j.Egypt.net.med.Assoc 80, no 2. 209 – 214 (2020)

TRANSCRIPT ABUNDANCE OF GAPDH, PGES, HSP70, PPAR AND SOD2 MRNA GENES EXPRESSION DURING THE DIFFERENT STAGES OF REPRODUCTION IN EGYPTIAN BUFFALOES

By

Seham S. Soliman[a], Abdoon A.S. [a]*, Nahed El-Touchy, Omima M.T. Kandil[a], Sabra.H.A. [a] and Attia M.Z

[a]Department of Animal Reproduction and Artificial Insemination, Veterinary Research Division, National Research Centre, Dokki 12622, Cairo, Egypt.

[b]Department of Physiology, Faculty of Veterinary Medicine, Cairo University, Egypt.

ABSTRACT

The buffalo's production has never been accomplished owing to many reproductive problems. Smooth inactive ovaries and low pregnancy rate are major constraint. Molecular genetics provide valuable information about genes underlying quantitative fertility traits. In order to understand better how the expression levels of PGES, HSP70, PPAR and SOD2 genes related to buffalo fertility. The present study investigated the expression levels of the genes in buffalo's endometrial and luteal tissues. Endometrial and luteal tissues collected from slaughtered female buffaloes (Cyclic, pregnant, smooth inactive ovaries) (n =7/group). Total RNA was extracted from such tissues and c-DNA was synthesized, RT-PCR was performed for five genes including GAPDH, PGES, HSP70, PPAR γ and SOD2. The results showing that, the PGES (essential for pregnancy maintenance) genes was significantly up regulated in pregnant animals endometrium and corpus luteum (CL) ($p < 0.05$) as compared to the smooth inactive ovaries. The expression of PPAR γ gene (essential for embryo development) was significantly up regulated ($p < 0.01$) in pregnant animal endometrium and CL comparing with the smooth inactive buffaloes ovaries , while, SOD2 (indicator of oxidative stress) gene was significantly up regulated in smooth inactive ovaries endometrium and down regulated in pregnant endometrium and CL ($p < 0.01$). HSP70 (indicator to several stress factors) expression was significantly ($P < 0.01$) up regulation in smooth inactive ovaries endometrium, down regulated in pregnant buffaloes endometrium and CL ($p < 0.01$). All genes expression are significantly ($P < 0.01$) higher in endometrium than in CL. In conclusion, PPAR γ and PGES genes were down regulated in infertile buffaloes (smooth inactive ovaries) and it up regulated in pregnant buffalo, while SOD2 and HSP70 genes were

down regulated in pregnant animals and up regulated in smooth inactive ovaries. These genes can be used to select fertile and exclude infertile buffaloes.

Keywords:

Buffaloes, Gene expression, Cyclic, Pregnant, Smooth inactive ovaries.

INTRODUCTION

Buffalo is a species of great economic potential, it provides meat and milk in Egypt. Productive and reproductive traits are affected by genetic factors. Improving dairy buffalo fertility by genetic selection is important, since declining fertility cannot only be arrested by improved management. Genomic selection is better than traditional breeding methods in the accuracy of choosing juvenile animals, it is the first point for reproductive performance improvement and new management strategies development **(Fooda *et al.*, 2010)**. Knowledge on genes is important to combat poor fertility, such as the development of biomarkers to identify non-pregnant versus pregnant, and non-cycling versus cycling animals **(Barbat *et al.*, 2010)**. Identify gene expression patterns in the endometrium could be used for differential diagnosis of fertility problems **(Bauersachs *et al.*, 2007)**.

The Prostaglandin E synthase (PGES) gene expression involved in prostaglandin synthesis and its expression increases with pregnancy **(Ankita *et al.*, 2018)**. This gene controls the female reproductive processes that include corpus luteum lifespan, follicular development, ovulation, parturition and pregnancy in the dairy animals **(Ravjibhai *et al.*, 2018)**. Peroxisome proliferator-activated receptors (PPARγ) play a main role in the function and development of uterus **(Szymanska and Blitek, 2018)**. It is important in pregnancy as it mediate fetal growth **(Matsuda *et al.*, 2013)**.

The Heat shock proteins (HSPs) are a cluster of greatly conserved proteins that are encouraged in both eukaryotes and prokaryotes by a diversity of cellular stresses **(Ross *et al.*, 2003).** Amongst the HSP, HSP70 has a major role in cell thermo tolerance **(Beckham *et al.*, 2004**), besides its expression acts as a possible sign of animal adaptation to severe environmental stress (**Hansen, 2004**). HSP70 gene is a preferred choice as "molecular chaperon" in stress management in anestrous animals **(Rosic *et al.*,2010)**.Gene's identification discussing cellular thermo tolerance gives the possibility of transferring these genes to heat-sensitive breeds towards reproduction improvement (**Hansen, 2004)**. Superoxide dismutase 2 (SOD2) is the main antioxidant enzyme responsible for maintaining and

protecting the anti-oxidative/oxidative balance, impaired antioxidant to oxidative status leads to oxidative damage resulting in immune suppression and aggravate the inflammatory conditions **(Bhattacharyya *et al*., 2014).**

Inactive ovaries represent 50% of the causes of summer infertility in Egyptian buffaloes **(Soliman *et al*., 2016)**. Pregnancy should be understood at molecular level, numerous processes which are critical for pregnancy establishment occur through pregnancy, counting implantation, placentation, initiation of placental and fetal growth **(Bairagi *et al*., 2016).**

Endometrium has an essential role in the conceptus elongation besides implantation and embryo nourishment **(Bazer *et al*., 2012)**. Making comparison between expression of genes between smooth inactive ovaries and pregnant animals may manipulate the reproduction **(Ankita *et al*., 2018).**

Until now, there is no enough literature present studying the mechanisms by which these genes regulate fertility in Egyptian buffalo. Consequently, it seems obligatory to define the molecular mechanisms that affect fertility.

The present work was planned to study the relative mRNA expression of PGES, HSP70, PPARγ and SOD2 genes using RT-PCR technique in buffalo endometrium and corpus luteum in cyclic, pregnant and smooth inactive ovaries.

MATERIAL AND METHODS

Sample Collection:

Genital tracts of apparently healthy female buffalo were collected at local abattoir (El-Waraq and El-Moneibe) immediately after slaughter. After screening about 21 genitalia, 7 were selected comprising cyclic non-gravid uterus, gravid uterus of pregnancy (n=7) and smooth inactive ovaries animals (n=7) and transported to the laboratory under aseptic conditions on ice. The different stages of reproductive status were distinct via macroscopic observation of the ovaries (corpus luteum stage, consistency, color, size and number of follicles) and the uterus (color, mucus and consistency).

Genital organs were washed twice in PBS. Uteri were dissected longitudinally for tissue collectionand cut open along the greater curvature on their longitudinal axis. The endometrial and luteal tissue from those stages were collected, washed with ice,cold physiological saline, about 200 -300 mg of endometrial and luteal tissue were collected and stored immediately in liquid nitrogen until used for RNA isolation.

Ribonucleic acid (RNA) extraction and complementary DNA (c-DNA) synthesis:

Total RNA was extracted from frozen luteal and endometrium tissues after homogenization using trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Total RNA samples were purified on Qiagen columns according to the manufacturer's protocol (RNeasy Mini kit, Qiagen) the quantity and quality of RNA were determined using the Agilent 2100 Bio analyzer (Agilent Technologies, Santa Clara, CA, USA) and the Nano Drop 1000 (Thermo Fisher Scientific, Inc., Wilmington, DE, USA) respectively. Only samples with RNA integrity number above 0.8 were used for gene expression analysis. Upon addition of RNase inhibitor (RNasin, Promega), total RNA was stored at - 80 °C.

Total RNA (1ug) was converted to c-DNA using Superscript II kit (Invitrogen) in a 20-uL volume. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed for four candidate genes and for GAPDH as housekeeping gene. Primers for candidate genes (Table 1) were designed using Primer 3 online software (http:// frodo.wi.mit.edu/primer3/, accessed January 2009) and subsequently entered in the Basic Local Alignment Search Tool to ensure specificity(BLAST;http://blast.ncbi.nlm.nih.gov/Blast.cgiaccessed January2009). Quantitative real-time PCR was performed on the 7000 Fast Real-Time PCR System (Applied Biosystems). Each reaction consisted of 20 ng c-DNA,forward and reverses primers (Both 300 nmol) and 7.5 mL SYBR green master mix (Applied Biosystems) made up to a final reaction volume of 15 mL with RNase- and DNase- free water. All reactions were performed in duplicate. Cycling conditions consisted of 50 °C for 2 min, 95 °C for 10 min and 45 cycles of 95 °C for 15 s, 60 °C for 1 min and followed by elongation at 72 °C for 1 min. A dissociation curve was included to ensure specificity of amplification. Based on the relative standard curve method, quantification of the amount of candidate genes mRNA relative to that of GAPDH were calculated **(Abdoon *et al.*, 2014)**.

Table (1): Primer sequences description used for gene quantification by real-time PCR.

Gene	Gene Symbol	accession no.	Primer sequence	Size	Tm. (ºC)
Glyceraldehyde 3 phosphate dehydrogenase.	GAPDH	U85042	F:50-CCCAGAAGACTGTGGATGG-30 R: 50-AGTCGCAGGAGACAACCTG-30	306	62.0 62.0
Heat shock pratein 70	HSP70	JN604432.1	F: 50-TTCGTGGAGGAGTTCAAGAG-30 R 50TGAAGATCTGCGTCTGCTTC-30	565	57.3 57.3
Superoxide dismutase 2	SOD2	NM_201527.2	F: 5'-ACGTGAACAACCTCAACGTC-3 R: 5'-AGTCACGTTTGATGGCTTCC-3'	201	57.3 57.3
Prostaglandin E synthase	PGES	AY032727	F:5- TGCAAAGTGGTACGATCGG -30 R:5- TAACCTTGGCCATGACTGG -30	103 bp	56.7 59.7
Peroxisome proliferator-activated receptors	PPARγ	Y12419	F: TTC AGA AGT GCC TTG CTG TG R: TCA GCG GGA AGG ACT TTA TG	186	60.2 60.2

Analysis of real-time PCR data:

After amplification the cycle threshold (Ct) values of both experimental and control groups with reference gene were taken for fold change calculating in gene expression target. Expression of GAPDH was taken as an endogenous reference**.** In negative controls, nuclease free water was substituted for template. Relative quantification of target gene was done by the $2^{\Delta\Delta CT}$ method **(Chaudharia *et al*., 2018)**.

Statistical Analysis:

All analyses were performed using one-way ANOVA followed by Tukey's post hoc test (Duncan, LSD) test using SPSS Statistics for Windows (Version 20). After normalization by GAPDH, means ± standard error of mean (SEM) were calculated and significance was set at $P < 0.05$ as illustrated in (Tables 2, 3).

RESULTS

The expression of five genes related to fertility in endometrial and luteal tissues was investigated in different reproductive status of buffalo. The expression of PGES, HSP70, PPARγ, and SOD2 genes significantly vary between cyclic, pregnant and smooth inactive ovaries of buffaloes.

Relative expression of Prostaglandin E synthase (PGES) gene:

Prostaglandin E synthase (PGES) gene expression was significantly up regulated in pregnant endometrium and corpus luteum ($p<0.05$) in comparison to non-pregnant and down regulated ($p<0.05$) in smooth inactive ovaries with fold change of7.4and2.2 respectivelywhen compared with 1 fold change in estrus animal. Its expression significantly increases in corpus luteum by 1.8 fold change. Its expression in endometrium is higher than in corpus luteum Fig. (1).

Relative expression of Heat shock protein 70 (HSP70) gene:

From Fig. (2) it can be seen that, the expression pattern analysis heat shock protein 70 gene was significantly higher ($p < 0.01$) in smooth inactive ovaries endometrium with 2.9-fold change. However, the difference was reported to be significant in the cases of pregnant and cyclic with 0.8- and 1-fold change respectively, when comparing with the smooth inactive ovaries buffaloes, although the variance between pregnancy and smooth inactive ovaries was found to be very significant ($p < 0.05$). In corpus lutum samples, expression of HSP70 gene decreased significantly ($p < 0.01$) with 0.6-fold change in pregnant. Its expression in endometrium was higher than in corpus luteum.

Relative expression of Peroxisome proliferator-activated receptors (PPARγ) gene:

There was significant difference for the expression of PPARγ gene in the endometrium and luteal samples of pregnant, cyclic comparing with the smooth inactive ovaries buffalo. The expression pattern analysis of PPARγ gene revealed significantly up regulated ($p < 0.05$) in pregnant buffaloes with 2.11-fold change and 1-fold change for cyclic one as compared to 0.54 in the smooth inactive ovaries buffalo as shown in Fig. (3). its expression in endometrium was higher than in corpus luteum. The pattern analysis for PPARγ and GPES expression have exposed similar expression trend and it displayed highly significant increase ($p < 0.01$) in fold change in pregnancy with fold change 2.1 and 7.4 respectively, both the genes showed no significant difference in cyclic one.

Relative expression of Superoxide dismutase 2 (SOD2) genes:

Data of the present work revealed that, the pregnant animals revealed highly significant ($p < 0.01$) difference when comparing with the non-pregnant one. SOD2 gene revealed significant down regulation ($p < 0.01$) with (Fold change of 0.4) in pregnancy and cyclic phase as compared to inactive ovaries buffalo (Fold change of 1.5) as in Fig. (4). The SOD2 and HSP70 expression had also revealed nearly similar trend designated high significant

($p < 0.01$) rise in smooth inactive ovaries buffaloes with fold change of 1.5 and 2.9 respectively. But HSP70 gene had non-significant ($p > 0.05$) down regulation in pregnant animals.

DISCUSSION

Understanding the mechanism of discriminates among fertile and an infertile buffalo by gene expression is essential for translation of research into practice for the reproductive efficiencies and fertility improvement in Egyptian buffaloes, scarce information is presented about the molecular mechanisms driving buffalo fertility.

The present work was directed to provide new insights about the endometrial and luteal gene expression in different reproductive status in Egyptian buffalo, a major contributor in the husbandry of animal as milk, meat and draft animal. The production potential is below expectations owing to certain innate reproductive features such as low pregnancy rate, prolonged calving interval, and high incidence of anestrus **(Zicarelli *et al.*, 2010)**. Poor reproduction considers as one of the limiting issues for quick genetic improvement in the buffalo population. Molecular genetics offers valuable information which could contribute to the genes knowledge underlying quantitative production traits **(Othman *et al.*, 2013)**. Biological markers are the indicators of the biological states through genes expression pattern that help as reference point in breeding for the genetic potential improvement **(Sejian *et al.*, 2017)**. In view of the shortage of studies about the expression of fertility genes in Egyptian buffaloes in different reproductive status, the present research objective was to identify the expression of PGES, HSP70, PPARγ, and SOD2 genes which are linked to the fertility in Egyptian buffaloes using RT-PCR technique.

A number of potential genes have been selected and identified for analyses depend on their association with reproduction traits such as prostaglandin E synthase (PGES), heat shock proteins (HSP70), peroxisome proliferator-activated receptors (PPARγ), and superoxide dismutases 2 (SOD2) genes are considered markers for the farm animals reproduction **(Othman *et al.*, 2013)**. The high HSP70 gene expression in the corpus luteum could be one of the inhibitory issues that cause low conception rates **(Yao *et al.*, 2011)**.

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) has been stated as a housekeeping gene, it involved in the glycolytic pathway and it plays a basic role in energy metabolism.

PGES gene was established to be up regulated in pregnant as compared to non-pregnant, also found to be up regulated in pregnant and down regulated in smooth inactive ovaries buffaloes. The expression of PGES gene was difference significantly in smooth inactive ovaries and pregnancy; this refers to the importance of this gene in pregnant animal. This result agrees with **Ankita *et al*. (2018)** who noticed that increase in PGES gene expression with pregnancy, suggesting comparatively higher production of PGE2 in pregnancy. Prostaglandins (PGs) are the main mediators of several female reproductive functions, including ovulation, lute lysis, implantation, fertilization, pregnancy, and parturition. PGES is terminal PG synthases, which display tissue specific distribution besides convert PGH2 into PGE2 and PGF2a **(Tithof *et al*., 2007)**. The PGF2a involved in lute lysis and PGE2 are involved in maintenance of pregnancy, both consider the major of PGs which refers to the importance of this gene in fertility **(Raheem, 2017)**. The result was supported by **McCracken *et al*., 2004** whom found that PGs have essential role in female reproduction from ovulation to parturition.

High temperature is a continuous challenge to buffaloes rearing in tropical climatic conditions. Heat shock proteins (HSPs) are highly conserved proteins that contribute to cell survival through stress different conditions, HSP70 could act as characteristic cellular and physiological indicators of high seasonal temperature in buffaloes **(Manjari *et al*., 2015).** In the present study, HSP70 gene significantly up regulated in smooth inactive ovaries compared with pregnant animals, which mean that this animal expose to a stress as confirmed by **Basirico *et al*. (2011)** who reported that cellular response to stress includes proteins synthesis called heat shock proteins (HSPs) which belonging to a subgroup of molecular chaperones.

These findings are in accordance with the previous studies of **Kumar *et al.* (2017) and Jerome *et al*.** (**2015)** that display a significant increase in expression of HSP70 in smooth inactive ovaries animals, HSP70 can be used as a marker for excluding smooth inactive ovaries buffaloes from herd in breeding programs, also HSP70 was significantly higher in acyclic buffaloes.

The expression of HSP70 and SOD2 can be used as good indicators of fertility in Egyptian buffaloes allowing them to be potential biomarkers for animal fertility, because they increased significantly ($P<0.05$) in smooth inactive ovaries while decreased significantly ($P<0.05$) in pregnant buffaloes. The work also reported that SOD2 gene displayed significant down

regulation ($p < 0.05$) with (fold change of 0.4) in pregnancy and cyclic as compared to smooth inactive ovaries of buffaloes (fold change of 1.5), this conform the role of the gene in antioxidant defense, because diminished antioxidant to oxidative status leads to oxidative damage causing immune suppression and aggravate the inflammatory conditions **(Bhattacharyya *et al*., 2014)**.

Superoxide dismutase 2 (SOD2) genes showed up regulation in smooth inactive buffaloes ovaries because corpus luteum-derived SOD2 was served as LH-release inhibitory factor **(Kawaguchi, *et al.*, 2013)**.

The Peroxisome proliferator-activated receptors transcript level was raised throughout the pregnancy compared with the level of the estrous animals this result agree with **(Nishimura *et al.*, 2011 and Yujing *et al.*, 2017)** expression of PPAR gene in the endometrium has been reported for cattle, **(Bogacka and Bogacki, 2011)**. PPARγ significance has been reported during the estrous and pregnancy and its expression was higher during estrous than smooth inactive ovaries as seen by **(Bogacka *et al.*, 2015)**. PPARs regulate proliferation of ovarian cells, tissue remodeling, and steroidogenesis, regulation of cytokines synthesis, prostaglandins and steroids also PPARs has role in maturation, trophoblast differentiation and invasion besides in the embryo development **(Bogacka, *et al.*, 2015)**. Aggregate evidence shows an important role for PPAR gene in female reproduction. These results indicate a possible role for this gene in the development of the placenta by increase PGE2 concentrations **(Szymanska and Blitek, 2018)**.

The transcript level of PPARγ was low in smooth inactive ovaries, which agrees with **Vitti *et al.* (2016)** who refer to PPAR gene as vital regulators of steroid synthesis in reproductive tissues.

CONCLUSION

The present study was planned to differentiate between fertile and infertile buffalo by gene expression, and provided insights on the expression of (PGES, HSP70, PPARγ and SOD2) genes in pregnancy, cyclic and smooth inactive buffalo's ovaries. Gene expression levels in endometrium and corpus lutum in these cases were compared; Main conclusions of this work carried out in Egyptian buffaloes are that (i) these genes are differ in their expression between pregnancy, cyclic, smooth inactive buffaloes ovaries. (ii) Differential expression for SOD2 and HSP70 in smooth inactive ovaries than pregnant and cyclic buffaloes. (iii) PGES and

PPARγ transcript level appeared significantly lower in smooth inactive ovaries compared to cyclic and pregnant, up regulation of mRNA expression of theses gene in pregnancy. The present results suggest that these genes could take part in regulation of Egyptian buffalo fertility, also it can be used to select fertile and exclude infertile buffalo by measuring the expression of these genes. These conclusions need to be confirmed via studying a larger number of buffalo to shed light on molecular mechanisms that drive its fertility.

Acknowledgement:

Grateful acknowledge for Prof. Dr. Maxima M. Kandil director to Embryo and Genetic Resource Conservation Bank in National Research Centre, financially supported by STDF (CB grant ID: 2339) for genetic and spectrophotometer equipment's necessary to gene analysis.

REFERENCES

Abdoon, S Ahmed., Christoph Gabler, Christoph Holder, Omaima M. Kandila, Ralf Einspanier. (2014): Seasonal variations in developmental competence and relative abundance of gene transcripts in buffalo (Bubalus bubalis) oocytes, Theriogenology, 82, p. 1055–1067.

Ankita, D. V., Manjit, P., Naseer, A. B., Sourabh, S., Sadam, Subhashree, P., Krishnaswamy, N., Arvind, A and Sonwane, B. B. (2018): Differential expression of ten candidate genes regulating prostaglandin action in reproductive tissues of buffalo during estrous cycle and pregnancy. Theriogenology, 105(1), p. 7-14.

Bairagi, S., Quinn, K. E., Crane, A. R., Ashley, R. L., Borowicz, P. P. and Caton, J. S. (2016): Maternal environment and placental vascularization in small ruminants. Theriogenology, 86, p. 288-305.

Barbat, A., Le Mezec, P., Ducrocq, V., Mattalia, S., Fritz, S. and Boichard, D. (2010): Female fertility in French dairy breeds: current situation and strategies for improvement. J Reprod Dev., 56, p.15-21.

Basirico, L., Morera, P., and Primi, V., Lacetera, N., Nardone, A. and Bernabucci, U. (2011): Cellular thermo tolerance is associated with heat shock protein 70.1 genetic polymorphisms in Holstein lactating cows. Cell Stress and Chaperones, 16, p. 441 - 448.

Bauersachs, S., Mitko, K., Blum, H. and Wolf, E. (2007): A Tailored tool for studying bovine endometrium biology and pathophysiology. J. Dairy Sci., 90, p. 4420 - 4423.

Bazer,F.W.,Song,G.andThatcher,W.W.(2012): Rolesofconceptus secretory proteins in establishment and maintenance of pregnancy in ruminants. Asian Australis. J. Anim. Sci., 25 (1), p.1.

Beckham, J. T., Mackanos, M. A., Crooke, C., Takahashi, T., O'Connell-Rodwell, C., Contag, C. H. and Jansen, E. D. (2004): Assessment of cellular response to thermal laser injury through bioluminescence imaging of heat shock protein 70. Photochemistry and Photobiology, 79, p. 76-85.

Bhattacharyya, A., Chattopadhyay, C., Mitra, S. and Crowe, S. E. (2014): Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiol. Rev. 94 (2), p. 329 -354.

Bogacka, I. and Bogacki, M. (2011): The quantitative expression of peroxisome proliferator activated receptor (PPAR) genes in porcine endometrium through the estrous cycle and early pregnancy. J. Physiol. Pharmacol., 62, p. 559-565.

Bogacka, I., Kurzynska, A., Bogacki, M. and Chojnowska, K. (2015): Peroxisome proliferator-activated receptors in the regulation of female reproductive functions. Folia. Histochem. Cytobiol, 53, p.189 -200.

Chaudhari, C. F., Derashri, H. J., Modi, L. C., Chaudhari, N. F., Khasatiya, C. T. and Tyag, K. K. (2018): Impact of ovarian status and harvesting method on oocyte collection from buffalo ovaries. Indian Journal of Animal Reproduction, 39 (2), p. 50-51.

Fooda, T., Mourad, K. A. and Gebreel, I. (2010): Phenotypic and genetic trends for milk production in Egyptian buffaloes. J. Am. Sci., 6, p.143-147.

Hansen, P. J. (2004): Physiological and cellular adaptations of zebu cattle to thermal stress. Animal Reproduction Science, 82-83, p. 349 -360.

Jerome, A., Srivastava, S.K. and Sharma, R.K. (2015): Expression profile of follicular genes vis-à-vis season and cyclicity in buffalo. Indian Journal of Animal Sciences 85 (5), p. 514 -516.

Kawaguchi, S., Sakumoto, R. and Okuda, K. (2013): Induction of the expressions of antioxidant enzymes by luteinizing hormone in the bovine corpus luteum.The Journal of Reproduction and Development, 59, p. 219 -224.

Kumar, R. , Ghosh, M ., Kumar, N., Balhara, A.K., Gupta, M., Sharma, R.K. and Singh, I. (2017) : Polymorphism in 5′ untranslated region of heat - shock protein 70 gene as marker of post- partum anoestrus in Murrah buffaloes. Reprod. Dom. Anim., 52, 505-512.

Manjari, R., Yadav, M., Ramesh, K., Uniyal, S., Rastogi, S.K., Sejian, V. and Hyder, I. (2015): HSP70 as a marker of heat and humidity stress in Tarai buffalo. Trop. Anim. Health Prod., 47(1), p.111–116.

Matsuda, S., Kobayashi, M.and Kitagishi, Y.(2013): Expression and function of PPARs in placenta, PPAR Res., p. 1-7.

McCracken, J.A. and Prostaglandin, F. (2004): The luteolytic hormone. Eicosanoids, p. 525-545.

Nishimura, K., Yamauchi, N. and Chowdhury, V. S.(2011) Expression of peroxisome proliferator-activated receptor isoforms in the rat uterus during early pregnancy. Cell Tissue Res., 345, p.275-284.

Othman, E., Mohamed, F. and Abdel-Samad R.F. (2013): Polymorphism of three fertility genes in Egyptian buffalo. Journal of Applied Biological Sciences 7 (2), p. 94-101, ISSN: 1307-1130.

Raheem, K. A. (2017): An insight into maternal recognition of pregnancy in mammalian species. J. Saudi Soc. Agric. Sciences, 16 (1), p.1- 6.

Ravjibhai, K., Chaudharia, Mahlaa, A. S., Singha, A. K., Sanjay, K., Singha, Pawdeb, A. M., Gandhamc, R. K., Gyanendra, S., Mihir, S., Harendra, K. and Narayanan, K. (2018) : Effect of dietary n-3polyunsaturated fatty acid rich fish oil on the endometrial prostaglandin production in the doe (Caprahircus). Prostaglandins and Other Lipid Mediators 1352, p.7–35.

Rosic,N. N., Pernice, M., Dove, S., Dunn, S. and Hoegh-Guldberg, O. (2010): Gene expression profiles of cytosolic heat shock proteins Hsp70 and Hsp90 from symbiotic dinoflagellate in response to thermal stress: Possible implications for coral bleaching. Cell Stress and Chaperones, 16 (1), p. 69-80.

Ross, O.A., Curran, M.D., Crum, K.A., Rea, I.M., Barnett, Y.A. and Middleton, D. (2003): Increased frequency of the 2437 T allele of the heat shock protein 70-Hom gene in an aged Irish population. Experimental Gerontology, 38, p. 561-565.

Sejian, V., Krishnan, G., Bagath, M., Vaswani, S., Pragna, P., Aleena, J., Lees, A.M. Maurya, V.P., Bhatta, R. (2017): Measurement of severity of heat stress in sheep. Sheep Production Adapting to Climate Change, p. 307-318.

Soliman, S. S., Attia, M. Z., Abdoon, A.S., El-Toukhey, N.E., Kandil, O. M. and Sabra, H. A. (2016): Seasonal variation in ovarian functions in Egyptian buffalo and cattle. International Journal of Pharm Tech Research CODEN (USA): IJPRIF, 9 (6), p. 34 - 42.

Szymanska, M. and Blitek, A. (2018): Prostacyclin synthesis and prostacyclin receptor expression in the porcine corpus luteum: evidence for a luteotropic role in vitro. Biology of Reproduction, 100 (1), p. 162–174.

Tithof, P.K., Roberts, M.P, Guan, W., Elgayyar, M. and Godkin, J.D. (2007): Distinct phospholipase A2 enzymes regulate prostaglandin E2 and F2alpha production by bovine endometrial epithelial cells. Reprod. Biol. Endocrinol, 5 (1), p.1.

Vitti, M., Di Emidio, G., Di Carlo, M., Carta, G., Antonosante, A., ArtiniPG, Cimini A, Tatone C and Benedetti E. (2016): Peroxisome proliferator-activated receptors in female reproduction and fertility. PPAR Res., ID 4612306.

Yao, Y.W., Zhang, G. H., Zhang, Y. Y., Li, W. D., Wang, C. H., Yin, C.Y. and Zhang, F. M. (2011): Lip polysaccharide pretreatment protects against ischemia/reperfusion injury via increase of HSP70 and inhibition of NF-kappaB, Cell Stress Chaperones, 16, p. 287-296.

Yujing, H., Jose, M., Garciab, Weiqun, S., Honghui, R., Lin Zhangc, Yanzhou, W., Yao, T., Hui, L., Hui, Z. and Chenc, J. (2018): Peroxisome proliferator activated receptor gamma in human placenta may mediate the adverse effects of phthalates exposure in pregnancy. Reproductive Toxicology 75, p. 121–126.

Zicarelli, L., Lucy, M. C., Pate, J. L., Smith, M. F. and Spencer, T. E. (2010) : Enhancing reproductive performance in domestic dairy water buffalo (Bubalus bubalis) Eighth International Symposium on reproduction in domestic ruminants, Nottingham University Press, Anchorage, Alaska, p. 443-455.

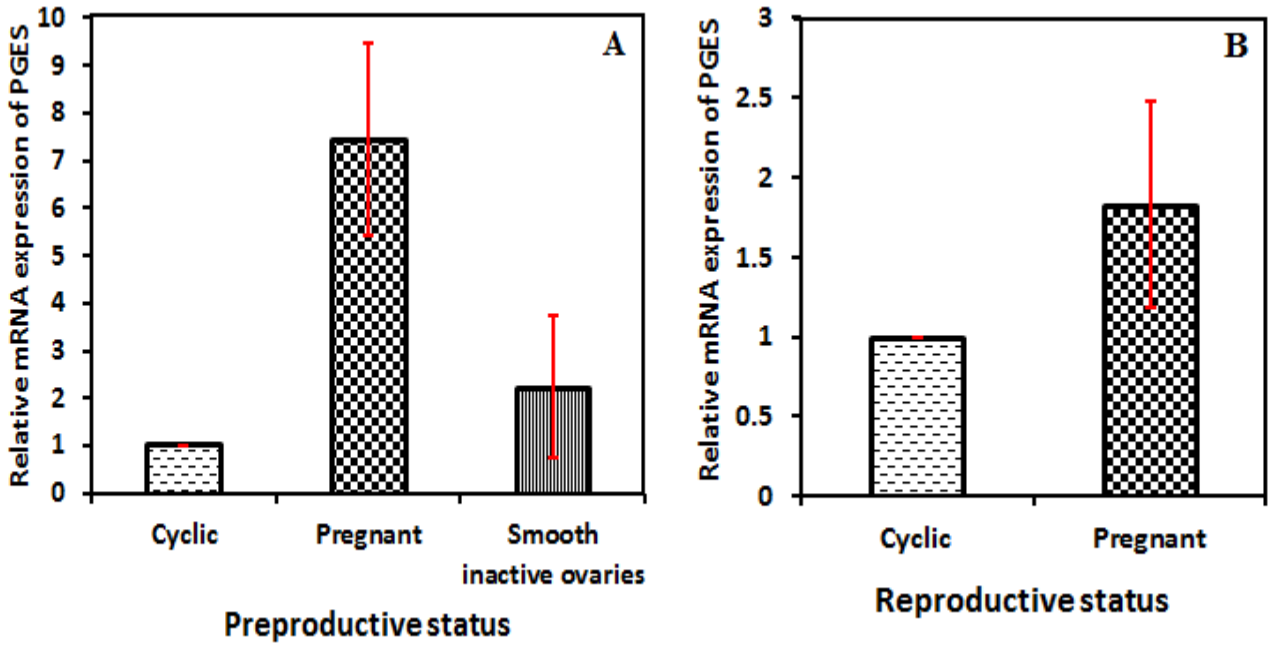

Fig.(1): Gene expression of PGES in buffaloes with different reproductive status.

A: Endometrium during cyclic, pregnant and smooth inactive ovaries.

B: Corpus luteum during cyclic, pregnant.

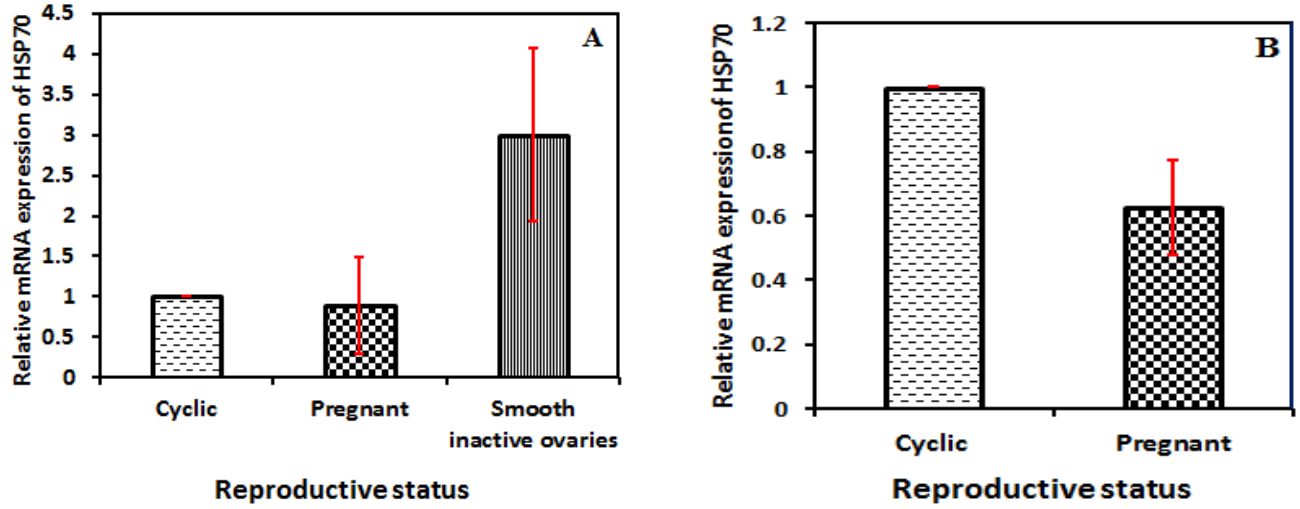

Fig. (2): Gene expression of HSP70 in buffaloes with different reproductive status.

A: Endometrium during cyclic, pregnant and smooth inactive ovaries.

B: Corpus luteum during cyclic, pregnant.

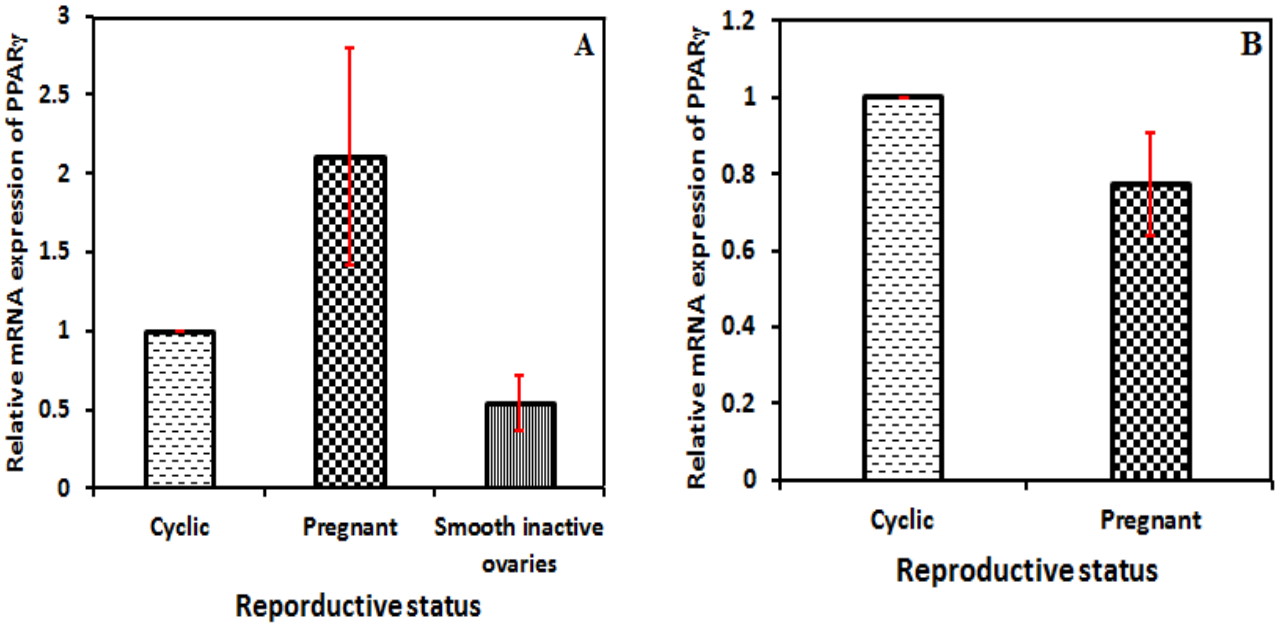

Fig. (3): Gene expression of PPARγ in buffaloes with different reproductive status.

A: Endometrium during cyclic, pregnant and smooth inactive ovaries.

B: Corpus luteum during cyclic, pregnant.

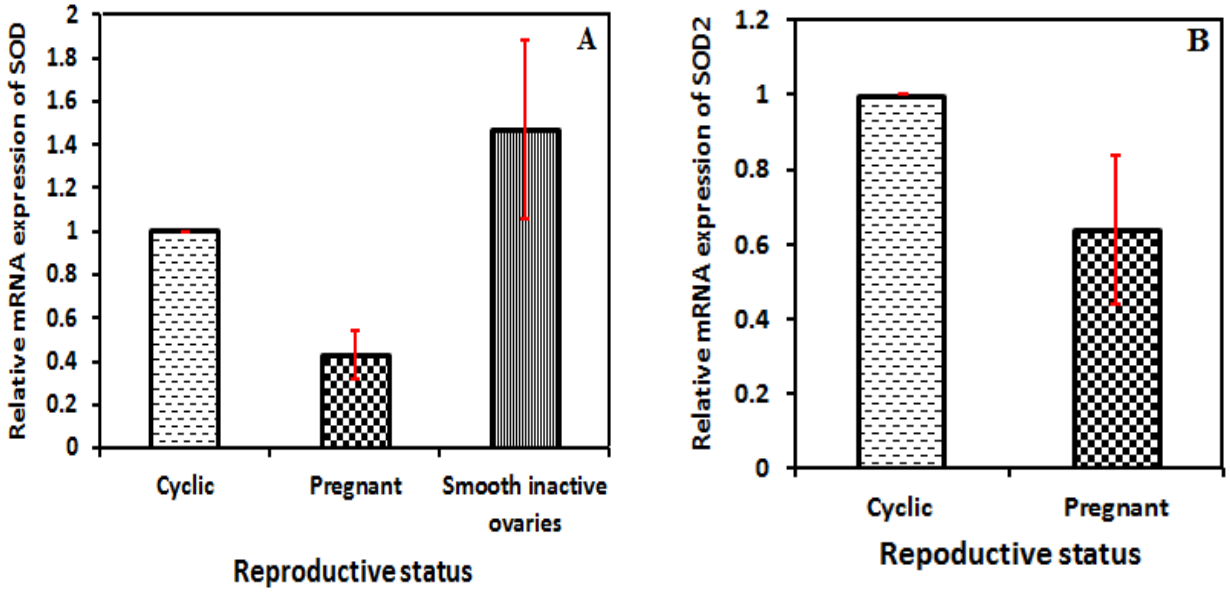

Fig. (4): Gene expression of SOD2 in buffaloes with different reproductive status.

A: Endometrium during cyclic, pregnant and smooth inactive ovaries.

B: Corpus luteum during cyclic, pregnant.

Table (2): The expression difference between HSP70, PGES, PPARγ and SOD2 in buffalo endometrium.

Gene		No.	Mean	Std. Deviation	Std. Error	Significant
HSP70	Cyclic	7	1	0	0	
	Pregnant	7	0.89	0.6	0.23	0.63
	Smooth inactive ovaries	7	2.99	1. 07	0.4	0.011
PGES	Cyclic	7	1	0	0	
	Pregnant	7	7.44	2. 02	0.8	0.01
	Smooth inactive ovaries	7	2.23	1.5	0.56	0.05
PPARγ	Cyclic	7	1	0	0	
	Pregnant	7	2.11	0.7	0.3	0.01
	Smooth inactive ovaries	7	0.54	0.17	0. 06	0.001
SOD2	Cyclic	7	1	0	0	
	Pregnant	7	0.43	0.11	0. 04	0.001
	Smooth inactive ovaries	7	1.5	0.41	0.15	0.011

Table (3): The expression difference between HSP70, PGES, PPARγ and SOD2 in buffalo corpus luteum.

Gene		No.	Mean	Std. Deviation	Std. Error	Significant
HSP70	Cyclic	7	1.0	0	0	0
	Pregnant	7	0.63	0.15	0.06	0.001
PGES	Cyclic	7	1	0.0	0	0
	Pregnant	7	1.83	0.65	0.24	0.005
PPARγ	Cyclic	7	1.0000	0.0	0	0
	Pregnant	7	0.77	0.14	0.05	0.001
SOD2	Cyclic	7	1	0	0	0
	Pregnant	7	0.64	0.2	70.0	0.001