Egyptian J. Anim. Prod. (2019) 56(3):93-99

EXPRESSION OF SELECTED CANDIDATE GENES DURING DIFFERENT STAGES OF CORPUS LUTEUM DEVELOPMENT IN CYCLIC EGYPTIAN BUFFALOES

Marwa S. Faheem[1,2] and Sh. M. Dessouki[1,2]

1- Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt, 2- Cairo University Research Park (CURP), Faculty of Agriculture, Cairo University, Giza, Egypt

Received: 16/6/2019 Accepted: 27/6/2019

SUMMARY

The current study was done to investigate the expression profile of selected candidate genes in corpus luteum of cyclic Egyptian buffaloes. A total number of nine corpora lutea (CLs) was collected from 9 cyclic slaughtered female Egyptian buffaloes. Based on their developmental phases and morphological features, CLs were classified into; growing (around days 3-5), static (around days 7-10) and regressed (around days 19-21 of the estrous cycle). Transcript abundance of genes regulating mitochondrial activity (SOD2, TFAM and CPT2), lipid metabolism (SREBP1), pro-inflammatory cytokine (TNFα) and oxidative stress (NFE2L2) was assessed relative to GAPDH as a housekeeping gene.

Relative gene expression profiles of TFAM and SOD2 increased ($P \leq 0.05$) in static phase compared to both growing and regressed phases. Transcript abundance of both CPT2 and SREBP1 was significantly higher at static phase, while was at minimum level of expression during regression phase. The expression profile of NFE2L2 revealed insignificant differences among the studied phases of CLs. Relative transcript abundance of TNFα was higher ($P \leq 0.05$) at regression phase compared to growing and static ones. Taken together, the pattern of genes regulating mitochondrial activity and lipid metabolism were higher during growing phase and reached a plateau in static phase and fully declined during regression phase. Meanwhile, the transcript abundance of pro-inflammatory cytokine gene denoted an opposite trend. Thus, gene expression profile reflected the cyclic changes of buffalo corpus luteum development.

Keywords: Corpus luteum, gene expression, mitochondrial activity, buffalo.

INTRODUCTION

Corpus luteum (CL) is an ovarian structure that formed from the ovulated Graafian follicle. It has distinct morphological and physiological changes during the estrous cycle of female farm animals. The appropriate functionality of CL is crucial for pregnancy maintenance and normal reproductive cyclicity of the cows (Quintal-Franco *et al*., 1999). Different studies have been conducted to understand the developmental changes as well as progesterone profile (Barkawi *et al*., 2002) during estrous cycle of buffaloes.

Early study on buffaloes reported that luteal tissue has been detected as hypoechoic patches inside the developed echogenic mass on days 3-6 of the buffaloes estrous cycle (Fadel, 2000). The mean diameter of the CL when detected on day 3 was reported to be 2.8 mm, 9.3 mm in mature phase (about day 12), and 5.0 mm in diameter on day 18 (Fadel, 2000 and Barkawi *et al*., 2002). The duration of CL development phases was 6.4, 11 and 4.5 days for growth, static and regression while, the diameter at these phases was 2.8, 9.3 and 5.0 mm (Fadel, 2000 and Barkawi *et al*., 2002). The day at which reached that diameter was around day 10 of the estrous cycle (Barkawi *et al*., 2002).

These anatomical changes were also accompanied with cellular and biological activities. Jainudeen and Hafez (1993) reported that, progesterone (P_4) levels in plasma and milk of buffaloes, as in cattle, reflect the endocrine activity of CL but with lower levels than in cattle. The maximum level of plasma P_4 was associated with higher number of small luteal cells (SLC) and large luteal cells (LLC) present in the CL and positively correlated with the luteal P_4 production at mid-luteal (ML) stage in buffaloes (Mishra *et al*., 2018). CL has distinctive types of cells (O'Shea *et al*., 1989), which classified as steroidogenic (small and large luteal cells) and non-steroidogenic (pericytes, fibroblasts, endothelial, macrophages, leukocytes and mast cells). Overall, there have been many studies done describing the physiological and cellular changes of CL during estrous cycle of different farm animal species. However, few investigations were done to detect developmental stages of buffalo CL at the molecular level. Therefore, the current study was performed to monitor gene expression profile during different developmental phases of cyclic Egyptian buffaloes.

MATERIALS AND METHODS

Unless stated, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Collection of CL samples:

Ovaries were collected from local slaughterhouse immediately after slaughtering. Three different developmental stages of CL: corpus hemorrhagicum: growth phase (around days 3-5), static phase (around days 7-10) and corpus albicans: regression phase (around days 19-21), were collected and classified according to their morphological features, color and size (Ireland *et al*., 1980 and Ambrose *et al*. 1993). From each developmental stage of CL, three samples were collected from three different animals.

RNA extraction:

The total RNA was extracted from all stages of corpus luteum samples using RNA isolation kit (Qiagen, Hilden, Germany). All steps of extraction were done at room temperature. A total of 30 mg of frozen CL tissue was grinded using a mortar pestle and homogenized thoroughly in 300 µl of lysis buffer, supplemented with 10 µl of β-mecaproethanol using conventional rotor-stator homogenizer for 10 sec. Afterwards, 600 µl of Proteinase K (10 µL of the included Proteinase K diluted in 590 µL of TE buffer) was added to lysate and mixed thoroughly by vortex then incubated for 10 min. The whole mix was centrifuged for 5 min at ≥12000 × g and the supernatant was transferred into a new RNase-free micro-centrifuge tube, 450 µL of ethanol (96-100%) was added and mixed by pipetting. The lysate (700 µL) was transferred to the GeneJET RNA purification column (GeneJET RNA Purification Kit, Thermofisher Scientific, CA, USA) and centrifuged for 1 min at ≥12000 × g and the flow-through was discard and this step for the rest of lysate was repeated. The GeneJET RNA purification column was placed into a new 2 mL collection tube, 700 µL of wash buffer1 was added to column, centrifuged for 1 min at ≥12000 × g. Th flow-through was discarded and this step was repeated using 250 µL of Wash Buffer 2. The collection tube containing the flow-through was discarded and column was transferred to a sterile 1.5 mL RNase-free micro-centrifuge tube. Finally, 50 µL of nuclease-free water was applied to the center of the column membrane and centrifuged for 1 min at ≥12000 × g to elute RNA, which was stored at -80°C until use. The concentration of RNA was measured using a Nano Drop 2000C (Thermo Scientific, Wilmington, DC, USA). An absorbance ratio of 1.8 to 2.0 was obtained for all samples, which indicated the high purity of RNA and free from DNA contamination.

cDNA synthesis

After adjusting RNA concentration of all samples (1µg), cDNA synthesis was performed using 2µl 10X RT random primer, 2µl 10X RT buffer, 0.8 µl 25X dNTP, 1µl multiscribe reverse transcriptase, 1µl RNase inhibitor (Applied Biosystems, , USA) and various volumes of nuclease free water to make 20 µl total volume. The following PCR program (25°C for 10 min, 37°C for 120 min and 85°C for 5 min) was used for cDNA synthesis

Quantitative real-time PCR analysis:

In order to perform the relative quantification of gene expression, specific primers of target genes were designed using Prime3 software as shown in Table (1) and according to Rozen and Skaletsky (2000) from sequences described in the GenBank database (www.ncbi.nlm.nih.gov). Quantitative analysis of cDNA samples was performed using a StepOnePlus™ Real-Time PCR instrument (Applied Biosystems, USA). Prior to quantification, the forward and reverse primers were optimized. Specific primer combinations with low threshold cycle (CT) values and that did not form primer dimers were selected for subsequent PCR. PCR was performed in a 20 uL reaction volume containing 10 uL of Power SYBR Green PCR Master Mix (Applied Biosystem). Universal thermal cycling parameters (10 minutes at 95 °C, then 40 cycles of 15 seconds at 95 °C, 20 seconds at 60 °C, and 30 seconds at 72 °C, followed by a final 60 seconds extension at 60 °C) were used to quantify each gene of interest (Table 1). Results were quantitatively analyzed with the delta delta Ct method and were reported as relative expression to the calibrator after normalization of the target transcript to the endogenous control.

Table 1. List of primers used for quantitative real-time PCR analysis

Gene Name	Gene bank accession number	Primer sequence	Fragment size (bp)
TFAM	NM_001034016	F: 5'-CTGGTCAGTGCTTTGTCTGC-3' R: 5'-CTAAAGGGATAGCGCAGTCG-3'	128
SOD2	NM_201527	F: 5'-GTGATCAACTGGGAGAATGT-3' R: 5'-AAGCCACACTCAGAAACACT-3'	163
GAPDH	NM_001034034.2	F: 5'- AGGTCGGAGTGAACGGATTC -3' R: 5'- GGAAGATGGTGATGGCCTTT -3'	219
CPT2	NM_001045889	F: 5'-CCGAGTATAATGACCAGCTC-3' R: 5'-GCGTATGAATCTCTTGAAGG-3'	152
SREBP1	NM_001113302	F: 5'-CCGTTTCTTCGTGGATGG-3' R: 5'-ATGCTGGAGCTGATGGAG-3'	264
TNFα	AF011927	F: 5'-GTGAAGTCGCTCAGTCGTGC-3' R: 5'-TCTACAAGGCGGGAGACCTG-3'	170
NFE2L2	NM_001011678	F: 5'-TAAAACAGCAGTGGCTACCT-3' R :5'-GAGACATTCCCGTTTGTAGA-3'	159

PCR (polymerase chain reaction) bp (base pair), F (Forward), R (Reverse)

Data analysis:

The data produced from the evaluation of gene expression profile from the three animals was analyzed using the General Linear Model procedure (SAS Institute Inc., 2011). The analysis was done to test the effect of stages of CL on the expression of two genes by the use of the following model: $Y_{ij} = \mu + C_i + e_{ij}$. Y_{ij} = Measured trait, μ= Overall mean, C_i = Stages of CL, and e_{ij} = Experimental error. The mean values were compared for statistical significance using Duncan's range test (Duncan, 1955). Differences were considered statistically significant at $P \leq 0.05$.

RESULTS

Expression profile of SOD2 (Figure 1), TFAM (Figure 2) and CPT2 (Figure 3) increased significantly (P≤0.000) in static phase compared to growing and regression ones. Meanwhile, the expression of SOD2 and TFAM genes was higher insignificantly during growth phase than regression phase. However, the transcriptional profile of CPT2 gene was significantly higher during growth phase than regression phase. Meanwhile, the transcript abundance of SREBP1 (Figure 4) increased (P≤0.000) at static phase while it was at minimum profile during regression phase of CL. The expression profile of NFE2L2 (Figure 5) was not significantly different among all stages of CL development, however; it reached the highest expression during static phase. On the other hand, relative transcript abundance of TNFα (Figure 6) was higher (P≤0.000) at regression phase than growth and static phases.

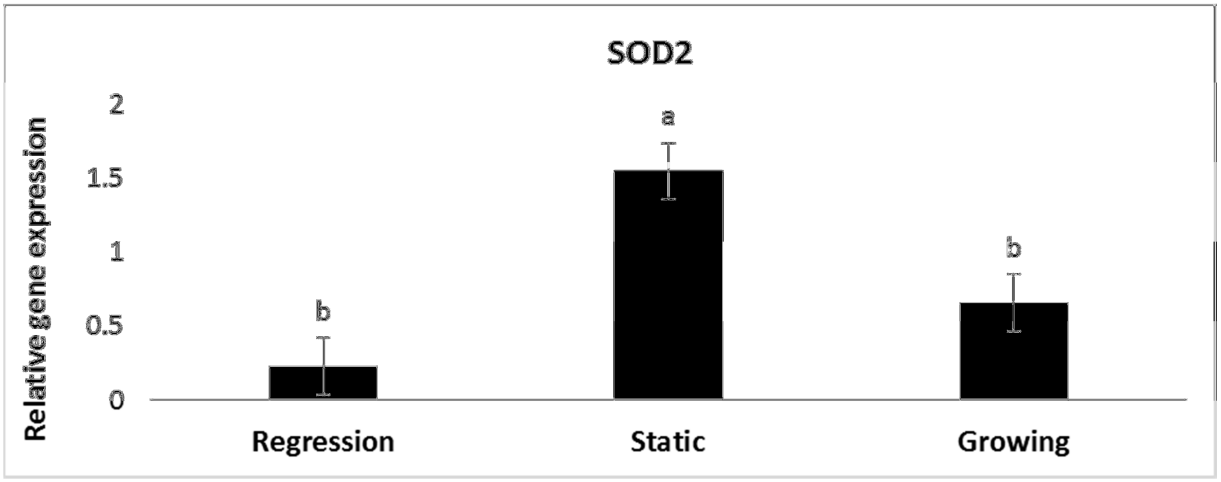

Figure 1. Transcriptional abundance of SOD2 in different developmental phases of cyclic Egyptian buffalo's corpus luteum

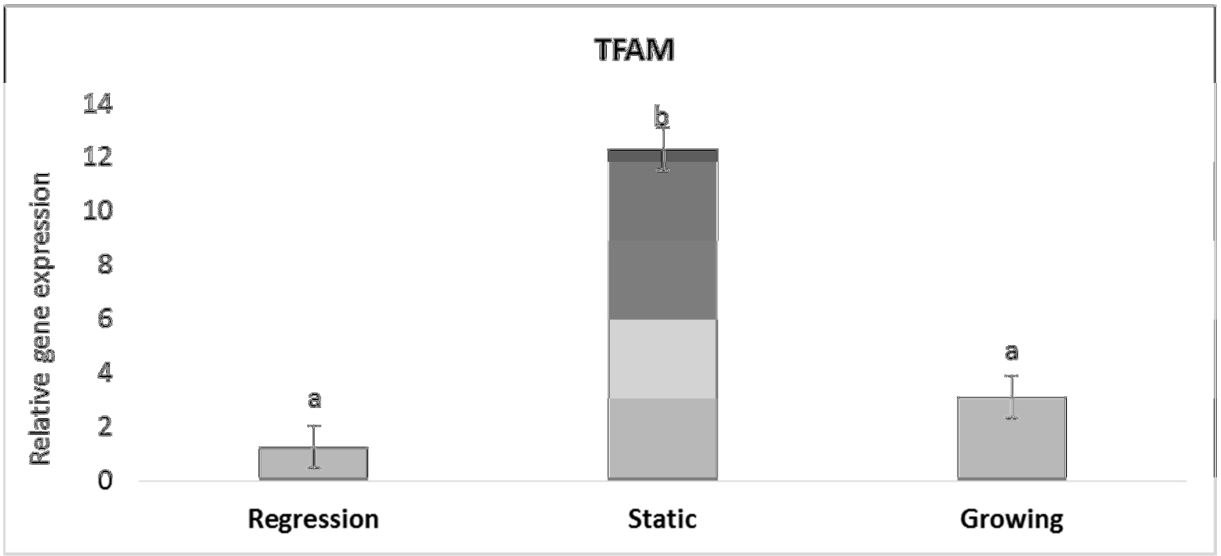

Figure 2. Transcriptional abundance of TFAM in different developmental phases of cyclic Egyptian buffalo's corpus luteum.

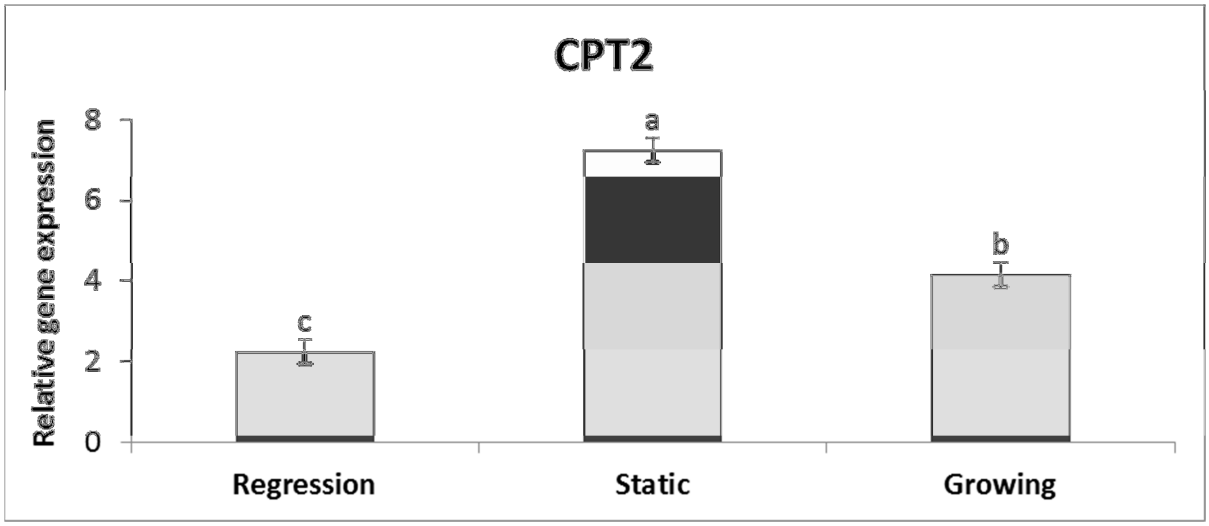

Figure 3. Transcriptional abundance of CPT2 in different developmental phases of cyclic Egyptian buffalo's corpus luteum.

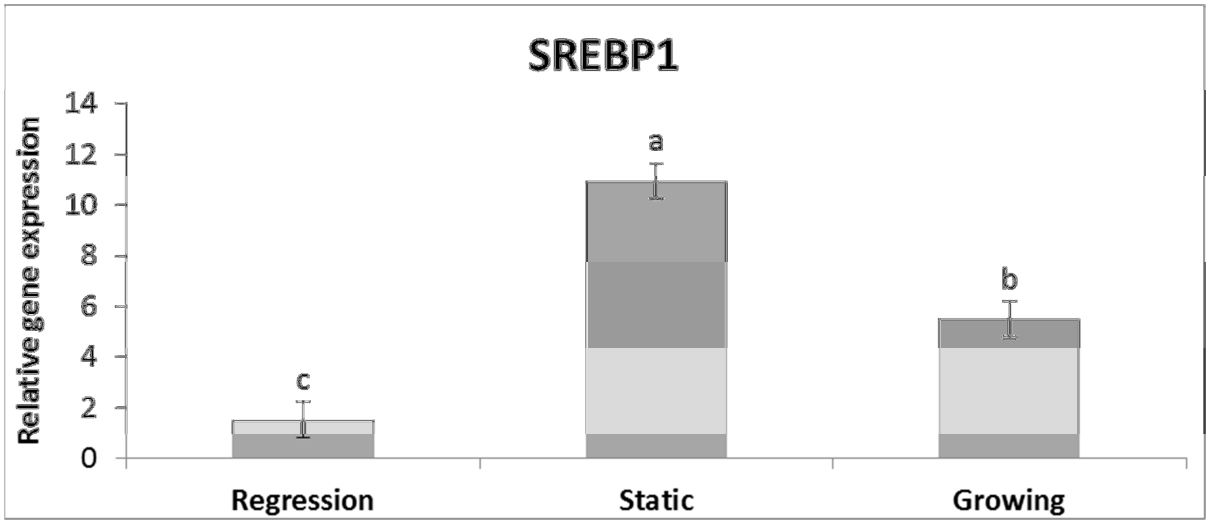

Figure 4. Transcriptional abundance of SREBP1 in different developmental phases of cyclic Egyptian buffalo's corpus luteum.

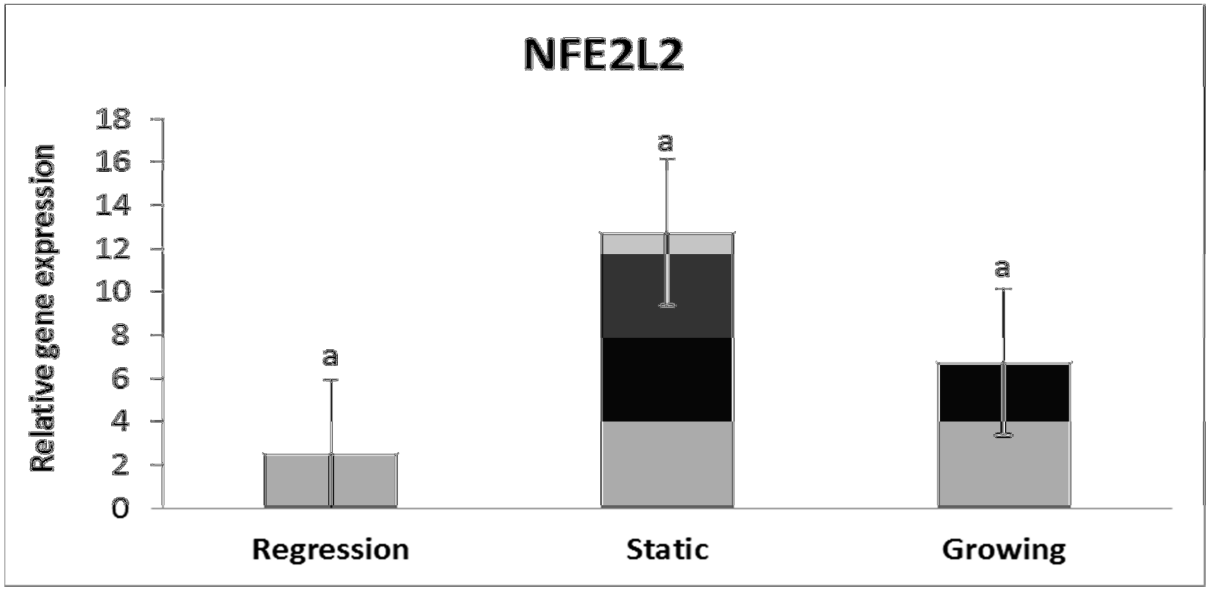

Figure 5. Transcriptional abundance of NFE2L2 in different developmental phases of cyclic Egyptian buffalo's corpus luteum.

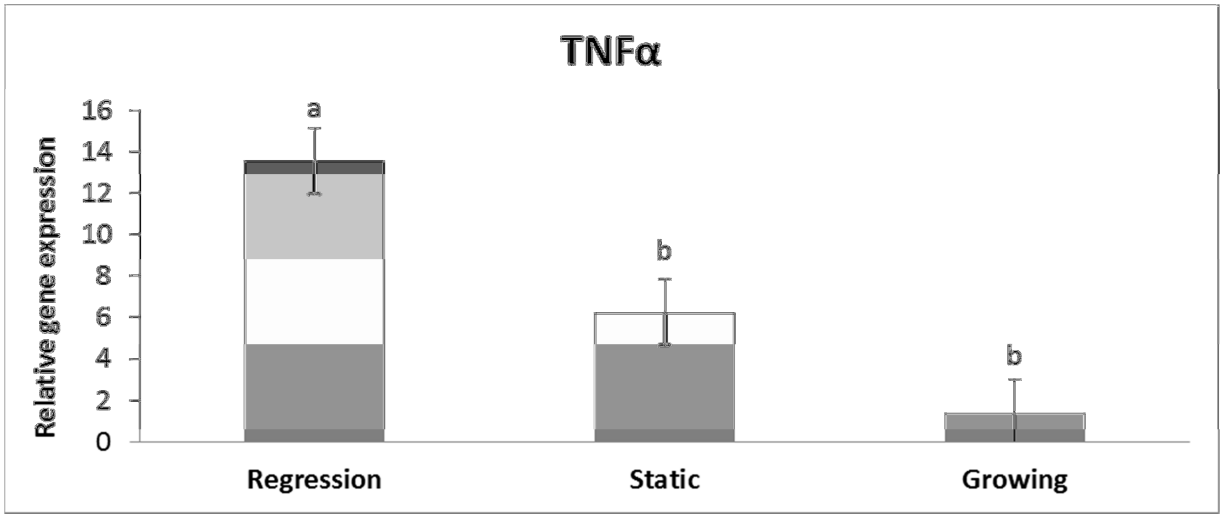

Figure 6. Transcriptional abundance of TNFα in different developmental phases of cyclic Egyptian buffalo's corpus luteum.

DISCUSSION

Mitochondria have intrinsic functions during ovarian follicle development and granulosa cells luteinization. Progesterone synthesis started inside the mitochondria and finally produced in the endoplasmic reticulum of lutein cells (Niswender *et al*., 2000). Moreover, mitochondrial transcripts represented approximately 14% of overall expressed sheep ovary transcriptome (Pokharel *et al*., 2018) which implied a high-energy demand tissue. Consistent with this idea, results of the current study revealed up-regulation of three mitochondrial transcripts (SOD2, TFAM and CPT2) in static compared to both growing and regressed phases of CL development. In mouse, Foyouzi *et al.* (2005) found that mRNA for all superoxide dismutase (SOD) enzymes (SOD1, SOD2, and SOD3) expressed in the CL. In addition, SOD2 was the most highly expressed gene in CL (Foyouzi *et al*., 2005). Kawaguchi *et al*. (2013) indicated that LH could increase the level of both mRNA protein of SOD1 and SOD2 in bovine CL, which reached the highest expression in the mid-luteal phase. The up-regulation of these two antioxidant enzymes (SOD1, SOD2) is crucial for sustaining cell viability and maintaining CL function during the luteal phase. Indeed, luteal cells generate oxygen radical species (ROS) as a result of normal cellular metabolism, which has been linked with decreased progesterone production and cell death (Riley *et al*., 1991). Thus, up-regulation of SOD2 might increase the ability of cells to neutralize ROS. On the other hand, the reduced expression of SOD2, TFAM and CPT2 in coincidence with the regressed phase could be explained by the reduction in the relative volume of mitochondria in the lutein cells during luteolysis process of CL (Pivko *et al*., 2016). It is known that β-oxidation of fatty acids depends on the activity of two key enzymes (CPT1B and CPT2) residing in outer and inner mitochondrial membranes (Sutton-McDowall *et al*., 2012).

The process of steroidogenesis in CL depends mainly on the supply of cholesterol as required precursor from intracellular biosynthesis and extracellular sources. Sterol regulatory element-binding proteins (SREBP) regulate cholesterol (Hua *et al.,* 1993) and fatty acids (Ye and DeBose-Boyd 2011) at intracellular level.

In the process of CL regression, there is a decline in steroidogenesis coupled with reduction of SREBP2 activity (Rennert *et al*., 1990 and Xu *et al*., 2018). Obtained results of higher expression of SREBP1 during static than both growing and regression phases revealed a possible action of SREBP1 in supporting P_4 synthesis and secretion by luteal cells during static phase. Similarly, high expression of PAPP-α as lipid candidate gene was detected in CL of buffalo ovary as compared to corpus albicans (Nayan *et al.,* 2013).

The tumor necrosis factor alpha (TNFα) is one of the apoptotic signals, which interact with certain cell surface receptors that induce cell death (Nagata, 1997). In accordance, the results of the current investigation revealed higher relative transcript abundance of TNFα at regression phase than growth and static ones of CL development. In 1995, Shaw and Britt observed that TNFα is released during both spontaneous and PGF2aα induced luteolysis of bovine CL. This also supports the idea that TNFα regulates CL regression via up-regulating its specific receptors (TNFR type-I) during bovine CL luteolysis (Sakumoto *et al*., 2014 and Neuvians *et al*., 2004). Moreover, Friedman *et al*. (2000) suggested that, the tumor necrosis factor α (TNFα) which induced programmed cell death during structural luteolysis is mediated by its type I receptor (TNFR I), affecting endothelial cells, and then the decline in P40 Preceding structural luteolysis, is a prerequisite for the initiation of apoptosis in endothelial cells. Furthermore, TNFα induces synthesis and release of endothelin 1 (EDN1) from endothelial cells that may induce CL regression (Okuda *et al*., 1999 and Friedman *et al*., 2000). Therefore, some researchers (Meidan *et al*., 1999 and Meidan *et al*., 2005) have

suggested that EDN1 is activated and subsequently regulating the structural regression of CL by promoting migration of leucocyte and stimulating release cytokines such as TNF-α from macrophages.

On the other hand, the expression of two genes (SOD2 and NFE2L2) that involved in cellular defense mechanism against any stress signals increased during static phase compared with growing and regression phases of CL cyclic changes. Because of normal metabolism, cells generate oxygen radical species and the luteal cells increased production of ROS that has been linked with reduced secretion of P_4 and apoptosis of its cells (Riley *et al*., 1991 and Foyouzi *et al*., 2005). As the mRNA for all SOD enzymes (SOD1, SOD2 and SOD3) expressed in CL of mice being moreover, SOD2 the most highly expressed gene (Foyouzi et al. 2005). Therefore, an explanation of higher expression of NFE2L2 and SOD2 genes is based on the highlighted crucial role of these genes in defense mechanism against oxidative stress as a recent study showed that downregulation of NFE2L2 expression has resulted in increased ROS level, reduced mitochondrial activity and cellular proliferation (Khadrawy *et al*., 2019). Interestingly, P_4 profile during the luteal phase was positively correlated with the activity of SOD1. Reduction in SOD1 during CL regression was accompanied by increased ROS levels (Behrman *et al*., 2001). Kawaguchi *et al*. (2013) observed increasing expression of the protein and mRNA levels of both SOD1 and SOD2 in the bovine CL reached the highest expression profile in the mid-luteal phase as a result of LH induction.

In conclusion, the profile of genes regulating mitochondrial activity, lipid metabolism, pro-inflammatory and stress response was coupled with morphological and biological changes of corpus luteum during estrous cycle of Egyptian buffaloes.

REFERENCES

Ambrose, J.D., R.S. Manik, S. K. Singla and M.L. Madan, 1993. A simplified laparoscopy technique for repeated ovarian observation in the water buffalo (Bubalus bubalis). Theriogenology, 40: 487-496.

Barkawi, A.H., Y.M. Hafez, S.A. Ibrahim, A.K. El-Asheeri and N. Ghanem, 2002. Progesterone profile in relation to corpus luteum development throughout the normal estrous cycle of Egyptian buffaloes. Egyptian Journal of Animal Production 39, 87-94.

Behrman, H. R., P. H. Kodaman, S. L. Preston and S. Gao, 2001 "Oxidative stress and the ovary," Journal of the Society for Gynecologic Investigation, vol. 8, pp. S40–S42.

Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics 11, 1-42.

Fadel, M.S., 2000. Uses of ultrasonography in monitoring the reproductive pattern in some farm animals. Ph.D Thesis, Fac. of Vet. Med., Cairo Univ., Giza, Egypt.

Foyouzi, N., Z. Cai, Y. Sugimoto and C. Stocco, 2005. Changes in the Expression of Steroidogenic and Antioxidant Genes in the Mouse Corpus Luteum During Luteolysis Biol Reprod. May;72 (5):1134-41.

Friedman, A., S. Weiss, N. Levy and R. Meidan, 2000. Role of tumor necrosis factor and its type I receptor in luteal regression introduction of programmed cell death in bovine corpus luteum-derived endothelial cells. Biology of Reproduction, 63, 1905-1912.

Hua, X., C. Yokoyama, J. Wu, M.R. Briggs, M.S. Brown, J.L. Goldstein and X. Wang, 1993. SREBP-2, a second basic-helix-loop-helix-leucine zipper protein that stimulates transcription by binding to a sterol regulatory element. Proc Natl Acad Sci U S A. 90:11603–11607.

Ireland, J.J., R.L. Muphee and P.B. Coulso, 1980. Accuracy of predicting stages of bovine estrous cycle by gross appearance of corpus luteum. Journal of Dairy Science, 63: 155- 160.

Jainudeen, M.R., and E.S.E. Hafez, 1993. Cattle and Buffalo. In: Reproduction in Farm Animals, by E.S.E. Hafez (ed), Lea & Febiger, Philadelphia, U.S.A. (Fifth edition).

Kawaguchi, S., R. Sakumoto and K. Okuda, 2013. Induction of the expressions of antioxidant enzymes by luteinizing hormone in the bovine corpus luteum. J Reprod Dev. 59(3):219-24.

Khadrawy, O., S. Gebremedhn, D. Salilew-Wondim, M.O. Taqi, C. Neuhoff, E. Tholen, M. Hoelker, K. Schellander and D. Tesfaye, 2019. Endogenous and Exogenous Modulation of Nrf2 Mediated Oxidative Stress Response in Bovine Granulosa Cells: Potential Implication for Ovarian Function. Int J Mol Sci. Apr 2;20(7). pii: E1635.

Meidan, R., N. Levy, T. Kisliouk, L. Podlovny, M. Rusiansky and E. Klipper, 2005. The yin and yang of corpus luteum-derived endothelial cells: Balancing life and death. Domest Anim Endocrinol 29, 318–328.

Meidan, R., R.A. Milvae, S. Weiss, N. Levy and A. Friedman, 1999. Intra-ovarian regulation of luteolysis. J Reprod Fertil Suppl 54, 217–228.

Mishra, G. K., M. K. Patra, P. A. Sheikh, A. S. Teeli, N. S. Kharayat, M. Karikalan, S. Bag, S. K. Singh, G. K. Das, K. Narayanan and H. Kumar, 2018. Functional Characterization of Corpus Luteum and its Association with Peripheral Progesterone Profile at Different Stages of Estrous Cycle in the Buffalo. Journal of Animal Research: v.8 n.3, p. 507-512. June.

Nagata, S., 1997. Apoptosis by death factor. Cell 88, 355-365.

Nayan, V., B. Anuradha and S. Dheer, 2013. PAPP-A in Indian Water Buffalo (Bubalus bubalis) Ovary: Phylogeny, Expression, Hormonal Regulation, and Sequence Characterization. Agric Res., 2(2):153–165.

Neuvians, T.P., D. Schams, B. Berisha and M.W. Pfaffl, 2004. Involvement of pro-inflammatory cytokines, mediators of inflammation, and basic fibroblast growth factor in PGF2ainduced luteolysis in bovine corpus luteum. Biol Reprod 70, 473–480.

Niswender, G.D., J. L. Juengel, P. J. Silva, M. K. Rollyson and E.W. McIntush, 2000. Mechanisms controlling the fuctions and life span of the corpus luteum. Physiol Rev. Jan; 80(1):1-29.

O'Shea, J. D., R. J. Rodgers and M. J. D'Occhio, 1989. Cellular composition of the cyclic corpus luteum of the cow. J Reprod Fertil, 85: 483–487.

Okuda, K., R. Sakumoto, Y. Uenoyama, B. Berisha, A. Miyamoto and D. Schams, 1999. TNFa receptors in microvascular endothelial cells from bovine corpus luteum. Biol Reprod 61, 1017–1022.

Pivko J., V.A. Makarevich, E. Kubovičova, J. Rafay and P. Chrenek, 2016. Ultrastructural changes in the cyclic corpus luteum of dairy cows with different body condition. Acta Veterinaria-Beograd 2016, 66 (2), 245-256.

Pokharel, K., J. Peippo, M. Honkatukia, A. Seppälä, J. Rautiainen, N. Ghanem, T. M. Hamama, M. A. Crowe, M. Andersson, M. H. Li and J. Kantanen, 2018. Integrated ovarian mRNA and miRNA transcriptome profiling characterizes the genetic basis of prolificacy traits in sheep (Ovis aries). BMC Genomics. 29;19(1):104.

Quintal-Franco, J. A., F. N. Kojima, E. J. Melvin, B. R. Lindsey, E. Zanella, K. E. Fike, M. E. Wehrman, D. T. Clopton and J. E. Kinder, 1999. Corpus luteum development and function in cattle with episodic release of luteinizing hormone pulses inhibited in the follicular and early luteal phases of the estrous cycle. Biol Reprod. Oct;61(4):921-6.

Rennert, H., R. T. Fischer, J. G. Alvarez, J. M. Trzaskos and J. F. Strauss, 1990. 3rd. Generation of regulatory oxysterols: 26hydroxylat ion of cholesterol by ovarian mitochondria. Endocrinology. Aug;127(2):738-46.

Riley, J. C., and H. R. Behrman, 1991. In vivo generation of hydrogen peroxide in the rat corpus luteum during luteolysis. Endocrinology. 128: 1749–1753.

Rozen, S., and H. Skaletsky, 2000. Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol.132:365-86.

Sakumoto, R., K. G. Hayashi and T. Takahashi, 2014. Different expression of PGE synthase, PGF receptor, TNF, Fas and oxytocin in the bovine corpus luteum of the estrous cycle and pregnancy. Reprod Biol. Apr;14(2):115-21.

SAS Institute Inc., 2011. SAS/STAT® 9.3 User's Guide. SAS Inst. Inc., Cary, NC, USA.

Shaw, D. W., and J. H. Britt, 1995. Concentrations of TNFa and P4 within the bovine corpus luteum sampled by continuousflow microdialysis during luteolysis in vivo. Biol Reprod 53, 847–854.

Sutton-McDowall, M. L., D. Feil, R. L. Robker, J. G. Thompson and K. R. Dunning, 2012. Utilization of endogenous fatty acid stores for energy production in bovine preimplantation embryos. Theriogenology. 77: 1632–41.

Xu, Y., S. M. Hutchison, J. J. Hernández-Ledezma, and R. L. Bogan, 2018. Increased 27-hydroxycholesterol production during luteolysis may mediate the progressive decline in progesterone secretion. Mol Hum Reprod. Jan 1;24(1):2-13.

Ye, J., and R. A. DeBose-Boyd, 2011. Regulation of Cholesterol and Fatty Acid Synthesis. Cold Spring Harb Perspect Biol. Jul 1;3(7).

التعبير عن الجينات المختارة خلال مراحل مختلفة من تطور الجسم الأصفر في الجاموس المصري

مروه سعيد فهيم[1,2] ، شريف محمد دسوقى[1,2]

١ - قسم الإنتاج الحيوانى، كلية الزراعة، جامعة القاهرة، ٢ - مجمع المعامل البحثية، كلية الزراعة، جامعة القاهرة

أجريت هذه الدراسة لبحث نمط التعبير الجينى لبعض الجينات المختارة فى الجسم الأصفر بالجاموس المصرى. تم جمع تسعة أجسام صفراء خلال الدراسة من الجاموس المصرى المذبوح. تم تقسيم الأجسام الصفراء بناءا على مراحل التطور والصفات المورفولوجية الى أجسام صفراء نامية (من اليوم ٣- إلى ٥)، أجسام صفراء مكتملة النمو (من اليوم ٧- إلى١٠) وأجسام صفراء مضمحلة (من اليوم ١٩- إلى٢١). تم تقييم مدى توفر النسخ من تعبير الجينات المتحكمة فى نشاط الميتوكوندريا (SOD2, TFAM and CPT2) والتمثيل الغذائى للدهون (SREBP1) والسيتوكين المرتبط بالإلتهاب (TNFα) والإجهاد التأكسدى (NFE2L2) مقارنة بالتعبير الجينى المرجع (GAPDH).

وجدت زيادة ($P \leq 0.05$) فى التعبير الجينى للجينات المرتبطة بنشاط الميتوكوندريا فيما يتعلق بكلا من TFAM و SOD2 فى مرحلة إكتمال النمو مقارنة بمرحلتى النمو والإضمحلال. فى حين حقق التعبير الجينى للجينات المرتبطة بتمثيل الدهون (SREBP1) و نشاط الميتوكوندريا (CPT2) إرتفاعا معنويا فى مرحلة إكتمال النمو بينما وصل مستوى التعبير الجينى لهما لأقل مستوياته خلال مرحلة الإضمحلال. لم يلاحظ وجود إختلافات معنوية فى مستوى تعبير الجين المسئول عن الإجهاد التأكسدى (NFE2L2) خلال المراحل المختلفة لتطور الجسم الأصفر. وعلى العكس أظهر التعبير الجينى للسيتوكين المرتبط بالإلتهاب إرتفاعا معنويا خلال مرحلة الإضمحلال مقارنة بمرحلتى النمو وإكتمال النمو. مما سبق يتضح أن هناك زيادة فى نمط التعبير الجينى للجينات المتحكمة فى نشاط الميتوكوندريا وتمثيل الدهون خلال مرحلة نمو الجسم الأصفر ثم بلغت أعلى مستوى أثناء مرحلة إكتمال النمو إلى أن وصلت لأقل تعبير لها خلال مرحلة الإضمحلال. فى حين أن أظهر جين السيتوكين المرتبط بالإلتهاب نمطا مختلفاً. وهكذا يعكس التعبير الجينى التغيرات الدورية أثناء تطور الجسم الأصفر بالجاموس المصرى.