

**Research Article****Association between Lipid Metabolites and Follicular Cyst Formation in Cattle**Hirotada Tsujii*, AG Miah¹, U Salma¹, Nobuhiko Hidaka and Hideaki Yaemori²

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ABSTRACT

The study was to investigate whether there was any association between follicular cysts and lipid metabolites. A total of 10 spontaneously occurring cystic cows were used as a treatment group and 10 normal estrous cyclic cows were used as a control group. The serum progesterone concentration in the cystic cows was below 1.0 ng/ml. Fatty acids in serum and cystic fluid or follicular fluid of cystic cows or normal estrous cyclic cows were determined by gas chromatography. The concentrations of glucose, cholesterol and triglycerides were determined by enzymatic colorimetric method and progesterone level by fluoroimmunoassay. The fatty acids, such as palmitic acid, stearic acid, oleic acid, linoleic acid and α -linoleic acid concentrations were significantly higher ($P<0.05$) in cystic fluid than the follicular fluid of the normal estrous cyclic cows. Among the three types of follicles, the concentrations of all fatty acids were highest in the small follicles than the medium or large follicles. Stearic acid and linoleic acid concentrations were higher ($P<0.05$) in serum of cystic cows compared to the serum of normal estrous cyclic cows. The overall concentration of fatty acids was higher ($P<0.05$) in the cystic fluid than the concentration in the serum of normal cyclic cows. The concentration of glucose was lowest ($P<0.05$) in follicular fluid and serum of the cystic cows than that of the normal estrous cyclic cows. Conversely, the cholesterol concentration was higher ($P<0.05$) in follicular fluid and serum of the cystic cows, while triglycerides was lowest ($P<0.05$) in serum of normal estrous cyclic cows. Therefore, it might be concluded that impaired lipid metabolism and its higher concentration resulted the follicular cyst formation in dairy cattle.

Key words: Cholesterol, Dairy cattle, Lipid metabolites, Follicular cysts

INTRODUCTION

Follicular fluid is a liquid fills the follicular antrum and surrounds the ovum in an ovarian follicle. It provides a very important microenvironment for the development of oocytes. Follicular fluid is a product of both the transfer of blood plasma constituents that cross the blood follicular barrier and of the secretory activity of granulosa and thecal cells (Fortune, 1994). It is reasonable to think that some biochemical characteristics of the follicular fluid surrounding the oocyte may play a critical role in determining oocyte quality and the subsequent potential to achieve fertilization and embryo development. The analysis of the follicular fluid components may also provide information on metabolic changes in blood serum, as the circulating biochemical milieu may be reflected in the composition of follicular fluid (Leroy *et al.*, 2004). Follicular cysts have been defined as thin-walled

structures of at least a 25-mm diameter, filled with follicular fluid that persist for ten days in the absence of a corpus luteum and fail to ovulate due to the interruption of normal estrous cycles (Kesler *et al.*, 1980). Follicular cysts are becoming a new economic problem for dairy producers since cows are infertile as long as the condition persists. The metabolic activity, together with the "barrier" properties of the follicular fluid is changing significantly during the growth phase of the follicle (Gosden *et al.*, 1988).

Though there is no clear consensus on the development of follicular cysts, it is believed to be associated with many factors, including periparturient stress, aging, nutritional inadequacies, and genetic predisposition (Day, 1991). Elevated non-esterified fatty acid levels of follicular fluid are associated with a negative energy balance (NEB). Jorritsma *et al.* (2000) suggested that differences in a NEB or the accumulation

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of triacylglycerol in the liver of postpartum dairy cows affect fertility performance. The increased lipid content of the follicular fluid is associated with oxidative stress, suboptimal mitochondrial function and reduced developmentally important gene transcripts of oocytes (Abe *et al.*, 1999; 2002). The association of follicular cysts with abnormal hormone metabolism was also previously assumed. In cows with cystic follicles, progesterone, luteinizing hormone and estradiol-17 β concentration in plasma remain at high levels even around the expected time of ovulation (Hatler *et al.*, 2003). However, which factors exert follicular cysts and have a predisposing effect on the development of the follicular cysts is still remain in dark. Before focusing on possible effects of metabolic changes on follicle and oocyte quality it is necessary to determine physiological concentrations of metabolites in the follicular fluid, cystic fluid and serum. Thus, the study was designed to investigate whether there are any associations between follicular cyst development and lipid metabolites in dairy cattle.

MATERIALS AND METHODS

Animals

A total of 20 Holstein cows (approximately 6-years-old) were selected based on the conditions of genitalia and ovaries examined by rectal palpation. Spontaneously occurring cysts were diagnosed in 10 cows were considered as treatment group. Another 10 cows exhibited normal estrous cycles were used as control group. All experimental procedures were performed in accordance with the regulations approved by the Institutional Animal Care and Use Committee of the Shinshu University, Nagano, Japan.

Detection of cysts

Gynaecological examination of cows was conducted in the herd in 14-day intervals since 60 \pm 7 days after parturition. Diagnostics of follicular cysts was conducted using transrectal ultrasonographic examination (DP-3300 Vet Digital Ultrasonic Imaging System, Midray Medical Corp. USA) with 5 MHz linear probe present on one or both ovaries. As a criterion for cyst identification was the size of follicle (\geq 2.5 cm) and the wall thickness of the follicle. An inactive ovary was diagnosed in cows that were not observed in estrus for two months postpartum and had small ovaries, which were either flat or smooth or rounded on rectal palpation, in the absence a corpus luteum.

Progesterone assay

Progesterone level in plasma samples was assayed in a solid phase fluoroimmunoassay based on the competition between europium-labeled steroids and sample steroids for polyclonal antisteroid antibodies derived from a rabbit. The DELFIA (Dissociation-enhanced lanthanide fluoroimmunoassay) progesterone reagent (Wallace Inc., Gaithersburg, MD) was used and the manufacturer's instructions were followed.

Ultrasonography, blood collection and sampling

The follicular cysts of each cow were aspirated by transvaginal ultrasound guidance of the aspiration. For

ultrasound guidance of the aspiration needle, an ultra scanner (SSD-500; ALOKA Co. Ltd., Tokyo, Japan) was used that was equipped with a 7.5 MHz transvaginal convex transducer (UST M15-21079; ALOKA Co. Ltd.) with an attached stainless steel needle guide. Before the follicle aspiration, the cows received a caudal epidural anesthesia, 50 ml of 2% liocaine (Xylocaine; AstraZeneca Co., Osaka, Japan), to prevent straining, and then their vulva and perineal areas were cleaned. The transvaginal convex transducer was inserted into the vagina, and the ovary containing the follicular cyst was positioned next to the transducer face by rectal manipulation so that the targeted follicle was displayed on the needle path of the monitor. An 18-gauge-single lumen stainless steel needle connected to a 10 ml disposable syringe was pushed into the needle guide and inserted into the antrum of the follicle through the vaginal wall. The follicular fluid was collected into a 15 ml plastic tube, brought to the laboratory in ice water, and centrifuged to remove follicular debris. The follicular fluid samples were kept at -40°C until the analysis of fatty acid, cholesterol and triglycerides concentrations. Blood samples were collected by caudal venipuncture just prior to each ultrasound scan, and plasma was obtained by centrifugation and then stored.

Determination of fatty acids

Lipids in 0.2 ml of serum were extracted with Folch's solution under a nitrogen atmosphere. After methyl esterification by 0.4 M potassium methoxide and 14 weight percentage boron trifluoride methanol, total fatty acids were measured using a gas chromatograph (Shimadzu, GC14B, Kyoto, Japan) equipped with an Omegawax 250 capillary column (30 m \times 0.25 mm i.d.; 0.25 μ m thickness; Supelco, Bellefonte, PA USA). Peaks were determined using a flame-ionization detector and were quantified with an electric integrator (Shimadzu, CR-7A, Kyoto, Japan) using pure standard mixtures (Sigma, St. Louis, Mo, USA). The weight percentage of each fatty acid was adopted in all detected fatty acids as a measurement value.

Enzymatic analysis

Total cholesterol, triglycerides and glucose concentrations in the follicular fluid, cystic fluid and serum samples were determined as enzymatic methods using commercially available reagent kits (Wako T-cho-M, Wako TG-M and Wako Glu-CII; Wako Pure Chemical Industries Ltd., Tokyo, Japan). Cholesterol and triglycerides concentrations in the follicular fluid, cystic fluid and serum samples were determined by spectrophotometer (APEL Co., Saitama, Japan) at 600 nm, and glucose concentration was determined at 505 nm.

Statistical analysis

Experimental data were assessed by one-way analysis of variance (ANOVA) followed by Fisher's least significant difference tests (LSD) using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS Institute Inc., Cary, NC, USA). The data were expressed as the mean \pm SEM. Differences were considered significant at $P < 0.05$ and $P < 0.01$.

RESULTS

The fatty acids concentration of the follicular fluid or cystic fluid and serum in normal estrous cyclic cows and cystic cows are shown in Figure 1. The concentration of palmitic acid was higher ($P<0.01$) in cystic fluid than the follicular fluid collected from any size of follicles of normal estrous cyclic cows. Accordingly, the concentration of palmitic acid was also higher ($P<0.05$) in serum of cystic cows compared to the normal estrous cyclic cows. Among the follicular fluids of three sizes follicles, the highest concentration of palmitic acid was observed in the small size follicle compared to the medium and large size follicles. The accumulation of palmitic acid was higher ($P<0.01$) in the cystic fluid than serum of the cystic cows (Fig. 1 A).

The concentration of stearic acid was higher ($P<0.01$) in the cystic fluid and serum of cystic cows than the follicular fluids and serum of the normal estrous cyclic cows, and highest amount was observed in the follicular fluid collected from small follicles. However, there was no significant difference between the accumulation rate of

stearic acid in cystic fluid and serum of cystic cows, though it was higher in follicular fluid than serum of normal estrous cyclic cows (Fig. 1 B).

Oleic acid concentration was higher ($P<0.05$) in the cystic fluid than the follicular fluids of the normal estrous cyclic cows, though there was no significant difference between serum of normal estrous cyclic cows and cystic cows. There were significant differences ($P<0.05$) of oleic acid concentrations among the different size of follicles, where the highest concentration was observed in small size follicles and 2nd highest in large size follicles. The concentration of oleic acid was higher in cystic fluid or follicular fluid than that of the serum of either cystic cows or normal estrous cyclic cows (Fig. 1 C).

The concentration of linoleic acid in cystic fluid was higher compared to follicular fluids collected from any size of follicles of normal estrous cyclic cows. Its concentration in serum was also higher ($P<0.05$) in cystic cows than that of the normal estrous cyclic cows. However, the higher concentration of linoleic acid was observed in serum than in follicular fluid or cystic fluid of either normal estrous cyclic cows or cystic cows (Fig. 1 D).

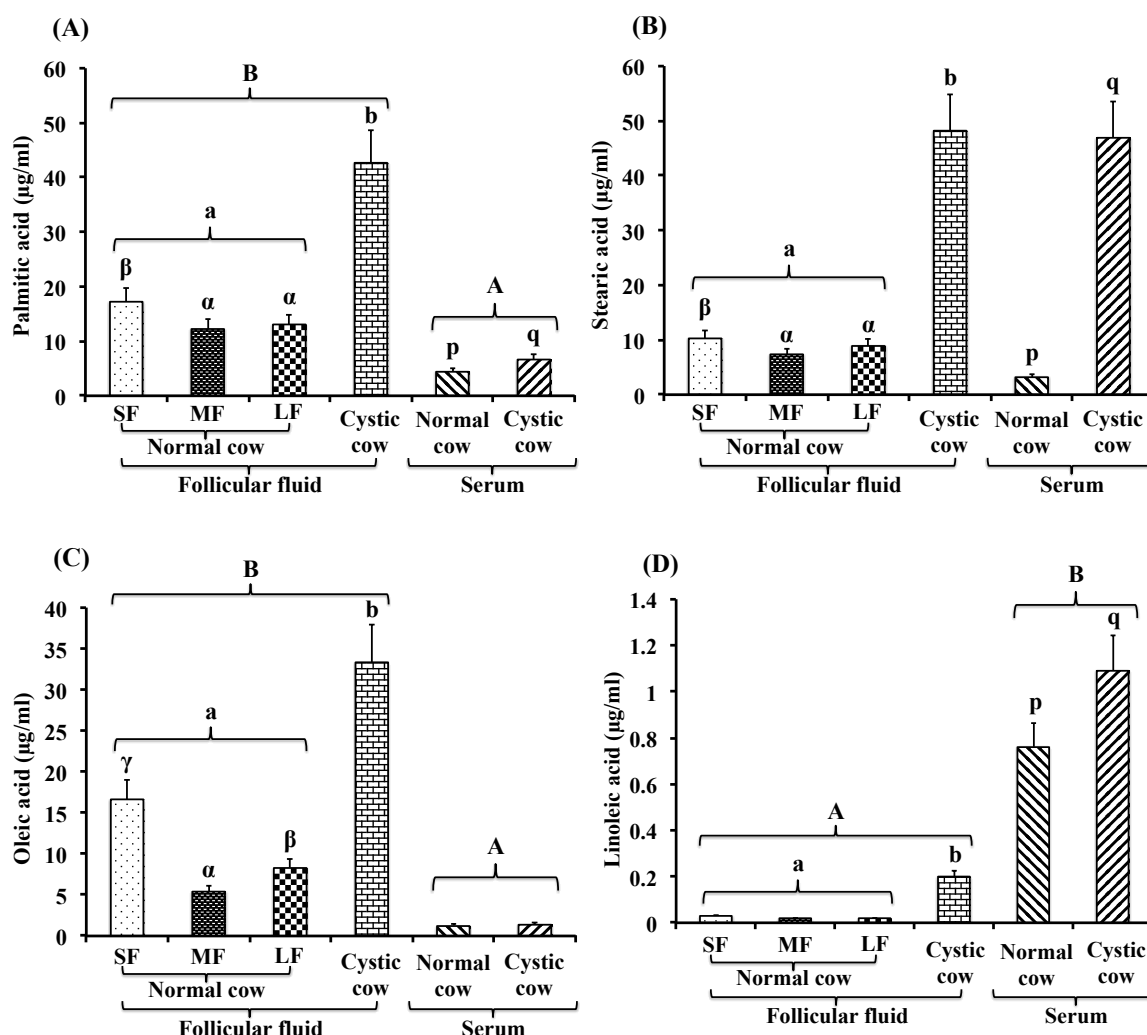


Fig. 1: The fatty acids concentration of the follicular fluid or cystic fluid and serum in normal estrous cyclic cows and cystic cows: (A) Palmitic acid, (B) Stearic acid, (C) Oleic acid and (D) Linoleic acid. Each bar with error bar represents the mean±SEM values; different letters above the error bars of the same factor indicate significant differences ($P<0.05$) or ($P<0.01$). Where, SF = small follicle; MF = Medium follicle; LF = large follicle; Cystic cow = cystic fluid or serum of cystic cows; and Normal cow = follicular fluid or serum of normal estrous cyclic cows.

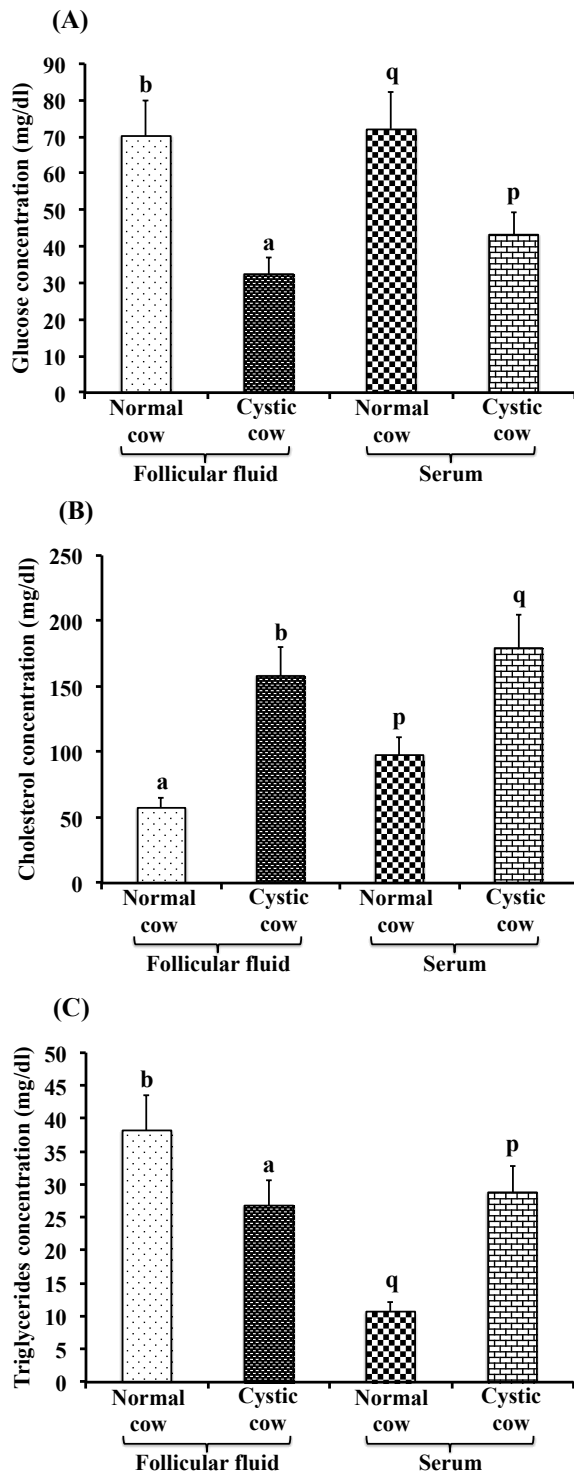


Fig. 2: The concentration glucose and lipid metabolites in the follicular fluid or cystic fluid and serum in normal estrous cyclic cows and cystic cows: (A) Glucose, (B) Cholesterol, and (C) Triglycerides. Each bar with error bar represents the mean \pm SEM values; different letters above the error bars of the same factor indicate significant differences ($P < 0.05$). Where, SF = small follicle; MF = Medium follicle; LF = large follicle; Cystic cow = cystic fluid or serum of cystic cows; and Normal cow = follicular fluid or serum of normal estrous cyclic cows.

The concentration of lipid metabolites in serum and follicular fluid or cystic fluid of normal estrous cyclic and cystic cows are shown in Figure 2. The concentration of glucose was significantly lower ($P < 0.05$) in cystic fluid compared to the follicular fluid of normal estrous cyclic

cows. Similarly, the glucose concentration in the serum of cystic cows was lower ($P < 0.05$) than the normal estrous cyclic cows (Fig. 2 A).

The concentration of cholesterol in cystic fluid as well as in serum were higher ($P < 0.05$) in cystic cows compared to the normal estrous cyclic cows (Fig. 2 B). Whereas, the concentration of triglycerides in follicular fluid was lower ($P < 0.05$) in cystic fluid than the follicular fluid of normal estrous cyclic cows, but higher ($P < 0.05$) in serum of cystic cows than that of the serum of normal cows (Fig. 2 C).

DISCUSSION

In the present study it was indicated that the high concentration of fatty acids and lipids in the cystic fluid and serum of cystic cows may be a partial explanation for the infertility in the modern high yielding dairy cows. Ketone bodies, free fatty acids and glucose have long been used in dairy cattle for energy status changes in the body condition score. In this study, the concentration of all the fatty acids in the cystic fluid was markedly increased in the cystic cows. Similarly, the fatty acids (especially, palmitic acid and stearic acid) concentration in serum of the cystic cows was higher than the normal estrous cyclic cows. It was reported that these saturated fatty acids were negatively correlated with the oocyte maturation, fertilization, cleavage rate and blastocyst formation. Thus, the result is in agreement with the present study. It is probable that non-esterified fatty acids (NEFA) also have an effect on fertility (Canfield and Butler, 1990). There is some evidence for NEFA uptake by the ovary as well as a strong correlation between the concentration of NEFA in plasma and the follicular fluid, which could explain possible harmful effects of NEFA on either granulosa cells or the oocyte (Rabiee *et al.*, 1997; Comin *et al.*, 2002; Jorritsma *et al.*, 2003). The NEFA uptake by the ovary is too low to be measured *in vivo* to explain an intra-ovarian effect (Rabiee *et al.*, 1997), but the important function of arachidonic acid during follicular development and ovulation suggests that uptake occurs (Boone *et al.*, 1993; Espey, 1994). Several studies confirmed the importance of fatty acids for embryo development, but the present study indicates that their impaired metabolism and high concentration might lead to the follicular cyst formation. Fatty acids can be regarded as relatively toxic, given the results from studies in sheep (Herdt, 1994). This study showed that an acute increase in serum fatty acid concentration might induce triglycerides accumulation in the liver by elevating cytosolic and microsomal phosphatidate phosphorhydrolase concentrations, which indicates the toxic potential of fatty acids. The study might suggest that the vulnerable oocytes should be protected from too high of an accumulation of fatty acids during the NEB in high-yielding dairy cows.

Glucose plays an important role in ovarian metabolism since it is the major energy source for the bovine, mouse and human ovary, which is also metabolized by the ovary through the anaerobic pathways, leading to lactate formation (Rabiee *et al.*, 1997; Leese and Lentua, 1999; Boland *et al.*, 1994). The present study observed that there was a significant

reduction of glucose concentration in the cystic fluid and in the serum of cystic cows was low. Leroy *et al.* (2004) found that the glucose concentration in follicular fluid was closely correlated with the serum levels and that it was consistently higher than in serum possibly due to an active inward transport. This finding strongly suggests that post partum changes in glycaemia are well reflected in the follicular fluid of dominant follicles but that the oocyte is more or less protected from low glucose concentrations. The present study suggests that the reduced concentration of glucose in the cystic fluid and serum cause both growth and functional impairment of oocytes, total cholesterol and triglycerides concentrations marked increased in the cystic fluid and the serum of the cystic cows. The accumulation of triglycerides or the existence of a severe NEB is hypothesized to have a negative impact on fertility. Postpartum accumulation of triglycerides in the liver is a possible consequence of a postpartum NEB. The presence of NEB, which is to a certain degree assumed to be physiological, evokes a mobilization of NEFA and an accumulation of triglycerides in the liver. Since every dairy cow in early lactation encounters NEB, and a fatty liver (high triglycerides concentrations in liver tissue) is not observed in every cow, NEB does not result in high liver triglycerides in all cows. A NEB is associated with changes in metabolites and metabolic hormones as recently reviewed by Van Knegsel *et al.* (2005). The mobilisation of body fat causes increased blood levels of NEFA and ketone bodies like acetoacetate, acetone and β -hydroxybutyrate. This is usually associated with low levels of blood glucose. At the same time blood levels of growth hormone are increased and that of insulin and IGF-1 are decreased (Boisclair *et al.*, 2006). These metabolic changes are negatively associated with fertility. They include disturbances in LH pulse frequency, growth rate and diameter of the dominant follicle, weight of the corpus luteum, and progesterone and oestradiol concentrations (Van Knegsel *et al.*, 2005). Cows with an ovulatory follicle had reduced triglycerides concentration in liver compared with non-ovulatory cows, in agreement with preliminary findings by Marr *et al.* (2002). Following parturition, the triglycerides concentration remains relatively low in the serum and the follicular fluid while the total cholesterol concentration doubles. This observation is partially caused by the mammary conversion of triglycerides-rich lipoproteins (Bauchart, 1993). Wehrman *et al.* (1991) demonstrated that the triglycerides concentration in the follicular fluid is relatively stable, regardless of an increase in the serum level due to physiological status or diet. In contrast, the present study observed that the triglycerides concentration in the follicular fluid did not change extensively. However, dietary fat supplementation is known to increase serum and cholesterol concentrations in follicular fluid (Wehrman *et al.*, 1991). The present study also found that total cholesterol levels in the follicular fluid raised when serum concentrations increased but both increases of total cholesterol were at different rates. Leroy *et al.* (2004) also found that the total cholesterol concentration in follicular fluid is only 43-48% (at day 14 and day 46 postpartum, respectively) of the serum concentration. The physiological explanation of a cystic ovary linked with total cholesterol and triglycerides

accumulation in embryos is not yet clearly known. This accumulation of lipid droplets is possibly due to an altered lipid metabolism in high-yielding dairy cows, which results in a cystic ovary. In the present study, progesterone concentration in the serum of a cystic cow was much lower than the normal cow and is negatively correlated with the lipid content (data is not shown). Low serum progesterone and high basal luteinizing hormone concentrations are characteristic of the cystic cows (Hamilton *et al.*, 1995). Hatler *et al.* (2003) observed that at the time of diagnosis, accompanied by suprabasal progesterone concentrations, which play a role in cyst turnover. These observations, together with the similarities between persistent follicles induced by suprabasal progesterone and naturally occurring cysts, suggest a role for progesterone in the pathogenesis of cystic ovarian follicles. However, many aspects of the follicular cyst disease, and especially pathogenesis, remain unclear and inconclusive, as for example, illustrated by the lack of a clear definition. Future research should also focus on the fatty acid-associated metabolic hormonal changes and energy utilization on follicular development and seroidogenesis. The present study results should be taken into account in planning further *in vivo* and *in vitro* research concerning fertility problems in high-producing dairy cattle by affecting the quality of the oocytes. Moreover, it might be concluded that impaired lipid metabolism and its higher concentration resulted the follicular cyst formation in dairy cattle.

REFERENCES

- Abe H, S Yamashita, T Itoh, T Satoh and H Hoshi, 1999. Ultrastructure of bovine embryos a developed from *in vitro* matured and fertilized oocytes: comparative morphological evaluation of embryos cultured either in serum-free medium or serum-supplemented medium. *Mol Reprod Dev*, 53: 325-335.
- Abe H, S Yamashita, T Itoh, T Satoh and H Hoshi, 2002. Accumulation of cytoplasmic lipid droplets in bovine embryos and cryotolerance of embryos developed in different culture systems using serum-free or serum-containing media. *Mol Reprod Dev*, 61: 57-66.
- Bauchart D, 1993. Lipid absorption and transport in ruminants. *J Dairy Sci*, 76: 3864-3881.
- Boisclair YR, SR Wesolowski, JW Kim, RA Ehrhardt, 2006. Roles of growth hormone and leptin in the periparturient dairy cow. K. Sejrsen, T. Hvelplund, M.O. Nielsen (Eds.), *Ruminant Physiology: Digestion, Metabolism and Impact of Nutrition on Gene Expression, Immunology and Stress*, Wageningen Academic Publishers, 327-344.
- Boland NI, PG Humpherson, HJ Leese and RG Gosden, 1994. The effect of glucose metabolism on murine follicle development and steroidogenesis *in vitro*. *Hum Reprod*, 9: 617-623.
- Boone DL, WD Currie and PC Leung, 1993. Arachidonic acid and cell signalling in the ovary and placenta. *Prostaglandins Leuk Essent Fatty Acids*, 48: 79-87.
- Canfield RW and WR Butler, 1990. Energy balance and pulsatile LH secretion in early postpartum dairy cattle. *Domest Anim Endocrinol*, 7: 323-330.

- Comin A, D Gerin, A Cappa, V Marchi, R Renaville, M Motta, U Fazzini and A Prandi, 2002. The effect of an acute energy deficit on the hormone profile of dominant follicles in dairy cows. *Theriogenology*, 58: 899–910.
- Day N, 1991. The diagnosis, differentiation and pathogenesis of cystic ovarian disease. *Vet Med*, 8: 735–760.
- Espey LL, 1994. Current status of the hypothesis that mammalian ovulation is comparable to an inflammatory reaction. *Biol Reprod*, 50: 233–238.
- Fortune JE, 1994. Ovarian follicular growth and development in mammals. *Biol Reprod*, 50: 225–232.
- Gosden RG, RHF Hunter, E Telfer, C Torrance and N Brown, 1988. Physiological factors underlying the formation of ovarian follicular fluid. *J Reprod Fertil*, 82: 813–825.
- Hamilton SA, HA Garverick, DH Keisler, ZZ Xu, K Loos, RS Youngquist, BE Salfen, 1995. Characterization of ovarian follicular cysts and associated endocrine profiles in dairy cows. *Biol Reprod*, 53: 890–889.
- Hatler TB, SH Hayes, LF Laranja da Fonseca and WJ Siliva, 2003. Relationship between endogenous progesterone and follicular dynamics in lactating dairy cows with ovarian follicular cysts. *Biol Reprod*, 69: 218–223.
- Herdth TH, T Wensing, HP Haagsman, LM Van Golde and HJ Breukink, 1983. Hepatic triacylglycerol synthesis during a period of fatty liver development in sheep. *J Anim Sci*, 66: 1997–2013.
- Jorritsma R, H Jarritsma, YH Schukken and GH Wentink, 2000. Relationships between fatty liver and fertility and some periparturient diseases in commercial Dutch dairy herds. *Theriogenology*, 54: 1065–1074.
- Jorritsma R, MW de Groot, PLAM Vos, TAM Kruip, T Wensing and JPM Noordhuizen, 2003. Acute fasting in heifers as a model for assessing the relationship between plasma and follicular fluid NEFA concentrations. *Theriogenology*, 60: 151–161.
- Leese EU and EA Lentua, 1990. Glucose and lactate in human follicular fluid: concentrations and interrelationships. *Hum Reprod*, 5: 915–919.
- Leroy JL, T Vanholder, JR Delanghe, G Opsomer, A Van Soom, PE Bols, J Dewulf and A de Kruif, 2004. Metabolic changes in follicular fluid of the dominant follicle in high-yielding dairy cows early post partum. *Theriogenology*, 62: 1131–1143.
- Leroy JLMR, T vanholder, JR Delanghe, G Opsomer, A Van Soom, PEJ Bols, J Dewulf and A de Kruif, 2004. Metabolic changes in follicular fluid of the dominant follicle in high-yielding dairy cows early post partum. *Theriogenology*, 62: 1131–1143.
- Marr AL, MS Piepenbrink, TR Overton, MC Lucy and WR Butler, 2002. The somatotrophic axis and lipid metabolism in transition dairy cows in relation to timing of first postpartum ovulation. *J Dairy Sci*, 85 (Suppl. 1): 66.
- Rabiee AR, IJ Lean, JM Gooden, BG Miller and RJ scaramuzzi, 1997. An evaluation of transovarian uptake of metabolites using arterio-venous difference methods in dairy cattle. *Anim Reprod Sci*, 48: 9–25.
- Van Kneegsel ATM, H. Van den Brand, J. Dijkstra, S. Tamminga and B. Kemp, 2005. Effect of dietary energy source on energy balance, production, metabolic disorders and reproduction in lactating dairy cattle. *Reprod Nutr Dev*, 45: 665–688.
- Wehrman ME, TH Welsh and GL Williams, 1991. Diet-induced hyperlipidemia in cattle modifies the intrafollicular cholesterol environment, modulates ovarian follicular dynamics, and hastens the onset of postpartum luteal activity. *Biol Reprod*, 45: 514–522.