregnant rabbits

Fig. 8. Effect of 20% haemorrhage on serum sodium concentration (Na⁺) in nonpregnant and pregnant rabbits

Fig. 9. Effect of 20% haemorrhage on serum potassium concentration (K⁺) in nonpregnant and pregnant rabbits
There was no difference in the RR between pregnant and non-pregnant rabbit. However, there was increase in RR of rabbits in response to bleeding. Acute blood loss may lead sequentially to haemodynamic instability, decreased tissue perfusion, stagnant hypoxia and increased hydrogen ions, which stimulates chemoreceptors afferents thus activating the respiratory center and induces tachypnoea [46]. An increase in RR was reported in rats subjected to critical hemorrhagic shock hypotension [47].

The moderate blood loss in the current study caused decrease in blood pressure, which was compensated by baroreceptor mediated rise in HR. The response to blood loss involves release of norepinephrine by the adrenal medulla which induces vasoconstriction and increases HR [48]. A significant increase in heart rate and total peripheral resistance was reported in animals subjected to 20% haemorrhage [49]. Also a similar increase in HR was reported in sheep [50,51] and goats [4].

4.2 Erythrocytic and Leukocytes Parameters

Erythrocytic parameters did not differ between pregnant and non-pregnant rabbits. However, the reduction in PCV and Hb concentration in both groups (Table 2) is related to the shift of fluids from interstitial space to the capillaries in order to restore the circulating blood volume during haemorrhage [46]. Similarly, a significant decrease in PCV values post-haemorrhage was reported in goats [4] and sheep [19]. A reduction in PCV associated with significant drop of cardiac output during haemorrhage was previously reported in pregnant rabbits [7]. In current study, the TLC values were increased (Fig. 1). This

Fig. 10. Effect of 20% of haemorrhage on serum calcium concentration (Ca\(^{2+}\)) in nonpregnant and pregnant rabbits

Fig. 11. Effect of 20% haemorrhage on plasma osmolality in nonpregnant and pregnant rabbits
reduction is presumably related to shifting of fluid to increase plasma volume during haemorrhage [46].

4.3 Platelet Count

There were no differences in the platelet count between pregnant and nonpregnant rabbit. However, the platelet counts were decreased in pregnant and nonpregnant (Fig. 2). The movement of fluid into vascular space from interstitium may explain partially the decrease of platelets in rabbits. The significant decrease in platelets count in pregnant animals could be related to expansion of blood volume during pregnancy. Wells et al. [52] reported that haemodilution was considered to be the cause of decrease of platelets during pregnancy in rabbits.

4.4 Prothrombin Time and Activated Partial Thromboplastin Time

Haemorrhage was associated with fluctuations in prothrombin time (PT) in nonpregnant rabbits, while significantly increased PT in pregnant rabbits (Fig. 3). APTT was significantly prolonged in pregnant and nonpregnant rabbits at 24 hrs post-haemorrhage (Fig. 4). The prolonged inadequate tissue perfusion during haemorrhage may induce shifting of cellular metabolism and produces lactic acid which triggers metabolic acidosis. Acidosis was associated with prolonged PT and APTT in swine [53]. A decrease in clotting factors during haemorrhage was reported in trauma patients [54]. The less influence of haemorrhage in PT and APTT in pregnant rabbits could be related to relative deficiency in clotting factors resulting from haemodilution in rabbits as consequence of shifting of cellular and interstitial fluid [55]. The current results agree with the findings of Honda et al. [56] who reported a prolonged APTT in pregnant rabbits.

4.5 Arterial Blood Gas Analysis

The current results showed an increase in partial pressure of arterial oxygen (PaO\textsubscript{2}) in pregnant and nonpregnant rabbits at 24 hrs and 48 hrs post–haemorrhage (Fig. 5). However, higher PaO\textsubscript{2} were observed in pregnant rabbits at 48 hrs post–haemorrhage. Maternal ventilation and blood gases undergo substantial changes in pregnancy. In humans, there was 40% increase in minute ventilation during gestation [57] resulting in a rise in PaO\textsubscript{2} and a fall in PaCO\textsubscript{2} and HCO\textsubscript{3} [58]. This increase in ventilation is thought to be mediated by progesterone which lowers the threshold and increases the sensitivity of the respiratory centers [59]. Miller et al. [60] reported that acute blood loss in rats resulted in respiratory alkalosis with an increase in blood pH, a decrease in PaCO\textsubscript{2} and increase in PaO\textsubscript{2} and increase in affinity of Hb for oxygen.

The PaCO\textsubscript{2} was significantly decreased at 30 min post-haemorrhage in pregnant rabbits, while nonpregnant rabbits had higher PaCO\textsubscript{2} at 30 min post-haemorrhage (Fig. 6). The significant decrease of PaCO\textsubscript{2} in pregnant rabbits could be related to maternal hyperventilation. Pregnancy is associated with increase in O\textsubscript{2} consumption and basal metabolic rate [61]. This extra demandis achieved via increase in resting minute ventilation which causes rise in PaO\textsubscript{2} and decrease in PaCO\textsubscript{2} and HCO\textsubscript{3} [62]. Sunyal et al. [63] reported that the PaCO\textsubscript{2} and HCO\textsubscript{3} were significantly lower in pregnant women.

There was no significant difference in HCO\textsubscript{3} level between pregnant and nonpregnant rabbit. The results indicated that the blood bicarbonate HCO\textsubscript{3} level was significantly increased in pregnant and nonpregnant rabbits at 24 hrs post-haemorrhage. The general trend indicates a slight increase in HCO\textsubscript{3} level in pregnant rabbits (Fig. 7). This increase could be related to renal compensation of respiratory acidosis in response to acute blood loss which leads to increase in HCO\textsubscript{3} level. Previous studies reported elevation of HCO\textsubscript{3} level as a result of complete compensation of respiratory acidosis [64].

4.6 Serum Electrolytes

The results showed that serum Na level was significantly increased in pregnant and nonpregnant rabbits at 30 min and 48 hrs post-haemorrhage (Fig. 8). This response could be related to the fact that haemorrhage induces secretion of angiotensin II which activates the release of aldosterone hormone. Aldosterone increases Na reabsorption and K excretion [65]. Reabsorption of Na ions occurs through epithelial Na channels on the apical membrane of the membrane of the distal tubules and cortical collecting duct. However, there was a mild increase in Na level in pregnant rabbits. An increase in post-haemorrhage Na level in pregnant rabbits was related to compensatory mechanism induced by renin-angiotensin system [66]. The serum K level was significantly decreased at 30 min, 24hrs and 48 hrs post-haemorrhage in pregnant and non-pregnant rabbits (Fig. 9). That could be related to shifting
of intracellular and interstitial fluid to plasma and induces haemodilution in response to haemorrhage [46]. However, the slight decrease in K level in this study agrees with previous study conducted in pregnant ewes [67].

The serum Ca level showed fluctuations in nonpregnant rabbits, while a slight increase was detected in pregnant rabbits post-haemorrhage (Fig. 10). These changes could be related to the fact that Ca is compensated by ionization of intracellular Ca. Honda et al. [56] reported increase in Ca level in pregnant rats. However, Ware et al. [20] noted that serum Ca level was not changed within 90 min post-haemorrhage in rats subjected to 20% bleeding.

**4.7 Plasma Osmolality**

Haemorrhage in rabbits was associated with significant decreases in plasma osmolality in pregnant rabbits, while the nonpregnant group maintained normal osmolality at 30 min, 24 hrs and 48 hrs post-haemorrhage (Fig 11). The decrease in plasma osmolality during pregnancy is the consequence of a decrease in the threshold for osmotic stimulation of vasopressin release [68]. Elevation of plasma vasopressin and decrease in plasma osmolality were reported during pregnancy in rats [69]. Vasopressin mediated water reabsorption in the collecting duct occurs via aquaporin II water channels by activation of vasopressin II receptors [70]. An increase of vasopressin concentration was reported in pregnant and nonpregnant rabbits subjected to 30% haemorrhage [7]. Studies in human pregnancy have shown comparable results [71,72]. This led to the assumption that in pregnancy, osmoregulation is reset at a lower osmolality plasma level, thereby promoting water retention [71].

**5. CONCLUSION**

The results of this study showed that controlled haemorrhage has an impact on various body systems. However, pregnancy induced modifications in some physiological responses to haemorrhage. The information generated could be used in monitoring maternal health during pregnancy and risks of changes associated with haemorrhage in mammals.

**ETHICAL APPROVAL**

The ethical issues were addressed adequately according to veterinary and institutional guidelines.

**COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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