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Effects of Kisspeptin-10 on the Steroidogenic Capacity and Metabolic Aspects of Bovine Granulosa Cells *in vitro*

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Abstract

Kisspeptin (Kp) potently stimulates the reproductive hormone secretion, modulates the cellular metabolic machinery, and induces the antioxidant defense mechanism *in vivo*. However, the data regarding steroidogenic, metabolic and antioxidant effects of Kp on granulosa cells on the level of *in vitro* studies are quite rare leaving a wide gap in literature and giving a strong driving force for the present study. Thus, this study aimed to clarify the effects of human kisspeptin-10 (Kp10) on these biological features in small and large bovine granulosa cells (BGCs). Upon preparation of the monolayer-BGCs, they were allocated to eight groups; two untreated-control groups, and six Kp10-treated groups according to the follicular size; three groups for each follicle size, supplemented with Kp10 at three different doses; 10^{-8}, 10^{-7} and 10^{-6} M [$KP(I\text{-}III)_S$ and $KP(I\text{-}III)_L$, respectively]. Spent media and BGCs were pooled after 24 hours from addition of Kp10. Kisspeptin stimulated the glucose consumption in media by BGCs obtained from the small sized follicles for production of estradiol 17-β and progesterone. In the small-sized follicles, the TC levels were significantly decreased in response to $KP(I, II)_S$, but not the $KP(III)_S$ compared to their control (C_S). On the other hand, the $KP(I\text{-}III)_L$ significantly increased the TC levels compared to their control (C_L). When Kp10 was used at the highest two doses, less glucose was consumed by BGCs collected from large sized follicles leading to low production of P_4 and preservation of cellular TC content. Improvement in the metabolic efficacy of BGCs in response to Kp10-treatment was evidenced by increased glucose utilization and decreased lactate production. Increased total antioxidant capacity versus decreased lipid peroxides in Kp10-treated groups could indicate that Kp10 induces cryoprotection by restoring the favorable redox status in BGCs. Those findings suggest that Kp10 causes a size- and dose- dependent physiological changes in the BGCs.

KEYWORDS
Kisspeptin, Bovine granulosa cells, Steroidogenesis, Metabolism, Redox status

INTRODUCTION

Kisspeptin (Kp) is the product of *KiSS-1* gene, which encodes a 145-amino acid peptide that is further processed into biologically active peptides of various peptide chains; 10-54 amino acids, termed kisspeptins (Caraty and Franceschini, 2008; Ahmed *et al.*, 2009). Kisspeptin-10 (Kp10) is a shorter variant of Kp retaining full biological activity (Ahmed *et al.*, 2009). Kp10 was experimentally used both *in vivo* (Ezzat *et al.*, 2015; Abou Khalil and Mahmoud, 2020; EL-sherry *et al.*, 2020) and *in vitro* (Ezzat Ahmed *et al.*, 2011) to investigate its pivotal role and cellular activity in improving fertility of both sexes from the physiological and neuro-/endocrine points of views. Granulosa cells (GCs) act as a scavenger of free radicals to protect and energize the involved oocyte (Tanghe *et al.*, 2002; Yuan *et al.*, 2005; Tiwari *et al.*, 2017). Kisspeptin and its receptors were detected in a variety of ovarian structures (Inoue *et al.*, 2009; Mishra *et al.*, 2019), indicating that its vital role in regulating the reproductive functions. In addition, several investigations revealed that interruption of Kp signaling pathway resulted in impaired ovarian functional activity even with preserved hypothalamic pituitary gonadal axis (Gaytan *et al.*, 2014). This proves the direct ovarian role of Kp and the possibility of its local signaling through specific ovarian functions. Although *in vitro* studies reported that Kp is involved in controlling the steroidogenesis in the GCs from preovulatory follicles of laying hen, ovarian tissue of fruit bats, corpora lutea of pseudopregnant rabbit and luteal cells of rat (Xiao *et al.*, 2011; Peng *et al.*, 2013; Maranesi *et al.*, 2019), no data is available about its effect in relation to the variability in the size of the follicles from which bovine granulosa cells (BGCs) are obtained. Kisspeptin widely affects the metabolic markers based on compelling data amassed from pancreatic islet, hepatocyte culture, and adipocyte cell line (Wu *et al.*, 2012; Schwetz *et al.*, 2014; Pruszyńska-Oszmałek *et al.*, 2017). It also possesses a highly promising antioxidant effects (Abou Khalil and Mahmoud, 2020; Huang *et al.*, 2021; Fatima and Qureshi, 2022) however, its potential *in vitro* antioxidant properties had not fully explored. The present study aimed to clarify the effect of Kp10 on BGCs and size-relative modulation in the view of steroidogenic secretory potential, antioxidant, and metabolic efficacy.

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MATERIALS AND METHODS

Chemicals

Kisspeptin-10 was purchased from Sigma-Aldrich Chemicals, St. Louis, MO, USA. Fetal bovine serum, Dulbecco's modified Eagle's medium (DMEM), and penicillin/ streptomycin were purchased from Bio west, France. Amphotericin B was supplied from Bio Whittaker, LONZA, Switzerland. Trypsin was obtained from Vac-Sera, Egypt.

Preparation of bovine granulosa cells monolayer

Bovine granulosa cells were prepared for culturing as described by Maeda *et al*. (1996). Briefly, forty bovine ovaries were collected from a local abattoir in Upper Egypt from healthy non-pregnant cows and immediately transported to the laboratory in a thermos with antibiotic-containing saline. Granulosa cells were obtained by aspiration of the follicular fluid from small follicles; < 8 mm, and those large follicles; ≥ 8 mm, by using 18-gauge needle. The follicular fluid was centrifuged at 3000 rpm for 10 minutes. The GCs' pellet was washed twice with 20 mL of calcium-/ magnesium-free phosphate buffer saline (PBS) containing 100 IU/mL of penicillin and 100 µg/mL of streptomycin and centrifuged again for 5 min. The cells were vigorously pipetted for separation, and then washed twice by centrifugation for another 5 min with 10 mL of the same buffer. Finally, the cells were re-suspended in the Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal calf serum, 100 IU/mL penicillin, 100 µg/mL streptomycin, and 1 % amphotericin B. Thereafter, 2 mL of the suspension was placed into 24-well culture plate (Thermo fisher scientific, USA) and incubated in a humidified atmosphere at 38 °C with 5 % CO2 for 5 days to produce a confluent monolayer. The culture medium was changed during the incubation period every 24 hours.

Experimental groups and sample collection

The size categories of follicles from which BGCs are obtained represent a cutoff point for the changes in the steroidogenic capacity, nitric oxide production and cell viability (Basini *et al*., 1998). Bovine granulosa cells were allocated into eight groups; two controls; C_S and C_L, incubated without addition of any treatment, and six Kp10-treated groups; three groups for each follicle size, supplemented with Kp10 at three doses: 10^{-8} M in $KP(I)_S$ and $KP(I)_L$ groups, 10^{-7} M in $KP(II)_S$ and $KP(II)_L$ groups, and 10^{-6} M in $KP(III)_S$ and $KP(III)_L$ groups (Kp10 was diluted in PBS as 0.013, 0.13 and 1.3 µg/mL respectively) (Novaira *et al*., 2009; Saadeldin *et al*., 2012). The maturation media were collected for estimation of estradiol 17-β (E_2), progesterone (P_4), glucose, lactate and nitric oxide (NO) levels. Bovine granulosa cells were isolated from the bottom of the plate by adding 1mL of trypsin to each well and incubated in the CO2 incubator (NüveSanayi-Malzemeleri Manufacturing and Trade A.S., EC160, Turkey) for 3-4 min waiting for separation of the cells. The fetal bovine serum was added as 1 mL to 10 mL of media, and then 1 mL of mixture was added to each well to neutralize the trypsin. Granulosa cells were centrifuged (Ortoalresa, digicen 21 R, version CE 113, Germany) at 3000 rpm for 10 min. The pellets were homogenized in 100 mL of Tris-HCl buffer (40 mmol/L, pH 7) and sonicated (MSE soniprep 150, labtech, USA) at 50 W for 1 min, centrifuged at 8000 rpm for 20 min at 4 °C, and the supernatants were collected for measurement of total cholesterol (TC), total antioxidant capacity (TAC), lipid peroxides (LPO) levels and lactate dehydrogenase (LDH) activity.

Estimation of estradiol 17-β and progesterone

Concentration levels of E_2 and P_4 were measured using ELISA kits obtained from BioCheck, Inc., South San Francisco, USA (catalog number: BC-1111 and BC-1113, respectively) according to kits instructions. The concentrations were estimated based upon a standard curve. The minimum detectable concentrations of E_2 and P_4 were estimated as 10 pg/ mL and 0.0625 ng/ mL respectively.

Estimation of metabolic parameters

Glucose and lactate levels were measured using calorimetric kits provided by the Egyptian Company for Biotechnology, Cairo, Egypt (250001 and 274001, respectively). Lactate dehydrogenase activity was measured by a kinetic kit, while TC level was measured by a calorimetric kit provided by Vitro Scient, Cairo, Egypt (134-EN and 113-EN, respectively) according to manufacture instructions. Total protein level was determined according to Lowry *et al*. (1951). The estimated total protein in BGCs was used to normalize the levels of cellular metabolic and redox parameters in BGCs.

Estimation of oxidant/antioxidant parameters

Total antioxidant capacity levels were measured using a calorimetric kit (TA 2513, Biodiagnostic, Giza, Egypt). The determination of the antioxidative capacity is performed by the reaction of antioxidants in the sample with a defined amount of exogenously provide hydrogen peroxide (H_2O_2). The antioxidants in the sample eliminate a certain amount of the provided H_2O_2. The residual H_2O_2 is determined colorimetrically by an enzymatic reaction which involves the conversion of 3,5,dichloro-2-hydroxy benzensulphonate to a coloured product. Lipid peroxides levels were estimated according to Ohkawa *et al*. (1979). Tetramethoxypropane was used as a standard, and the level of LPO was calculated from standard curve. The NO levels were measured according to Ding *et al*. (1988). In brief, phosphoric acid is converted to a diazonium salt by reaction with nitrite in acid solution. The diazonium salt is then coupled to naphthylene diamine dihydrochloride forming an azo dye that can be spectrophotometrically quantitated using a standard curve. Glucose, lactate, TC, LDH, TAC, NO and LPO were measured using the spectrophotometer kits (S1200, Unico, USA).

Statistical analysis

All data were presented as mean±standard error of means (SEM). The difference between groups were analyzed by using one-way analysis of variance (ANOVA) and Duncan was used as a post-hoc test. GraphPad-Prism software was used (San-Diego, Co., USA, V.5).

RESULTS

Effects of Kisspeptin-10 on estradiol 17-β and progesterone levels in the spent media of bovine granulosa cells obtained from follicles of different sizes

The changes in E_2 and P_4 levels in the spent media following the incubation of BGCs obtained from small and large sized follicles with three doses of Kp10; 10^{-8}, 10^{-7} and 10^{-6} M were shown in Table 1. Kisspeptin-10 significantly increased the levels of E_2 in both the small- and large-sized follicles with variant sensitivities.

In KP(I-III)$_S$, E_2 level significantly increased compared to that of respective control (C_S). However, the levels of E_2 in KP(II)$_L$ and KP(III)$_L$, but not in KP(I)$_L$, were significantly greater than those of the respective control (C_L). It is worthy to note that BGCs collected from the large follicles had higher basal levels of E_2 and P_4 than those collected from the small follicles. The small follicles showed higher sensitivity to Kp10 than those large follicles, and that the medium dose could be the highly potent one regardless the follicular size. In Kp groups, the levels of P_4 significantly increased in the small-sized follicles, but not from those large follicles, compared to their respective controls. However, in the small-sized follicles, the P_4 secreting sensitivity was inversely proportional to the different treatments of Kp10, where it is highly potent in KP(I)$_S$ and the potency dramatically decreased in in KP(II)$_S$ and KP(III)$_S$. Interestingly, the P_4 secretion significantly decreased in KP(II)$_L$ and KP(III)$_L$ compared to their respective control (C_L).

Effects of Kisspeptin-10 on some metabolic parameters in BGCs of different sized-follicles

The changes in glucose, lactate, LDH, and TC levels in the spent media produced by BGCs obtained from the small- and large-sized follicles following incubation with the three different doses of Kp10 were shown in Table 2. The concentrations of glucose in the spent media in KP(I-III)$_S$ significantly decreased compared to the control of small-sized follicles (C_S). However, the glucose concentrations in KP(II-III)$_L$ significantly increased compared to that in the control of the large-sized follicles (C_L). On the other hand, there was a significant decrease in the levels of glucose concentrations in KP(I)$_L$ compared to that in the control (C_L). Kisspeptin-10 significantly decreased the concentration levels of lactate in both the small and large sized follicles compared to their respective controls (C_S and C_L, respectively). Lactate dehydrogenase activity was significantly increased in KP(II)$_S$ and KP(III)$_L$ compared to each respective controls; C_S and C_L. The different sized follicles showed variant sensitivities of TC in response to Kp10. In the small-sized follicles, the TC levels were significantly reduced in response to KP(I, II)$_S$, but not the KP(III)$_S$ compared to their control (C_S). However, the large-sized follicles were more responsive to Kp10, where the TC levels significantly increased in the KP(I-III)$_L$ compared to its control (C_L).

Effects of Kisspeptin-10 on some oxidant-antioxidant parameters in bovine granulosa cells obtained from follicles of different sizes and its spent media

The changes in TAC, LPO and NO levels in the spent media produced by BGCs obtained from the small- and large-sized follicles following incubation with the three different doses of Kp10 were shown in Table 2. Although all Kp groups, except the KP(I, III)$_L$, showed non-significant changes up to significant decrement in TAC compared to their respective controls, Kp10 in the various groups except KP(II)$_S$ and KP(III)$_L$ showed a significant decrease in the LPO in the different-sized follicles compared to each respective control. On the other hand, Kp10 showed non-significant effects on the NO of the small- and large-sized follicles.

DISCUSSION

In the present study, the increased E_2 levels in the spent media of BGCs derived from small and large sized follicles incubated with the three doses of Kp10 was similar to that found in a cultured ovarian tissue of a fruit bat (Cynopterus sphinx), and could be attributed to increased luteinizing hormone receptor, steroidogenic acute regulatory protein (STAR) and 3β-Hydroxysteroid dehydrogenase (3β-HSD) (Krishna, 2017). The reactive oxygen species inhibit steroidogenesis through reduction of STAR and 3β-HSD protein levels and disruption of mitochondrial membrane potential (Allen *et al*., 2004), and so Kp10 could enhance the production

Table 1. Effects of kisspeptin-10 (Kp10) on the secretions of E_2 and P_4 in the spent media of BGCs obtained from follicles of different sizes.

	Small follicles				Large follicles			
	C_S	KP(I)$_S$	KP(II)$_S$	KP(III)$_S$	C_L	KP(I)$_L$	KP(II)$_L$	KP(III)$_L$
E_2 (pg/mL)	297.30±2.01^c	531.04±7.68^a	526.83±6.98^a	470.62±3.97^b	460.64±2.37^b	477.80±17.61^a	508.67±8.18^a	507.05±16.69^a
P_4 (ng/mL)	8.10±0.140^b	22.75±0.85^a	19.45±1.86^a	18.81±1.38^a	23.91±0.21^a	23.68±0.26^a	16.24±1.14^b	16.48±1.79^b

All data were presented as Mean±SEM for six wells per group (N=6).
C_S and C_L denote untreated BGCs obtained from small- and large-sized follicles, respectively. KP(I)$_S$, KP(II)$_S$, and KP(III)$_S$ denote Kp10 treatments for small follicles, as; 10^{-8} M, 10^{-7}M, and 10^{-6} M, respectively. KP(I)$_L$, KP(II)$_L$, and KP(III)$_L$ denote the same Kp10 treatments but for large follicles. Values with different superscript letters in the same row between treatments in the same group of follicles denote significance at P<0.05.

Table 2. Effects of Kp10 on the different metabolic parameters secreted in the spent media from BGCs obtained from small- and large-sized follicles

	Small follicles				Large follicles			
	C_S	KP(I)$_S$	KP(II)$_S$	KP(III)$_S$	C_L	KP(I)$_L$	KP(II)$_L$	KP(III)$_L$
Glucose	81.7±16.90^a	10.67±0.5^d	22.96±2.40^b	13.91±0.50^c	145.43±3.30 ^{c}	15.25±0.10^d	216.45±5.10^a	180.19±2.01^b
Lactate	50.4±1.40^a	44.71±1.3^b	29.37±1.40^c	25.38±2.50^c	49.80±1.30^a	37.18±2.90^b	31.38±1.60^b	38.31±3.10^b
LDH	0.05±0.01^b	0.06±0.01^b	0.11±0.02^a	0.07±0.01^{ab}	0.11± 0.04 ^{b}	0.11±0.10^b	0.05±0.01^b	0.83±0.01^a
TC	0.35±0.03^a	0.22±0.02^b	0.11±0.004^c	0.35±0.04^a	0.21±0.030^d	0.30±0.01^c	0.58±0.003^b	0.65±0.04^a
TAC	726.0±59.6^a	8.3±0.30^c	259.9±37.40^b	544.6±115.70^a	593.6±92.10^c	1302.8±166.1^b	268.1±38.0^d	8655.3±88.3^a
LPO	4.58±0.40 ^{a}	1.17±0.20^b	5.29±0.80^a	4.003±0.10^a	7.95±0.90^a	2.196±0.9^c	5.36±0.60^b	5.65±0.10^b
NO	2.03±0.10	1.99±0.10	1.84±0.10	1.94±0.13	1.65±0.10	1.79±0.12	1.71±0.14	1.89±0.11

All data were presented as Mean±SEM for six wells per group (N=6).
Glucose (mg/dL), lactate (mg/dl), lactate dehydrogenase (LDH; U/mg protein), total cholesterol (TC; mg/mg protein), total antioxidant capacity (TAC; µmol/mg protein), lipid peroxides (LPO; nmol/mg protein) and nitric oxide (NO; nmol/mg protein). Values with different superscript letters in the same row between treatments in the same group of follicles denote significance at P<0.05. Other explanations were given in Table 1.

of steroidogenic hormones like E_2 (Aslan *et al*., 2017; Güvenç and Aksakal, 2018). On the other hand, Kp10 did not cause any significant change in E_2 level in cultured rat luteal cells (Peng *et al*., 2013). The differences in animal species from which the cells were obtained, culture model and dose of Kp10 could be the underlying factors behind this controversy.

The present increment in P_4 in the spent media from small sized follicles when incubated with Kp10 is in harmony with that observed in chicken GCs, rat luteal cells, rabbit corpora lutea, and fruit bat ovarian tissue (Xiao *et al*., 2011; Peng *et al*., 2013; Krishna, 2017; Maranesi *et al*., 2019). The increase in PGE_2/$PGF_2\alpha$ ratio, the key steroidogenic enzymes transcription levels, free cholesterol transport were considered the mechanistic pathways of the present finding (Xiao *et al*., 2011; Peng *et al*., 2013; Krishna, 2017; Maranesi *et al*., 2019; Guo *et al*., 2022). In the same line with results from the present study, Peng *et al*. (2013) hypothesized that Kp acts in a similar manner to human chronic gonadotropin which stimulate the P_4 secretion. Kisspeptin had a dose-dependent effect on both E_2 and P_4 levels in parallel to up-regulation of the kisspeptin/GPR54-receptors by the physiological levels of KP and their down-regulation and desensitization by the supra-physiological high doses of KP (Krishna, 2017). The size and maturity-related changes in the expression of Kp receptors and the downstream signaling response could explain the pathway of the detected reduction in P_4 in response to $KP(II)_L$ and $KP(III)_L$ compared to their respective control.

According to a previous study, Kp is suggested as a strong driver of the glycolytic pathway by up-regulating the glucose transporter-1 gene expression and the phosphofructokinase-1 which confirmed the present glucose depletion in the spent media and enhancement of the cellular glucose utilization as a source of energy (Liu *et al*., 2014). However, the significant elevation noticed in glucose levels of $KP(II)_L$- and $KP(III)_L$-treated groups compared to CL group indicate that the cellular uptake of glucose under the effect of Kp10 is dependent on the follicular size where the glucose metabolism is less intensive in the large follicles compared to those small-sized ones (Nandi *et al*., 2007; El-Shahat *et al*., 2018). Thus, suppression of glucose consumption by BGCs, relative to the doses of Kp10 and the follicular size used in the present experiment could be the cause of accumulated glucose in the spent media.

The significant reduction in lactate relative to the control groups in this study is similar to what happened in human breast cancer cell line incubated with Kp10 as the aerobic glycolysis was shifted to oxidative phosphorylation with enhancement of the mitochondrial biogenesis (Liu *et al*., 2014; Song and Zhao, 2016). Furthermore, the significant increment in LDH activity of the large follicles-treated group compared to the control may be due to up-regulation of the lactate dehydrogenase-B gene in parallel to KP-1 expression in the C8161.9 cell line (Liu *et al*., 2013). Lactate dehydrogenase activity is measured on the basis of lactate conversion to pyruvate increased LDH activity in the large follicles and accelerate the turnover of lactate to pyruvate as evident by decrease in lactate content of spent media which reduce the acidification of extracellular microenvironment (Jain *et al*., 2018). This reduction in acidification could repair the DNA and metabolic pathway and modulate the cellular sensitivity to growth regulatory signal which raises the possibility that the highest dose of Kp10; 10^{-6} M, could improve the activity and energetic potential of BGCs (Raghunand *et al*., 2003; Chen *et al*., 2009; Liu *et al*., 2014).

The significant increase in TC levels of the kisspeptin-treated groups compared to the control were compatible to those detected in chicken embryo hepatocyte tissue culture (Wu *et al*., 2012). Kisspeptin was suggested to stimulate lipogenesis via up-regulation of the sterol regulatory element binding protein-1, fatty acid synthetase, 3-hydroxy-3-methylglutaryl coenzyme A reductase and apolipoprotein VLDL-II gene expression (Wu *et al*., 2012; Zuxiang *et al*., 2012; Wu *et al*., 2013). On the other hand, the deceased levels of TC in the small follicles-treated groups was consistent with those detected in mouse adipocyte cell line; 3T3-L1, and that C8161.9 cells following incubation with Kp leading to down-regulation of peroxisome proliferator-activated receptor gamma and CCAAT enhancer binding protein, and up-regulation of periliphin and hormone sensitive lipase gene expression, and inhibition of Acetyl-CoA carboxylase (Pruszyńska-Oszmałek *et al*., 2017; Dudek *et al*., 2018).

In the present study, the GCs of the small-sized follicles incubated with Kp10 consumed most of the glucose from the spent media, and so the energy produced was used for production of high amount of E_2 and P_4 in parallel to the decreased amounts of the steroidogenic precursor TC. On the other hand, less glucose was consumed by the large-sized follicles leading to less production of P_4, with accumulation of TC in those cells.

Since the antioxidants work in concert through chain breaking reactions, estimation of TAC was used as a bio-diagnostic indicator of antioxidant status in the BGCs following Kp exposure denoting the cumulative synergistic action of all antioxidants (Ghiselli *et al*., 2000; Brock *et al*., 2004; Konuganti *et al*., 2012). In comparison with the respective control groups, TAC levels of $KP(I)_S$, $KP(II)_S$ and $KP(II)_L$ groups showed a significant reduction while those of $KP(I)_L$ and $KP(III)_L$ groups showed a significant elevation. Several *in vivo* studies had reported the antioxidant role of Kp as consistent to the obtained results (Aydin *et al*., 2010; Akkaya *et al*., 2014; Hou *et al*., 2017; Güvenç and Aksakal, 2018). Kisspeptin was suggested to play a pivotal role in scavenging of the free radicals, stimulating the antioxidant expression and reduction of the pro-oxidant enzymes (Forester and Lambert, 2011). The differences in the doses of Kp10 and the follicular sizes could explain the role of KP as pro-oxidant or antioxidant showing a double-faced effect on the cellular redox state relative to its concentration (Bouayed and Bohn, 2010; Forester and Lambert, 2011; Yordi *et al*., 2012). Furthermore, the differences in the levels of cellular antioxidants based on variation in the size of follicles and the stage of the estrous cycle could explain the differential response of BGCs to KP and thus explain the increasing levels of TAC as the follicle increased in size during folliculogenesis (Gupta *et al*., 2011). Similarly, in a previous study large follicles obtained from buffaloes at the luteal phase had a significant higher concentration of glutathione than those obtained from those small follicles, however the superoxide dismutase was higher in the large follicles than small ones obtained during the follicular phase and the opposite state was observed in the luteal phase (El-Shahat and Kandil, 2012).

Lipid peroxides are structural and functional biomarkers for the intracellular oxidative stress (Asadi *et al*., 2017). Those peroxides are produced as a consequence of free radicals attack to polyunsaturated fatty acids and can modify the physical properties of cellular membranes, proteins and nucleic acids (Gaschler and Stockwell, 2017). In the current study, LPO levels of Kp10-treated BGCs obtained from small and large sized follicles in $KP(I)_S$, $KP(I)_L$, $KP(II)_L$ and $KP(III)_L$ groups were significantly lower than those of their respective control. This finding supports the protective capacity of Kp against lipid peroxidation in several experimental tissue models including ischemia/reperfusion injured ovary and uterus, and the methotrexate-induced testicular dysfunction as evidenced by levels of MDA (Aydin *et al*., 2010; Akkaya *et al*., 2014; Aslan *et al*., 2017; Hou *et al*., 2017; Güvenç and Aksakal, 2018).

Kisspeptin-10, according to the obtained findings, succeeded in modulating the functional microenvironment of BGCs after incubation *in vitro* regarding its endocrine, metabolic and antioxidant capacity. The current study could pave the way towards the applicable use of Kp10 as a main component in the culture maturation media providing suitable hormonal conditions for successful fertilization in addition to treatment of fertility- relevant problems.

CONCLUSION

Culture of ovarian tissues incubated with Kp10 could enhance its developmental capacity and preserve its fertility potential based

on the improvement in metabolic and oxidative stress markers. Findings from this study could be applied in the field of cryopreservation which adversely changes the cellular membrane fluidity and induces excess electron production, mitochondrial ATP deprivation, and reactive oxygen species overproduction. Further studies are required to clarify the transcription factors related to sex hormones, metabolic pathways, and the redox balance to investigate the mechanistic tools underlying behind those findings.

CONFLICT OF INTEREST

The authors declares that they have no conflict of interests to disclose.

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