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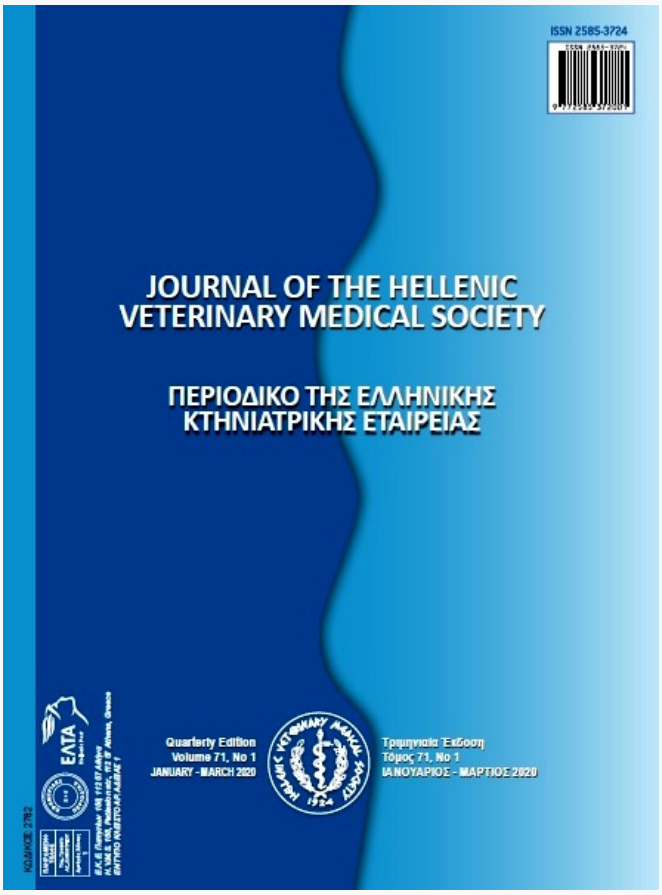

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Research article
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The Effect of Capsaicin on IGF-I and IGF-IR Expression in Ovarian Granulosa Cells

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ABSTRACT. Capsaicin (trans-8-methyl-N-vanillyl-6-noneadamide) is a pungent ingredient in red peppers from the Capsicum family. Insulin-like growth factor-I (IGF-I) is expressed in granulosa cells and has an important role in ovarian development. However, there are no data about the IGF-I expression in ovarian granulosa cells after capsaicin treatment. The aim of this study was to investigate the expression of IGF-I and its receptor (insulin-like growth factor-I receptor [IGF-IR]) in primary rat ovarian granulosa cells after low and high doses of capsaicin treatment. For this, granulosa cells were isolated and cultured from ovaries of 30-day-old female Sprague-Dawley rats. Granulosa cell plates were divided into four groups as cell control (C), vehicle control (V), and 50 µM and 150 µM capsaicin groups. In experimental groups, granulosa cells were exposed to capsaicin for 24 hours and immunocytochemistry was performed afterwards using anti-IGF-I and anti-IGF-IR antibodies. Both IGF-I and IGF-IR expressions were found to be significantly increased in parallel to the capsaicin doses. Elevated levels of IGF-I may be a risk factor for ovarian development. Because of the crucial role of IGF-I in ovary development, capsaicin treatment can be effective on follicular development and/or disorders characterized by high IGF-I levels.

Keywords: ovary, capsaicin, IGF-I, IGF-IR

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INTRODUCTION

Insulin-like growth factor-I (IGF-I) is a basic peptide comprised of 70 amino acids and promotes differentiation of various cell types with its anti-apoptotic activity and anabolic effect (Daughaday and Rotwein 1989). In addition to ubiquitous distribution in various tissues, the ovary is a major site of IGF-I production in mammalians (Daughaday and Rotwein 1989). IGF-I is highly expressed in the ovary and its mRNA is concentrated in all stages of developing follicles' granulosa cells (Kadakia *et al.,* 2001). IGF-I and its receptor (IGF-IR) are important factors that regulate ovarian cells' proliferation and differentiation as well as follicular development and ovulation (Armstrong and Webb 1997; Zhou *et al.*, 2013; Baumgarten *et al.,* 2017). IGF-I enhances FSH-stimulated estrogen and progesterone production by increasing steroid biosynthetic enzyme activities and induce LH receptors (Davoren and Hsueh 1884; el-Roeiy *et al.,* 1993). In vitro studies demonstrated that IGF-I stimulates granulosa cell proliferation in the ovary of rat (Adashi *et al.,* 1985), human (Wood *et al.,* 1993), pig (Xia *et al.,* 1994), sheep (Campbell *et al.,* 1995) and cow (Armstrong *et al.,* 1996; Stubbs *et al.,* 2013). IGF-I null mice are infertile with an arrest at the preantral follicle stage similar to FSHβ- and FSHR-deficient ovaries (Baker *et al.,* 2000). In addition, IGF-IR stimulates the development, transformation and differentiation of cells (Baserga 1995 and 2000; Chen and Sharon 2013). Previous studies showed that removal of the cell membrane IGF-IR by the abolition of the IGF-IR gene, suppression of cell expression or inhibition of function could lead to cell transformation (Baserga 1995; Baumgarten *et al.*, 2017).

Capsaicin (CAP) is the pungent ingredient in hot chili peppers of the family Capsicum. It is widely consumed as food additive and topical analgesic (Surh and Lee 1996; Arora *et al.*, 2011). Besides, CAP is currently being utilized for therapeutic treatment of various clinical conditions such as pain relief, rheumatoid arthritis, diabetic neuropathy, obesity, cardiovascular and gastrointestinal conditions (Josse *et al.*, 2010; Sharma *et al.*, 2013). CAP excites sensory neurons by binding to its receptor (TRPV1- capsaicin-sensitive receptor transient receptor potential, vanilloid type 1), localize on primary afferent neurons (Wardle *et al.*, 1997; Nagy *et al.,* 2004; Nakagawa and Hiura 2006). CAP-sensitive sensory neurons are nociceptive neurons that are known to activate ligand-gated, nonselective cation channels such as CGRP, substance P (SP) and neurokinin A (Jessell *et al.,* 1978; Saria *et al.,* 1987). Some researchers have suggested that CAP-sensitive sensory nerves could play a role in regulating the fertility and follicle development in females (Traurig *et al.,* 1984; Pintado *et al.,* 2003). Little is known about the effects of CAP on the female reproductive system. Pintado *et al.* (2003) treated female rats neonatally with a high dose of CAP (50 mg/kg) and found that rats exhibited an apparently normal courtship behavior but a lower reproductive success and litter size compared with control animals. On the contrary, low dose CAP protected the follicles from apoptosis and atresia, and stimulated follicular development (Zik *et al.,* 2010). Ozer *et al*. (2005) fed laying hens with a diet containing red hot pepper and demonstrated that follicular development was stimulated and laying performance was improved.

Previous studies have identified a link between the insulin family of trophic factors and TRPV1, showing that IGF-I sensitize TRPV1 receptors (van Buren *et al*, 2005). But to our knowledge, the mechanism of the effect of capsaicin in connection with IGF-I and IGF-IR in rat ovarian granulosa cells has not been reported. The objectives of this *in vitro* study, thus, were to examine (1) the expression of IGF-I and IGF-IR in rat ovarian granulosa cells, (2) effect of low and high dose CAP treatment on the expression of IGF-I and IGF-IR in rat ovarian granulosa cells.

MATERIALS-METHODS

Animals

Female Sprague-Dawley rats (30 days old) obtained from the Bursa Uludag University Experimental Animals Breeding and Research Center were used throughout the experiments. All procedures were performed after the approval of the Bursa Uludag University Animal Research Local Ethics Committee (Approval No. 2011-09/03). Handling and euthanasia of rats were performed according to the National Institute of Health Guide for the Care and Use of Laboratory Animals. The animals were housed five per cage at 20-24°C with a 60-70% humidity and 12/12 h light/dark cycle, and fed ad libitum. Euthanasia was performed by cervical dislocation after ether inhalation and the ovaries were harvested for culturing.

In Vitro Culture and Treatment

Granulosa Cell Culture

Granulosa cells were prepared as described previously with some modification (Uzumcu and Lin 1994; Zachow and Uzumcu 2006). Briefly, ovaries from the animals were rinsed in cold Hanks' Balanced

Salt Solution (HBSS) medium (PAN-Biotech GmbH; P04-34500; Germany) supplemented with 1% 1,000 U/ml penicillin G, 10 mg/ml streptomycin sulfate (Bio-Ind; 03-031-1B; CT, USA). Afterwards, the ovaries were cleaned of all connective tissues and fat and were moved into Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12) medium (Gibco™;11039-021) supplemented with 10% fetal bovine serum (FBS) (Bio-Ind; 04-007-1A) and 0.1% 1,000 U/ml penicillin G, 10 mg/ml streptomycin sulfate. Ovaries were punctured using a non-enzymatic needle puncture method with 27-gauge needle to extrude granulosa cells and the extract was then filtered through a 70 μm filter. The cell suspension was centrifuged at 200 g for 5 minutes, resuspended in a culture containing DMEM/F-12 medium supplemented with 10% FBS, and 0.1%, 1,000 U/ml penicillin G, 10 mg/ml streptomycin sulfate and then plated (Uzumcu and Lin 1994; Zachow and Uzumcu 2006).

The following day (day 0), the media were replaced with fresh media. Four groups were assigned: (1) cell control group (C), (2) media containing vehicle solution (0.01% DMSO; Ambresco; N182) for vehicle control (V), (3) low dose (50 μM) and (4) high dose (150 μM) of CAP (Sigma-Aldrich; M2028) diluted in vehicle solution. The cells were cultured at 37°C in a humidified atmosphere of 5% CO_2. Twenty-four hours after treatment, the experiments were terminated.

Immunocytochemistry

Granulosa cells were grown on coverslips in 24-well plates. The cells on the coverslips were washed three times in PBS. The cells were then fixed in 4% paraformaldehyde at room temperature for 15 min and permeabilized with 0.1% Triton X-100 for 10 min. The cells were blocked (Vector Lab., MP7401) for 20 min and incubated with anti-IGF-I (1:200), and anti-IGF-IR (1:250) antibodies at 4°C overnight and with the secondary antibody (Vector Lab.; MP7401) for 30 min. Cells were then treated with 3,3'-diaminobenzidine (DAB) for 5 min, counterstained with Harris Hematoxylin for 2 min, and the slides were then examined under Nikon Eclipse 80i microscope at a magnification of x400. Five microscopic areas were randomly counted and the percent value of stained cells was calculated for each experiment groups by both investigators. The average of two readings was taken.

Immunoflorescence

The cells were blocked with 5% BSA (in PBS) for 1 h followed by incubation with IGF-I (1:200) and IGF-IR (1:250) antibodies at 4°C overnight. Cells were then incubated with bovine anti-rabbit IgG-FITC (Santa Cruz; sc2365) secondary antibody (1:200) for 1 h in a dark room. Then coverslips were mounted and cells were visualized under Nikon Eclipse 80i microscope.

Statistical Analysis

The data were analyzed using the IBM SPSS Statistics 22. The normality of the data were determined by the Shapiro-Wilk test. Statistical significance between the groups was analyzed by the Kruskal-Wallis Test, followed by Mann-Whitney U posthoc test. Bonferroni correction was applied in order to control of alpha (α/k=0,008). All experimental data are expressed as mean±SD of three separate experiments, each carried out in replicate. A value of $p \leq 0.05$ was taken as statistically significant.

RESULTS

Granulosa cells in well plates were examined 24 h after CAP addition. Regular epithelioid structure and compact cell-cell interaction were observed in low dose CAP (50 μM) and control groups (Figure 1A). However, in the high dose group (150 μM), the structure of granulosa cells appeared deformed and cell-cell interactions were disrupted (Figure 1B).

The expression of IGF-I and IGF-IR was observed in the cytoplasm of granulosa cells ; the intensity was more prominent in the perinuclear area (Figures 2, 3). Rat ovarian granulosa cells with/without CAP addition were able to release the IGF-I and IGF-IR (Figures 2-4). Dose of CAP (50 and 150 μM) had a significant ($p \leq 0.05$) influence on IGF-I and IGF-IR expression. Highest number of cells expressing both IGF-I and IGF-IR was found after CAP treatment at the highest dose when compared to control and vehicle groups ($p \leq 0.05$) (Figure 4).

There was no statistical significance between the control and the vehicle group ($p>0.05$) regarding IGF-I immunoreactive cells, while CAP treated groups had significantly higher immunoreactive cells compared to the control groups ($p \leq 0.05$) (Figures 2, 4). Also, there is statistical significance between the groups administered dose 50 μM and 150 μM of CAP ($p \leq 0.05$) (Figures 2, 4). When IGF-IR results were evaluated, there was no significant difference between the control groups, but the difference was significant between the control and CAP groups difference of CAP groups. ($p \leq 0.05$) (Figures 3, 4). The number of granulosa cells expressing IGF-IR was more than IGF-I positive cells in all groups (Figure 4).

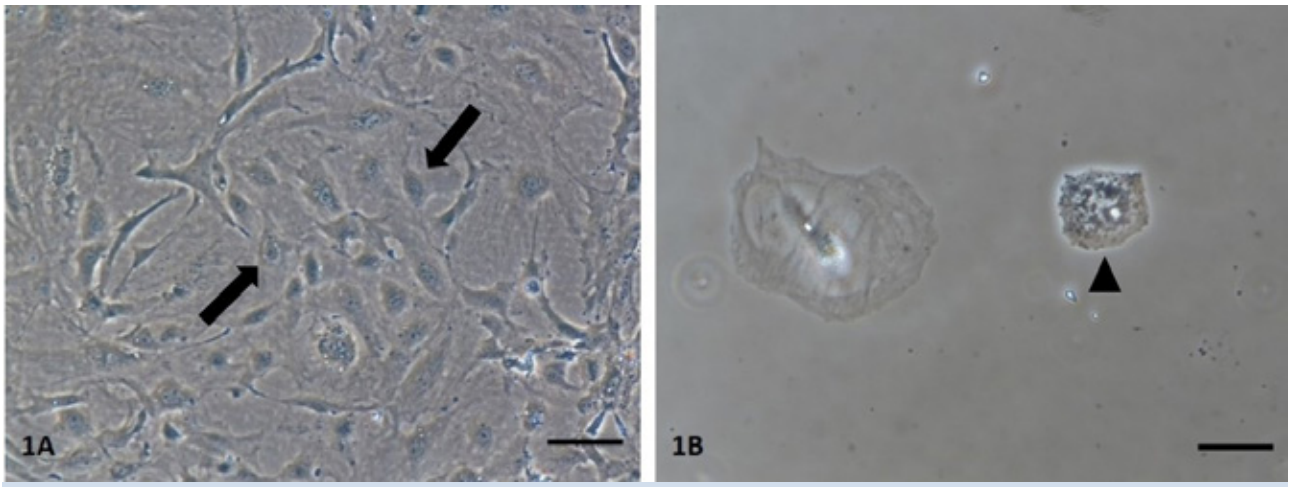

Figure 1. Morphological structure of ovarian granulosa cells A. Healthy ovarian granulosa cells (arrow) after 24 h, 50 µM CAP treatment, (Bar: 100µM), B. Apoptotic ovarian granulosa cells (arrowhead) after 24 h, 150 µM CAP treatment, (Bar: 100µM).

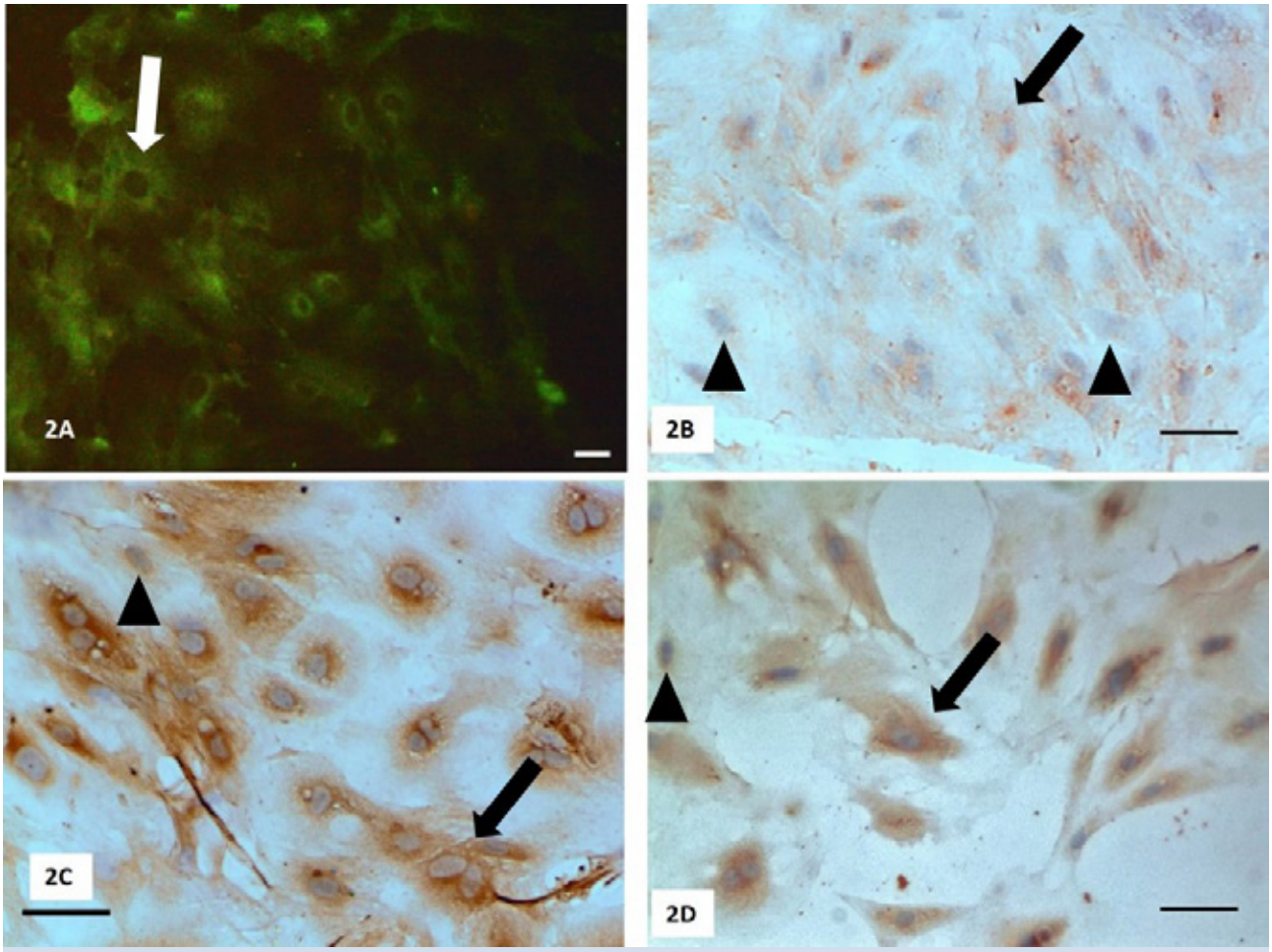

Figure 2. A. Positive (arrow) IGF-I expression in ovarian granulosa cells; 24 h, 50 µM CAP treatment (IF method) (Bar: 25µM). B. Positive (arrow) and negative (arrowhead) IGF-I expression in ovarian granulosa cells; control group (ICC method), (Bar: 50µM), C. Positive (arrow) and negative (arrowhead) IGF-I expression in ovarian granulosa cells; 24h, 50 µM CAP treatment (ICC method), (Bar: 100µM), D. Positive (arrow) and negative (arrowhead) IGF-I expression in ovarian granulosa cells; 24h, 150 µM CAP treatment (ICC method), (Bar: 50µM).

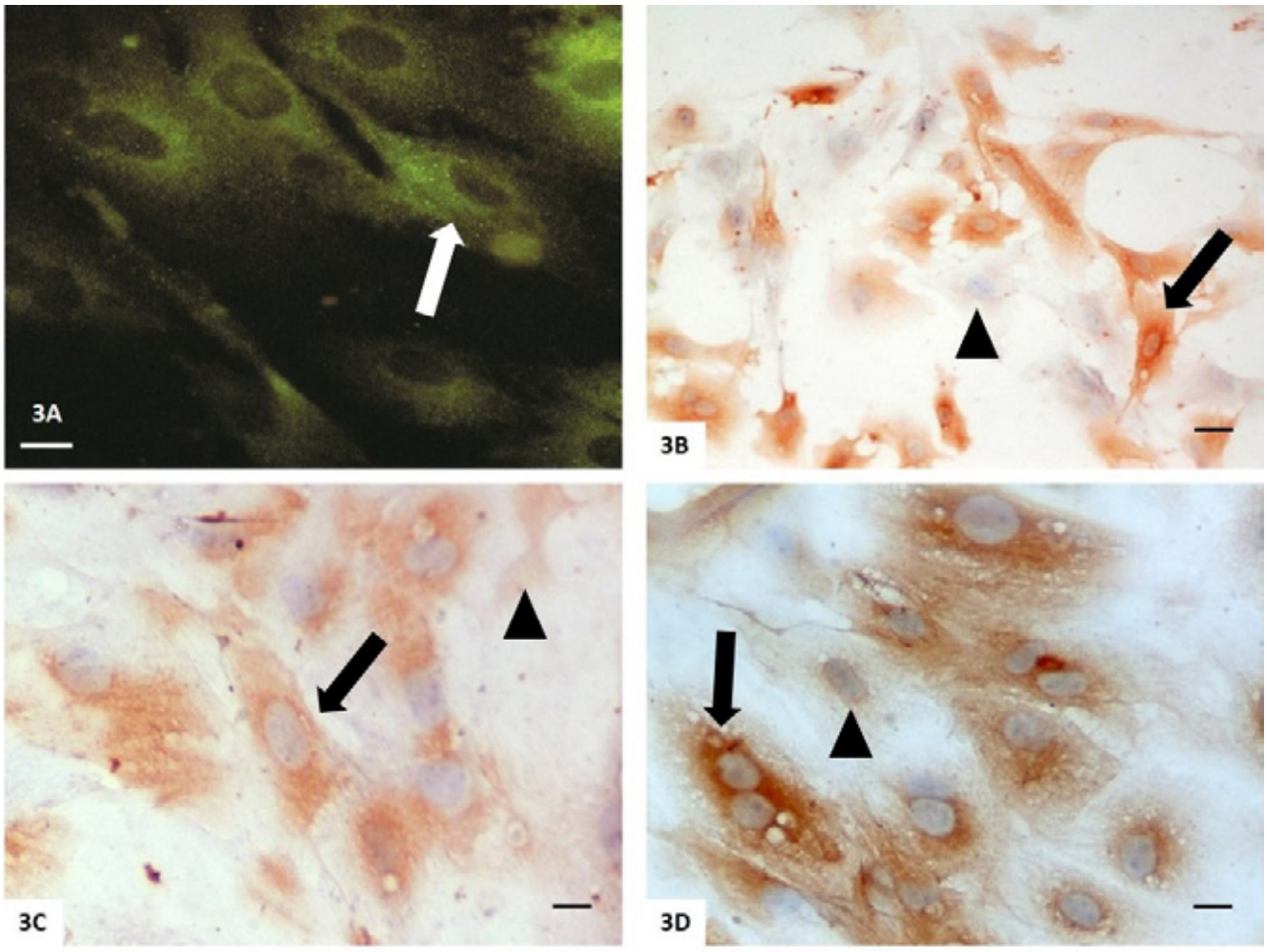

Figure 3. A. Positive (arrow) IGF-IR expression in ovarian granulosa cells; 24 h, 50 µM CAP treatment (IF method) (Bar: 25µM), B. Positive (arrow) and negative (arrowhead) IGF-IR expression in ovarian granulosa cells; control group (ICC method), (Bar: 25µM), C. Positive (arrow) and negative (arrowhead) IGF-IR expression in ovarian granulosa cells; 24h, 50 µM CAP treatment (ICC method), (Bar: 25µM), D. Positive (arrow) and negative (arrowhead) IGF-IR expression in ovarian granulosa cells; 24h, 150 µM CAP treatment (ICC method), (Bar:25 µM).

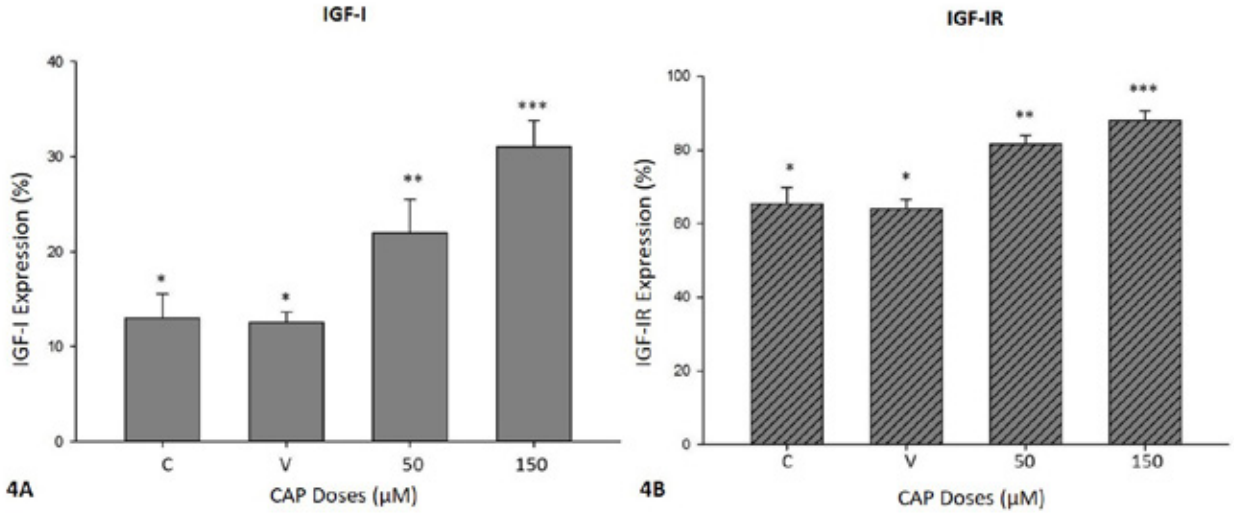

Figure 4. A. IGF-I expression, B. IGF-IR expression in ovarian granulosa cells.
*, **, ***; difference between groups, p≤0.05. C, cell control ; V, Vehicle control.

DISCUSSION

Many growth factors have been reported to participate in ovary physiology (Sirotkin 2011). IGF-I is one of them that amplifies gonadotropin action in granulosa and theca interstitial cells by acting on the IGF-IR (Maestro *et al.,* 1997). Stimulating effect of IGF-I on granulosa cell proliferation has been shown in many in vitro studies (Adashi *et al.,* 1985; Campbell *et al*., 1995; Armstrong *et al.,* 1996; Surh and Lee 1996). IGF-I stimulates initiation of preantral follicles development by revealing mRNA and protein levels. The presence of IGF-IR was demonstrated in the same study (Stubbs *et al.,* 2013). Baumgarten *et al.* (2017) reported that IGF-I and IGF-IR expression were found in granulosa cells of follicles from the primary to the antral stage and expression of IGF-IR in granulosa cells is essential for reproduction and lack of IGF-IR leads to apoptosis in granulosa cells. IGF-I gene expression in cell type-spesific in ovary, 10-fold greater abundance of IGF-I transcripts in granulosa cells as compared with theca-interstitial cells (Hernandez *et al.,* 1989). IGF-I staining localization and intensity in granulosa cells in our study also support previous data.

Effect of capsaicin on various type of cells is influenced by the administered dose (Pintado *et al.,* 2003; Ozer *et al.,* 2005; Zhang *et al.,* 2008; Zik *et al.,* 2010; Alatriste *et al.,* 2013). Many researchers have suggested that high dose of CAP affects steroidogenesis by creating degeneration in the sensory nerves of the hypothalamus-pituitary-ovarian pathway (Moran *et al.,* 2003; Pintado *et al.,* 2003; Alatriste *et al.,* 2013). Moran *et al*. (2003) injected CAP (50 mg/kg, sc) at birth and in 3 day-old rats which resulted in a significant delay of puberty and first vaginal estrus, as well as lower preantral and antral follicles. The studies about the effect of the low dose of CAP are limited (Ozer *et al.,* 2005; Zik *et al*., 2010). Zik *et al.* (2010) observed that low dose of CAP inhibits apoptosis and stimulates follicular development and proliferation of the granulosa cells. In addition, Ozer *et. al*. (2005) determined that low dose of red hot pepper (10 g/kg) added in diet of laying henscaused a significant increase in ovarian weight, follicle number and earlier onset of puberty compared to the control group.

In our previous studies, we found that low doses of CAP (10, 50 and 100 μM) increased cell proliferation in granulosa cells, but high doses (150 and 200 μM) induced apoptosis (Guler and Zik 2018). However, there are no studies on the expression of IGF-I and IGF-IR after CAP treatment on ovarian granulosa cells.

Many researchers have observed that IGF levels increase by applying toxic substances to ovarian cells (Holloway *et al.,* 2007; Cansu *et al.,* 2008; Ozden-Akkaya *et al*., 2017). Holloway *et al.,* (2007) administered dichlorodiphenylchloroethylene (DDT), a pesticide that can negatively affect ovarian function, and investigated IGF expression in vivo in rat ovaries and in vitro primary culture of human granulosa cells. They observed that IGF-I expression was increased in parallel to the increasing concentration of DDT. Ozden-Akkaya and *et al*., (2017) injected methoxychlor to fetal and neonatal rats and they found that IGF-I expression increased in granulosa cells. On the other hand, the increase in IGF-I expression in interstitial cells was directly related to the polycystic ovary syndrome (Schildkraut *et al.,* 1996; King *et al*., 2013). To our knowledge, the studies have been conducted on the expression of IGF-I and IGF-IR after CAP treatment on ovarian granulosa cells have not been reported. In some studies, the relationship between IGF-I and TRPV1 and IGF-IR and TRPV1 have also been demonstrated in different tissues and cells (Caprodossi *et al.,* 2011; Li *et al.,* 2013; Lilja *et al.,* 2007). Li and *et al.* (2013) showed that IGF-I expression increased in tibia bone marrow and it is responsible for the up-regulation of TRPV1 expression and function in the peripheral nerves. In another study IGF-I and insulin enhance TRPV1 protein expression in neublastoma cell line (Lilja *et al,* 2007).

In our experiments, the cell culture lasted 24 h with two different doses of CAP. Our results showed that the higher dose of CAP increased number of cells expressing IGF-I and IGF-IR. The expression results of IGF-I in other studies is consistent with our results (Halloway *et al.,* 2007; Cansu *et al.,* 2007; Ozden-Akkaya *et al.,* 2017). Several researchers observed co-existence of suppressed IGF-I expression and increased IGF-IR expression (Ozden-Akkaya *et al.,* 2017). But in our study, number of IGF-I and IGF-IR immunopositive granulosa cells increased with increasing doses of CAP.

Our results show that IGF-I and IGF-IR expressions were CAP dose-dependent. As the application dose of CAP increased, IGF-I and IGF-IR expressions increased in parallel, but morphological deformation was observed in cells.

CONCLUSION

In conclusion, this study demonstrates for the first time that CAP, a widespread food additive, can increase the expression of the essential ovarian growth factor, IGF and its receptor IGF-IR. High dose of CAP may be a risk factor and result in adverse reproductive outcomes. Our results are expected to lead to a focus of future in vivo studies about ovarian infertility in connection with IGF and CAP treatment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

Adashi EY, Resnick CE, Svoboda ME, Van Wyk JJ (1985) Somatomedin-C synergizes with follicle-stimulating hormone in the acquisition of progestin biosynthetic capacity by cultured rat granulosa cells. Endocrinology 116:2135-2142.

Alatriste V, Herrera-Camacho I, Martinez MI, Limon ID, Gonzalez-Flores O, Luna F (2013) Sensory denervation with capsaicin reduces ovarian follicular development and delays the onset of puberty in guinea pigs. Advances in Reproductive Sciences 1(3):29-37.

Armstrong DG, Webb R (1997) Ovarian follicular dominance: the role of intraovarian growth factors and novel proteins. Rev Reprod 2:139-146.

Armstrong DT, Xia P, De Gannes G, Tekpetey FR, Khamsı F (1996) Differential effects of insulin-like growth factor-I and follicle-stimulating hormone on proliferation and differentiation of bovine cumulus cells and granulosa cells. Biol. Reprod 54:331-338.

Arora R, Gill NS, Chauhan G, Rana AC (2011) An overview about versatile molecule capsaicin. International Journal of Pharmaceutical Sciences and Drug Research 3(4):280-286.

Baker DM, Davıes B, Dıckhoff WW, Swanson P (2000) Insulin-like growth factor I increases follicle-stimulating hormone (FSH) content and gonadotropin-releasing hormone-stimulated FSH release from coho salmon pituitary cells in vitro. Biol Reprod 63(3):865-871.

Baserga R (1995) The insulin-like growth factor I receptor: a key to tumor growth?. Cancer Res 55(2): 249-252.

Baserga R (2000) The contradictions of the insulin-like growth factor 1 receptor. Oncogene 19(49): 5574-5581.

Baumgarten SC, Armouti M, Ko C, Stocco C (2017) IGF1R expression in ovarian granulosa cells is essential for steroidogenesis, follicle survival, and fertility in female mice. Endocrinology 158:2309-2318.

Campbell BK, Scaramuzzi RJ, Webb R (1995) Control of antral follicle development and selection in sheep and cattle. J Reprod Fertil Suppl 49:335-350.

Cansu A, Giray SG, Serdaroglu A, Erdogan D, Coskun KZ, Korucuoglu U, Biri AA (2008) Effects of chronic treatment with valproate and oxcarbazepine on ovarian folliculogenesis in rats. Epilepsia 49(7):1192-1201.

Chen HX, Sharon E (2013) IGF-1R as an anti-cancer target-trials and tribulations. Chin J Cancer 32(5): 242-252.

Caprodossi S, Amantini C, Nabissi M, Morelli MB, Farfariello V, Santoni M, Gismondi A, Santoni G (2011) Capsaicin promotes a more aggressive gene expression phenotype and invasiveness in null-TRPV1 urothelial cancer cells. Carcinogenesis 32 (5) : 686-694.

Daughaday WH, Rotwein P (1989) Insulin-like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures, serum, and tissue concentrations. Endocr Rev 10: 68-91.

Davoren JB, Hsueh AJW (1984) Insulin enhances FSH-stimulated steroidogenesis by cultured rat granulosa cells. Mol Cell Endocrinol 35(2-3): 97-105.

el-Roeiy A, Chen X, Roberts VJ, LeRoith D, Roberts CT Jr, Yen SS (1993) Expression of insulin-like growth factor-I (IGF-I) and IGF-II and the IGF-I, IGF-II, and insulin receptor genes and localization of the gene products in the human ovary. J Clin Endocrinol Metab 77 : 1411-1418.

Guler S, Zık B (2018) Effects of capsaicin on ovarian granulosa cell proliferation and apoptosis. Cell and Tissue Research 372(3): 603-609.

Hernandez ER, Roberts CT, LeRoith D, Adashi EY (1989) Rat Ovarian Insulin-Like Growth Factor I (Igf-I) Gene Expression is Granulosa Cell-Selective: 5′-Untranslated mRNA Variant Representation and Hormonal Regulation. Endocrinology 125 (1) 572-574.

Holloway AC, Petrik JJ, Younglai EV (2007) Influence of dichlorodiphenylchloroethylene on vascular endothelial growth factor and insulin-like growth factor in human and rat ovarian cells. Reprod Toxicol 24(3-4): 359-364.

Jessell TM, Iversen LL, Cuello AC (1978) Capsaicin-induced depletion of substance P from primary sensory neurones. Brain Res 152:183-188.

Josse AR, Sheerffs SS, Holwerda AM, Andrews R, Staples AW, Philips SM (2010) Effects of capsinoid digestion on energy expenditure and lipid oxidation at rest and during exercise. Nutr Metab 7: 65.

Kadakia R, Arraztoa JA, Bondy C, Zhou J (2001) Granulosa cell proliferation is impaired in the Igf1 null ovary. Growth Horm IGF Res 11: 220-224.

King SM, Modi DA, Eddie SL, Burdette JE (2013) Insulin and insulin-like growth factor signaling increases proliferation and hyperplasia of the ovarian surface epithelium and decreases follicular integrity through upregulation of the PI3-kinase pathway. J Ovarian Res 6(1): 12.

Li Y, Cai J, Han Y, Xiao X, Meng XL, Su L, Liu FY, Xing GG, Wan Y (2014) Enhanced function of TRPV1 via up-regulation by insulin-like growth factor-1 in a rat model of bone cancer pain. Eur J Pain 18(6): 774-784.

Lilja J, Laulund F, Forsby A (2007) Insulin and insulin-like growth factor type-I up-regulate the vanilloid receptor-1 (TRPV1) in stably TRPV1-expressing SH-SY5Y neuroblastoma cells. Journal of Neuroscience Research 85(7): 1413-1439.

Maestro MA, Planas JV, Moriyama S, Gutiérrez J, Planas J, Swanson P (1997) Ovarian receptors for insulin and insulin-like growth factor I (IGF-I) and effects of IGF-I on steroid production by isolated follicular layers of the preovulatory coho salmon ovarian follicle. Gen Comp Endocrinol 106(2): 189-201.

Morán C, Morales L, Razo RS, Apolonio J, Quiróz U, Chavira R, Domínguez R (2003) Effects of sensorial denervation induced by capsaicin injection at birth or on day three of life, on puberty, induced ovulation and pregnancy. Life Sci 73(16): 2113-2125.

Nagy I, Santha P, Jancso G, Urban L (2004) The role of the vanilloid (capsaicin) receptor (TRPV1) in physiology and pathology. Eur J Pharmacol 500: 351-369.

Nakagawa H, Hiura A (2006) Capsaicin, transient receptor potential (TRP) protein subfamilies and the particular relationship between capsaicin receptors and small primary sensory neurons. Anat Sci Int 81: 135-155.

Ozden-Akkaya O, Altunbas K, Yagcı A (2017) Effects of methoxychlor on IGF-I signaling pathway in rat ovary. Biotech Histochem 92(3): 230-242.

Ozer A, Erdost H, Zik B (2005) Histological investigations on the effects of feeding a diet containing red hot pepper on the reproductive organs of the chicken. Phytother Res 19: 501-505.

Pintado CO, Pinto FM, Pennefather JN, Hidalgo A, Baamonde A, Sanchez

T, Candenas ML (2003) A role tachykinins in female mouse and rat reproductive function. Biol Reprod 69: 219-226.

Saria A, Ma RC, Dun NJ, Theodorsson-Norheim E, Lundberg JM (1987) Neurokinin A in capsaicin-sensitive neuronsof the guinea-pig inferior mesenteric ganglia: an additional putative mediator for the non-cholinergic excitatory postsynaptic potential. Neuroscience 21: 951-958.

Schildkraut JM, Schwingl PJ, Bastos E, Evanoff A, Hughes C (1996) Epithelial ovarian cancer risk among women with polycystic ovary syndrome. Obstet Gynecol 88: 554-559.

Sharma SK, Vij AS, Sharma M (2013) Mechanisms and clinical uses of capsaicin. Eur J Pharmacol 720(1-3): 55-62.

Sirotkin AV (2011) Growth factors controlling ovarian functions. J Cell Physiol 226(9): 2222-2225.

Surh YJ, Lee SS (1996) Capsaicin in hot chili pepper: carcinogen, co-carcinogen or anticarcinogen? Food Chem Toxicol 34: 313-316.

Stubbs SA, Webber LJ, Stark J, Rice S, Margara R, Lavery S, Trew GH, Hardy K, Franks S (2013) Role of Insulin-like growth factors in initiation of follicle growth in normal and polycystic human ovaries. J Clin Endocrinol Metab 98(8): 3298-3305.

Traurig H, Saria A, Lembeck F (1984) The effects of neonatal capsaicin treatment on growth and subsequent reproductive function in the rat. Naunyn-Schmiedeberg's Arch Pharmacol 327(3): 254-259.

Uzumcu M, Lin YC (1994) Characterization of the stimulatory actions of thymic factor(s) on basal and gonadotropin-induced steroidogenesis in cultured rat granulosa cells. Mol Cell Endocrinol 105: 209-216.

Van Buren JJ, Bhat S, Rotello R, Pauza ME, Premkumar LS (2005) Sensitization and translocation of TRPV1 by insulin and IGF-I. Mol Pain 1: 17.

Wardle KA, Ranson J, Sanger GJ (1997) Pharmacological characterization of the vallinoid receptor in the rat dorsal spinal cord. British Journal of Pharmacology 121:1012-1016.

Wood AM, Lambert A, Higham V (1993) Insulin-like growth factor-I stimulated growth and progesterone production by granulosa-lutein cells. Lack of interaction with physiological concentrations of luteinizing hormone and follicle stimulating hormone. Hum Reprod 8: 1813-1818.

Xia P, Tekpetey FR, Armstrong DT (1994) Effect of IGF-I on pig oocyte maturation, fertilization, and early embryonic development in vitro, and on granulosa and cumulus cell biosynthetic activity. Mol Reprod Dev 38: 373-379.

Zachow R, Uzumcu M (2006) The methoxychlor metabolite, 2,2-bis-(p-hydroxyphenyl)-1,1,1-trichloroethane, inhibits steroidogenesis in rat ovarian granulosa cells in vitro. Reproductive Toxicology 22(4): 659-665.

Zhang R, Humphreys I, Sahu RP, Shi Y, Srivastava SK (2008) In vitro and in vivo induction of apoptosis by capsaicin in pancreatic cancer cells is mediated through ROS generation and mitochondrial death pathway. Apoptosis 13(12): 1465-1478.

Zhou P, Baumgarten SC, Wu Y, Bennett J, Winston N, Hirshfeld-Cytron J, Stocco C (2013) IGF-I signaling is essential for FSH stimulation of AKT and steroidogenic genes in granulosa cells. Mol. Endocrinol 27(3): 511-523.

Zik B, Ozguden Akkoc CG, Tutuncu S, Tuncay I, Yilmaztepe OA, Ozenci CC (2010) Effect of low dose capsaicin (CAP) on ovarian follicle development in prepubertal rat. Revue Med Vet 161: 288-294.