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Reproductive Characteristics and Oxidative Stress Markers in Female Cavies (*Cavia Porcellus*) Exposed to High Ambient Temperature

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Abstract

Heat stress affects animal reproductive characteristics, and subsequencely induce low productivity and enormous economic loss. The aim of this study is to investigate the effects of heat stress on reproductive function and hepatic oxidative stress markers of female Guinea pig (Cavia porcellus). Forty-eight (48) adult female cavies 4 months old, averagely weighing 400 ± 10 g were divided into four groups of 12 cavies each. One group (control) was maintained to ambient temperature (20 - 25 °C), while other groups (Groups 2, 3 and 4) were exposed daily for 6 h to temperature at 32 ± 1 °C, 39 ± 1 °C and 46 ± 1 °C respectively. At the end of the 90 days of exposure including 60 days of gestation, animals were sacrificed and data collected on reproductive characteristics and hepatic oxidative stress markers. Analysis showed a significant ($p < 0.05$) decreased of the relative weight of ovary, fœtus weight and length of rump in heat stress animals compared to control. Inversely, no significant difference was noted in serum content of progesterone. Heat stress significantly ($p < 0.05$) increased antioxidant enzymes activities (superoxide dismutase and catalase) and serum level of malondialdehyde (MDA), nitric oxide (NO) and HSP-40. In conclusion, exposition of female guinea pig to 46 ± 1 °C for 90 days had induced oxidative stress that subsequencely affected reproductive paramaters.

Keywords: Guinea Pig; Heat Shock Proteins; Heat Stress; Oxidative Stress; Reproduction

Introduction

Temperature is an environmental factor affecting animal growth and reproduction. For most farm animals, a mean daily temperature is ranged from 10 to 20 °C. Above previous temperature, warm-blooded animals reduce caloric intake and increase respiratory frequency that provoke an oxidative stress.

Heat stress is a physiological condition where animals cannot longer regulate their internal temperature and this means that animals are not able to regulate their heat homeostasis passively [1]. It mainly occurs when animals are exposed to high ambient temperatures, high direct and indirect solar radiation [2]. Heat stress is one of the most important stressors especially in hot regions of the world. In many mammals such as cow [3], rabbit [4] and sheep [5], high environmental stress contributes to biological functions. Guinea pigs have little functional sweet gland [6] and lack the ability to manufacture their own vitamin C [7] that acts as antioxidant in the body in oxidative stress situation. These conditions expose them to heat stress which is considered as an important factor influencing their fertility, reproductive and physiological traits [8]. Negative effects of prolonged exposition of male cavies to heat stress have been demonstrated to impair sperm characteristics, increase the oxidative stress markers [8].

Inversely, no study has been done to evaluate the effects of heat stress on female guinea pig reproductive and physiological traits. Nevertheless, several female species such as cow [3], rabbit [1,4] and sheep [5] have been well studies for a long time. Therefore, the physiological response such as biochemical parameters and antioxidant markers were significantly increased in indigenous sheep exposed to hyperthermia [5]. Heat stress has been found to decrease serum progesterone levels, leading to implantation failures and pregnancy loss in mice [9]. Wolfenson, *et al.* [10] reported the effect of heat stress on cattle ovarian functions. Lushchak, *et al.* [11] and Oyinloye, *et al.* [12] showed that the implantation site and embryonic development are impaired due to heat stress.

Regarding the adverse effects of heat stress on physiological parameters in mammals, the present study is to examine the effects of heat stress on the reproductive parameters and oxidative stress markers in female guinea pig (*Cavia porcellus*).

Materials and Methods

Experimental Animal and Feeding

A total of forty-eight (48) 4 months' female cavies, averagely weighing 400 ± 10 g were obtained from the Teaching and Research Farm (TRF) of the University of Dschang, Cameroon. Eight adult males were used only as sires and were not treated. Throughout the experimental period (90 days), drinking water and commercial complet feed from Cameroon Feed Company (S P C) containing 16% crude protein, 2350 kcal/kg metabolisable energy and 7% crude fiber were providing *ad libitum.*

Experimental Design

Before starting the experiment, the animals were weighed and identified using numbered earrings. The 48 female cavies were divided into 4 groups of 12 cavies each, with comparable average body weight. The animals were randomly assigned to temperature controlled cages (control group 1: ambient temperature 20 - 25 °C, group 2: 32 ± 1 °C, group 3: 39 ±1 °C and group 4: 46 ±1 °C). During 90 days of exposure, all groups were exposed to heat from 8.00 am to 2.00 pm (6 h) daily [13]. Exactly at 30 days of experiment, mating was done by placing one non-treated male into cages containing six treated females.

Sacrifice of Cavies and Organs Collection

At the end of the trial, each animal was anesthetized using ether vapor before sacrifice. Blood was collected from the ventral aorta and stored at room temperature. Serum was subsequently isolated 8 hours later for the estimation of serum level of progesterone and heat shock proteins (HSP-40). Fetuses and organs (liver and ovaries) were carefully removed and weighed separately using a scale of 160 g and 10^{-3} g precision. The same section of liver of each sacrificed animal was crushed in a corresponding volume of NaCl 0.9% to obtain a 15% homogenate. The resulting homogenate was centrifuged at 3000 rpm for 30 min and aliquot supernatants were used for biochemical estimation of hepatic oxidative stress markers (superoxide dismutase (SOD), catalase (CAT), totals peroxidases (POX) activities, malondialdehyde (MDA) and Nitric oxide (NO) concentrations). The following characteristics were also evaluated: number, weight and length of fetuses, viability and mortality index.

Serum Progesterone and Heat Shock Protein (HSP) and Hepatic Oxidaive Stress Markers Evaluation

The serum progesterone and heat shock protein-40 (HSP 40) levels were determined using respectively appropriate commercial Elisa kits: Omega diagnostics kit (Scotland, United Kingdom) and Guinea Pig Heat Shock Protein 40 (HSP-40) ELISA kit ABclonal Technology. Oxidative stress markers like superoxide dismutase (SOD), catalase (CAT), totals peroxidases (POX) activities; malondialdehyde (MDA) and nitric oxide (NO) concentrations were measured using the spectrophotometer (GENESYS 20.0) and according to the respectively methods [14-18].

Histology of Ovaries

The left ovary of each female was fixed in formol solution (10%) for one week and then washed, dehydrated in ascending grade alcohol bath, clarified in xylene immersion, and embedded in paraffin. Sections of 5 µm were stained with hematoxylin-eosin for histological observations under a light microscope (HE: 200 X).

Statistical Analyses

Statistical analyses were performed with SPSS for Windows software 20.0 software. Difference between treatments was assessed using one-way ANOVA followed by Duncan post hoc test. The limit of signification was 5% and the results were expressed as mean ± standard deviation.

Results

Effects of High Ambient Temperature on Fœtus Toxicity Parameters in Guinea Pig

Fetuses' toxicity parameters in guinea pig exposed to hyperthermia for 90 days are shown in Table 1. No statistical differences ($p > 0.05$) in the number of fetuses per dam, number of corpora lutea, number of implantation sites, number of pre- and post- resorptions, number of alive or dead fetuses and number of placentae were observed. Nevertheless, the number of fetuses per dam, number of corpora lutea, number of implantation sites, number of alive or dead fetuses, and number of placentae are decreased with the higher level of temperature (46±1°C). In the reference to the control, ovaries weight decreased significantly ($p < 0.05$) in the group of female exposed to the highest level of temperature (46±1°C). Inversely, the placenta weight increased significantly ($p < 0.05$). In comparison to the control, ovaries weight decreased significantly ($p < 0.05$) and placenta weight increased significantly ($p < 0.05$) in the group of female exposed to the highest level of temperature (46 °C ± 1).

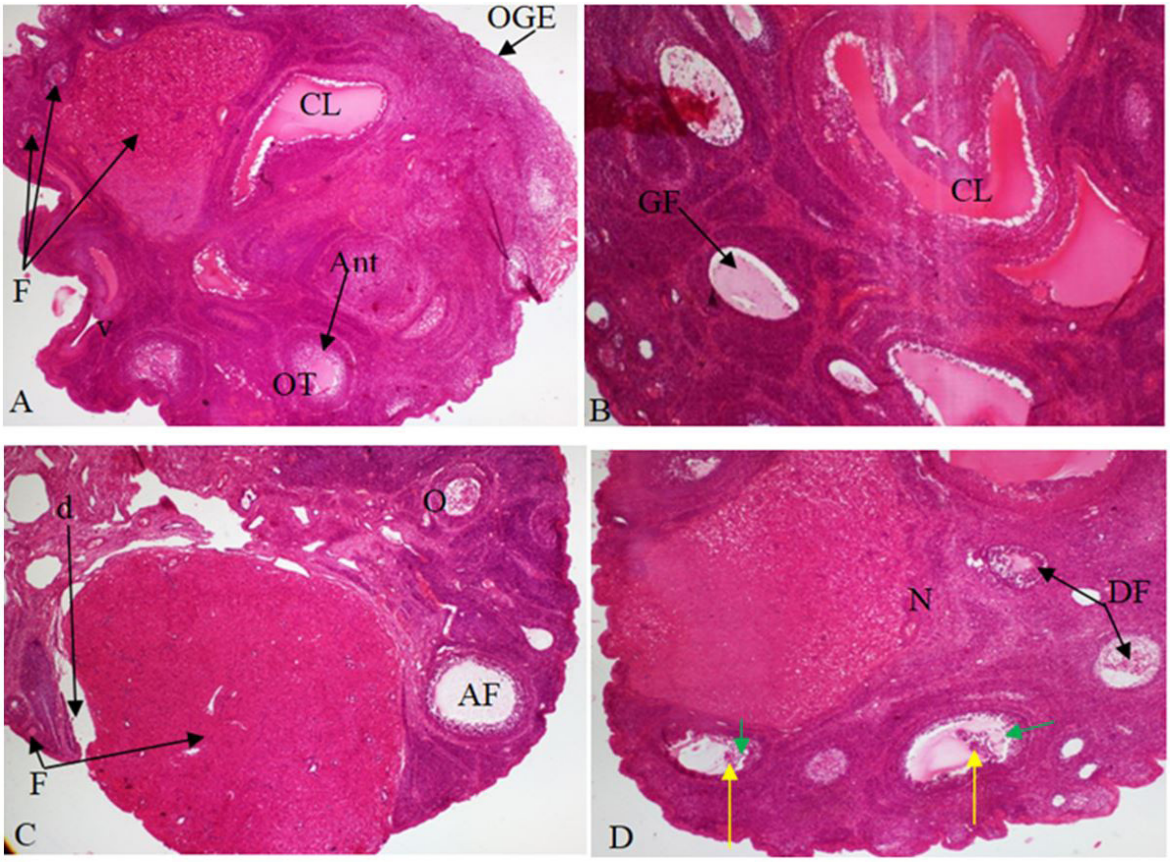
Parameters	Level of temperature (°C)				P-value
	20-25	32 ± 1	39 ± 1	46 ± 1	
Number of fetuses/dam	2.25 ± 0.50	2.25 ± 0.50	2.17 ± 0.41	2.00 ± 0.00	0.58
Number of corpus luteum/dam	2.25 ± 0.50	2.25 ± 0.50	2.17 ± 0.41	2.00 ± 0.00	0.58
Ovaries weight	0.013 ± 0.001[a]	0.014 ± 0.002[a]	0.012±0.001[ab]	0.010 ± 0.03[b]	0.04
Number of implantation sites	2.25 ± 0.50	2.25 ± 0.50	2.17 ± 0.41	2.00 ± 0.00	0.6
Number of pre-implantation resorption	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1
Number of post-implantation resorption	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1
Number of Viable fœtus	2.25 ± 0.50	2.25 ± 0.50	2.17 ± 0.41	2.00 ± 0.00	0.6
Number of death fœtus	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1
Number of placenta	2.25 ± 0.50	2.25 ± 0.50	2.17 ± 0.41	2.00 ± 0.00	0.66
Placenta weight (g)	4.64 ± 0.94[c]	6.25 ± 0.95[b]	5.60 ± 0.89[b]	10.00 ± 1.66[a]	0

[a,b] On the same line, means with the same letter were not significantly different ($P > 0.05$)

Table 1: Ovary weight and foetus index in female guinea pig exposed to hyperthermia

Histological Structure of Ovaries in Guinea Pig Exposed to Hyperthermia

The histopathological examination (Figure 1) of ovaries of guinea pigs exposed to 20-25 °C (A) and 32 ± 1 °C (B), showed a normal tissue and ovocytes, ovaries structure of heat stressed guinea pigs 39 ± 1 °C (C) and 46 ± 1 °C (D) exhibited evident signs of tissular injury such as degeneration and necrosis of the follicular cells and loss of vascularization in the stroma with reduced presence of oocytes.



(A): Ovary of guinea pig exposed at 20 - 25 °C; **(B):** Ovary of guinea pig exposed to 32 ± 1 °C ; **(C):** Ovary of guinea pig exposed to 39 ± 1 °C; **(D):** Ovary of guinea pig exposed to 46 ± 1 °C ; (OGS) ovarian germinal epithelium; (F) different stage of follicles development; (OT) tertiary oocyte; (GF) Graafian follicle; (DF) degenerated follicle; (Ant.) antrun; (AF) artretic follicle; (V) Vascularisation; (d) degeneration; (N) Necrosis; (yellow arrows) mature Graffian follicle containing degenerated oocyte; (green arrow) abnormal arrangement of granulosa cells.

Figure 1: Histological structure of ovaries

Effects of High Ambient Temperature on Foetus Growth Characteristics of Female Guinea Pig

The results presented in Table 2 shows that foetus weight and rump length significantly ($p < 0.05$) decreased in the higher hyperthermia treated groups (46 ± 1 °C) as compared to the control group (20-25 °C). Inversely fœtus length and head length no significantly ($p > 0.05$) affected with increasing level of temperature.

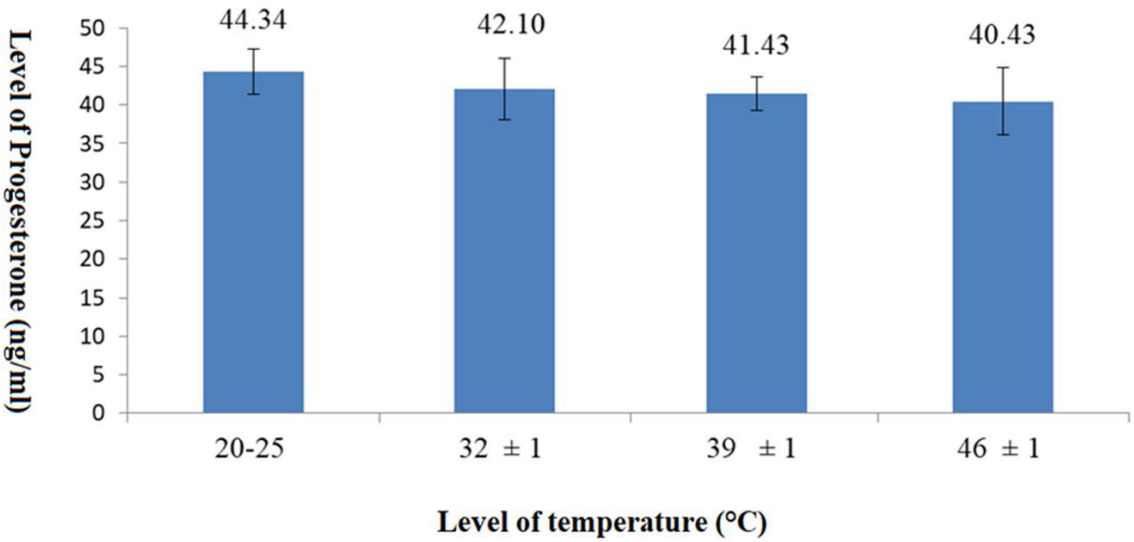
Parameters	Level of temperature (°C)				P-value
	20-25	32 ± 1	39 ± 1	46 ± 1	
fetuses weigth (g)	31.89 ± 3.56^{a}	27.78 ± 3.85^{ab}	23.50 ± 3.77^{b}	24.50 ± 3.04^{b}	0.04
fetuses length (mm)	6.03 ± 1.50	5.17 ± 1.01	6.08 ± 1.08	5.70 ± 0.78	0.7
Head length (mm)	2.50 ± 0.66	2.13 ± 0.27	2.20 ± 0.27	2.30 ± 0.17	0.64
Rump length (mm)	2.23 ± 0.81^{a}	1.27 ± 0.15^{b}	1.86 ± 0.42^{ab}	1.33 ± 0.29^{b}	0.04

^{a,b} On the same line, means with the same letter were not significantly different ($P > 0.05$)

Table 2: Effects of hight ambient temperature on fœtus growth characteristics

Effects of High Ambient Temperature on Serum Level of Progesterone in Female Guinea Pig

As shown in Figure 2, the serum level of progesterone decreased with increasing level of temperature. Nevertheless, no significant ($p > 0.05$) difference was registered among treatments.



^{a,b} On the same line, means with the same letter were not significantly different ($P > 0.05$)

Figure 2: Effects of hight ambient temperature on serum level of progesterone

Effects of High Ambient Temperature on Oxidative Status of Female Guinea Pig

The results of Table 3 presented the major oxidative stress markers. Except the activity of hepatic totals peroxidases (POX) and the concentration of malondialdehyde (MDA) which are comparable ($p > 0.05$) among all groups. The enzymatic activities of superoxide dismutase (SOD), catalase and nitric oxide (NO) increased significantly ($p < 0.05$) in heat stress groups with reference to the control (20 - 25 °C).

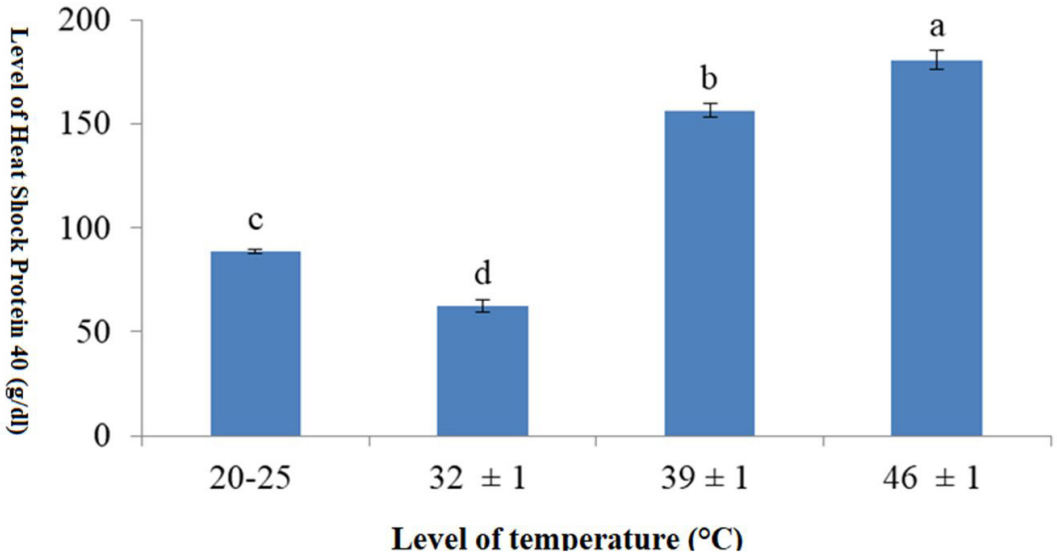
Oxidative Stress Markers	Level of Temperature (°C)				P-value
	20-25	32 ± 1	39 ± 1	46 ± 1	
SOD (U/min/g of hepatic proteins)	0.33 ± 0.04^{c}	0.47 ± 0.09^{b}	0.55 ± 0.06^{b}	0.71 ± 0.08^{a}	0
CAT (µM/min/g of hepatic proteins)	0.90 ± 0.12^{c}	1.32± 0.18^{b}	1.76 ± 0.24^{a}	1.55 ± 0.19^{ab}	0
(POX) (mM/min/ g of hepatical proteins)	36.51 ± 11.46	36.72 ± 7.70	43.52 ± 10.91	37.18 ± 9.81	0.69
MDA (µM/g of liver)	2.01 ± 0.49	1.96 ± 0.20	2.07 ± 0.39	1.75 ± 0.19	0. 71
NO (µM/g of liver)	25.54 ± 4.32^{b}	30.15 ± 6.04^{b}	38.51 ± 6.96^{a}	38.43 ± 3.69^{a}	0

$^{a, b, c}$: means within the same line with different letters are significantly different ($p > 0.05$). SOD: superoxyde dismutase; CAT: catalase; POX: totals peroxidases; MDA: malondialdehyde; NO: nitric oxide p-value = probability value

Table 3: Oxidative stress markers in female guinea pig exposed to hyperthermia

Effects of High Ambient Temperature on Serum Level of Heat Shock Protein 40 (HSP- 40) in Female Guinea Pig

The serum level of heat shock protein 40 (HSP- 40) increased significantly ($p < 0.05$) with the level of temperature in 39 ± 1 and 46 ± 1°C stressed groups when compared to control group (20-25 °C) as shown in Figure 3. When compared only elevated temperature treated groups, the females exposed to 46 °C ± 1 have shown the significant ($p < 0.05$) high level of heat shock protein 40 (HSP- 40).



[a,b] On the same line, means with the same letter were not significantly different ($P > 0.05$).

Figure 3: Effects of hight ambient temperature on serum level of heat shock protein 40 (HSP-40)

Discussion

The result of this study has revealed the ability of heat stress to induce oxidative stress in female guinea pig and its impact on reproductive parameters. The decrease in the ovaries weight of the group of females exposed to 46 ± 1 °C was recorded. This result corroborates that of Wegner, *et al.* [19] which showed lower ovarian weight, diameter and volume of the largest follicles. Ngoula, *et al.* [13] also observed a reduced weight of reproductive organs in males' guinea pig exposed at 46 ± 1 °C during 60 days. In the present study, a slightly reduced numbers of corpus luteum and implantation sites in the group of animal exposed to 46 ± 1 °C were recorded. These results agreed with those obtained by Ullaha, *et al.* [9] and Yhamid, *et al.* [20] who recorded a significant reduce number of implantation sites in rats exposed respectively to 33 ± 2 °C and 40.5 ± 0.2 °C during day 6 to 8 of gestation. Reduced implantation sites may be due to the effects of elevated temperature on the embryonic development or/and uterine receptivity. High temperature decrease blood flow, nutrient uptake and hormonal release by the follicles [21].

Progesterone is required in all mammals for support the survival and development of the conceptus including embryo/fetuses and attached membranes [22]. In normal conditions, the progesterone level during gestation is found to increase significantly. In this study, the serum progesterone of pregnant does decrease slightly in group of female exposed to high level of ambient temperature. This result is in agreement to those obtained by Ullaha, *et al.* [9] and Yhamid, *et al.* [20] in rats exposed respectively to 33 ± 2 °C and 40.5 ± 0.2 °C during day 6 to 8 of gestation. The decreased level of progesterone in heat stress animals would be the consequence of reduces number of corpus luteum and placenta. These organs are the principal sources of progesterone that act as a gestation hormone. According to Roth [23], the corpus luteum cells markedly degenerate in high temperature condition.

Histological of the ovaries in cavies exposed to high temperature (39 ± 1°C and 46 ± 1°C) exhibited evident signs of tissular injury such as degeneration, necrosis of the follicular cells, loss of vacuolars in the stroma with reduced presence of oocytes. This effect sbsequencely decrease the ovary weight. These results are in accordance with those reported by Emara, *et al.* [24] in goat during summer season, where decreased follicular development and increased degeneration of ovaries were noted.

In this study, the number of placenta decreased with the increasing level of temperature but the placenta weight increased significantly. These results corroborate those obtained by Bell, *et al.* [25] when exposed ewe to 38 - 40 °C for 9 h daily between 45 to 120 days of gestation. During gestation, reducing blood flow to the uterus and reduction of uterine clearance of oxygen (O2) are positively correlated with the reduced number of placenta [25]. The increased placenta weight observed in this study could be explained by the way that, exposition of animals to heat shock induced ischeamia necrosis at the level of cells. This necrosis could be due to the lowering clearance of placenta to oxygen and nutriment during heat stress [26]. In addition, during heat shock, the overproductions of reactive oxygen species (ROS) are responsible of lipid peroxidation (MDA) at the level of cell membrane leading to loss of membrane integrity and cell death. This loss of cell content causes an inflammatory response in the surrounding tissues. One of the specific markers of inflammatory cell is the nitric oxide (NO) and the results of this study showed an increased level of nitric oxide (NO) in the groups of female guinea pigs exposed to 39 or 46 °C.

The numbers of fetus per dam and viable fetuses decreased with the high level of ambient temperature (46 °C). These results corroborate those registered by Vatnick, *et al.* [27] with ewes exposed to 40 °C during 9 h/day between days 50 to 75 of gestation. These results could be explained by the high demand of the fetuses at late stages of pregnancy [4]. In addition, malondialdehyde (MDA) is the most major metabolite of lipids peroxidation and oxidative stress endpoint product that reveals essentially the impacts on cell membrane [28]. The slightly decreased in the level of MDA observed in the present study could be used as a possible explanation to the decreased number of viable fetuses and their body weight, as a consequence of lipids peroxidation at the level of foetus cells' membrane.

Hyperthermia is known to induce fetal growth retardation in terms of body dimensions. The results of this study revealed that, heat stress induced significant reduction of rump length in group of female exposed to 46 °C. These results agreed those of Vatnick, *et al.* [27] in ewe exposed at 40 °C during 9 h/day between days 50 to 75 of gestation but disagreed those obtained by Bell, *et al.* [25] who have observed no change in rump length with unheated group (48.0 cm) after heat stress. Foetal growth retardation observed can be directly related to the insufficient blood flow to the uterus and as consequence of nutrients reduction during pregnancy.

Pregnancy is a state of increased generation of ROS, arising from the heightened placental metabolic and steroidogenic activities involved in the increased oxygen consumption by the foeto-placental unit [29]. In the present study, the hepatic enzymatic activities of SOD, catalase and total peroxidases increased with heat stress exposure, inversely, the concentration of MDA decreased with heat stress exposure, suggesting that heat shock lead to oxidative stress. Abdoon, *et al.* [30] observed the same results in hot season in egyptian buffaloes. In fact, SOD, catalase and total peroxidases are antioxidant enzymes that protect cells from oxidative damage. Their significant increases registered in this study were to fight against overproduction of ROS and then to keep the embryo and their mother from oxidative damage. Their significant increases registered in this study were to fight against overproduction of ROS that attack the animal cell membranes.

Another protective mechanism used in the organism against heat stress is the expression of heat shock proteins (HSPs) which is closely associated with adapted thermotolerance [31]. The present study also revealed that the level of HSP-40 in the animal serum after 90 days of heat stress increased significantly in the groups exposed to 39 ± 1 °C and 46 ± 1 °C. These results disagreed those observed by Ngoula *et al.* [20] in male cavies exposed to 46 ± 1 °C during 60 days. These contradictory results can be directly related to the sex and the physiological status of the animals.

Conclusion

The results of this study revealed that exposition of female cavies to heat stress (46 ± 1 °C) negatively affects ovaries weight and fœtus characteristics. Inversely, it increases antioxidant enzymes activities (SOD and CAT), serum level of nitric oxide (NO) and HSP-40.

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