Original Research

Effect of Adiponectin on Proliferation of Granulosa and Luteal Cells of Buffalo Ovary

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Abstract

Adiponectin is an adipocyte derived cytokine involved in various metabolic functions. The present study was conducted to determine the effect of adiponectin on proliferation and apopotosis of granulosa and luteal cells of buffalo ovary. Granulosa and luteal cells were cultured in vitro and treated with adiponectin each at 1 and 10 ug/mL concentrations for 48h after obtaining 75% to 80% confluence. Real time PCR was used to demonstrate the relative expression of proliferation marker PCNA and apoptosis marker BAX and signaling molecule PDK and Akt. The results showed that adiopnectin at the dose 10 ug/mL increased mRNA expression of PCNA, Akt, PDK ($P<0.05$) and decreased mRNA expression apoptosis marker BAX in presence of FSH (30 ng/mL) and IGF-I (10ng/mL). In luteal cell culture adiponectin did not affect the proliferation or apoptosis at both doses. To conclude, this study provides the evidence that adiponectin have positive effect on cell proliferation and it varies in different tissues.

Key words: Adiponectin, Apoptosis, Buffalo, Proliferation

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Introduction

Adiponectin is cytokine hormone mainly produced from adipocytes of white adipose tissues (Scherer *et al*., 1995). Many studies have demonstrated that adiponectin is also produced from other tissues including skeletal muscles (Delaigle *et al*., 2004), hypothalamus, pituitary glands (Psilopanagioti *et al*., 2009), ovary, uterus and placenta (Caminos *et al*., 2005). Adiponectin plays an important role in lipid and carbohydrate metabolism and increases insulin sensitivity and improve glucose metabolism (Yamauchi *et al*., 2002). Adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (adipoR2) plays the role of receptors for adiponectin (Kadowaki and Yamauchi, 2005). Both AdipoR1 and AdipoR2 are seven trasmembrane

domain containing receptor and are different from G protein coupled receptor (Yamauchi *et al*., 2003). Growing body of evidences indicated that adipionectin in central and peripheral regulation of reproduction. Centrally it regulates reproductive process by affecting hypothalamo pituitary gonadal axis (Wena *et al*., 2008). Adiponectin exerts direct peripheral effect on reproduction reproductive tissues like uterus, ovary, oocyte and embryo (Mitchell *et al*., 2005). Recent studies have demonstrated the expression of adiponectin and its receptors in rat, bovine, porcine, caprine and chicken species (Chabrolle *et al*., 2007a; Chabrolle *et al*., 2007 b; Tabandeh *et al*., 2010; Maleszka *et al*., 2014; Oliveira, *et al*., 2017). It has demonstrated that adiponectin affect steroidogenesis in ovarian cells in rat, bovine, porcine and chicken species (Chabrolle *et al*., 2007 b; Lagaly, *et al.*, 2008; Maillard *et al*., 2010; Maleszka *et al*., 2014).

Reproductive cycle is the process of sequential proliferation, differentiation and transformation of ovarian follicular cells followed by formation and regression of the corpus luteum (CL) in a cyclic manner. The cellular proliferation and apoptosis plays an important role in this cellular remodeling process. Some of the studies have reported that adiponectin affects proliferation and apoptosis of the normal ovarian cells as well as cancer cells (Lagaly *et al*., 2008; Kim *et al*., 2010; Feng *at al*., 2018). However, no study has been reported in bubaline species that shows the role of adiponectin in proliferation and apoptosis of ovarian cells. The present study was therefore designed to identify the In-vitro effect of adiponectin on proliferation and apoptosis of granulosa cells and luteal cells of buffalo ovary.

Materials and Methods

Granulosa Cell Culture

To evaluate the effect of adiponectin on granulosa cell prolifereation, the granulosa cell culture was followed as described in earlier study (Gupta *et al*., 2015). Briefly, ovaries were washed properly with physiological saline solution and granulosa cells (GCs) were collected from pre ovulatory follicles separately by aspiration of follicular fluid (FF) using a 18 gauze needle and syringe and by gently scraping the inner wall of follicle. Aspirants were transferred to a petri dish, containing 0.1% solution of PBS, and all cumulus oocyte complexes were recovered. The remaining cells and fluids were centrifuged in 15 ml conical tubes at 300 ×g for 5 min, and the GC pellet was resuspended in 10 mL of 1X PBS prior to a second centrifugation. Finally, GCs were resuspended and washed in culture medium with DMEM/F12 media (supplemented with 10% FBS, antibiotic and antimycotic solution). Viable cells were counted using trypan blue exclusion. The cells were centrifuged, resuspended and plated out at 1.5×10^5 viable cells per well in a 24-well plate (total volume: 1ml containing 10% Fetal Bovine Serum (Gibbco) and Antibiotic & Antimycotic solution (10,000 units Penicillin, 10mg Streptomycin, 25µg Amphotericin B per mL (Sigma-aldrich) in a humidified CO2 (5%) incubator at 37.5 °C. The cells were allowed to attach and grow (75-80% confluent) for 48h and there after the media was replaced with fresh media (without serum) containing

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different concentrations (1, and 10 ug/mL) of adiponectin alone and with porcine FSH (30 ng/mL) and IGF-I (10 ng/mL) were maintained for 48 h. The doses of the adiponectin were determined based on the earlier report (Chabrolle *et al.*, 2007, Lagaly *et al.*, 2008; Maleszka *et al.*, 2014). Control cells were grown in media without adiponectin. The cells were allowed to attach for 24h and after a further 24 h period the medium was changed. After 48 h, the medium was separated and cells were collected for mRNA isolation. Each treatment was tested in quadriplicate wells for each experiment.

Luteal Cell Culture

In order to evaluate effect of the adiponectin on luteal cell proliferation function, a luteal cell culture model was developed with cells isolated from fresh CL. Luteal cells were cultured as described in earlier studies (Gupta *et al*., 2014). Briefly, ovaries were collected from a local abattoir and transported back to the laboratory in 1XPBS at 37°C. The second stage CL (days 5-10) were used for luteal cell culture and these were selected based on criteria applied previously (Gupta *et al*., 2014). The minced luteal tissue was washed 3 times for 5 min at 12000 × g with dispersing medium. All cells (including luteal, endothelial, pericytes and fibroblasts) were dispersed by incubating the luteal tissue in DMEM/F12 medium (Himedia, India) containing 2mg/mL collagenase I type 1A, 25 µg/mL DNase I (Sigma-aldrich) and 0.5% BSA Fraction for 2×45 min in a incubator at 37°C and shaking manually at 10 min interval. The dispersed cells from each incubation were pooled together and then filtered through 70 µm cell strainer to remove nondissociated tissue fragments. The filtrate was washed twice by centrifugation for 5 min at 250×g with DMEM/F12 media. Supernatant was discarded. Later, erythrocyte lysis was accomplished by washing the pellet with RBC lysis buffer and further one washing step was performed.

Cells were resuspended in DMEM/F12 medium containing 10% Fetal Bovine Serum (FBS) (Gibco life technologies, South America) and antibiotic & antimycotic solution (10,000 units penicillin, 10mg streptomycin, 25µg amphotericin B per ml). Cell viability, determined by trypan blue exclusion dye and was higher than 90%. The cells were then plated out at 1.5×10^5 viable cells per well in a 24-well plate in a humidified CO_2 (5%) incubator at 37.5°C. The cells were allowed to attach and grow till 75-80% confluence was obtained with replacing of the media at every 48h. Then cells were maintained in DMEM/F12 media without FBS for 24h and then cells were treated with fresh media (without FBS) containing different concentrations (1 and 10 ug/ml) of recombinant adiponectin (Biovendor) and were maintained for 48 h. The doses of the adiponectin were selected based on the earlier report. Control cells were grown in media without adiponectin. Four replicates were tested for each experimental condition. After 48 h, the spent media was separated and RNA was isolated from cells by TRIZOL reagent as described above.

RNA Isolation and RT-PCR

Total RNA was isolated from the attached granulosa and luteal cells by TRIzol reagent (Invitrogen) as per manufacturer instructions. RNA was treated with Dnase 1 (Sigma-aldrich, USA) to remove any possible

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DNA contamination. The integrity of total RNA was checked on 1.5% agarose gel using 1× TAE as electrophoresis buffer and gel stained with ethdium bromide. The purity and concentration of total RNA was checked using nanodrop (Eppendorf, USA). The isolated RNA samples were free from the protein contamination as the OD 260: OD 280 values were more than 1.8. RT-PCR was used to detect PCNA, BAX, PDK and AKT expression luetal and granulosa cultured cells. 1µg of total RNA was reverse transcribed in 20µl of final volume of reaction containing 4 µL 5X reaction buffer, 3 µL $MgCl_2$, 1 µL PCR nucleotide mix, 1 µL RNase inhibitor, 0.5 µL reverse transcriptase, 1.5 µL Oligo (dt 15) primer, 1µL RNA template, 8 µL nuclease free water followed by incubation for 15 minutes at 50°C and 2 minutes 30 seconds at 42°C and finally hold at 4°C. The cDNA was stored at -20°C for long term use.

Primers

Primer of PCNA, BAX and ribosomal protein 15 (RPL15) were designed using the Fast PCR (Version: 6.2.73) software. Published primers were used for beta actin, PDK and Akt (details of the primers used are given in Table 1.

Table 1: Gene transcript, primer sequence (5′-3′) and resulting fragment size

Gene	Sequences 5′-3′	Amplicon Length (bp)	EMBL Accession No. or Reference
PCNA	For: ACCTGCAGAGCATGGACTCGTC	160	NM_001034494.1
	Rev: CATGCTGGTGAGGTTCACGCCCA		
BAX	For: TCTGACGGCAACTTCAACTG	250	NM_173894.1
	Rev: AAGTAGGAGAGGAGGCCGTC		
PDK	For: TGAAGACGAGAAGAGGTTGTTG	104	Mishra *et al*., 2016
	Rev: GCTTGTCTACTGGACCCATTT		
Akt	For: GCGTGACCATGAATGAGTTTG	113	Mishra *et al*., 2016
	Rev: CAGGATCTTCATGGCGTAGTAG		
Beta actin	For: TCTCACGGAGCGTGGCTACAG	100	Gupta *et al*., 2014
	Rev: CTGCTCGAAGTCCAGGGCCACGTA		
RPL15	For: TGGGCTACAAGGCCAAACAA	140	MG969348
	Rev: GCTTCGAGCAAA CTTGAGCTGG		

Quantitative RT-PCR analysis

Targeted cDNAs were quantified by real time using GoTaq® qPCR master mix (Promega, USA) and specific primers in a total volume 15 µL reaction containing 7.5 µL GoTaq® qPCR master mix, 0.5 µL forward primer (0.5 mM), 0.5 µL reverse primer (0.5 mM), 1.0 µL cDNA template, 5.5 µL nuclease free water. PCR was performed with specific primer pairs of PCNA, BAX, Akt, PDK, beta actin and RPL15. The samples were initially denatured at 95°C for 2 min, then 40 PCR cycles were processed denaturation at 95° C for 20 sec, Annealing at 58°C for beta actin and 60° C for PCNA, BAX, PDK, Akt and RPL15 for 25 sec, Extension at 72° C for 30 sec, with a final extension at 72° C for 15 sec and final hold at 4° C. PCR

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products were migrated on 1.5 % agarose gel stained with ethidium bromide. Optical data were collected at end of each extension step and relative expression of PCR product was determined by the $2^{-\text{Delta Delta Ct}}$ method. The geometric mean of Ct of beta actin and RPL15 was taken as reference/ internal control.

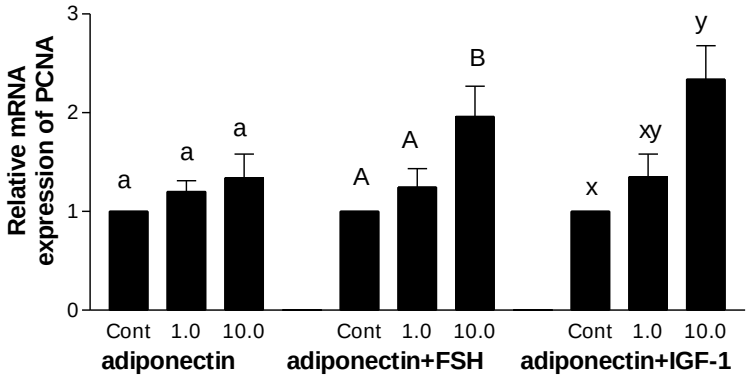
Statistical Analysis

The data was analyzed by SPSS 17.0 software. All experimental data has been shown as mean ± SEM. The statistical significance of difference in mRNA expression of PCNA, BAX, PDK and AKT in granulosa and luteal cells was assessed by one-way ANOVA followed by the Ducan as a multiple comparison test. Difference were considered if $P<0.05$.

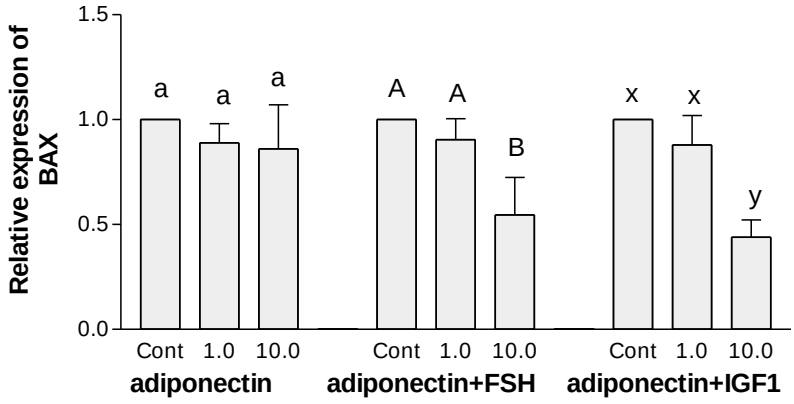
Results and Discussion

Effect of Adiponectin on mRNA Expression of PCNA, BAX, PDK and Akt in Granulosa Cells

The relative mRNA expression of PCNA, BAX, PDK and Akt in granulosa cell culture is shown in Fig.1.



(A)



(B)

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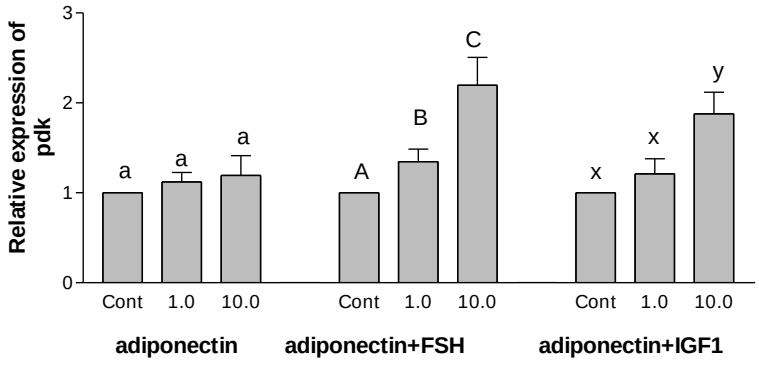
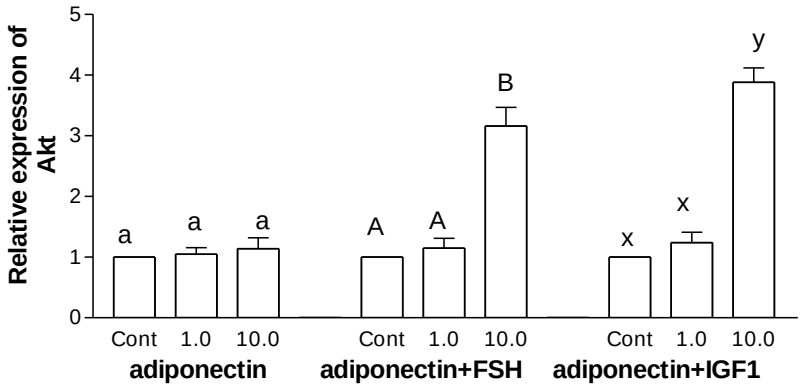


(C)



(D)

Fig. 1: Relative mRNA expression of PCNA, BAX, PDK and AKT from GC cultures treated with adiponectin for 48 h at dose rate 1 µg and 10µg/mL, adiponectin + FSH (30ng/mL) and adiponectin + IGF-I (10ng/mL) (n =4 follicle); (A) expression of PCNA mRNA; (B) expression BAX mRNA; (C) expression of PDK mRNA ; (D) expression of Akt mRNA. All values are shown as mean ± standard error of the mean. Different superscripts denote statistically different values ($P < 0.05$). PCNA, proliferating cell nuclear antigen; BAX, Bcl-2 associated X protein; PDK, phosphoinositide-dependant kinase; AKT, protein kinase B; mRNA,messenger RNA.

The mRNA expression of PCNA (Fig.1A) did not differ significantly ($P>0.05$) in control group and adiponectin treated group at both doses (1 and 10 ug/mL) however the mRNA expression of PCNA increased significantly ($P<0.05$) at 10 ug/mL adiponectin in presence of FSH (30 ng/mL) and IGF-I (10ng/mL). In presence of FSH and adiponectin at 10ug/mL the PCNA expression increased by 1.96 fold as compared to control and in presence of IGF-I and adiponectin at 10ug/mL it was increased by 2.34 fold. The relative expression of BAX (Fig. 2B) has shown the reverse trend than PCNA. In presence of adiponectin the BAX expression did not change but in presence of FSH and IGF-I, the expression of BAX decreased significantly at 10ug/mL of adiponectin dose. The mRNA expression of PDK (Fig. 1C) and Akt (Fig. 1D) showed a similar pattern of expression in granulosa cells as that of PCNA. At 10ug/mL dose of

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adiponectin in presence of FSH and IGF-I and the expression of PDK and AKT increased (P< 0.05) to 2.19 fold, 1.88 fold and 3.16 fold, 3.88 fold respectively. The representative gel images of all the amplified genes (PCNA, BAX, PDK, Akt) in granulosa cell culture are shown in Fig. 2 A to D.

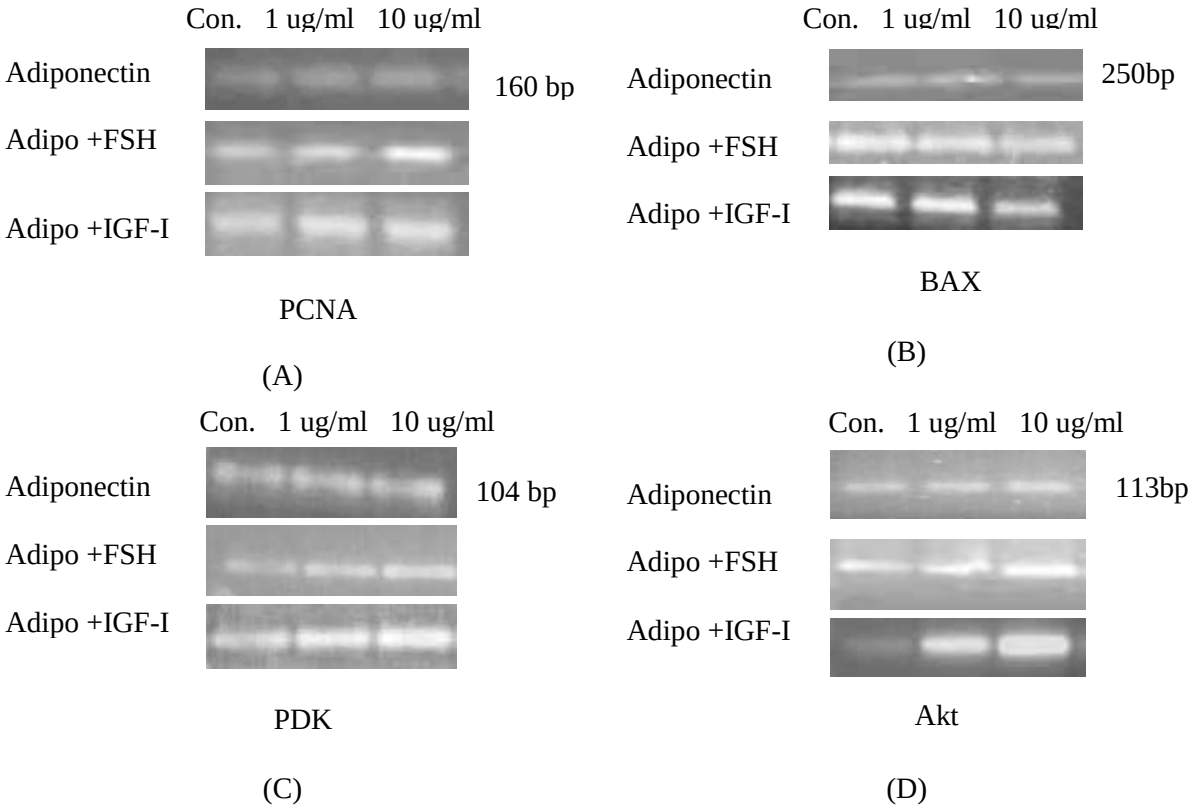

Fig. 2: Gel image of amplification by RT-PCR of PCNA (160 bp), BAX (250 bp), PDK (104 bp) and Akt (113 bp) in granulosa cells cultured in presence of adiponectin, adiponectin+FSH and adiponectin+IGF-I (*n* = 4).

The results observed in present study in granulosa cell has indicated that adiponectin has positive effect on cellular proliferation and it down regulates the apoptosis in granulosa cell in presence of FSH and IGF-I and adiponectin alone does not affect the cell proliferation or apoptosis in bubaline granulosa cells. In-vitro studies on effects adiponectin in granulosa and theca cells of ovary has been carried out earlier in bovine, porcine, caprine and chicken species (Chabrolle *et al*., 2007b; Lagaly *et al*., 2008; Maillard, *et al*., 2010; Maleszka, *et al*., 2014; Oliveira *et al* ., 2017). In these studies it was observed that adiponectin affect the steriodogenesis in ovarian cells and the effect varies in presence of FSH, LH and IGF-I. In bovine granulosa cells it has been demonstrated that adiponectin increased IGF-1 induced GC proliferation but not basal or insulin induced proliferation (Maillard *et al*., 2010). In another study in bovine revealed that adiponectin had no effect on insulin-induced proliferation of theca cells from large follicles (Lagaly *et al*.,

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2008). It has been demonstrated that Adiponectin receptor 1 (adipoR1) plays an important role in cell survival and a strong reduction in adipoR1 in human KGN granulosa cells abolishes cell proliferation and increases apoptosis (Pierre *et al*., 2009). In primary human osteoblasts, adiponectin have positive effect on proliferation and differentiation through AdipoR1signalling (Luo *et al*., 2005). In colon cancer cells it was observed that adiponectin inhibit the growth and proliferation of colon cancer cells through stimulating AMPK activity (Kim *et al*., 2010) however, in ovarian cancer cells adiponectin exhibits proliferative and anti-apoptotic effects on ovarian cancer cells via PI3K/Akt and Raf/MEK/ERK pathways (Feng *et al*., 2018). In human aortic smooth muscle cells adiponectin inhibits cell proliferation by interacting with several growth factors in an oligomerization-dependent manner (Wang *et al*., 2005). Also in human MKN45 and NUGC3 cell lines adiponectin (10ug/mL) suppressed cell prolifereation (Tsukada *et al*., 2011). The results of the present study are in conformity with the findings of Maillard *et al*., 2010. Further, adiponectin in presence of FSH and IGF-I increased the expression of cellular proliferation factors AKT and PDK. The possible explanation could be due to, as adiponectin act via PI3K-AKT pathway and activates PDK (Feng *et al*., 2018) along with AKT (as PDK phosphorylates AKT) to upregulate the downstream signaling factors for the proliferation of cultured GCs in vitro.

Effect of Adiponectin on mRNA Expression of PCNA, BAX, PDK and Akt in Luteal Cells

The relative mRNA expression of PCNA, BAX, PDK and Akt in luteal cell culture is shown in Fig. 3 and the gel images of amplification of PCNA, BAX, PDK, Akt and housekeeping genes β-actin and RPL 15 are shown in Fig. 4.

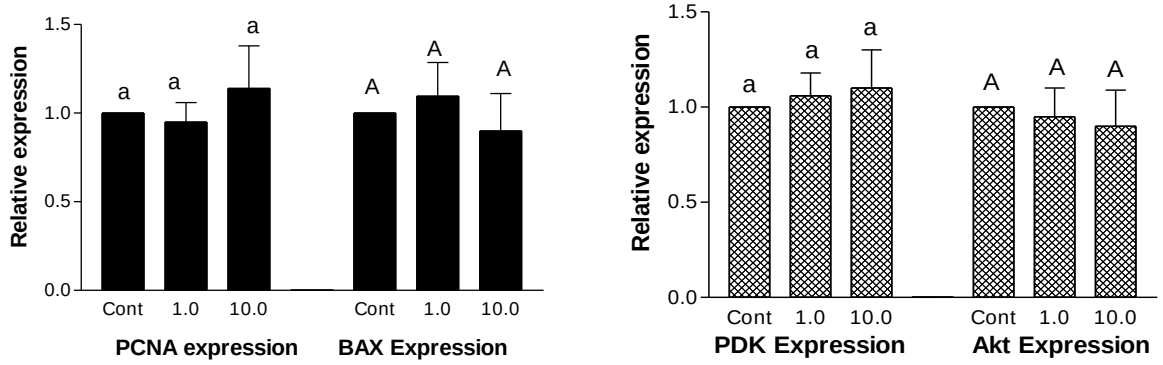

Fig. 3: Relative mRNA expression of PCNA, BAX, PDK and AKT from luteal cell cultures treated with adiponectin for 48 h at dose rate 1 µg and 10µg/mL (n =4 CL); (A) expression of PCNA and BAX mRNA; (B) expression PDK and Akt mRNA. All values are shown as mean ± standard error of the mean. Different superscripts denote statistically different values ($P < 0.05$).

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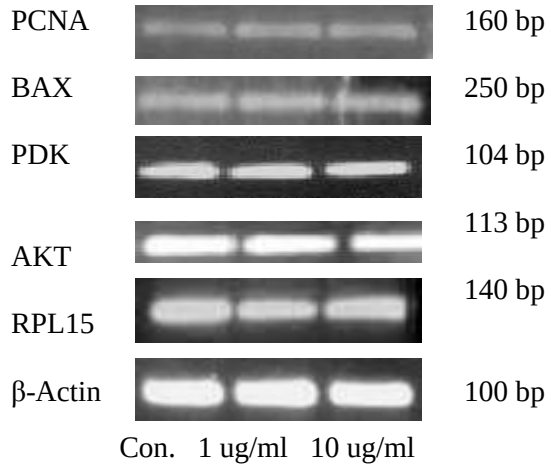

Fig. 4: Gel image of amplification by RT-PCR of PCNA (160 bp), BAX (250 bp), PDK (104 bp), Akt (113 bp), RPL 15 (140 bp) and β-Actin (100 bp) in luteal cells cultured in presence of adiponectin at dose rate of 1 and 10 ug/mL (n = 4).

The expression of neither marker of proliferation PCNA nor the marker of apoptosis BAX and the signaling molecules PDK and Akt differ significantly in luteal cells. Few studies have demonstrated the effect of adiponectin on progesterone secretion from cultured luteal cells (Maleszka, *et al*., 2014) through modulation of steroidogenic enzymes and steroidogenic acute regulatory protein (StAR) gene expression. The microarray analysis also indicated that adiponectin is having modulatory effect on the porcine ovarian cells during the luteal phase of the oestrous cycle (Szeszko *et al*., 2016). The evidences are lacking regarding the effect of adiponectin on survival/proliferation and apoptosis of luteal cells. A study has been conducted in theca cells wherein wherein it was observed that adiponectin does not change the proliferation of bovine theca cells (Lagaly *et al*., 2008). The results of the study on luteal cells are in accordance with the findings of the Lagaly *et al*., 2008. From the study it revealed that effect of adiponectin is tissue specific and FSH and IGF potentiate the proliferative effect of adiponectin.

Conclusion

From the present study it can be concluded that adiponectin regulate the cellular proliferation and apoptosis in presence of FSH and IGF-I and the effect is dose dependent and, the role of adiponectin for cell survival varies with the cell type in buffalo.

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References

1. Chabrolle C, Tosca L, Crochet S, Tesseraud S and Dupont J. 2007a. Expression of adiponectin and its receptors (AdipoR1 and AdipoR2) in chicken ovary: potential role in ovarian steroidogenesis. *Domestic Animal Endocrinology*, 33: 480– 487.
2. Chabrolle C, Tosca L and Dupont J. 2007b.Regulation of adiponectin and its receptors in rat ovary by human chorionic gonadotrophin treatment and potential involvement of adiponectin in granulosa cell steroidogenesis. *Reproduction;* 133:719–731.
3. Caminos JE, Nogueiras R, Gallego R, Bravo S, Tovar S, Garcia-Caballero T, Casanueva FF and Dieguez C. 2005. Expression and regulation of adiponectin and receptor in human and rat placenta. *Journal of Clinical Endocrinology and Metabolism*, 90:4276 – 4286.
4. Delaigle AM, Jonas JC, Bauche I B, Cornu O, and Brichard SM. 2004. Induction of adiponectin in skeletal muscle by inflammatory cytokines: in vivo and in vitro studies. *Endocrinology*, 145 (12): 5589–5597.
5. Feng Y, Hao F, Wan W, and Wang X. 2018. Adiponectin exhibits proliferative and anti-apoptotic effects on ovarian cancer cells via PI3K/Akt and Raf/MEK/ERK pathways. *Tropical Journal of Pharmaceutical Research*, 17 (11): 2141-2149.
6. Gupta M, Dangi SS, Chouhan VS, Babitha V, Yadav VP, Singh G, Das GK, Mitra A, Bag S and Sarkar M.. 2014. Expression and localization of ghrelin and its functional receptor in corpus luteum during different stages of estrous cycle and the modulatory role of ghrelin on progesterone production in cultured luteal cells in buffalo. *Domestic Animal Endocrinology*, 48, 12-21.
7. Gupta. M, Dangi SS, Singh G, Sarkar M. 2015. Expression and localization of ghrelin and its receptor in ovarian follicles during different stages of development and the modulatory effect of ghrelin on granulosa cells function in buffalo. *General and Comparative Endocrinology*, 210: 87–95.
8. Kadowaki T and Yamauchi T. 2005. Adiponectin and adiponectin receptors. *Endocrine Review*, 26:439-451.
9. Kim AY, Lee YS, Kim KH, Lee JH, Lee HK, Jang SH, Kim SE, Lee GY, Lee J W, Jung SA, Chung HY, Jeong S and Kim JB. 2010. Adiponectin represses colon cancer cell proliferation via AdipoR1- and -R2-mediated AMPK activation. *Molecular Endocrinology*, 24(7): 1441-1452.
10. Lagaly DV, Aad PY, Grado-Ahuir JA, Hulsey LB and Spicer LJ. 2008. Role of adiponectin in regulating ovarian theca and granulosa cell function. *Molecular and Cellular Endocrinology*, 284:38 – 45.
11. Luo XH, Guo LJ, Yuan LQ, Xie H, Zhou HD, Wu XP and Liao EY. 2005. Adiponectin stimulates human osteoblasts proliferation and differentiation via the MAPK signalling pathway. *Experimental Cell Research*, 309:99– 109.
12. Maillard V, Uzbekova S, Guignot F, Perreau C, Ramé C and Coyral-Castel S. 2010. Effect of adiponectin on bovine granulosa cell steroidogenesis, oocyte maturation and embryo development. *Reproductive Biology and Endocrinology*, 8 :23.
13. Maleszka A, Smolinska N, Nitkiewicz A, Kiezun M, Chojnowska K, Dobrzyn K, Szwaczek H and Kaminski T. 2014. Adiponectin expression in the porcine ovary during the oestrous cycle and its effect on ovarian steroidogenesis. *International Journal of Endocrinology*, 2014: 1-9.
14. Mishra SR, Thakur N, Somal, A. Parmar, MS, Yadav VP, Bharati J, Bharti MK, Paul A, Verma MR, Chouhan VS, TaruSharma G. Singh G., González LA, D'Occhio MJ, and Sarkar M.. 2016. Expression and localization of angiopoietin family in buffalo ovarian follicles during different stages of development and modulatory role of angiopoietins on steroidogenesis and survival of cultured buffalo granulosa cells.
15. Mitchell M, Armstrong DT, Robker RL, Norman RJ. 2005. Adipokines: implications for female fertility and obesity. *Reproduction*, 130:583–597.
16. Oliveira BSP, Costa JAS, Gomes ET, Silva DMF, Torres SM, Monteiro PLJ Jr, Santos Filho AS, Guerra MMP, Carneiro GF, Wischral A and Batista AM. 2017. Expression of adiponectin and its

 DOI 10.5455/ijlr.20181229032418

receptors (AdipoR1 and AdipoR2) in goat ovary and its effect on oocyte nuclear maturation *In vitro. Theriogenology, 104:127*-133.
17. Pierre P, Froment P, Negre D, Rame C, Barateau V, Chabrolle C, Lecomte P, and Dupont J. 2009. Role of adiponectin receptors, AdipoR1 and AdipoR2, in the steroidogenesis of the human granulosa tumor cell line, KGN. *Human Reproduction*, 24 (11): 2890–2901.
18. Psilopanagioti A, Papadaki H, Kranioti EF, Alexandrides TK and Varakis JN. 2009. Expression of adiponectin and adiponectin receptors in human pituitary gland and brain. *Neuroendocrinology*, 89:38 –47.
19. Scherer P E, Williams S, Fogliano M, Baldini G, and Lodish H F. 1995. A novel serum protein similar to C1q, produced exclusively in adipocytes. *Journal of Biological Chemistry*, 270 (45): 26746–26749.
20. Szeszko K, Smolinska N, Kiezun M, Dobrzyn K, Maleszka A, and Kaminski T. 2016. The influence of adiponectin on the transcriptomic profile of porcine luteal cells. F*unctional & Integrative Genomics*, 16 (2).101–114.
21. Tabandeh M R, Hosseini A, Saeb M, Kafi M and Saeb S. 2010. Changes in the gene expression of adiponectin and adiponectin receptors (AdipoR1 and AdipoR2) in ovarian follicular cells of dairy cow at different stages of development. *Theriogenology*,. 73 (5), 659–669.
22. Tsukada T, Fushida S, Harada S, Terai S, Yagi Y, Kinoshita J, Oyama K, Tajima H, Ninomiya I, Fujimura T and Ohta, T. 2011. Adiponectin receptor-1 expression is associated with good prognosis in gastric cancer. *Journal of Experimental & Clinical Cancer Research*, 30(1), 107.
23. Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, *et al*. 2002. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nature Medicine*, 8 (11): 1288–1295.
24. Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K and Tsunoda M. et.al. 2003. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature, 423: 762 – 769.
25. Wang Y, Lam KS, Xu JY, Lu G., Xu LY, Cooper GJ and Xu A. 2005. Adiponectin inhibits cell proliferation by interacting with several growth factors in an oligomerization dependent manner. *Journal of Biololgical Chemistry*, 280(18): 18341-18347.
26. Wena JP, Lv WS, Yang J, Nie AF, Cheng XB, Yang Y, *et al*. 2008. Globular adiponectin inhibits GnRH secretion from GT1-7 hypothalamic GnRH neurons by induction of hyperpolarization of membrane potential. *Biochemical and Biophysics Research Communications*, 371: 756–61.

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