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A Role of Unsaturated Fatty Acid in Animal Reproductive Cells and Biology

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ABSTRACT

As a one of unsaturated fatty acid, polyunsaturated fatty acids (PUFAs) have multiple actions: as precursor of prostaglandins (PGs), steroid hormone synthesis and energy production in animal reproduction. PUFAs, which include omega-3 (n-3) and omega-6 (n-6), are derived from the diet and changed by diet, species, breed and season. The plasma membrane of spermatozoa in mammals contain various PUFAs. These composition of PUFAs regulate the membrane fluidity and cause lipid peroxidation via generation of reactive oxygen species (ROS). Induced lipid peroxidation by ROS decreased viability and motility of spermatozoa, and it is reduced by addition of antioxidant and low concentration of PUFAs. Because oocytes of animal have a high lipid components, process of oocyte maturation and embryo development are influenced by PUFAs. In *in vitro* study, oocyte maturation, embryo development, intracellular cAMP and MAPK activity were increased by treatment of n-3 α-linolenic acid (ALA) during maturation, whereas n-6 linoleic acid (LA) negatively influenced. Also, inhibition of fatty acid metabolism in oocyte influenced blastocyst formation of cattle. PGs are synthesized from PUFAs and various PUFAs influence PGs via regulation of PG-endoperoxide synthase (PTGS). Steroid hormone synthesis from cholesterol is regulated by expression of steroid acute regulator (StAR) protein and mRNA. Exogenous n-3 and n-6 PUFAs altered sex hormone in animal through stimulate or inhibit StAR activity. Because PUFAs altered PG and steroid hormone synthesis, follicular development was influenced by PUFAs. This effect of unsaturated fatty acid could provide information for improvement of reproductive ability in animals.

(Key words : Unsaturated fatty acid, Reproductive biology, Sperm, Oocyte, Animals)

INTRODUCTION

A fatty acids, which are important components of cells, are a carboxylic acid with a long aliphatic chain that consist of carbon atoms. Generally, it are derived from triglycerides or phospholipids, and synthesized from acetyl- and malonyl-CoA by the action of fatty acid synthesis (FAS) in cytoplasm of the cells. And energy production by β-oxidation and the citric acid cycle is a major role of fatty acids in animal metabolism. These fatty acids are classified into three types: free fatty acid, unsaturated fatty acid and saturated fatty acid.

Unsaturated fatty acids have a one or more double and triple bond between carbon atoms and it can be saturated by adding hydrogen atoms, converting the multiple bonds to single bonds. Because of this multiple bonds between carbon atoms, unsaturated fatty acids have a cis- and trans- isomer form. Unsaturated fatty acid chain is monounsaturated fatty acids (MUFAs) if it contains on double bond, and polyunsaturated fatty acids (PUFAs) if it contains more than one multiple bond. PUFAs are classified into three groups by location of first double bond from the methyl end of the molecule: omega-3 (n-3), omega-6 (n-6) and omega-9 (n-9). Alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are included n-3 PUFA and n-6 PUFA include linoleic acid (LA), gamma-linoleic acid (GLA), and arachidonic acid (AA). Especially, LA and ALA are not synthesized in animals and need to be provided from the diet (Wa-

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thes *et al*., 2007).

An important role of unsaturated fatty acids is the components of phospholipids in cell membrane. Different type of cells, membranes, and phospholipids have different composition of unsaturated fatty acid and these fatty acid composition may be influenced by metabolism, state of cell activation, hormone, and genetic factors (Calder, 2015). The physical features of cell membrane that include fluidity and permeability are influenced by unsaturated fatty acids composition of cell membrane. Furthermore, the unsaturated fatty acids as a precursors are related to prostaglandins (PGs) synthesis and steroidogenesis (Needleman *et al*., 1986; Stocco *et al*., 2005). Because of various roles of unsaturated fatty acids, general and specific reproductive events are regulated in reproductive cell and tissue. Therefore, this review will focus on effects of unsaturated fatty acids on male and female reproduction in animals.

UNSATURATED FATTY ACIDS AND SPERMATOZOA

The spermatozoa of various mammalians including man, ram, bull and boar have a long chain PUFAs (Poulos *et al*., 1986; Alvarez and Storey, 1995). The PUFA components (n-3 and n-6 PUFAs) in spermatozoa are change by PUFA sources in the diet, species and breeds (Maldjian *et al*., 2005; Waterhouse *et al*., 2006). Unsaturated fatty acids in sperm plasma membrane regulate the fluidity that need to participate in the membrane fusion between oocyte and spermatozoa. However, these PUFA molecules are susceptible to attack by reactive oxygen species (ROS) which can initiate a lipid peroxidation cascade (Alvarez and Storey, 1995). Because of lipid peroxidation by ROS, the structural and functional integrity of cells are damaged. Such oxidative damages will be expected to disrupt the fusogen in membrane of sperm and membrane-bound enzymes including ATPase. In addition, the activation of signal transduction pathways that important in fertilization could be delayed by changes in membrane fluidity. The levels of lipid peroxidation derived from ROS in sperms correlate negatively with semen quality in human and livestock animals (Kasimanickam *et al*., 2006).

Because internal antioxidant enzymes in spermatozoa are depleted during cytoplasmic extrusion, extracellular antioxidants in seminal plasma are important for reduction of oxidative damage in mammalian spermatozoa. The spermatozoa is exposed to antioxidant enzymes, such as members of glutathione peroxidase (GPx) and superoxide dismutase (SOD), and small molecular mass free radical scavengers, which include carnitine, typrosine and vitamin C, secreted by male reproductive tract during their passage (Dacheux *et al*., 2006; Drevet, 2006). Since seminal plasma provide the powerful antioxidant environment into spermatozoa, deficiencies of this protective milieu are associated with oxidative stress and male infertility (Sanocka *et al*., 1997). During the liquid preservation of boar semen, supplementation of SOD in extender improved semen quality, and decreased lipid peroxidation and intracellular levels of hydrogen peroxide (Zhang *et al*., 2016). And supplementation of cysteine and glutathione in extender during the cryopreservation of bull semen decreased DNA damage (Tuncer *et al*., 2010). Spermatozoa in golden hamster, which was surgically ablated the male accessory glands, increased oxidative stress, levels of DNA damages and rates of embryonic loss in mated female (Wai-Sum *et al*., 2006).

A relationship between high level of unsaturated fatty acid and ROS had been shown by recent study. Ollero *et al*. (2000) reported that spermatozoa in subfertile man contains high amounts of unsaturated fatty acids, especially DHA and AA. And generation of free radical, DNA damage, and lipid peroxidation in human spermatozoa were occurred by exposure of LA, AA and DHA (Aitken *et al*., 2006). Immature spermatozoa in defective human that have abnormal retention of cytoplasm and high levels of unsaturated fatty acid generated the high levels of ROS (Ollero *et al*., 2001). The excess residual cytoplasm caused not only increased contents of unsaturated fatty acid but also corresponded to abundance of cytoplasmic enzymes. These enzymes can stimulate the NADPH generation in the cytoplasm of spermatozoa, and ROS are produced through NADPH oxidase (Gomez *et al*., 1996). The induced lipid peroxidation by ROS generation should activate phospholipase A2 (PLA2), facilitating the release of unsaturated fatty acids from the phospholipid in sperm plasma membrane. Thus, excessive unsaturated fatty acids in spermatozoa could cause structural and functional damages. Despite the negative effects of high concentrations of PUFAs on mammalian spermatozoa, experiments related to supplementation of various unsaturated fatty acid in livestock animal have been conducted for improvement of fertility. Addition of LA, AA, and oleic acid during incubation improved motility, viability and acrosome reaction in boar sperm (Hossain *et al*., 2007). Maldjian *et al*. (2005) reported that supplements of 3% fish oil to the daily boar feed increased the DHA in the spermatozoa and the number of sperm in ejaculate, however, freezing ability was not altered. In another study, addition of low concentration of ALA during cryopreservation improved post-thawed bull sperm characteristics including motility, membrane integrity, and viability (Kaka *et al*., 2015)

UNSATURATED FATTY ACID AND OOCYTE AND EMBRYO DEVELOPMENT

Follicular fluid plays a regulatory role in oocyte maturation and development in ovarian follicle. PUFAs are accumulated in follicular fluid through the concentration gradient of serum levels (Fouladi-Nashta *et al*., 2009). The composition of PUFAs in follicular fluid are altered by dietary and oocytes absorb PUFAs from the follicular fluid. Thus, composition of fatty acid in oocyte was changed by various factors including species (McEvoy *et al*., 2000), quality of oocyte (Kim *et al*., 2001) and season (Zeron *et al*., 2001). The abundant amount of lipid in oocyte cytoplasm were higher in livestock animals than human and mouse (Sutton-McDowall *et al*., 2012). In pre-maturation development, the oocyte tends to accumulate the lipid (Sturmey *et al*., 2009) and accumulation of lipids was occured at several growth period during the progression to mature oocyte in metaphase Ⅱ. It was considered that the final stages of *in vitro* maturation are sensitive to modification of cytoplasmic lipid content (McKeegan and Sturmey, 2011). In the oocyte, resumption time of nuclear maturation and cumulus cell expansion can be influenced by change of fatty acid composition and it is important for oocyte development after fertilization (Marei *et al*., 2010). Porcine embryos contain a lot of lipid as intracellular lipid droplets form compared with other mammals (Nagashima *et al*., 1994). The lipid contents of porcine oocyte are mostly constituted form of triacylglycerol (Homa and Brown, 1992). It is inducer of glycerol having an acyl group to the three oxygen derived from a fatty acid and play an important role to intracellular energy store. Oleic acid, which is one of the unsaturated fatty acid, was founded in porcine oocytes (McKeegan and Sturmey, 2011). As a main fatty acid in bovine follicular fluid, LA activate protein kinase C that plays an important role in cell growth and differentiation (Murakami *et al*., 1986; Marei *et al*., 2010). Supplementation of dietary PUFA can influence reproductive potential in cattle (Mattos *et al*., 2000; Robinson *et al*., 2002; Bilby *et al*., 2006; Wathes *et al*., 2007; Santos *et al*., 2008). And Fouladi-Nashta *et al*. (2007) have shown that blastocyst quality and development of oocyte to the blastocyst stage were improved by high level of dietary rumen protectant fatty acid mixture. Also lipid content and fatty acid composition in bovine oocyte influenced oocyte maturation and development competence (Kim *et al*., 2001; Castaneda *et al*., 2013). In many *in vitro* studies, supplementation of n-3 ALA during maturation improved oocytes maturation rate and subsequent embryo development in cow (Marei *et al*., 2009), whereas n-6 LA was detrimental (Marei *et al*., 2010). Similarly, Veshkini *et al*. (2012) reported that maturation, cleavage rates and blastocyst formation of goat embryo were increased by ALA treatment.

Energy metabolism is important for maturation of oocyte because dynamic processes require high level of energy from various substrates that include amino acids, carbohydrates, and lipids (Songsasen, 2012; Collado-Fernandez *et al*., 2012). Triglycerides that the major component of lipid in oocyte act as an important preservatory of energy (Algriany *et al*., 2007; Sturmey *et al*., 2009). In the bovine oocytes, inhibition of fatty acid metabolism and β-oxidation during *in vitro* maturation reduced blastocyst development (Ferguson and Leese, 2006). Also, treatment of exogenous LA inhibited germinal vesicle breakdown and influenced mitochondrial activity and ROS generation in oocyte (Homa and Brown, 1992; Marei *et al*., 2012). In another study, intracellular cAMP concentration and phosphorylation of MAPK 1 and 3 were increased by ALA supplementation to oocyte maturation medium (Marei *et al*., 2009).

UNSATURATED FATTY ACIDS AND REPRODUCTIVE EVENTS

Prostaglandin Synthesis

For various physiological activities, the endometrium secreted PGs that were synthesized from n-3 and n-6 PUFAs (Wathes *et al*., 2007). As a precursor and substrates, PUFAs influence PG production in animal reproductive tract. Free PUFA, such as AA, ALA and dihomo-γ-linoleic acid (DGLA), were released from membrane phospholipids by PLA2, and metabolism of released PUFAs was induced by two types of PG-endoperoxide synthase (PTGS1 and 2). Produced PGH_2 in endometrium, which is synthesized from n-6 fatty acids, converted to PGE_2 and $PGF_{2\alpha}$ by each Prostaglandin E synthase (PGES) and Prostaglandin F synthase (PGFS) respectively (Arosh *et al*., 2002). In the biosynthetic pathway of PGs, ALA change to 3-series PGs through EPA and DHA, LA change to 1-series PGs through GLA and DGLA, and continuously change to 2-series PGs through DGLA and AA. Among the PGs that synthesized from n-3 and n-6, PGE and PGF series are closely associated with uterine physiology (Cheng *et al*., 2005). In variety of species including pigs, PGs synthesis is suppress before embryo implantation and reduced PG synthesis lead to unsuccessful pregnancy (Herath *et al*., 2009). Like these two factors have each purpose as different as mainly secreted sites, PGE_2 and $PGF_{2\alpha}$ acted on the uterus as the luteotrophic and luteolytic factor, respectively (Herath *et al*., 2009). And it

has been reported that at the bovine estrus cycle, production of endometrial PGE_2 was higher in mid and late luteal phase (Arosh *et al*., 2003). In addition, PGE_2 and $PGF_{2\alpha}$ secreted from corpus luteum (CL) were involved in CL function. These function of PGs are important for various reproductive phenomenon such as uterine activity, follicle development, luteal development and luteolysis (Wathes *et al*., 2013). PUFAs also act as inhibitor of PTGS for regulation of PG production. Coyne *et al*. (2008) reported that PTGES mRNA expression in endometrium was increased in beef cattle fed fish oil. In the cattle, supplementation of LA by diet decreased production of 2-series PGs (Cheng *et al*., 2001). Similarly, 2-series PGs production in ovine endometrial cells was decreased by LA treatment, in contrast, supplementation with GLA and AA increased PG synthesis (Cheng *et al*., 2004). Especially, production of PGE_2 in ovine endometrial cells was higher than $PGF_{2\alpha}$ production by treatment of n-6 PUFAs (Cheng *et al*., 2004).

Steroid Synthesis

PUFAs are related to regulation of steroid hormone synthesis through both direct and indirect mechanism. Steroids including progesterone, estradiol, and testosterone are synthesized from cholesterol and PUFAs could influence to cholesterol metabolism via regulation of transcription factors (Abayasekara *et al*., 2009). Production of steroid, as a sex steroid hormone, is also influenced by PGs. PGI_2 that derived from AA stimulate the progesterone synthesis at the early stage of CL (Hansel*et al*., 1987), in contrast, $PGF_{2\alpha}$ suppress the progesterone by luteolysis at later stage (Poyser, 1995). Steroid acute regulator protein (StAR) and cytochrome P_{450} directly effect to steroidogenic mechanism. Both of PUFAs and its metabolites stimulate or inhibit StAR expression and activity associated with increase/decrease of steroid synthesis (Stocco *et al*., 2005). For example, addition of exogenous AA enhanced dibutyryl cAMP-induced steroid synthesis as well as StAR expression and activity (Wang *et al*., 2000). Inhibition of PTGS2, also known as cyclooxygenase-2 (COX-2), was also related with steroid synthesis and StAR expression (Wang *et al*., 2003). With regard to reproductive physiology, estrogen concentration and estradiol signaling pathways were altered by fish oil supplemented diet (Witt *et al*., 2010; Cao *et al*., 2012). The ability to synthesize the EPA and DHA that are converted from ALA is greater in female mammals than males (Childs *et al*., 2008). During estrous cycle and pregnancy, the female reproductive tract is exposed to estradiol and progesterone. Estrogen increases the conversion of ALA and LA to PUFAs by up-regulation of fatty acid desaturases (Burdge, 2004), in contrast, progesterone inhibits eicosanoid synthesis in uterus (Lewis, 2003; Patel *et al*., 2003).

Follicle Development

As a one of important reproductive phenomenon, periodic ovarian follicle development and ovulation are crucial for estrous cycle and successful pregnancy. A variety of hormone including pituitary and sex steroid hormones are associated with change of ovarian physiology. As a precursor of steroid hormones and PGs, unsaturated fatty acids would affect to changes of ovarian environment, and a number of researchers had reported that both of n-3 and n-6 PUFAs could influence to development of ovarian follicle and ovulation. In the sheep, high levels of n-3 PUFAs by dietary increased concentration of progesterone in the follicular fluid (Wonnacott *et al*., 2010) and it was related an increase of StAR expression (Hughes *et al*., 2011). And supplementation of ALA and n-6 PUFAs increased the steroid secretion by granulosa cells in cattle (Wehrman *et al*., 1991; Robinson *et al*., 2002). Similar to sheep and cattle, women that consumed more ALA by their diet had a higher concentrations of estradiol (Hammiche *et al*., 2011). Moreover, ovarian PG synthesis that could alter the follicular capacity for ovulation is changed by PUFAs. The level of PGE in follicular fluid of large follicles was reduced in dairy cattle that fed the high n-3 PUFUs diet (Zachut *et al*., 2011). Similarly, feeding of fish oil, which contain n-3 PUFAs, to mice decreased both of PGE and PGF by reduction of PTGS2 in ovary (Yi *et al*., 2012). And Broughton *et al*. (2006a, b) reported that production of PGE and PGF in rat ovaries increased by DHA and EPA, whereas ALA increased PGF but reduced PGE (Broughton *et al*., 2010).

CONCLUSION

Unsaturated fatty acids have multiple actions in body and it could influence to fertility as a precursor and energy resource. Many researchers have been reported that role of PUFAs from dietary and *in vitro* treatment affected to male and female reproductive biology including characteristics of sperm, oocyte physiology, PG and steroid synthesis. Also, energy production by fatty acid metabolism is one of important factors in animal reproductive system. Proper concentration of unsaturated fatty acids could improve the function of reproductive cells and biological phenomenon, however, excessive fatty acids caused detrimental effect such as decrease of sperm motility and viability, oocyte maturation and development through lipid peroxidation and

ROS generation. Despite the harmful effect of unsaturated fatty acid, many study for improvement of animal reproduction were conducted. Therefore, this review would contribute to study of animal reproduction *in vivo* and *in vitro*.

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