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Folliculogenesis and follicular fluid adiponectin in cows: its alterations and relationships with ovarian function

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ABSTRACT

The objectives of the present study were to study the dynamic changes in adiponectin concentration in the growing luteal as well as the preovulatory follicles in dairy cows. In the first study, the ovaries and blood of 15 Holstein dairy cows in the luteal phase were collected from a slaughterhouse. Clear antral follicles were divided into three diameter groups (small, 3-5 mm; medium, 6-9 mm and large, ≥10 mm) and their fluid was aspirated. In the second study, the coccygeal blood and fluid of the preovulatory follicles of eight live Holstein dairy cows were aspirated transrectally, using a transrectal-guided fine-needle. Concentrations of adiponectin in the serum, and follicular fluid and progesterone in the serum were measured. Serum adiponectin concentrations in both luteal and follicular phases were higher than the follicular fluid adiponectin concentrations in all types of follicles ($P<0.05$); but the trend of a reduction in adiponectin in the serum from the luteal to the follicular phase was similar to the follicular fluid. The follicular fluid adiponectin concentrations of luteal phase follicles in the luteal phase did not differ ($P>0.05$), and the reduction was seen in preovulatory follicles in comparison with small follicles ($P = 0.001$). In the luteal phase, a significant positive correlation was observed between the adiponectin concentrations in different sized follicles, and also in the serum progesterone and follicular fluid adiponectin of follicles ($P<0.05$). In conclusion, lower adiponectin concentrations in blood serum and preovulatory follicles in comparison to luteal growing follicles reflect the effect of ovarian stage on adiponectin alterations.

Key words: adiponectin; progesterone; follicular fluid; folliculogenesis; cow

Introduction

Adipose tissue secretes hormones known as adipokines, reflecting their origin and their effects as cytokines on target tissues. Adiponectin is an adipokine that is known for its important role in the regulation of energy homeostasis. In dairy cattle, the negative energy balance recorded during early lactation causes significant loss in body weight and prolongs the intervals between parturition to first estrus (WATHES et al., 2007). Adiponectin has receptors such as AdipoRI, AdipoRII and T-cadherin (YAMAUCHI et al., 2003). The presumable occurrence of AdipoRI and RII in the pituitary suggests that adiponectin may affect reproductive functions by controlling the hypothalamic-

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pituitary-gonadal axis (KIEZUN et al., 2013) and/or peripheral actions on the ovary (CAMPOS et al., 2008; MITCHELL et al., 2005). Adiponectin is primarily synthesized by adipocytes, but has also been detected in other tissues, including ovaries (CHABROLLE et al., 2007a). On the other hand, adiponectin may be an important signal, indicating the adequacy of nutritional status for reproductive function (CAMPOS et al., 2008).

Beside circulatory adiponectin action on ovarian function, the peripheral actions of adiponectin in the ovary recently led some researchers to indicate another role of this hormone in ovarian function. In some studies the function and sites of adiponectin receptors in the ovaries are mentioned. For instance, in farm animals, AdipoRI expression in theca cells were higher than in granulosa cells of pig and cattle ovaries, and expression of AdipoRII in theca cells of the large follicles was higher than in granulosa cells of large follicles in rats (CHABROLLE et al., 2007a; CHABROLLE et al., 2007b). In pig granulosa cells, the expression of AdipoRI and AdipoRII was the same in all small, medium and large follicles (LEDOUX et al., 2006).

On the other hand, other groups of studies have shown the interaction of adiponectin with gonadotropins and insulin in coordination of ovarian follicular growth. Adiponectin alone, or in combination with gonadotropins and insulin, induced the expression of effective genes as cyclooxygenase-2, prostaglandin E synthase and vascular endothelial growth factor in porcine granulosa cells (LEDOUX et al., 2006).

Regarding the relationship between adiponectin and gonadotropins in humans, treatment with human chorionic gonadotropin (hCG) or follicle stimulating hormone (FSH) increased AdipoRII mRNA in ovarian granulosa cells but did not affect the Adipo RI mRNA (WICKHAM III et al., 2013). Additionally, treatment of women with hCG during a follicle stimulating protocol increased the adiponectin concentration in the follicular fluid (GUTMAN et al., 2009). Treatment of chicken granulosa cells with recombinant human adiponectin increased the insulin growth factor-I (IGF-I), and induced progesterone secretion, but it decreased progesterone secretion under the influence of luteinizing hormone (LH) or FSH (CHABROLLE et al., 2007a). However, in the absence of IGF-I or gonadotropins, adiponectin had no effect on progesterone secretion from the granulosa cells of the first and third/fourth largest ovarian yellow follicles (CHABROLLE et al., 2007a). In cattle, adiponectin reduced the insulin-dependent steroidogenesis, and increased the IGF-I induced proliferation of granulosa cells cultured via involvement of ERK1/2 MAPK pathway (MAILLARD et al., 2010). In addition, treatment of immature rats with PMSG, followed by HCG, increased the protein of adiponectin, and the protein and mRNA of AdipoRI in ovarian cells (CHABROLLE et al., 2007b). In these conditions, the protein and mRNA of AdipoRII remains unchanged, but the amount of adiponectin in the plasma decreases (CHABROLLE et al., 2007b). Mice with a mutation in the adiponectin gene were fertile, which indicates that this protein lonely is not necessary for ovarian activity in mice. Its role may be in the strength of insulin and gonadotropin messages involved in ovulation (MAEDA et al., 2002). Transgenic mice that have 2-3 times higher adiponectin were infertile (COMBS et al., 2004). On the other hand, modification or partial deletion of the adiponectin gene in mice also induced infertility in female mice (BAUCHE et al., 2006, COMBS et al., 2004). In women, this co-function of adiponectin and insulin is also shown, Decreased insulin sensitivity plays an important role in impaired final maturation and ovulation in women with polycystic ovary syndrome (PCOS) (CARMINA et al., 2005).

In women, changes in body fat and its distribution in different periods before and after menopause showed significant differences, and in the course of menopause is associated with changes in FSH (SUMINO et al., 2004). In addition, adiponectin levels in obese women was lower than in normal women during menopause (SUMINO et al., 2004). Furthermore, a relationship was recently shown between body condition score, as an index of fat tissue reserve, and serum adiponectin concentrations and their combined effects on dairy cow reproductive function during the postpartum period (KAFI et al., 2012a; KAFI et al., 2015).

Therefore, regarding the functions of insulin and gonadotropins in regulation of the estrus cycles of dairy cows (HEIN et al., 2015; SCHAMS et al., 1978), evaluation of the hypothesis that alterations in adiponectin during the estrus cycle in dairy cows may play an effective role in follicular growth, was the first aim of this study. Information about changes in follicular fluid and blood serum adiponectin concentrations during a dairy cow's estrous cycle is limited. Therefore, the objective of the present study was to determine adiponectin concentrations in the serum and follicular fluid of different-sized follicles in the follicular and luteal phases of a dairy cow's estrous cycle.

Materials and methods

Animals. The Committee for Research and Animal Experiments of Shiraz University approved the experimental protocol. Follicular fluid samples were collected from slaughtered Holstein cows in the luteal phase. To obtain follicular fluid samples from presumptive ovulatory follicles we used live cows whose estrous cycles had been synchronized.

Collection of follicular fluid in the luteal phase. The ovaries and blood of 18 Holstein dairy cows in the luteal phase were collected from the local slaughterhouse. Samples were collected from healthy cows with an apparently normal reproductive tract (without uterine infection, ovarian cysts, or any other gross structural disorders). Blood samples (2 mL) were collected from the heart ventricles immediately after the heart was removed. The stage of the estrus cycle was determined on the basis of the ovarian structures and serum progesterone level. Only ovaries with normal color and consistency, with the presence of different sized follicles and a corpus luteum, and without follicular or luteal cysts (≥20 mm) were collected. The blood and ovaries were transported in cold conditions (1 °C) to the laboratory. The clear antral follicles were classified into three diameter groups (small, 3-5 mm; medium, 6-9 mm and large, ≥10 mm) and their fluid was aspirated using 26 gauge needles attached to 5 mL syringes. The blood serum was separated by centrifugation (for 10 min at 3,000 g). The supernatant of the aspirated follicular fluid was separated by centrifugation (for 7 min at 10,000 g). The follicular fluid supernatant and blood serum were stored at -22 °C until assayed for hormone determination.

Collection of preovulatory follicular fluid. The coccygeal blood and preovulatory follicle follicular fluid of eight live Holstein dairy cows were collected. Estrus was induced using a single intramuscular administration of 500 µg of $PGF_2\alpha$ (estroPLAN, Parnell laboratories, Alexandria. NSW, Australia). The standing heats were detected by observing the cows three times a day for 30 minutes each time. After detecting standing heat, the presence of preovulatory follicles (13-19 mm) was confirmed using a real-time B-mode ultrasound scanner (Ultra Scan 900, Ami, Medical Alliance Inc., Montreal, QC, Canada) equipped with a 5 MHz, linear-array transducer (TAMADON et al., 2010). Blood samples were collected (venipuncture of the coccygeal vein) from all cows before follicular fluid aspiration. Follicular fluid was aspirated using a rectal-guided fine-needle via the rectum (a training video is available here: https://youtu.be/OGZTpVS4FFY). Briefly, the cows were restrained and epidural anesthesia was induced with 2% lidocaine HCl (0.22 mg/kg). Twelve hours after detecting standing heat, ovarian ultrasonography was performed to assess the presence of a preovulatory follicle (10-17 mm diameter). Then, a sample of follicular fluid was aspirated transrectally using a long fine-needle, covered by a hard plastic tube. All follicular fluid samples used for hormone assay were free from blood contamination. Confirmation of proper sampling and follicular fluid drainage was approved using trans-rectal ultrasonography.

Blood and aspirated follicular fluid were transferred to the laboratory in cold conditions. The serum was separated by centrifugation (for 10 min at 3,000 g). The supernatant of the aspirated follicular fluid was separated by centrifugation (for 7 min at 10,000 g). Follicular fluid supernatant and blood serum were stored at -22 °C until assayed.

Progesterone analysis and determination of ovarian stage. Serum P_4 concentrations were determined using a validated commercial radioimmunoassay kit (Immunotech kit, France) (KAFI et al., 2012b). The intra- and inter-assay coefficients of variation (CVs) of the assays were

5.8%, and 9.0%, respectively. The sensitivity of the test was 0.05 ng/mL, and the recovery rate of the assay ranged from 85 to 110%. Cows with serum P_4 concentrations ≥1 ng/mL were considered to have luteal activity (TAMADON et al., 2010).

Determination of serum and follicular fluid adiponectin concentrations. Blood serum and follicular fluid adiponectin concentrations were determined using a validated commercial ELISA kit (Bovine Adiponectin, Cusabio Biotech Co. Ltd., China). The analytical sensitivity of the test was typically less than 3.12 µg/mL. The inter assay coefficients of variability for low and high bovine adiponectin (4.2 and 14.3 µg/mL, respectively) were 6.2 and 5.9, respectively. The intra assay variations for the two above samples were 3.4 and 4.7, respectively.

Statistical analysis. After confirmation of exact luteal phase by progesterone assay, the blood serum and follicular fluid of 15 slaughtered cows in the luteal phase and follicular fluid of six live cows were included in the study on the basis of the serum P_4 concentrations; $P_4 \leq 1$ ng/mL was considered as the follicular phase group and $P_4 > 1$ ng/mL was analyzed as the luteal phase group (KAFI et al., 2015). Cows with BCS between 2.75 to 3.25 as normal BCS cows (TAMADON et al., 2011) were included in the study. Normal distributions of the serum and follicular fluid adiponectin concentrations and serum progesterone concentrations were evaluated using a Kolmogorov-Smirnov test (SPSS for Windows, version 11.5, SPSS Inc, Chicago, Illinois).

Statistical differences in serum and follicular fluid adiponectin concentrations between the different groups were analyzed using an independent sample *t*-test (two groups) or one way ANOVA and the Bonferroni post hoc test (three groups). Correlation coefficients between adiponectin concentrations in the follicular fluid of different sized follicle, or between adiponectin concentrations in the follicular fluid and serum concentrations of adiponectin or progesterone were analyzed using Spearman's test and shown using scatter plots (GraphPad Prism version 5.01 for Windows, GraphPad software Inc., San Diego, CA, USA). $P \leq 0.05$ was considered significant and data are presented as the mean ± SD.

Results

Dynamic changes in follicular fluid adiponectin concentration. There were no differences between adiponectin concentrations in the follicular fluid of different sizes of follicles in the luteal phase ($P > 0.05$, Fig. 1). There was a positive correlation between adiponectin concentrations in different sized luteal phase follicles ($P = 0.01$, Table 2). Adiponectin concentration in preovulatory follicles was lower than in small, medium and large luteal phase follicles ($P < 0.05$, Fig. 1).

Table 1. Correlation between progesterone, adiponectin serum concentrations and large luteal, preovulatory follicular fluid adiponectin concentrations in dairy cow (n = 8)

Stage of cycle		Serum adiponectin	Serum progesterone
Follicular	Follicular fluid adiponectin	r = -0.009, P = 0.98	r = 0.43, P = 0.11
	Serum progesterone	r = 0.08, P = 0.79	
Luteal	Follicular fluid adiponectin	r = -0.37, P = 0.47	r = -0.14, P = 0.79
	Serum progesterone	r = -0.14, P = 0.79	
Overall	Follicular fluid adiponectin	r = 0.14, P = 0.56	r = 0.55, *P = 0.009
	Serum progesterone	r = 0.32, P = 0.15	

* $P < 0.05$

Serum adiponectin and its correlation with follicular fluid adiponectin concentration. Adiponectin concentration was higher in the serum than in the follicular fluid of large and preovulatory follicles ($P < 0.01$, Fig. 1) in both follicular and luteal phases. However, the adiponectin concentrations of serum and follicular fluid of large follicles did not differ, either in the follicular phase or the luteal phase ($P > 0.05$, Figure 1). The serum adiponectin concentration in the luteal phase was higher than

Table 2. Correlation between serum and follicular fluid adiponectin and serum progesterone concentrations in different sized luteal phase follicles in dairy cows (n = 15)

	Follicular size	Follicular fluid adiponectin		Serum adiponectin	Serum progesterone
		Small	Medium		
Follicular fluid adiponectin	Small			r = 0.13, P = 0.65	r = 0.60, *P = 0.02
	Medium	r = 0.87, *P = 0.001		r = 0.32, P = 0.25	r = 0.69, *P = 0.004
	Large	r = 0.60, *P = 0.02	r = 0.54, *P = 0.04	r = -0.009, P = 0.98	r = 0.43, P = 0.11

* P<0.05

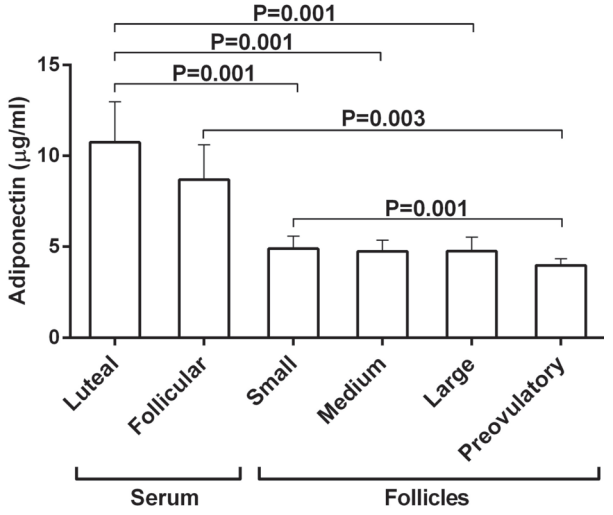

Fig. 1. Serum, large luteal and preovulatory follicular fluid adiponectin concentrations. Significant differences between columns are shown by lines (P<0.05).

follicular fluid concentration in small, medium and large follicles (P<0.001, Fig. 1). There was no correlation between adiponectin follicular fluid concentration in different sized luteal phase follicles and serum adiponectin concentration (Table 2).

Correlation between serum progesterone and follicular fluid adiponectin concentration. The serum progesterone concentration in the luteal phase (5.44 ± 2.63 ng/mL, n = 15) was higher than in the follicular phase (0.86 ± 0.21 ng/mL, n = 6; P = 0.004). There was a positive correlation between adiponectin concentration in the follicular fluid of large follicles, and the progesterone concentration of serum in both luteal and follicular phases (P = 0.05, Table 1). There was a negative correlation between serum adiponectin concentration and serum progesterone concentration in the luteal group (P = 0.04). In the other cases, the correlation coefficient between these factors was not significant. In addition, there was a positive correlation between serum progesterone concentration and adiponectin concentration in the follicular fluid of medium luteal phase follicles (P = 0.03).

Discussion

Serum adiponectin and ovarian activity. The present study showed the presence of an inverse relationship between serum adiponectin concentration and serum progesterone concentration in both luteal and follicular phases. It was shown previously that the concentration of circulating adiponectin was higher after parturition in Holstein dairy cows, but it remained constant during different stages of lactation (OHTANI et al., 2012). The serum adiponectin concentration was stable during the menstrual cycle in humans (DAFOPOULOS et al., 2009). Furthermore, in women, removing the effect of ovarian hormones or administration of estrogen had no effect on serum adiponectin levels (CHALVATZAS et al., 2009). In contrast, the present study recorded higher concentrations of serum adiponectin during the luteal phase. Furthermore, our previous report showed that serum adiponectin and progesterone concentrations changed in the estrous cycle of high-producing dairy cows (KAFI et al., 2015). Therefore, there is a relationship between the stage of the estrous cycle and serum adiponectin concentrations in dairy cow. Also, there was less adiponectin in preovulatory follicles than serum adiponectin in the luteal phase, which may perhaps show that a reduction in adiponectin is involved in the progression of ovarian ovulation.

On the other hand, evidence obtained from other species demonstrates that the interaction between adiponectin, gonadotropins and insulin regulates

ovarian function. KIEZUN et al. (2013) showed different expressions of adiponectin receptors mRNA in pituitary stages during the porcine estrus cycle. For instance, during the luteal phase, the expression of AdipoRII increased in the dorsal pituitary (KIEZUN et al., 2013). In addition, this inverse activity of progesterone and adiponectin can be altered by exogenous administration of sex steroid hormones and gonadotropins. In normal monkeys, administration of a progesterone and estrogen combination of contraceptive pills increased serum adiponectin levels (SHAW et al., 2013). Furthermore, women with high adiponectin concentrations showed a better response to gonadotropin stimulation in LβT2 immortalized mouse gonadotroph cells (LIU et al., 2005). Adiponectin inhibits the release of the LH hormone, and reduced expression of the GnRH receptor (LU et al., 2008). Therefore, although this is not completely understood, it seems that alterations in adiponectin concentrations in the circulatory level may affect steroidogenesis and control ovarian activity in dairy cows.

Serum and follicular fluid adiponectin. In the current study, the only significant correlation between serum progesterone concentration and adiponectin concentration in follicular fluid was the positive correlation between adiponectin concentration in the follicular fluid of medium follicles and serum progesterone concentration. Information about changes in and the interactions of adiponectin of follicular fluid and serum during the estrous cycle is limited. In the current study, in the luteal phase and follicular phase of parous dairy cows, the adiponectin concentrations of follicular fluid were on average 44.3% and 46.0% of the serum adiponectin concentration, respectively. It has been shown that the concentrations of total adiponectin in the follicular fluid were on average 62% (HEINZ et al., 2015), 80% to 90% (LEDOUX et al., 2006), and 22% to 29% (BANGA et al., 2008; BERSINGER et al., 2006) of the corresponding serum adiponectin concentration in heifers, swine and humans, respectively. However, consistent with our findings, the adiponectin concentrations in the serum and follicular fluid were not correlated in heifers (HEINZ et al., 2015). Although this weak correlation may be due to the small number of animals sampled, it seems that serum adiponectin concentrations may affect the follicular fluid concentration of adiponectin in dairy cows, and they may both play a role in ovarian activity. Furthermore, the decrease in the adiponectin concentration in preovulatory follicles in comparison to large luteal phase follicles indicates that the ovulation related changes in adiponectin concentrations shows the possible role of adiponectin in ovulation. On the other hand, in women evaluated for outcome of intracytoplasmic sperm injection/embryo transfer cycles, BERSINGER et al. (2006) showed that the group with positive efficiency of pregnancy compare to groups that have no pregnancy, including more serum adiponectin but non-different follicular fluid adiponectin. Therefore, it may be concluded that the combination of both serum and follicular fluid adiponectin concentrations may play a role in the regulation of bovine ovarian activity, but further investigation is necessary to clarify the nature of this rule.

Follicular fluid adiponectin and ovarian activity. In the current study, there was a significant correlation between adiponectin concentration in the follicular fluid of large follicles and adiponectin concentration in the follicular fluid of small and medium follicles in the luteal phase. Consistent with our findings, no changes in adiponectin receptors were detected in rats during the normal development of follicles by the immunohistochemistry technique (CHABROLLE et al., 2007b). Furthermore, in porcine granulosa cells, the expressions of AdipoRI and AdipoRII in small, medium and large follicles were similar (LEDOUX et al., 2006). In addition, in the current study, there was no significant correlation between adiponectin concentration in preovulatory follicles and serum progesterone concentration. Adiponectin decreased the insulin dependent, but not the IGF-I dependent steroidogenesis of bovine granulosa cell culture (MAILLARD et al., 2010). In addition, in rat granulosa cells, adiponectin had no effect on the base production of progesterone and estrogen, in the presence of FSH, but production of these hormones increased in the presence of IGF-I by adiponectin (CHABROLLE et al., 2007b). However, adiponectin inhibited the production of

progesterone in the presence of LH or FSH in rats (CHABROLLE et al., 2007b). Furthermore, the study by CHABROLLE et al., (2007a) on chicken granulosa cells showed that adiponectin can inhibit progesterone in the presence of FSH and LH, but increase progesterone in the presence of IGF-I. In addition, adiponectin has different pathways to transmit different messages in the ovary. Adiponectin reduces progesterone production in rat ovarian cells through activation of 5'AMP-activated protein kinase (AMPK) (TOSCA et al., 2005). It was shown that adiponectin reduced the expression of CYP19A1 in porcine granulosa cells (LEDOUX et al., 2006). In most mammalian granulosa cells, androstenedione is converted to estradiol due to the action of CYP19A1 and HSD17B1 (LEDOUX et al., 2006). In addition, adiponectin reduced the expression of LH receptors and caused the reduction of CYP19A1 and CYP11A1 and finally caused progesterone and androstenedione reduction in bovine granulosa cells (LAGALY et al., 2008). Metformin from bovine granulosa cells severely decreased the secretion of progesterone and estrogen (TOSCA et al., 2007). AMPK activation by metformin decreased production of progesterone in the presence of FSH (TOSCA et al., 2007). Therefore, changes in ovarian steroids during the luteal phase have no effect on follicular adiponectin, and during follicular growth adiponectin remain unchanged throughout preovulatory changes.

In addition, a reduction in adiponectin concentration in preovulatory follicles was observed in the present study. Adiponectin alone or in combination with insulin and gonadotropins, induces expression of genes that are involved in prostaglandin and vascular endothelial growth factor synthesis, in porcine preovulatory follicle granulosa cells (LEDOUX et al., 2006). In contrast, the increase in adiponectin and its receptors in large human follicles was related to the ability of large follicles to respond to LH treatment (GUTMAN et al., 2009). The frequency of LH mRNA in cow granulosa cells was reduced by adiponectin (LAGALY et al., 2008). Adiponectin reduction increased the frequency of LH mRNA in granulosa cells, and led to follicular ovulation (LAGALY et al., 2008). Adiponectin reduction removed the inhibitory effect of adiponectin on insulin induced progesterone and androstenedione production, and therefore androstenedione increased (LAGALY et al., 2008). The current study findings probably indicate that the significant reduction in adiponectin in preovulatory follicles may indicate the regulatory role of adiponectin in the progression of the ovary into the ovulatory stage.

Furthermore, in granulosa cells, androstenedione metabolized into estrogen (WOOD and STRAUSS III, 2002) and the increased estrogen led to follicular ovulation. PPAR (one of the adiponectin signal transduction pathways in ovaries) is expressed in theca cells and in the early stages of follicular development (DUPONT et al., 2007). PPAR expression simultaneously increased with large follicle development and reduced after the LH surge (DUPONT et al., 2007). The reduction in PPAR expression after the LH surge led to the removal of the inhibitory effect of PPAR on these genes' expression, and the arrangement of essential proteins before ovulation (DUPONT et al., 2007). After LH reduction, adiponectin expression is increased in the theca cells that are converting to the corpus luteum and also the expression of PPAR receptors is increased (KOMAR 2005). Therefore, simultaneous with the adiponectin reduction in the follicular fluid, granulosa cells, as a major producer of estrogen in the ovary, metabolize the androstenedione to estrogen and this leads to an increase in estrogen and the progression of follicular ovulation.

Conclusions

The results of this study show that, in luteal phase of the estrous cycle, there was a significant correlation between serum progesterone and follicular adiponectin of medium follicles. In addition, the adiponectin concentration in the blood serum was higher during the luteal phase, significantly more than in the follicular phase. Furthermore, small, medium and large size luteal phase follicles had constant levels of adiponectin but adiponectin decreased significantly in preovulatory follicles. It may be concluded that adiponectin may have lesser effect on follicular growth but may play a role during ovulation.

Conflict of interest

Authors have no conflict of interest to declare.

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SAŽETAK

Cilj rada bio je istražiti dinamičke promjene u koncentraciji adiponektina u žutom tijelu tijekom njegova razvoja te predovulacijskim folikulima u mliječnih krava. U prvom su istraživanju u klaonici prikupljeni jajnici i krv 15 mliječnih krava holštajnske pasmine u lutealnoj fazi. S obzirom na poprečni presjek antralni folikuli podijeljeni su u tri skupine (mali: 3 - 5 mm, srednji: 6 - 9 mm i veliki: ≥ 10 mm) te je iz njih aspirirana tekućina. U drugom je istraživanju transrektalno, tankom iglom, uzeta krv iz repne vene i tekućina iz predovulacijskih folikula osam živih mliječnih krava holštajnske pasmine. Izmjerena je koncentracija adiponektina, folikularne tekućine i progesterona u serumu. Koncentracije serumskog adiponektina i u lutealnoj i u folikularnoj fazi bile su veće od koncentracija adiponektina u folikularnoj tekućini u svim tipovima folikula ($P < 0,05$), no trend smanjenja koncentracije adiponektina u serumu od lutealne do folikularne faze bio je sličan onomu u folikularnoj tekućini. Koncentracije adiponektina u folikularnoj tekućini žutih tijela nisu se razlikovale ($P > 0,05$), a smanjena je koncentracija uočena u predovulacijskim folikulima u usporedbi s malim folikulima ($P = 0,001$). U lutealnoj fazi znakovita je pozitivna korelacija zapažena između koncentracija adiponektina u folikulima različite veličine, a također i serumskog progesterona te adiponektina u tekućini folikula ($P < 0,05$). Zaključeno je da niže koncentracije adiponektina u serumu i predovulacijskim folikulima u usporedbi s folikulima u lutealnoj fazi odražavaju učinak stadija jajnika na promjene u koncentraciji adiponektina.

Ključne riječi: adiponektin; progesteron; folikularna tekućina; folikulogeneza; krava