Reproductive functions of hyperthermic male rabbits:

The ameliorative effects of virgin olive oil

Thesis Submitted to

Zoology Department, Faculty of Science,

Assiut University, Assiut

For

The Master degree of Science (M.Sc.) in Zoology (Physiology)

By

Reem Ahmed Mahmoud Ahmed

Bachelor of Science, Faculty of Science, Sohag University, Egypt-2012
Research Assistant, Animal Health Research Institute, Sohag Regional Lab.

Supervised by

Prof. Dr. Hossam El-Din Mohamed Omar

Prof. of Physiology and the Head of Zoology Department,
Faculty of Science, Assiut University

Prof. Dr. Abd El-Razek Kalifa Abd El-Razek

Prof. of Theriogenology and Vice Dean for Education and Student Affairs,
Faculty of Veterinary Medicine, Assiut University

Prof. Dr. Khaled Abd El-Latif Aziz El-Din

Prof. of Poultry and Chief Researcher,
Animal Health Research Institute, Sohag Regional Lab

Prof. Dr. Emad Abd El-Aziz Ahmed

Prof. of Molecular Physiology, Zoology Department,
Faculty of Science, Assiut University

Zoology Department
Faculty of Science, Assiut University
2019

Contents

List of Tables

List of Figures

Summary

Rabbitfarming plays an important role not only in solving meat production shortfall but also in providing support for low-income households and reducing poverty as a small-scale or backyard farming, particularly, in developing countries. However, nowadays, HS in rabbits represents a problem of great concern as climate change affects seasons producing long periods of heatwaves in summer followed by severe winters. Moreover, rabbits are very susceptible to heat, negatively affecting their physiological condition, and most importantly, resulting in suboptimal reproductive efficiency. Recently, the usage of natural safe dietary additives to alleviate the effects of high temperatures becomes a new welcomed trend rather than giving up rearing during summer seasons.

In this context, the current study aimed to investigate the impacts of chronic cyclic HS and the possible ameliorative effects of VOO supplementation on the hematological parameters, and mainly on the reproductive functions of NZW male rabbits through the estimation of oxidative stress marker in the testicular tissues,and semen analysis, and comparing both effects at different periods of HS exposure that were 10 days, 20 days and 30 days, in addition to the detection of some relevant reproductive hormonal parameters. Moreover, the present experiment also sought to study the ability of HS exposure to elicit DNA damage and germ cell apoptosis and its consequences on Hsps-70 expression in the testicular tissues, and their histological features. Also, the study explored if VOO inclusion in rabbits' diet could mitigate HS effects in these regards too.

For the purpose of this study, 2° mature male NZW were divided into three groups: control group (n=6) that was reared in favorable environmental temperature and humidity and fed with usual balanced ration. HSgroup (n=10) that was exposed to high ambient temperature (>32°C) for 2 hrs daily along 30 days and was also provided with the usual ration. And HS+VOO (n=9) that was also subjected to HS conditions just like the HS group, however, it was supplemented with VOO via oral gavage at fixed dosage of 3ml/day after the application of HS. The obtained results can be summarized up in the following:

- **The THI :**

  The THI values were 30.75, 32.08 and 33.45 for the 10-day, 20-day and 30-day periods of HS application respectively, which indicates a condition of very severe HS.

- **Hematological parameters:**

WBCs, lymphocytes, monocytes, neutrophils, and eosinophils reached the significantly lowest values in the HS bucks compared with the control. However, the VOO-supplemented group was not significantly different from control regarding the lymphocytes and monocytes counts. Moreover, HS+VOO group showed significant enhancement in the monocytes count compared with the HSgroup. In addition, regarding the effect of exposure time, the shortage was generally higher in the HS group in the studied traits. Whereas HS+VOO achieved significant improvements in the 10-day period in both lymphocytes and monocytes counts, and in the 20-day period in both WBCs and neutrophils counts.

As for the erythrograms and platelet count, no significant differences were observed among the three studied groups. However, only at 30 days of HS exposure, significant differences were detected with respect to RBCs, HB, and Hct, where the HS bucks had significantly higher mean values in comparison with the HS+VOO group, except for MCHC that was found to be significantly higher in HS+VOO group.

- **Oxidative stress markers:**

The estimation of the oxidative status revealed that HSbucks suffered from oxidative stress reflected in the significantly elevated levels of both LPO and NO compared with the control. These increments in LPO and NO contents were accompanied by significant declines in the levels of some endogenous antioxidants due to their consumption under very severe HS conditions. HS significantly decreased Vit.E and GSH levels, while it insignificantly reduced SOD activity but it did not affect vit.C level as compared with the control group.

However, VOO supplementation exerted a significant influence as both LPO and Vit.E levels of HS+VOO group were relatively unchanged compared with the control. While vit.C level was even significantly higher than that of the control. In addition, SOD activity was insignificantly higher than the control, whereas, it was significantly higher than that of HS group. On the contrary, NO level was significantly higher compared with the control. However, the overall GSH content was significantly lower than the control group in both groups, whereas, CAT activity did not change in the studied groups.

Regarding the effect of exposure time to HS, it was found that LPO showed a marked increasing pattern by increasing the time of HS exposure in HS bucks while it was not the case in HS+VOO group as a significantly lower LPO level in HS+VOO group was detected in 10 days and 30 days of HS exposure. However, both groups followed a similar pattern regarding the NO level at different periods of HS exposure, but generally speaking, NO levels were almost lower in the HS+VOO group.

More importantly, significant differences were detected between HS and HS+VOO groups for the levels of Vit.E and SOD in 10-day period, and the level of Vit.C in the 20-day period since the estimated levels were significantly increased for HS+VOO. While GSH behaved differently to some extent as it was initially significantly higher in HS group at the 20-day period, thereafter, it became significantly higher in favor of HS+VOO group at the 30-day period.

- **Hormonal parameters:**

HS bucks suffered from hypogonadism reflected in the significantly lower mean value for serum LH compared with the controlones. Whereas, HS+VOO bucks recorded the highest mean values for both serum LH and serum T compared with the other two groups where both parameters were significantly elevated compared with HS group. Whereas, only serum T level was even significantly higher than that of the control group.

- **Semen analysis:**

On one hand, HS bucks had the least number of successful semen samples collection relative to the HS+VOO and control groups as semen collection trials failed for 50% of HS bucks in the 30-day period of HS exposure, in addition, they took a longer time to produce ejaculate. On the other hand, routine semen analysis revealed that HSbucks produced semen of the lowest quality characterized by significantly reduced volume, sperm motility, and sperm concentration with significantly elevated pH, increased percentage of abnormal and dead sperms relative to the control. Whereas HS+VOO bucks produced semen of a moderate quality since the measured parameters showed a significant increment in the sperm motility and sperm concentration, while the percentage of both morphologically abnormal sperms and dead sperms were decreased significantly compared with HSgroup.

Regarding the effect of the exposure time, semen pH tended to be alkaline whereas the semen volume showed a decrement with the extended periods of HS exposure in both HS and HS+VOO groups. However, HS+VOO group improved significantly in the sperm motility, the percentage of both abnormal and dead spermatozoa in the 10-day and 20-day periods of HS exposure. It also had significantly higher mean values of sperm concentration in the 20-day and 30-day periods of HS exposure compared with HS group.

- **DNA DSBs estimation :**

The immunofluorescence staining of the testicular tissues by γ-H2AX antibodies showed that HS was able to induce DNA DSBs in the germ cells of HS rabbits, which exhibited a significantly elevated number of γ-H2AX positive early meiotic prophase cells in spermatocytes compared with the other groups in the study. While testicular sections from HS+VOO bucks had a moderate number of earlier meiotic prophase cells compared to the control.

- **Induction of germ cells apoptosis:**

The immunofluorescent TUNEL staining of the testicular tissues indicated that HS can trigger testicular germ cells death via apoptosis while VOO can mitigate this effect since HS bucks get a significantly highest number of apoptotic germ cells per tubule in TUNEL-stained testicular sections compared with both HS+VOO and control groups. It was found that spermatocytes present in the testis at the time of heating were the most affected cells.

- **Expression of Hsps-70:**

Hsp-70 antibodies immunosating of the testicular tissues showed that testicular tissues recovered from HS bucks showed elevated expression of Hsp-70, while in those of VOO-supplemented bucks; Hsp-70 was moderately expressed, in comparison with the control groups. The elevated expression of Hsp-70 represents a heat tolerance strategy to survive high internal tissues temperature and a cellular response to stress on the molecular level.

- **H&E histopathological investigations:**

Applied HS has elicited an overt impairment in the spermatogenic cycle, abnormal germ cell morphology, and loss of germ cells populating seminiferous tubules in the testicular sections. Damages in the spermatogonia, spermatocytes,

spermatids or spermatozoa present in the testis at the time of heating were easily noticed. However, spermatocytes and rounded spermatids or spermatozoa were the most affected cells. The formation of intratubular multinucleate giant cells was also observed. However, testicular tissues from HS+VOO bucks were still keeping their tissue architecture and seminiferous tubules were well-populated with few damaged cells.

Finally, the negative effects of HS on reproductive functions of male rabbits that were pronounced in the decreased semen quality, increased incidence of DNA DSBs and apoptosis in the germ cells, in addition to altered histology of testicular tissues, may be returned primarily to oxidative stress and disturbance of reproductive hormones. While, the induction of elevated expression of Hsp-70 is a cellular adaptive strategy to stress. However, the study concluded that VOO administration at a dosage of 3ml/day largely mitigated these detrimental influences of HS on the reproductive functions by enhancing the oxidative status and boosting the hormonal secretions of reproductive hormones.