WALIA journal 30(S2): 109-112, 2014
Available online at www.Waliaj.com
ISSN 1026-3861

Effects of conjugated linoleic acid (CLA) on hormones and factors involved in urine ovulation

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Abstract: Conjugated linoleic acid is composed of polyunsaturated fatty acids (PUFA) that found in dairy products, beef and lamb. The aim of this study was to determine the effects of different doses of dietary CLA on systemic and local hormones and factors involved in ovulation. In this case-control study, 80 (50±2-day old) female mice were randomly divided into four groups. There were four replicates in each group and there were 5 mice in every replicate (20 mice, in total). The mice in the control group were fed with no CLA in their diet but the ones in the treatment group received 0.1, 0.3 and 0.5g/kg of CLA (replacing corn oil in the diet), respectively for 120 days. Later on, blood samples were obtained from the tails of animals that displayed estrus signs and estradiol, progesterone, LH, FSH, NO, leptin and TNFα were measured. Furthermore, the effects of CLA on the ovarian production of prostaglandins and NO were investigated. The data were analyzed by SAS software. CLA significantly decreased serum levels of FSH ($p<0.05$), LH, estradiol, NO, leptin and TNFα ($p<0.01$). In addition, CLA decreased progesterone levels but this effect was statistically in significant. The significantly negative effects of CLA were seen on the ovarian production of PGE_2 and $PGF_{2\alpha}$ ($p<0.01$). It seems that CLA may play an effective role in reducing the ovulation rate in mice as CLA adversely affected female reproduction and it had negative effects on systemic and local hormones involved in ovulation.

Key words: *Conjugated linoleic acid; Nitric oxide; Ovary; Ovulation; Prostaglandin; Gonadotropin*

1. Introduction

It has been known for many years that LH peak in the middle of estrous cycle and its increasing effect on estradiol and increasing concentrations of enzymes that convert plasminogen to plasmin in follicle graph fluid cause the follicle wall becomes weak and ovulation occurs (Squires; 2003). In 1980 Espey called ovulation as an inflammatory and immunological phenomenon (Espey; 1980). Inflammation is a complex process that internal factors such as tumor necrosis are involved in and prostaglandins are exogenous variables that are effective in inflammation (Espey; 1994).

After peaking in the follicular Granulosa cells and the CAMP, LH The result is increasing in production of prostaglandins. Prostaglandins as well as in mammalian significantly increased ovulation rate (Tokuyama et al; 2001; Staud; 2000; Wu et al; 2001; Espey;1983). Inflammatory cytokines such as TNF and IL-1 and eicosanoids such as prostaglandins are important mediators of inflammation that are Leukotrienes production and their levels are influenced by long-chain saturated fatty acids in the diet (Nagao et al; 2008). Research has shown that certain combinations of nutrients such as fatty acids on ovarian physiological processes, including processes that affect ovulation (Ferguson et al; 1999). Conjugated linoleic acid is a mixture of 28 isomers of linoleic acid, which is one of the essential fatty acids (Aydin; 2005). Among the various isomers, the isomers cis-9, trans-11(C_9t_{11}) and trans-10, cis-12 ($t_{10}c_{12}$) are very active in terms of biology (Pariza et al; 2001).

The most important way to the formation of conjugated linoleic acid bacteria Butyro vibrio fibro solvens depends only on the digestive system (rumen) ruminants such as cattle can be found users (Fritsche et al; 1998). CLA has been shown to have many positive effects on physiology and health protection. Since the anti-cancer effects, anti-atherosclerosis, anti-hollow bones, anti-obesity, anti-diabetes, lower blood pressure, maintain cell membrane and increases the immune function has been emphasized (Collomb et al; 2006; Schmid et al; 2006; Nagao et al; 2005; Bhattacharya et al; 2006). A number of researchers believe that the fatty acids in the diet on hormonal regulation of body weight homeostasis and energy balance, leptin affects. Based on the above CLA can influence the confounding variables involved in ovulation (Rahman et al; 2007; Harris et al; 2001).

Leptin is an important regulator of reproductive function and the effect of increasing the secretion of gonadotropin releasing hormone of hypothalamic neurotransmitter nitric oxide has been demonstrated to mediate (Squires; 2003). Based on the above confounding variables affecting the ability of CLA to the ovulation. However, due to the origin of conjugated linoleic acid, which is produced in the

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rumen ruminants, Few studies have examined the effect of CLA on reproduction in ruminants is discussed, and the specific effects of CLA on the appropriate variables have been studied separately ovulation. We investigated the effects added to the diet, CLA concentrations of the systemic and local hormonal factors influencing the process of ovulation in mice is discussed.

2. Materials and Methods

2.1. Animals and sampling

A total of 80 lab female rats (mice) 2±50 days of age, Balb / C was prepared from Tehran Pasteur Institute. The design of this experiment, rats were randomly divided into four groups (one control group and three treatment groups) so there were 4 repeats in each group (which received the same treatment) and 5 replicates in each experimental unit(mice). Thus, of each group there were 20 rats. Treatments diet using quality raw material in animal sciences houses linked by a technician and was overseen by the project manager. Treatments were applied as follows:

1-Control group, which received 50 kg of corn oil in the diet

2-The first treatment group received 0/1 g/Kg of corn oil (10% Replacing corn oil diet)

3- Treatment group 2 received 0/3 g/Kg of corn oil (30% Replacing corn oil diet)

4- Treatment group 3 received 0/5 g/Kg of corn oil (50% Replacing corn oil diet)

CLA used (Sigma, Germany) and its purity, % 99/6 for c_9 t_{11} and 96/7 t_{10} c_{12} and 12% for the ratio of the two isomers to be 50:50.

measured variables to measure the concentrations of progesterone, estradiol, FSH, LH serum leptin, necrosis factor alpha, (NO) by nitric oxide 120 days after starting the experiment,10 mice from each group (total of 40 mice) were selected estrus symptoms after discontinuation of the infusion, blood samples were taken. Serum was separated after centrifugation. Estradiol and progesterone ELISA (Raidim, Italy) and TNF_{α} and leptin, FSH and LH were measured by ELISA (DRG, Germany) using the mouse. Nitric oxide was measured by Griess reaction was improved (Akahoshi et al; 2003). To measure the concentration of PGF2α, PGE2 and NO in the ovarian tissue, the tissue samples according to Harris and et al were prepared (Yamasaki et al; 2003).

2.2. Statistical analyses

The experimental design was a completely randomized design. Data were analyzed by SAS 96 software package. ANOVA table on each of the parameters included in the tourism and the difference between control and treatment groups were analyzed by Duncan's multiple range test.

3. Results and discussion

3.1. Effects on pituitary and ovarian hormones

The results of the present experiment showed a significant effect of conjugated linoleic acid on FSH of serum. Compared to the control group, the concentration was reduced. The comparisons showed that he mean (Table 1) had the lowest serum levels of FSH occur in the second treatment. Serum LH decreased compared to the control group. This reduction treatments compared to the other groups was significant. Significant effect on serum estradiol was that obtained in the third treatment decreased it. Reply progesterone concentrations compared to CLA decreased (Table 1), but this reduction was not statistically significant.

Table 1: effects on pituitary and ovarian hormones

Variables	control	Treatment1	Treatment2	Treatment3	P Value
LH (mIU/ml)	2.87^{a}	2.91^{a}	2.73^{a}	2.03^{b}	0.01
FSH (mIU/ml)	1.01^{a}	0.96^{ab}	0.91^{b}	0.99^{a}	0.05
E2 (nmol/L)	5.23^{a}	5.72^{a}	6.01^{a}	5.18^{b}	0.01
P4 (nmol/L)	0.09^{a}	0.11^{a}	0.08^{a}	0.09^{a}	0.1
NO (nmol/L)	0.17^{a}	0.23^{ab}	0.18^{ab}	0.19^{b}	0.05
Leptin (ng/ml)	0.91^{a}	0.82^{ab}	0.90^{ab}	0.87^{b}	0.05
TNF_{a}(pg/ml)	0.37^{a}	0.41^{ab}	0.36^{bc}	0.42^{c}	0.05

Common letters in each row indicate no significant difference (P<0.05)

3.2. Effect of paracrine factors

Treated with increasing concentrations of all measured variables which affect the local levels are significantly decreased compared to the control group. Serum nitric oxide, serum leptin and tumor factors in treatments were at their lowest levels (Table 1).

3.3. Effects on measured variables in ovary

Nitric oxide concentration in ovarian tissue in the treated groups has been declined compared to the control group. However, the reduction was not statistically significant (Table2).Both productions Prostaglandins measured relative to the control group decreased. Decrease in prostaglandin production in treatments with other treatment differences was statistically significant.

The process of ovulation depends on the coordinated function of the axis hypothalamus-pituitary-ovary. Some local factors and immunological factors are also extremely important

in this regard. Nutrition is an important factor affecting the hormones and factors. Study on mice showed that the treatment effect of dietary CLA concentration, the concentration is greater measure. The main hormone in the ovulatory process in E_2 and LH were significantly affected by dietary conjugated linoleic acid and decreased serum concentrations.

The LH-lowering effect may be related to the effects of nitric oxide and leptin; why past research shows that leptin and nitric oxide control LH release are important (Squires; 2003; Khodaei et al; 2007). Producing neurons have been shown to secrete nitric oxide (NO) have a direct effect on GnRH release (SAS Institute; 1997).This study showed that serum nitric oxide by conjugated linoleic acid treatment significantly reduced. In this study, serum leptin concentrations were reported by previous studies confirmed that this is (Harris et al; 2001). Reduced production of leptin justifies the possible loss of brain nitric oxide production because it has been proven completely stimulatory effect of leptin on nitric oxide-producing neurons is significant (Squires; 2003).

Probably due to reduced leptin on the main ligand, CLA is. Natural ligand, Peroxisome Proliferators-activated receptors in cells (Chatterjee et al; 1997). Studies show that activation of PPARS, quite significantly reduces leptin gene expression (Yamasaki et al; 2003; Tamanini et al; 2003). Thus, low leptin production might explain the decreased production of nitric oxide in the brain, as it has proved quite stimulatory effect of leptin on nitric oxide-producing neurons is significantly (Squires; 2003). Conjugated linoleic acid significantly reduced the estradiol. Probability is lower due to the reduced NO production by the ovaries. Many of its activity by binding to nitric oxide do metal proteinase enzymes.

However, research shows that certain concentrations of nitric oxide in ovarian steroid al have the ability to inhibit have not been in vest gated in this study. With increasing nitric oxide concentration in ovarian Conjugated linoleic acid concentration was significantly reduced. Conjugated linoleic acid has been reported to inhibit iNOS enzyme (Flint et al; 2002). But recently reported in goat conjugated linoleic acid has an additive effect on NO enzymes (Castro et al; 2007).

Decrease in prostaglandin production by ovarian tissue that was seen in this study may be related to the effects of CLA on PPARS. There is research to search for the possible mechanisms of the effects of conjugated linoleic acid on prostaglandins production are discussed. Preliminary findings indicated that some of the omega-3 long-chain fatty acid supplementation during pregnancy in humans and animals increases (Castañeda et al; 2007).

Since the beginning of the birth of increasing concentrations of prostaglandins act as a stimulant, increasing the duration of pregnancy may be related to the inhibitory effect of conjugated linoleic acid on prostaglandins produced. A recent study confirmed the inhibitory effect of conjugated linoleic acid on prostaglandins and is associated with activation of PPAR know (Tamanini et al; 2003). Studies on the effect of conjugated linoleic acid on the enzyme nitric oxide products are in consistent.

There is research to search for the possible mechanisms of the effects of conjugated linoleic acid on production prostaglandins are paid. In the present experiment, ovarian prostaglandin many variables affecting the process of ovulation are significantly reduced as well. TNF_{α} may be reduced, leading to a decrease in prostaglandin production. It seems that different mechanisms of CLA reduce ovarian prostaglandin production.

Table 2: effects on variables measured in ovary

Variables	Control	Treatment1	Treatment2	Treatment3	P Value
NO (nmol/L)	0.11^{a}	0.11^{a}	0.12^{a}	0.13^{a}	0.09
PGE2 (pg/mg)	9.92^{a}	8.63^{ab}	7.91^{ab}	7.77^{b}	0.01
PGF2α (pg/mg)	5.32^{a}	5.11^{ab}	5.88^{bc}	6.66^{c}	0.01

Common letters in each row indicate no significant difference (P<0.05)

4. Conclusions

the results of this study showed that not only conjugated linoleic acid had no stimulatory effect on ovulation rate in mice did not produce, but it also reduces the effective concentration of the variables (Fuentes et al; 2008, Castañeda et al; 2009). Increases in rat's variables affecting ovulation were observed (difference Non-ruminant and ruminant mammal with a breast).

Acknowledgements

Research group considers itself to Laboratory of Dr. Jalayer (Isfahan) Appreciating offers.

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