Theriogenology 96 (2017) 136–141

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Quercetin supplemented diet improves follicular development, oocyte quality, and reduces ovarian apoptosis in rabbits during summer heat stress

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ARTICLE INFO

Article history:
Received 29 September 2016
Received in revised form
10 March 2017
Accepted 14 March 2017
Available online 7 April 2017

Keywords:
Oocyte
Follicle
Quercetin
Heat stress
Rabbit

ABSTRACT

The present study was designed to test the modulatory effect of dietary quercetin on follicle population, apoptosis, *in vitro* maturation rate and quality of oocytes in heat stressed female rabbits. A total of thirty-four New Zealand White heat stress (HS) exposed female rabbits were either fed with quercetin supplemented diet (QU-HS) or non-supplemented (HS) diet. Firstly, laparotomy was performed for oocyte retrieval and then, oocyte grading and COCs dimensional assessments were conducted. The A and B-grade oocytes were submitted for *in vitro* maturation. Thereafter, the ovaries were collected from rabbits and were processed for follicular population estimation and granulosa cells apoptosis. The results showed that follicle number, retrieved oocytes and A-grade oocytes were higher in QU-HS, comparatively. A significant difference was observed in A-grade oocytes dimensions between QU-HS and HS treatment groups. The oocyte maturation rate was same across the groups. The quercetin supplementation significantly improved primordial and antral stage follicles. A greater number of apoptotic cells were observed in primary and antral follicles in the HS group. In conclusion, the quercetin provision improves the follicular development, minimize granulosa cells apoptosis, and maintain the oocyte competence in HS rabbits.

1. Introduction

Heat stress (HS) jeopardizes the reproductive efficiency by altering the spermatogenesis, follicular and oocyte development or maturation in animals. During the hot summer months, some variations occurring at the level of hypothalamic–pituitary–gonadal (HPG) axis are responsible for low fertility [1,2]. It has been described that the inappropriate follicular growth rate [3], changes in estradiol concentrations, reduced LH receptors expression and low aromatase activity delay the ovulation process under HS [4]. In addition, the exposure of female animals to HS during preovulatory phase reduce the oocyte quality by the involvement of increased reactive oxidative species (ROS) production [5]. Although, HS has no influence on germinal vesicle breakdown (GVBD) during oogenesis, it hinders in resumption of metaphase I (MI), metaphase II (MII) and subsequent blastocyst development [6,7]. The variations in fatty acids ratio, heat shock proteins, and antioxidants level decrease the oocyte maturation rate [8] under HS. Higher apoptosis rate in granulosa cells in response to HS, is another factor responsible for reduction in oocyte competence [9].

The oocyte maturation rate and embryonic development are negatively affected due to oxidative and heat stress. The antioxidants have the capacity to improve oocyte survival rate and reduce the damages induced by HS. In this regard the flavonoids may impede with biologically destructive chemical reactions in the reproductive organs and scavenge the ROS production [10]. Amongst the various classes of flavonoids, the quercetin is the

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http://dx.doi.org/10.1016/j.theriogenology.2017.03.029
0093-691X/

most common flavonoid, naturally existing in vegetables, fruits, tea, and red wine. It works as a strong antioxidant and has ROS scavenging ability that rely on its molecular configuration. The quercetin (3,5,7,3′,4′-pentahydroxyflavone) reacts with unpaired radical by donating a proton and become a free radical itself. However, the resulted unpaired radical is removed by resonance that makes the quercetin radical weak and less reactive with minimum energy [11]. Usually, three structural groups (B ring *o*-dihydroxyl groups, the 4-oxo group in conjugation with the 2,3-alkene, and the 3- and 5-hydroxyl groups) stabilize the quercetin molecule and help to act as an antioxidant [12]. The quercetin is supposed as a potential regulator of reproduction by mediating mTOR signalling system which is required for cell growth, proliferation and survival [13].

Generally, the ovarian dynamics results in overproduction of ROS during final stages of follicular development and at the time of ovulation. The over production of ROS is halted by the active enzymatic antioxidant system in the cells. A slight increase in ROS is necessary for the meiotic resumption from diplotene arrest in mammalian oocytes; however, superfluous of ROS in ovary or imbalance in redox state lead to oxidative stress state. The increased oxidative stress lead to irreversible changes in cytoplasm and nuclear regions of oocyte encased by the follicle that further affect the fertilization capacity of oocytes [14]. Quercetin protects the cumulus cells by culminating the lipid peroxidation, maintain the antioxidant enzymes and reduce the apoptosis through modifying the XIAP, BAX, BCL2 and caspase-3 signal expression [15].

To the best our knowledge, there is not a single report regarding the effect of quercetin supplemented diet feeding on reproductive efficiency of female rabbit under HS has been published yet. It is hypothesized that the feeding quercetin enriched diet could be a way to improve the reproductive performance of female rabbit by alleviating the oxidative stress, especially during hot summer months. Therefore, the objective of this study was to evaluate the impact of quercetin supplementation of feed on follicular development, apoptosis, oocyte production or maturation during the hot summer period in rabbits.

2. Materials and methods

The present study was carried out at Laboratory Animal Unit, Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Adnan Menderes University during the hot summer months (July to September 2015). The experiments were carried out according to the protocol approved by the animal care committee and were in compliance with institutional guidelines (ADÜ-HADYEK No. 64583101/2014/153).

2.1. Feeding and management of female rabbits

A total of thirty-four female rabbits of age about 8–9 months and body weight (3.2 ± 0.3 kg) were selected from the pure line of New Zealand White rabbits which was developed at faculty's lab animal unit. All the selected females were nulliparous and have never been bred. The rabbits were housed in individual cages ($70 \times 50 \times 35$ cm^3) in an air conditioned and properly ventilated room for a period of one year. To acclimatize with heat stress, the animals were deprived of air condition facility, at least one week before the start of study, during very hot summer month (July). The heat imposed females rabbits were then divided into two groups ($n = 17$/each group) and either fed with basal diet (HS group) or quercetin supplemented (Quercetin hydrate; 30 mg/kg of body weight, 337951, Sigma-Aldrich, St. Louis, USA) (QU-HS group) diet for successive 18–20 days.

2.2. Heat stress assessment

The ambient temperature and relative humidity were recorded twice a day through digital thermohygrometer. The temperature-humidity index (THI) was calculated through equation adapted by Marai et al. [16].

$$THI = db°C - [(0.31–0.31 \times RH) \times (db \times °C - 14.4)]$$

The heat stress level was categorized based on the THI values which are as follows:

No heat stress ≤ 27.8 THI
Moderate heat stress = 27.9 to 28.9 THI
Severe heat stress = 28.9 to 30.0 THI
Extreme heat stress ≤ 30.0 THI.

2.3. Oocyte collection

Oocytes were collected through laparotomy. Animals were kept off-feed for a period of 18 h prior to surgical procedure. The animals were administered intravenous pre-anaesthetic medication (Xylazine) and followed by anesthesia (Ketamine). A midline incision was made to approach the abdominal cavity. The ovaries were located, held by thumb forceps and washed with sterile PBS. All the follicles of size ranges between 1 and 3 mm were selected for oocyte aspiration by using insulin syringe. Afterwards, the incisions were sutured and animals were kept under intensive care for three days.

2.4. Grading and measurement of cumulus oophorous complex (COCs)

After collection, the oocyte were washed thrice in TCM-199 medium (Medium-199 M4530 Sigma-Aldrich, St. Louis, USA). The collected compact COCs were classified into three basic categories with respect to surrounding cumulus oocyte complex layers: The largest oocytes surrounded by a maximum number layers of cumulus cells were graded as A. The oocytes covered with 3–4 layers of cumulus cells were graded as B, whereas, oocytes without cumulus layer (denuded cell) were graded as C. The COCs dimensions including zona to zona distance (μm), whole COCs in the horizontal and perpendicular plane (μm) were measured under stereomicroscope (Olympus SZ51, Japan) at ×40 magnification.

2.5. In-vitro oocyte maturation

The A and B grades oocytes were selected and about 8–10 oocytes were kept in the maturation media for 24 h under sterile mineral oil covering. The maturation media (TCM199) was supplemented with 50 mM Hepes (HEPES sodium salt H3784 Sigma-Aldrich, St. Louis, USA), 0.3 mM sodium pyruvic (Sodium pyruvate P5280 Sigma-Aldrich, St. Louis, USA), 10% fetal calf serum (FBS F0804 Sigma-Aldrich, St. Louis, USA), 10 μg/mL follicle stimulating hormone (FSH, Folltropin 700 IU Bionichi Animal health Europe ltd Dublin Ireland), 5 IU/mL human chorionic gonadotrophin hormone (hCG Pregnyl 1500 IU NV Organon Oss-Holland), 100 IU/mL penicillin G potassium, 50 μg/mL streptomycin sulphate.

2.6. Oocyte maturation assessment: cumulus expansion and nuclear staining

The oocyte maturation was assessed by cumulus cells expansion rate in COCs and nuclear staining. Cumulus cells expansion in COCs,