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Effect of Pregnancy on Thermoregulation, Blood Constituents, Serum Bio-chemicals and Cortisol Level in *(Oryctolagus cuniculus)* Rabbit Model

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Authors' contributions

This work was carried out in collaboration among all authors. Author KAOS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors Abdalla Mohammed Abdelatif and Altiab Mohammed Alfaki managed the analyses of the study. Author Etayeb Mohamed Alfaki managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was conducted to evaluate the effects of pregnancy on the physiological responses in rabbit model in early, mid and late gestation periods.
Materials and Methods: The experiment was performed according to the complete randomized design. Fourteen (14) pregnant and non pregnant rabbits, 7 in each group, were used in this study. Pregnancy was ascertained by vaginal swabs taken after copulation by mature fertile male rabbits. Blood and serum constituents' responses were investigated at days 0, 7, 14, 21 and 28 of gestation.
Results: The obtained results revealed significant ($P<0.05$) increase in respiration rate (RR) in mid-gestation and in heart rate (HR) at late gestation. The packed cell volume (PVC), erythrocyte count, haemoglobin concentration (Hb) and total leukocyte count (TLC) were significantly ($P<0.05$) reduced in mid and late gestation. The serum levels of total protein decreased and cholesterol increased significantly ($P<0.05$) in early, mid and late gestation. The plasma osmolality decreased

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significantly (*P<0.05*) at mid gestation. Cortisol level increased significantly *(P<0.05*) during mid and late gestation.
Conclusions: The study concluded that the pregnancy altered HR, hematological, serum parameters and cortisol level in pregnant rabbit model.

Keywords: Pregnancy; thermoregulation; rabbit; haemetological parameters.

1. INTRODUCTION

Pregnancy induces extensive adjustments in maternal physiology that are often reflected in characteristic alterations in thermoregulation [1] and blood parameters in rabbits [2]. The changes include cardiovascular [3], respiratory [4] as well as biochemical and endocrine changes [5,6]. The cardiovascular system is progressively stressed during pregnancy and parturition; many changes appear during the first trimester of gestation including increases of cardiac output and decreases in vascular resistance [7].

In female mammals, thermoregulation and reproduction involve major energy expenditure [8]. As both of these processes are under hormonal regulation, interactions between thermoregulation and gestation, and glucocorticoid levels are expected [9,10]. Previous studies reported effects of pregnancy on blood cortisol levels [11] and thermoregulation in rabbits [12].

Rabbits are used as a model for mammalian reproduction and early embryology studies [13]. Many investigations identified the haemetological indices as factors affecting gestation in humans [14,15]. Anaemia is a widely reported blood disorder associated with adverse pregnancy outcome [16]. However, details of maternal thermoregulation and blood parameters, especially those related to blood coagulation and cortisol level are still obscure. This experiment adopted a rabbit model to investigate the effects of stage of gestation on thermoregulation, blood constituents, serum biochemical constituents and cortisol level.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Fourteen healthy, sexually mature pregnant and nonpregnant rabbits (*Oryctolagus cuniculus*) were used in the studies. The animals were kept in an animal house at the Department of Physiology. The rabbits were obtained from local market. The animals were aged 9−12 months with average body weight 1.50±0.30 Kg. 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. 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 stages of pregnancy in domestic rabbit model. The rabbits were assigned to two experimental groups of 7 rabbits each, group A served as nonpregnant control while group B rabbits were time mated with certified fertile male rabbits in cages on 1:1bases and pregnancy was confirmed by vaginal swab was taken after copulation. The presence of sperms in vaginal smear under light microscope was regarded as day 0 of gestation (GD0). Blood samples were collected for the determination of haematological and biochemical parameters and serum cortisol level at gestation day 7(GD7), day 14 (GD14), day 21 (GD21) and day 28 (GD28). Simultaneous measurements of rectal temperature (Tr), respiratory rate (RR), heart rate (HR) were performed during the course of the experiment.

2.3 Rectal Temperature (Tr)

The rectal temperature (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 respiratory rate (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 heart rate (HR) was measured by auscultation using a stethoscope on the left ventral chest wall, performed twice for one minute. During the experimental period, the HR was also measured by Pulse-oximetry Patient Monitor (General Meditech Inc, Shenzhen, China).

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 15min (Hettich-Zentrifugen-German) and haemolysis-free serum samples were pipetted into clean vials and immediately frozen at -20 ℃ 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.

2.6.1 Erythrocytic and Leukocytes parameters

The standard methods described by Jain [17] were used for the determination of the parameters of erythrocyte series, erythrocytic count, Packed cell volume (PVC), Haemoglobin concentration (Hb), Total leukocyte count (TLC) and Deferential leukocyte count (DLC).

2.7 Biochemical Parameters

2.7.1 Serum total protein

Serum total protein concentration was determined by the colorimetric standard calculator [18] using a kit (Cromatest Linear Chemicals S.L, Spain).

2.7.2 Serum albumin

Serum albumin concentration was determined by the colorimetric method [19] using a kit (Cromatest Linear Chemicals S.L, Spain).

2.7.3 Serum cholesterol

Serum cholesterol concentration was determined by the enzymatic-colorimetric method [20] using a kit (Spinreact, Spain).

2.7.4 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 directly measures the osmolality.

2.8 Serum Cortisol Concentration

Serum cortisol was determined by radioimmuno-assay I125 - F RIABULK reagents produced (China IMR 484).

2.9 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 design (CRD). Analysis of variance (ANOVA) test was used to evaluate the effect of pregnancy on thermoregulation, haematological, biochemical parameters and cortisol level in rabbits. The means values were compared significance at $P \leq 0.05$ and the group results were presented as mean ± SD.

3. RESULTS

3.1 Thermoregulation, Heart Rate and Respiratory Rate are Presented in Table 1

3.1.1 Rectal temperature (Tr)

The general trend indicates that there was no significant change in Tr values on gestation days GD7, GD14, GD21 and GD28. However, a lower Tr value was recorded on GD28 of pregnancy (Fig. 1).

3.1.2 Respiratory rate (RR)

The mean RR values of pregnant rabbits were significantly ($P<0.05$) lower in GD0 and GD7 and increased in GD14 and GD21 compared to the

respective nonpregnant values. Higher RR values detected on GD21 in pregnant group (Fig. 2).

3.1.3 Heart rate (HR)

The mean values of HR values were significantly (P<0.05) decreased in GD0, 7, 14 and GD21and increased in GD28 in pregnant rabbits compared to control values and higher HR during pregnancy obtained in GD28 (Fig. 3).

3.2 Haematological Parameters

The effects of stage of pregnancy on haematological parameters are shown in Table 2.

3.2.1 Packed Cell Volume (PCV)

Fig. 4 projects the effect of stage of gestation on mean values of PCV in rabbits. The pregnant group maintained significantly (P < 0.05) lower PCV values at GD7, GD14, GD21 and GD28 compared to the control group (Fig. 4).

3.2.2 Erythrocyte count

The values were significantly (P < 0.05) lower in GD7, GD14, GD21 and GD28 in pregnant group compared to control (Fig. 5).

3.2.3 Hemoglobin concentration (Hb)

The pregnant group maintained significantly (P<0.05) lower Hb concentrations during the course of gestation in GD0, GD21 and GD28 compared to the respective control values and the lowest Hb concentration obtained during pregnancy at GD28 shown in (Fig. 6).

3.2.4 Total Leukocyte Count (TLC)

Fig. 7 shows the effect of pregnancy on TLC. For both groups, there were fluctuations in TLC during the course of the experiment. The pregnant group had significantly (P < 0.05) higher value of TLC at GD14 and lower values at GD21 and GD28, compared to the respective control group values (Fig. 7).

3.2.5 Differential Leukocyte Count (DLC)

The pregnant rabbits had significantly (P<0.05) higher lymphocytes ratio at GD0, GD14, GD 21 and GD 28 compared to nonpregnant rabbits (Fig. 8).

The neutrophil ratio significantly (P<0.05) lower at GD0, GD14 and GD28 compared to nonpregnant rabbits values (Fig. 9). The pregnant group had significantly (P < 0.05) lower monocyte ratio in GD7 compared to nonpregnant rabbits (Fig. 10). The pregnant group maintained significantly (P < 0.05) lower of eosinophils ratio at GD7 and increased in GD0 and GD21compared to respective nonpregnant rabbits value (Fig. 11). No significant deference between pregnant and nonpregnant groups in basophiles ratio in rabbits during the course of the experiment (Fig. 12).

3.3 Serum Metabolites

The effects of stage of gestation on biochemical constituents in rabbits are shown in Table 3.

3.3.1 Serum total proteins

The total plasma proteins value was significantly (P < 0.05) lower in pregnant rabbits at GD7, GD14, GD21 and GD28 compared to respective nonpregnant group values shown in (Fig. 13).

3.3.2 Serum albumin

The pregnant group significantly (P < 0.05) lower albumin concentration on GD28 compared to the respective values of control rabbits (Fig.14).

3.3.3 Serum cholesterol

The pregnant group had significantly (P<0.05) higher cholesterol values at GD0, GD7, GD14 and lower values at GD21 compared to respective nonpregnant group values (Fig.15).

3.3.4 Plasma osmolality

The pregnant group maintained significantly (P<0.05) lower osmolality value at GD14 and higher values at GD0, GD7, GD21 and GD28, compared to respective nonpregnant group values (Fig.16).

3.3.5 Serum cortisol

The pregnant group had significantly (P < 0.05) higher serum cortisol levels at GD0, GD7, GD14, GD21 and GD28 compared to the nonpregnant group values (Fig. 17).

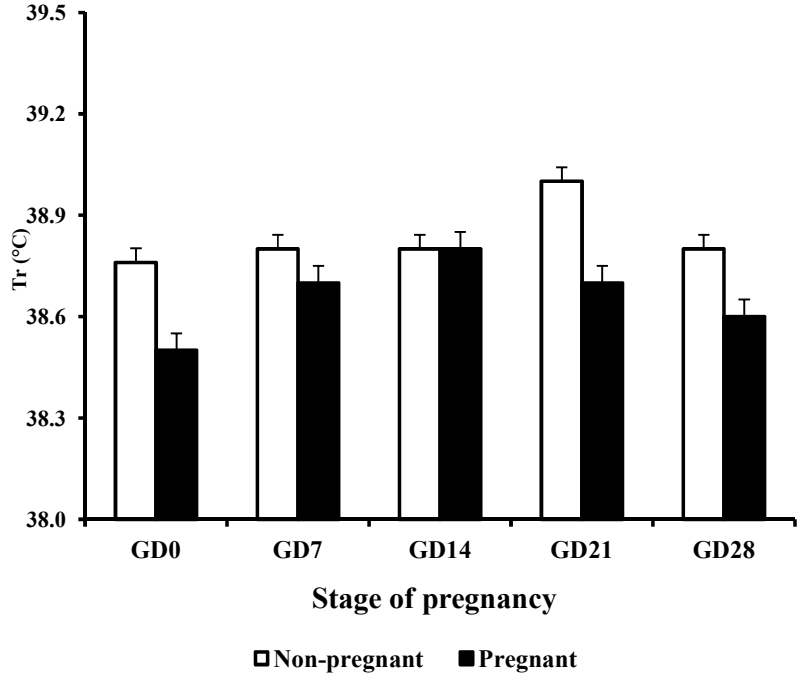

Fig. 1. Effect of stage of pregnancy on rectal temperature (Tr) in rabbits

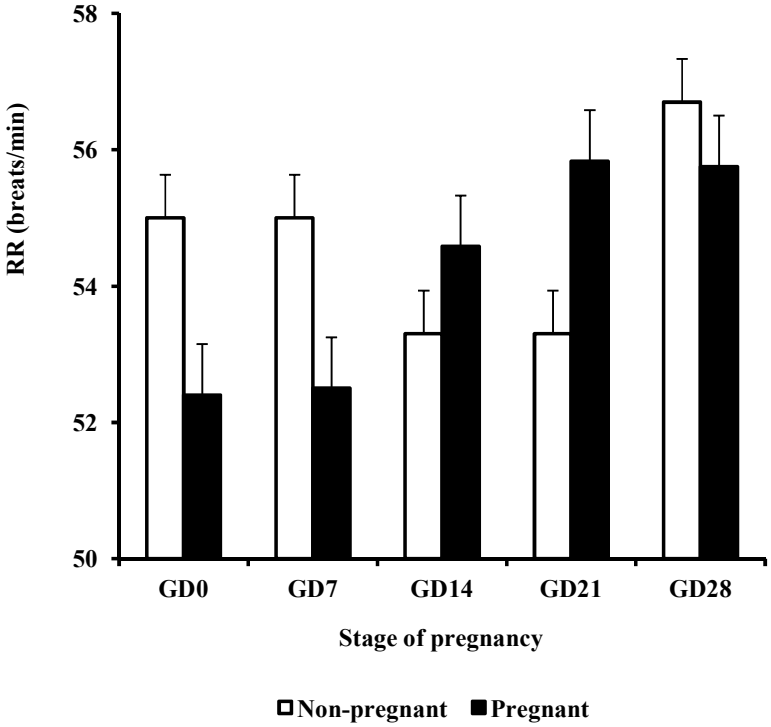

Fig. 2. Effect of stage of pregnancy on respiratory rate (RR) in rabbits

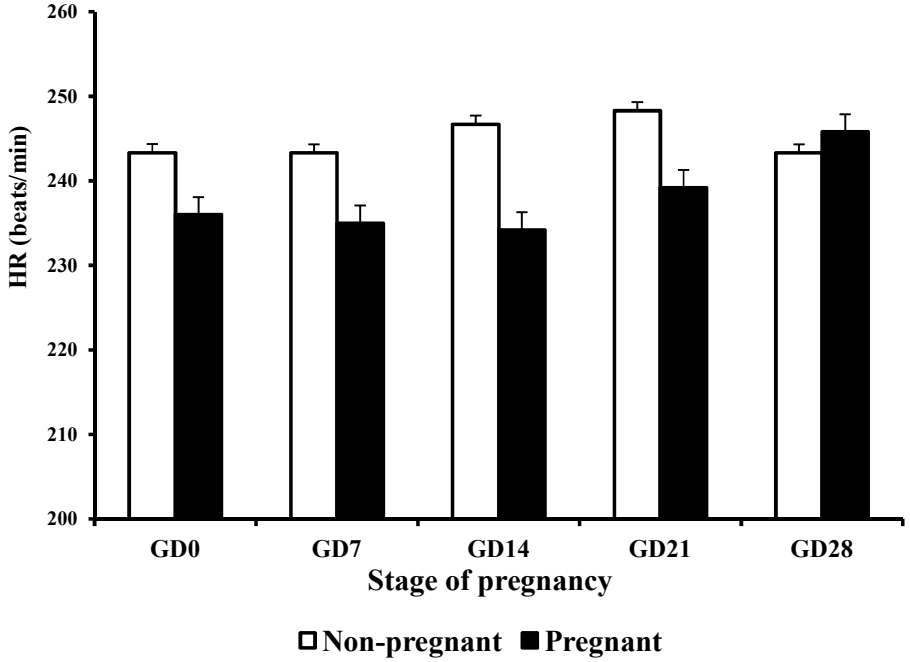

Fig. 3. Effect of stage of pregnancy on heart rate (HR) in rabbits

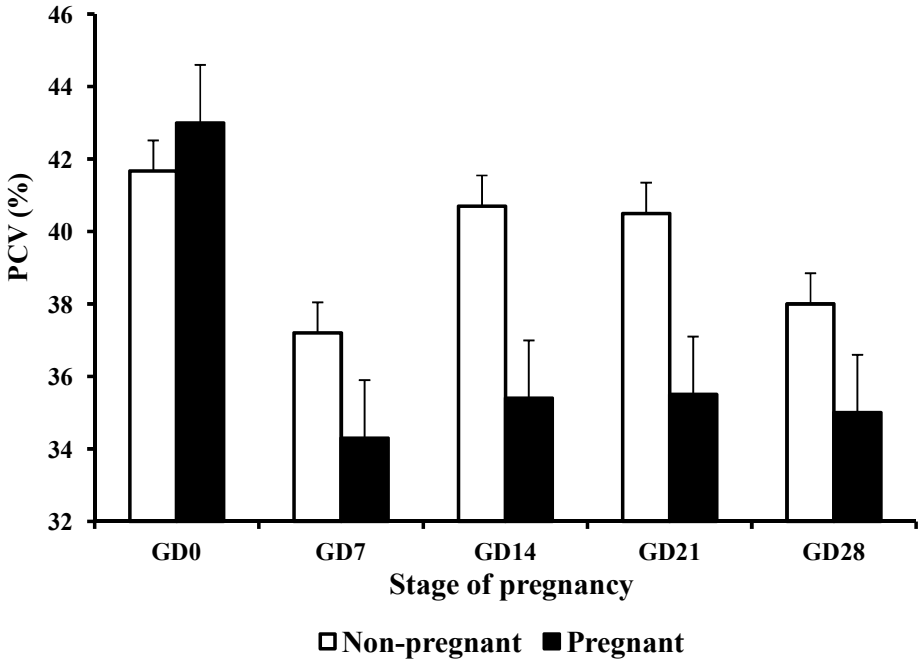

Fig. 4. Effect of stage of pregnancy on packed cells volume (PCV) in rabbits

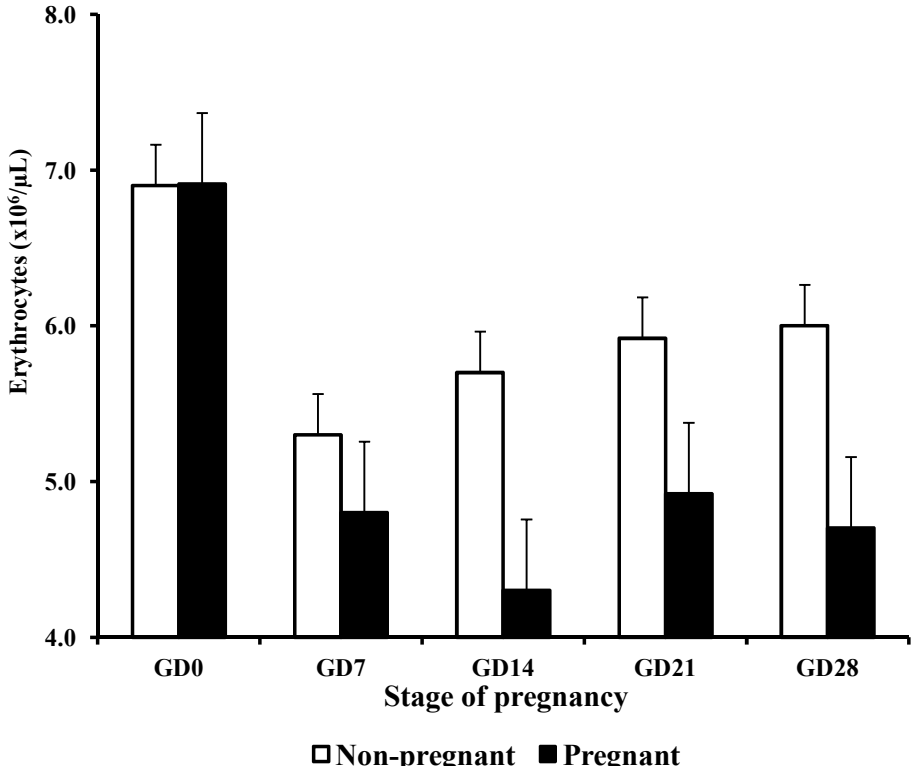

Fig. 5. Effect of stage of pregnancy on erythrocytes count in rabbits

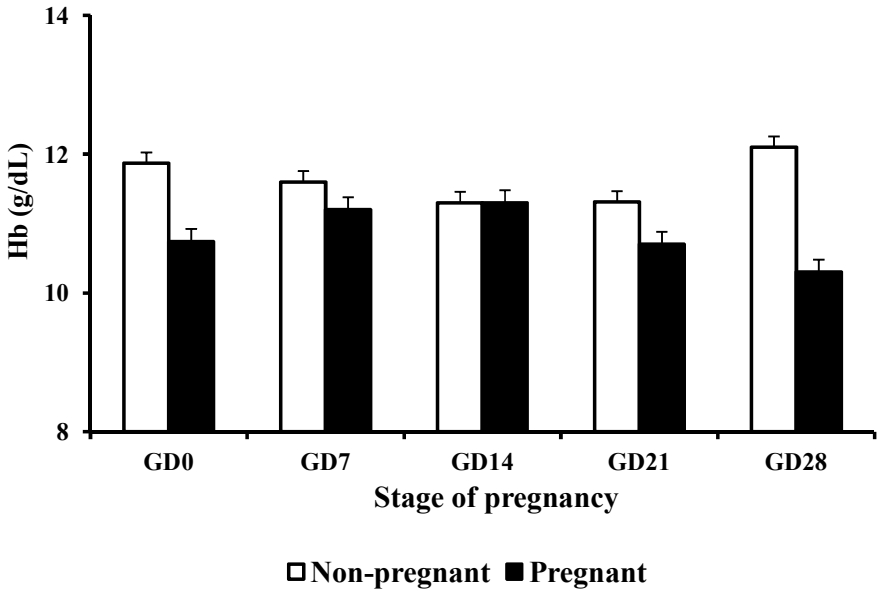

Fig. 6. Effect of stage of pregnancy on haemoglobin concentration (Hb) in rabbits

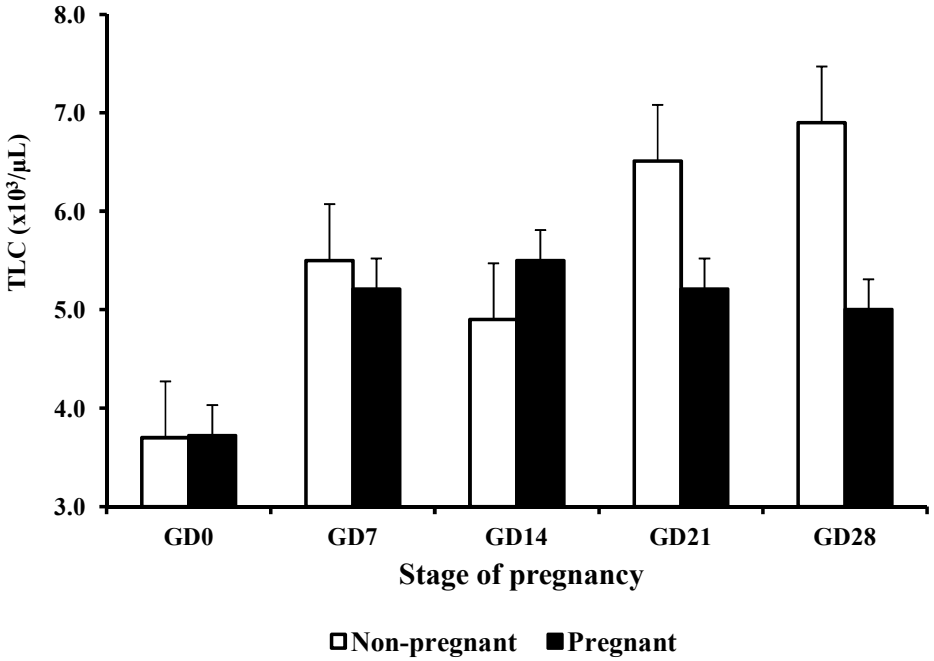

Fig. 7. Effect of stage of pregnancy on total leukocyte count (TLC) in rabbits

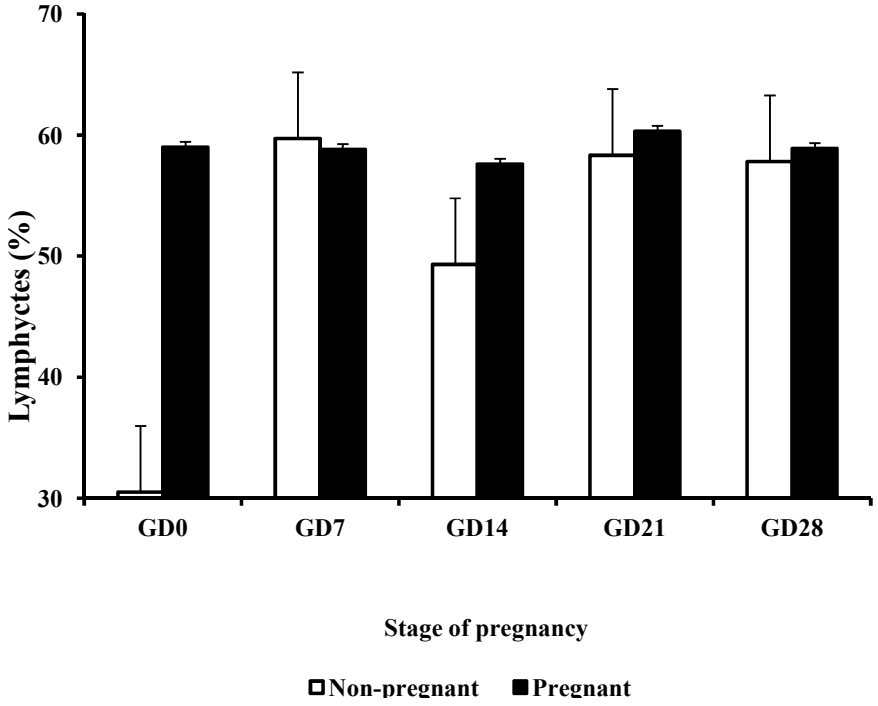

Fig. 8. Effect of stage of pregnancy on the lymphocytes ratio of rabbits

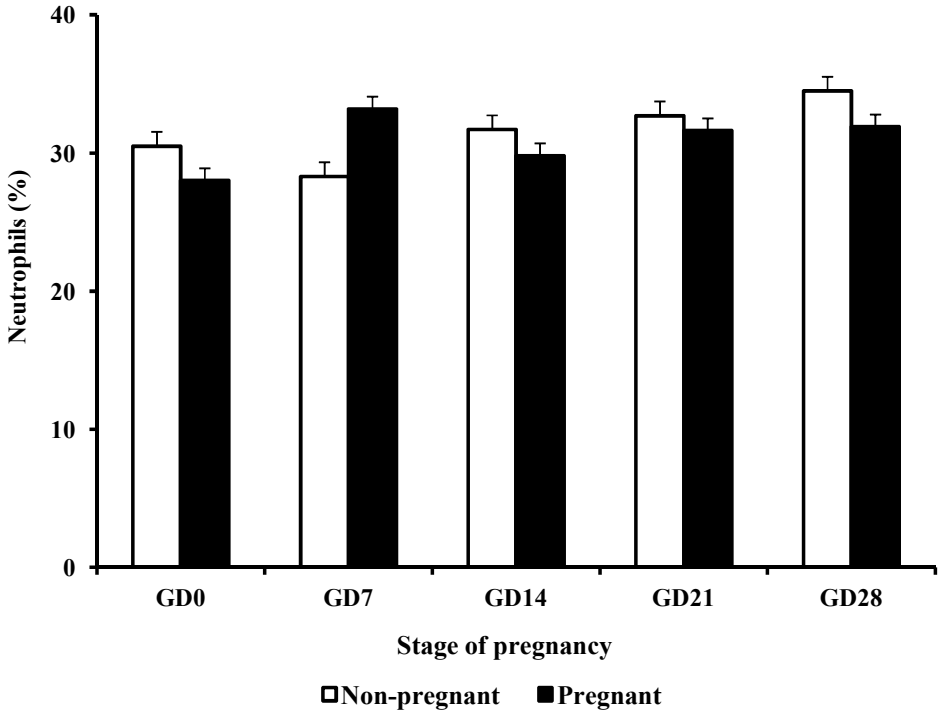

Fig. 9. Effect of stage of pregnancy on the neutrophils ratio of rabbits

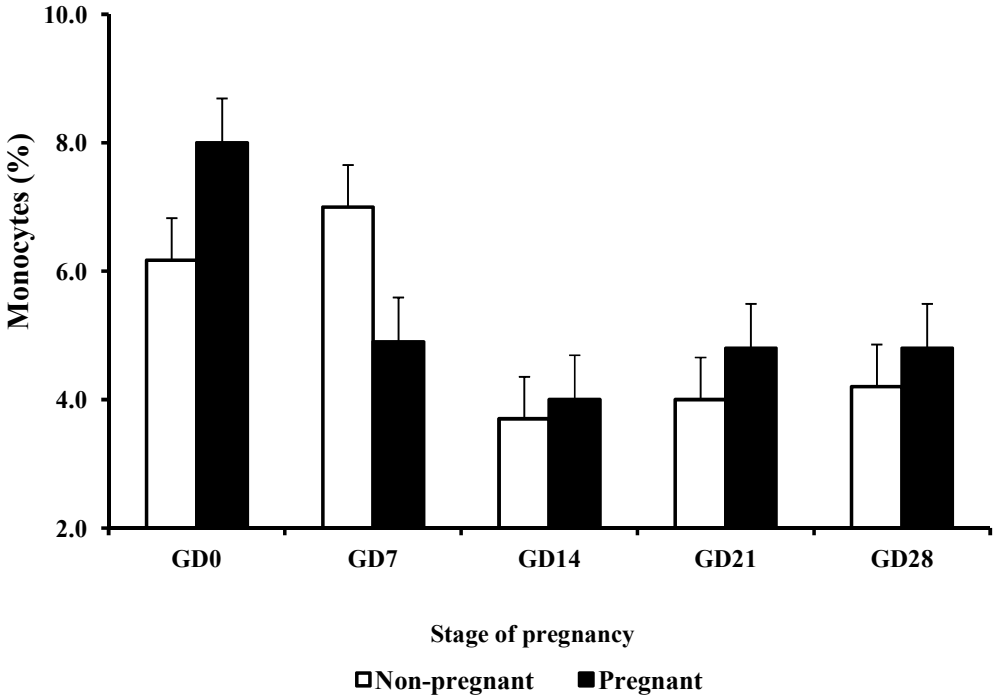

Fig. 10. Effect of stage of pregnancy on the ratio of monocytes in rabbits

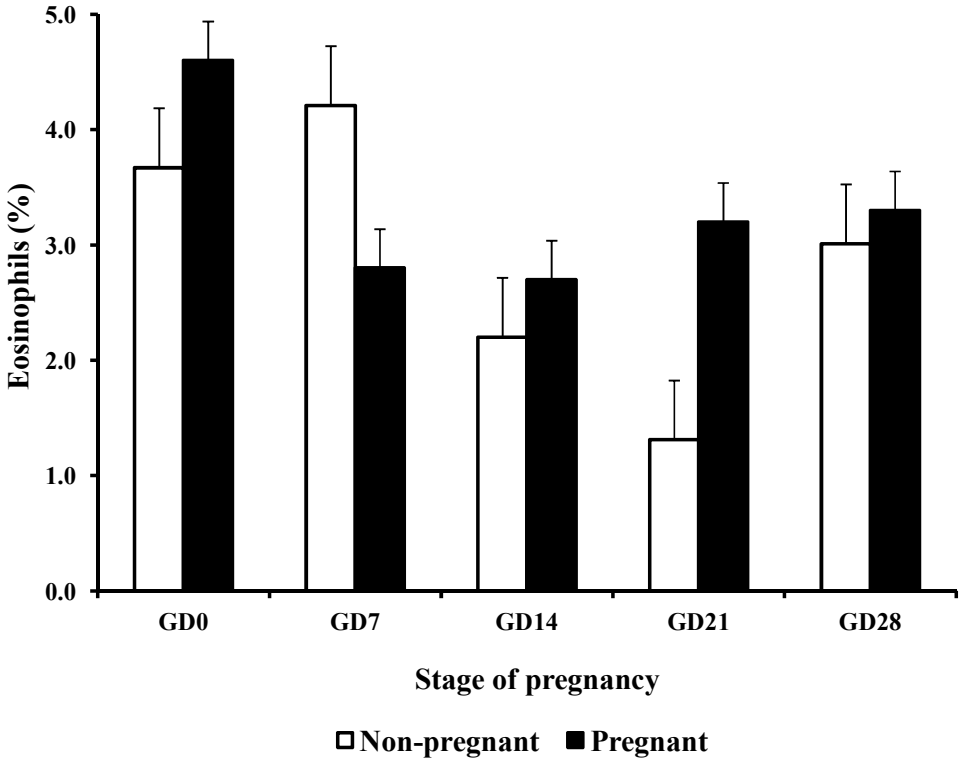

Fig. 11. Effect of stage of pregnancy on the ratio eosinophils in rabbits

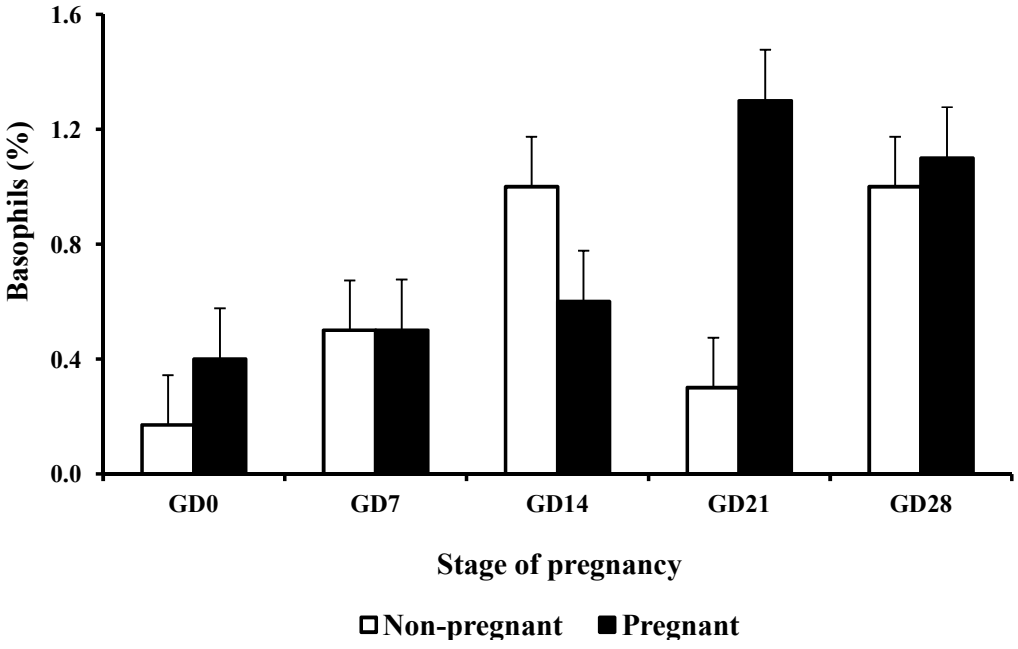

Fig. 12. Effect of stage of pregnancy on basophils in rabbits

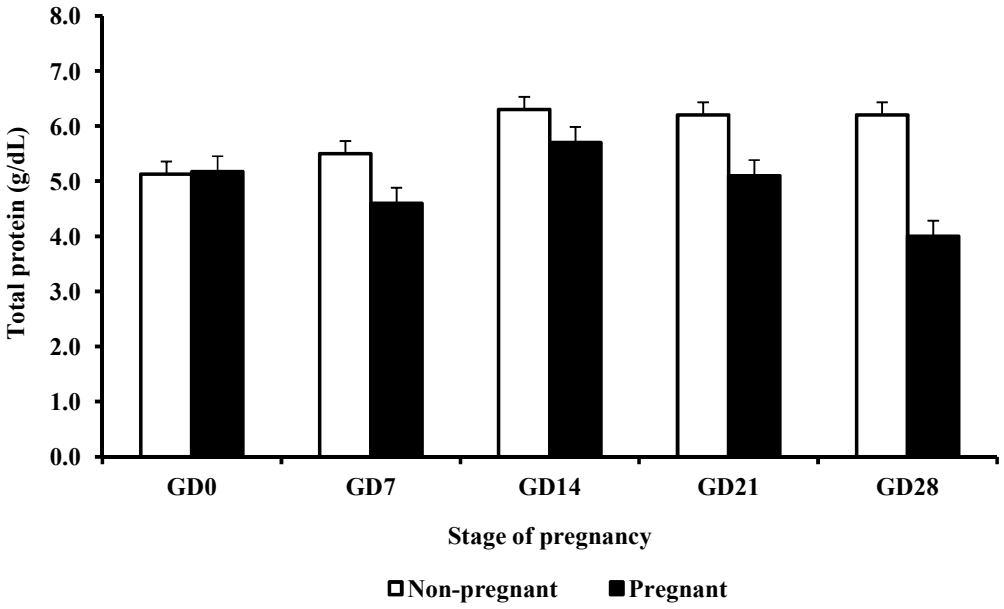

Fig. 13. Effect of stage of pregnancy on serum total protein concentration in rabbits

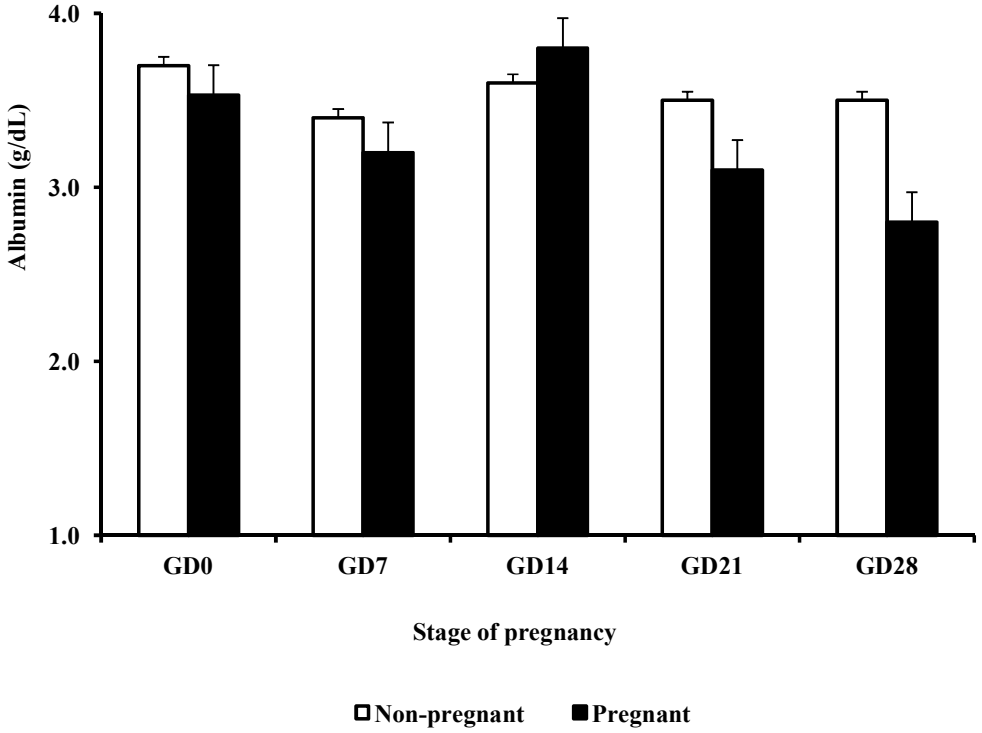

Fig. 14. Effect of stage of pregnancy on serum albumin concentration in rabbits

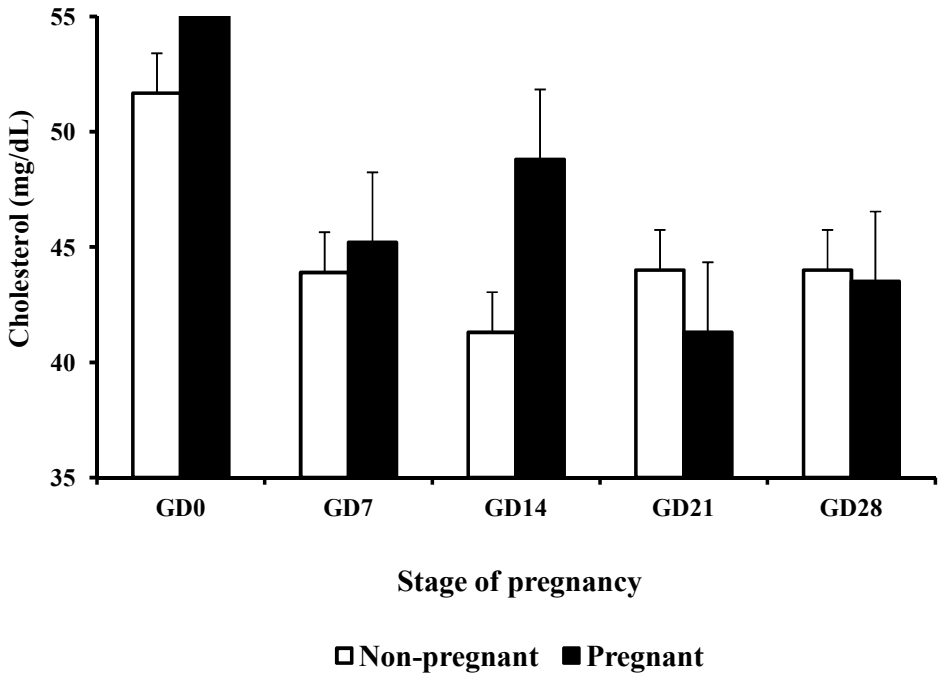

Fig. 15. Effect of stage of pregnancy on serum cholesterol level in rabbits

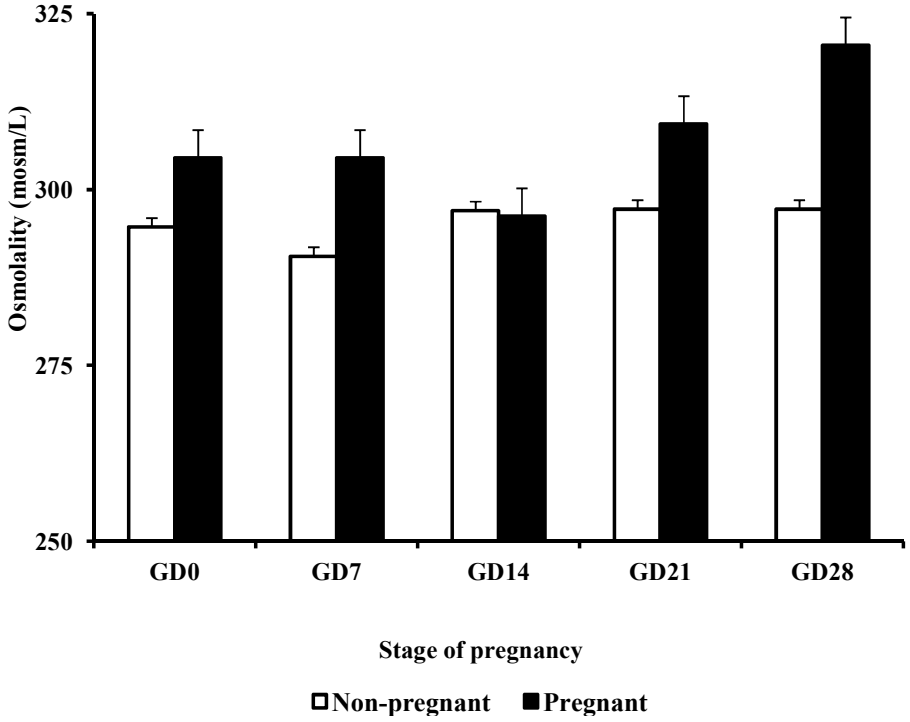

Fig. 16. Effect of stage of pregnancy on serum osmolality in rabbits

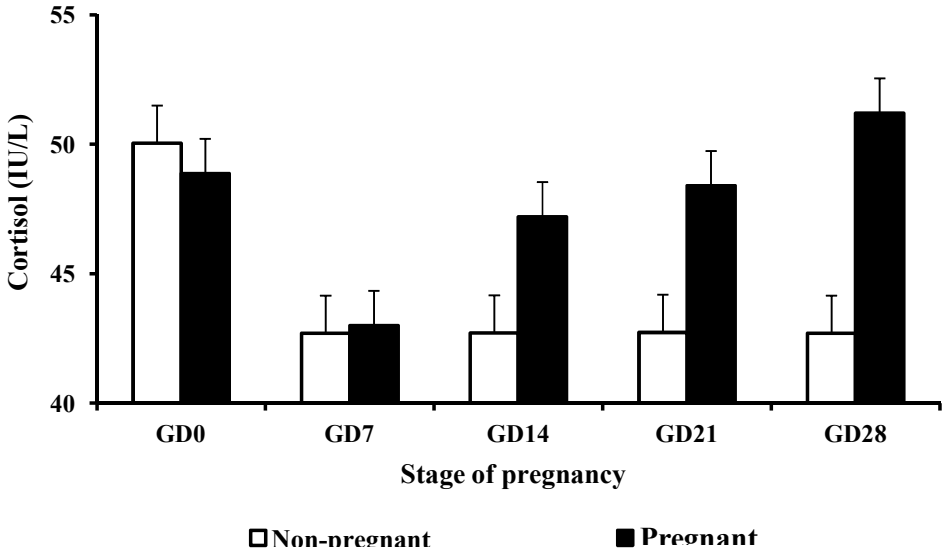

Fig. 17. Effect of stage pregnancy on serum cortisol level in rabbits

4. DISCUSSION

In this study, the effects of pregnancy on thermoregulation, haematological parameters, serum biochemical parameters and cortisol level were investigated on gestation days GD0, GD7, 14, 21 and GD28 in rabbits. The results showed that the rectal temperature (Tr) in pregnant rabbits tended to be lower at GD28. The decrease in Tr near term may have resulted from changes of thermogenic hormones and metabolic rate. The most important endocrine factors modulating obligatory thermogenesis are thyroid hormones [21,22]. The increase in thyroxin binding globulin (TBG) by liver during pregnancy and decreases in free T3 and T4 level during second and third trimester was reported in pregnant women [23]. Basal metabolic rate reflects the activity of mitochondria and is coupled the generation of high energy in ATP [24]. Thyroid hormones are major regulators of oxidative metabolic processes [21,22]. The current results are generally in line with the findings of Jilge et al. which reported that the core body temperature of rabbits gradually decreased during the last two third of pregnancy [25]. A decrease in body core temperature was reported in pregnant rats [26,27].

The respiratory rate (RR) values of rabbits increased significantly ($P<0.05$) during the course of pregnancy at GD14 and GD21. Increases in RR during gestation most likely occur in response to progesterone related sensitization of respiratory centre to carbon dioxide [28]. Minute ventilation is increased at term pregnancy mainly due to increase of tidal volume (40%) and respiratory rate (15%) [29]. However, Chesnutt reported that RR was not altered during pregnancy in humans [4]. The difference may be related to species specific. The present results indicate that the heart rate (HR) values were significantly ($P<0.05$) decreased in GD0, GD7, GD14 and GD21 and increased during the course of pregnancy in GD28. The inhibition of resting parasympathetic activity and increases of sympathetic excitation during gestation might be the mechanisms involved in increase of HR during normal pregnancy [30]. Pregnancy induces sympathetic stimulation and decreases basal parasympathetic tone in rats [31]. Sympathetic excitation increases slope of pacemaker potential by influencing the sodium and T-type of calcium currents. This causes the S.A node cells to reach threshold more rapidly and accelerates heart rate [32]. The heart rate increases progressively throughout the pregnancy to reach maximum rate in third trimester, the overall change in heart rate represents 20% to 25% increase over baseline [33]. Baroreflex dysfunction during pregnancy has been documented in many species including rabbits, rats and dogs [34]. Furthermore, in humans' pregnancy increases in sympathetic nerve firing and decreases in both basal parasympathetic activity and Baroreflex gain; these changes are exaggerated in women with pre-eclampsia [35].

Table 1. Effects of stage of pregnancy on rectal temperature (Tr), respiratory rate (RR) and heart rate (HR) in rabbits

Parameters	GD0		GD7		GD14		GD21		GD28	
	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant
Tr. (°C)	38.76±0.22^{a}	38.50±0.07^{a}	38.80±0.26^{a}	38.70±0.23^{a}	38.80±0.28^{a}	38.80±0.34^{a}	39.00±0.14^{a}	38.70±0.32^{a}	38.80±0.11^{a}	38.60±0.30^{a}
RR (breaths/min)	55.00±1.67^{a}	52.40±2.61^{b*}	55.00±1.67^{a}	52.50±2.28^{b*}	53.30±3.93^{b}	54.58±2.43^{a*}	53.30±2.42^{b}	55.83±2.89^{a*}	56.70±1.64^{a}	55.75±2.06^{a}
HR (beats/min)	243.33±10.33^{a}	236.00±5.48^{b*}	243.30±10.33^{a}	235.00±6.74^{b*}	246.70±12.11^{a}	234.20±12.40^{b*}	248.30±13.29^{a}	239.20±11.64^{b*}	243.30±13.66^{a}	245.80±10.84^{a}

*Mean values within the same row bearing different superscripts are significantly different at P<0.05. * P≤0.05*

Table 2. Effects of stage of pregnancy on haematological parameters in rabbits

Parameters	GD0		GD7		GD14		GD21		GD28	
	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant
PCV (%)	41.67±5.57^{b}	43.00±3.61^{a*}	37.20±1.94^{a}	34.30±2.10^{b*}	40.70±4.50^{a}	35.40±3.40^{b*}	40.50±3.15^{a}	35.50±2.20^{b*}	38.00±1.90^{a}	35.00±2.70^{b*}
Erythrocytes (x10^{6}/μL)	6.90±0.02^{a}	6.91±0.02^{a}	5.30±0.69^{a}	4.80±0.58^{b*}	5.70±0.60^{b}	4.30±0.43^{b*}	5.92±0.22^{a}	4.92±0.91^{b*}	6.00±0.43^{a}	4.70±0.72^{b*}
Hb (g/dL)	11.87±0.47^{a}	10.74±0.91^{b*}	11.60±0.58^{a}	11.20±0.83^{a}	11.30±0.85^{a}	11.30±0.92^{a}	11.31±1.07^{a}	10.70±1.23^{b*}	12.10±1.05^{a}	10.30±0.99^{b*}
TLC (x10^{3}/μL)	3.70±0.09^{a}	3.72±0.08^{a}	5.50±1.11^{a}	5.21±0.56^{a}	4.90±0.42^{b}	5.50±1.38^{a*}	6.51±1.30^{a}	5.21±0.81^{b*}	6.90±0.61^{a}	5.00±1.01^{b*}
Lymphocytes (%)	30.50±2.73^{b}	59.00±2.00^{a*}	59.71±2.66^{a}	58.81±1.23^{b*}	49.30±6.92^{b}	57.60±5.18^{a*}	58.32±2.16^{b}	60.32±2.84^{a*}	57.80±4.26^{b}	58.90±1.44^{a*}
Neutrophils (%)	30.50±2.34^{a}	28.00±2.34^{b*}	28.30±2.07^{b}	33.20±1.64^{a*}	31.70±3.08^{a}	29.80±5.17^{b*}	32.70±2.50^{a}	31.62±1.78^{a}	34.50±3.08^{a}	31.90±2.23^{b*}
Monocytes (%)	6.17±1.33^{b}	8.00±0.71^{a*}	7.00±00.89^{a}	4.90±00.79^{b*}	3.70±1.21^{a}	4.00±0.89^{a}	4.00±0.89^{a}	4.80±0.75^{a}	4.20±0.75^{a}	4.80±0.62^{a}
Eosinophils (%)	3.67±1.37^{b}	4.60±0.55^{a*}	4.21±1.17^{a}	2.80±0.72^{b*}	2.20±1.17^{a}	2.70±0.89^{a}	1.31±0.52^{b}	3.20±0.72^{a*}	3.01±0.63^{a}	3.30±0.65^{a}
Basophils (%)	0.17±0.41^{a}	0.40±0.55^{a}	0.50±0.55^{a}	0.50±0.52^{a}	1.00±0.89^{a}	0.60±0.79^{a}	0.30±0.52^{a}	1.30±0.96^{a}	1.00±0.89^{a}	1.10±0.67^{a}

Mean values within the same row bearing different superscripts are significantly different at P<0.05

Table 3. Effects of stage of pregnancy on serum biochemical constituents in rabbits

Parameters	GD0		GD7		GD14		GD21		GD28	
	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant
Total protein (g/dL)	5.13±0.81^{a}	5.17±1.46^{a}	5.50±1.19^{a}	4.60±0.22^{b*}	6.30±1.28^{a}	5.70±0.89^{b*}	6.20±0.43^{a}	5.10±0.88^{b*}	6.20±0.43^{a}	4.00±0.93^{b*}
Albumin (g/dL)	3.70±0.26^{a}	3.53±0.41^{a}	3.40±0.31^{a}	3.20±0.36^{a}	3.60±0.30^{a}	3.80±0.50^{a}	3.50±0.40^{a}	3.10±0.26^{b*}	3.50±0.40^{a}	2.80±0.83^{b*}
Cholesterol (mg/dL)	51.67±9.07^{b}	58.67±8.08^{a*}	43.90±5.1^{b}	45.20±10.7^{a}*	41.30±3.27^{a}	48.8±12.81^{b*}	44.00±3.95^{a}	41.30±4.18^{b*}	44.00±3.95^{a}	43.5±5.28^{a}
Osmolality (moms/L)	294.67±6.81^{b}	304.50±14.70^{a}*	290.50±2.18^{b}	304.50±14.70^{a*}	297.00±9.21^{a}	296.20±11.70^{b*}	297.20±15.29^{b}	309.30±5.92^{a*}	297.20±15.29^{b}	320.50±8.99^{a*}
Cortisol (IU/L)	50.04±9.76^{a}	48.87±5.58^{b*}	42.70±12.19^{a}	43.00±3.28^{a}	42.71±12.19^{b}	47.20±4.34^{a*}	42.74±12.19^{b}	48.40±4.52^{a*}	42.70±12.19^{b}	51.20±5.72^{a*}

*Mean values within the same row bearing different superscripts are significantly different at P<0.05. * P≤0.05*

The experimental data showed significant reduction in erythrocyte count, Hb concentration and PCV values at GD14, GD21 and GD 28 during the course of pregnancy. The reductions in these parameters could be related to expansion of plasma volume resulting in haemodilution [36,37]. This is associated with hormonal changes which promote fluid retention and iron deficiency during pregnancy [38]. The renin-angiotensin system regulates salt and water homeostasis in the body. There is an increase in both renin and angiotensin levels during pregnancy [39]. Similarly, reduction in erythrocyte count, Hb and PCV values were reported during pregnancy in *New Zealand White* rabbits [40]. An increase in plasma volume in the second trimester associated with progressive decline in Hb concentration and PCV values was reported during gestation in women [28], rats [41]. The current results are consistent with the previous reports in *Angora* rabbits [42].

The present results showed that the TLC, lymphocytes, and eosinophils values increased significantly at GD0, GD14 and GD21during the course of pregnancy. The increase in TLC could be related to bone marrow granulopoiesis. Luppi et al. reported that pregnancy alters the representation of leukocyte and ratios associated with peripheral leukocyte activation [43]. The current results are in agreement with the findings of Wells et al. and his friends who found a significant increase in lymphocyte and monocyte ratios in mid-gestation and a decrease in late gestation in rabbits [44]. Many researchers reported increase in TLC in early gestation in rats [45,46,47] and humans [48,49,50]. The significant reduction in TLC count, neutrophil and monocyte ratios observed at GD7, GD14, GD21and GD 28 in the present study may be secondary to haemodilution. These results are in line with the findings of Wells et al. [44] and Kim et al. [40] in pregnant rabbits and rats Kim et al. [51].

The serum concentrations of total protein and albumin significantly decreased during the course of pregnancy at gestation days GD21, and GD28. This pattern could be related to active transport of amino acids across the placenta to fulfill the needs of the developing foetus [23] reported that during gestation, protein catabolism is deceased as fat store are used to provide for energy [52]. Decreased albumin level has been reported in liver and renal disorders and malnutrition [2]. Similarly, decreases were recorded in *New Zealand White* rabbits and Angora rabbits [42]. Pregnant women, after 20 weeks of gestation, increase total proteins and albumin excretion [53]. The current results confirm findings previously reported in pregnant rabbits [54], rats [55] and pregnant women [56].

The serum cholesterol values were significantly ($P<0.05$) reduced in GD21 during pregnancy in the present study. This decrease in cholesterol level could be associated with decline in food consumption with the advance of pregnancy. A reduction in all types of lipids during the course of pregnancy was reported in rabbits [42] and rats [41]. Also, the present study was reported significant increased in pregnant rabbits at GD0, GD7 and GD14. That could suggest that changes in lipid metabolism to accommodate the needs of developing foetus. Bartels et al. reported that, in women, cholesterol levels were elevated in all trimesters of pregnancy [57]. That could be spices specific. The results indicate that serum osmolality increased in gestation days GD21 and GD28 and decreased in GD 14 in rabbits. This may suggest that the control of blood volume and salt metabolism is unstable during pregnancy in rabbits. The decrease in serum osmolality in GD 14 suggests water and salt retention in mid gestation and increase in plasma volume that reduced osmolality. Changes in the regulation of water and salt metabolism were observed in pregnant women [58,59]. The current results are consistent with the findings reported previously in women [60].

The results showed significant increase in serum cortisol level during mid and late gestation period. The increase of cortisol levels could be related to stimulation of production of corticotrophin releasing factor by the placenta in pregnant rabbits [61]. Total cortisol levels increase at the end of first trimester and third times compare to nonpregnant values at the end of pregnancy [62]. The current results are in agreement with the finding of Michel et al. [63] who found that gestation in guinea pigs increased baseline cortisol levels. Kammerer et al., reported that, stimulation of production of placental corticotrophin releasing factor increased in pregnant women [64].

5. CONCLUSIONS

The study concluded that pregnancy induced modifications in some physiological responses and cortisol levels in mid and late stage of pregnancy, furthermore pregnant rabbit model can be suitable for maternal health monitoring 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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