Fatty Acid Compositions of Oocytes, Follicular, Oviductal and Uterine Fluids of Pig and Cow

M. A. M. Yahia Khandoker, H. Tsujii[1] and D. Karasawa
Department of Agricultural Biotechnology, Faculty of Agriculture, Shinshu University, Nagano-ken, Japan

ABSTRACT : The fatty acid compositions of oocytes, follicular, oviductal and uterine fluids of pig and cow were analyzed using gas chromatography. Myristic (C 14 : 0), palmitic (C 16 : 0), palmitoleic (C 16 : 1), stearic (C 18 : 0), oleic (C 18 : 1), linoleic (C 18 : 2), linolenic (C 18 : 3) and arachidonic (C 20 : 4) acids were identified as the common fatty acid constituents with little exception. Oleic acid composition was the highest (21.90 to 36.24%) in both pig and cow followed by palmitic (18.61 to 31.90%) and stearic (10.34 to 20.39%) acid. The three polyunsaturated fatty acids like linoleic, linolenic and arachidonic acids were detected in both pig and cow reproductive fluid samples. Myristic acid was not detected in pig oviductal fluid. Similarly, in cow oocytes myristic, palmitoleic and linolenic acids were not detected. Moreover, palmitic, stearic, oleic and linoleic acid comprised about 80% (73.74 to 88.00%) of the total fatty acids in the different samples analyzed in both animals.
(**Key Words**: Fatty Acid, Oocyte, Reproductive Fluid, Pig, Cow)

INTRODUCTION

Lipids not only provide a source of nutrient to the cell, but also play a vital role in modifying the physical properties and functions of biological membranes, and have potential effects on cell-cell interactions, cell proliferation and transport (Stubbs and Smith, 1984). In addition, the activities of membrane proteins depends on their immediate lipid environment, and the ability of hormone receptor complexes to bind to the effector molecules may be modulated by the headgroup and fatty acid composition of membrane lipids (Hirata et al., 1979 and Galo et al., 1981). The principal component, associated with most lipids is fatty acids and usually they are esterified to glycerol and glycerophospholipids. The importance of fatty acids in maintenance of normal cell function is therefore unequivocal.

It was reported that exogenously supplied fatty acids are beneficial for growth and continued development of rabbit ova (Kane, 1979; Kane and Headon, 1980). It has been shown that mouse ova synthesize sterols and lipids (Pratt, 1978) and metabolize fatty acids (Flynn and Hillman, 1980). Crystallized bovine serum albumin (BSA) is commonly used as the macromolecular component in media used for culture of mammalian eggs. The information in the literature on the binding of low molecular weight compounds to albumin indicate that commercial BSA is a heterogeneous and ill-defined product and normal BSA contain 0.17 mol fatty acid/mol (Kane and Headon, 1980). The chromatographic results of BSA indicate that it contains several saturated and unsaturated fatty acids (Menezo et al., 1982 and Khandoker et al., 1995) and it is reasonable to assume that albumin in culture media and different reproductive fluids in *in vivo* supplies different fatty acids. Moreover, recent studies revealed that BSA binding fatty acids had considerable effect on mouse and rat (Khandoker et al., 1995) and cow (Ohboshi et al., 1996) embryo development. The follicular, oviductal and uterine fluids of mammals are of physiological interest, because they provide environment to the oocytes and preimplantation embryo *in vivo*. For *in vitro* maturation of oocytes and embryo development the culture environment is important (Bavister, 1995) and the culture media, usually devised based on the composition of reproductive tract fluids (Stubbs and Smith, 1984 and Quinn et al., 1985). Data concerning amino acids, proteins, RNA, DNA and sugars are available for numerous species. There is little information related to lipids nevertheless to date (Ishida, 1960; Loewenstein and Cohen, 1964; Holman and Hofstetter, 1965; Hamilton and Seamark, 1978; Yao et al., 1980; Biswas et al., 1986; Homa et al.,1986 and Khandoker et al., 1996). The present study sought to envisage the fatty acid environment of reproductive samples of pig and cow.

[1] Address reprint requests to H. Tsujii.
Received October 29, 1996; Accepted February 10, 1997

MATERIALS AND METHODS

Sample collection

Reproductive ducts of pigs and cows were collected from local slaughterhouse immediately after slaughtering the animals and brought to the laboratory in chilled condition with ice and the samples were prepared immediately. For each sample, reproductive ducts were collected from 15-20 animals and dissected free of other tissuse. They were rinsed thoroughly in 0.9% (w/v) NaCl, blotted dry on paper towel and ovary, oviducts and uterus were separated. Oocytes were aspirated along with follicular fluids from small ovarin follicles (2-5 mm in diameter) using a 21 gauge needle attatched to 5 ml disposable syringe. Oocytes and all other tissue debris were separated by centrifugation at 280 × g for 5 min and the supernatant was collected as follicular fluid. Oocytes were collected from the solid portion and cumulus cells were removed from the oocytes by using hyaluronidase (1 mg/ml phosphate buffer saline; PBS) and by repeated pipetting through a 150 to 200 μm bore pipette. The oocytes were then washed three times in PBS and 400 to 500 morphologically normal oocytes were collected for each sample. The oviductal and uterine fluids were flushed with PBS and were collected without contamination. The oviductal and uterine fluids were then filtered, using 75 μm filter paper (Toyo Roshi Co Ltd. Tokyo, Japan). The samples were stored at −40℃ until further preparation for analysis.

Sample preparation:

Lipid extraction of all samples were carried out according to the method of Bligh & Dyer (1959). Total lipid extracts were evaporated to dryness by vacuum aspiration and weighed. The fatty acid methylation was performed according to the method described by Stoffel et al. (1959). Briefly, 1 ml benzene and 1 ml sodium methoxide (GL Science, Tokyo, Japan) were added for each 10-30 mg total lipid extract in a stoppered centrifuge tube and the tube was heated to 80℃ and maintained for 15-20 min in a water bath for complete methylation. It was then allowed to cool at room temperature, and 3 ml each of diethyl ether and distilled water were added and mixed well. The lower aqueous layer was removed and the top solvent layer washed more than twice with 2-3 ml distilled water and dried overnight by adding anhydrous Na_2SO_4 at 4℃. After drying by vacuum aspiration, the residue containing the fatty acid methyl esters were solubilized in ethyl acetate (5 mg/ml) and aliquots of this were used for further analysis.

Gas chromatography:

The fatty acid methyl esters of the extracted total lipids were analyzed with a GC-14 A gas chromatograph (Shimadzu Co., Ltd. Kyoto, Japan) equipped with a capillary column (liquid phase, film thickness 0.25 μm, 30 m long and 0.25 mm i. d; split ratio 1/100; GL. Science, Tokyo, Japan). The chromatograms were recorded on a data processing recorder (Chromatopac, C-R 1 B, Shimadzu, Kyoto, Japan). The column temperature was initial, 150℃ and 210℃, helium was used as the carrier gas at a constant flow rate of 25 cm/min. Individual fatty acids were identified on the basis of their retention times with that of authentic lipid standards (Sigma Chemical Co., USA). Fatty acid peaks determined by gas chromatography were then used to calculate amounts of fatty acids as described by Slover and Lanza (1979).

RESULTS

The fatty acid profiles of pig samples are shown in table 1. Myristic (C 14:0), palmitic (C 16:0), palmitoleic (C 16:1), stearic (C 18:0), oleic (C 18:1), linoleic (C 18:2), linolenic (C 18:3) and arachidonic (C 20:4) acids were the common fatty acids detected in all the reproductive fluids, whereas myristic acid was not detected in oviductal fluid. In all cases, the oleic acid compositions was the highest (32.99 to 36.24%), followed by palmitic (21.09 to 31.90%) and stearic acid (10.34 to 17.30%). Myristic, palmitoleic and linoleic acid composition were comparatively lower in oocytes, oviductal and uterine fluids (1.05 to 4.57%).

Linoleic, linolenic and arachidonic acid were the three poly-unsaturated fatty acid detected and their levels were moderate in follicular (3.48 ± 0.19, 9.07 ± 0.55 and 10.82 ± 1.83%), oviductal (4.32 ± 0.21, 8.77 ± 0.42 and 9.51 ± 0.69%) and uterine fluids (4.38 ± 0.20, 9.85 ± 0.58 and 8.61 ± 0.31%) but was lower in oocytes (4.57 ± 0.33, 2.21 ± 0.24 and 4.93 ± 0.27%).

In case of cow samples the fatty acid content was almost similar to that of pig, but there was difference in the composition (table 2). The mono-unsaturated fatty acid, oleic acid was the highest (21.90 to 33.17%) followed by palmitic (18.61 to 28.07%), stearic (10.86 to 20.39%) and linoleic (7.64 to 16.80%) acid. Myristic, palmitoleic and linolenic acid were not detected in oocytes and had a lower percentage in follicular (1.60 to 3.38%), oviductal (0.90 to 12.87%) and uterine fluid (1.02 to 13.37%) but palmitoleic acid was moderate in oviductal (12.87%) and uterine fluids (13.37%). Arachidonic acid composition was also moderate in all samples analyzed (9.68 to 15.10%) except in follicular fluid (4.47 ± 0.67%).

Table 1. Fatty acid constituents and composition of several reproductive samples of pig

Fatty acid	Methylated Sample			
	Oocytes	Follicular fluid	Oviductal fluid	Uterine fluid
Myristic	4.23 ± 0.93	1.40 ± 0.45	UD	1.05 ± 0.25
Palmitic	31.90 ± 0.40	26.48 ± 1.99	21.09 ± 0.82	23.79 ± 1.00
Palmitoleic	3.30 ± 0.17	3.13 ± 1.07	2.76 ± 0.12	3.34 ± 0.26
Stearic	16.06 ± 0.18	10.34 ± 0.52	17.30 ± 0.58	14.03 ± 0.16
Oleic	32.99 ± 1.12	35.28 ± 0.29	36.24 ± 0.91	34.91 ± 0.70
Linoleic	4.57 ± 0.33	3.48 ± 0.19	4.32 ± 0.21	4.38 ± 0.20
Linolenic	2.21 ± 0.24	9.07 ± 0.55	8.77 ± 0.42	9.85 ± 0.58
Arachidonic	4.93 ± 0.27	10.82 ± 1.83	9.51 ± 0.69	8.61 ± 0.31
P+S+O+L	85.59	75.58	78.95	77.11

P, S, O, and L represents palmitic, stearic, oleic and linoleic acid, respectively UD = Undetectable.
Values are the means ± S.E.M. of three analyses and are expressed as weight % of the total fatty acid.

Table 2. Fatty acid constituents and composition of several reproductive samples of cow

Fatty acid	Methylated Sample			
	Oocytes	Follicular fluid	Oviductal fluid	Uterine fluid
Myristic	UD	3.38 ± 0.10	0.90 ± 0.08	1.02 ± 0.19
Palmitic	24.08 ± 0.34	28.07 ± 0.14	18.61 ± 0.86	21.28 ± 0.29
Palmitoleic	UD	2.54 ± 0.05	12.87 ± 0.54	13.37 ± 0.33
Stearic	10.86 ± 0.03	14.40 ± 1.34	20.39 ± 0.41	17.80 ± 0.64
Oleic	33.17 ± 0.33	32.27 ± 2.85	21.90 ± 0.30	27.02 ± 0.44
Linoleic	16.80 ± 0.08	13.26 ± 2.99	14.06 ± 0.18	7.64 ± 0.27
Linolenic	UD	1.60 ± 0.08	1.60 ± 0.12	2.13 ± 0.11
Arachidonic	15.10 ± 0.09	4.47 ± 0.67	9.68 ± 0.58	9.79 ± 0.93
P+S+O+L	84.91	88.00	74.96	73.74

P, S, O and L represents palmitic, stearic, oleic and linoleic acid respectively UD = Undetectable.
Values are the means ± S.E.M. of three analyses and are expressed as weight % of the total fatty acid.

The long chain fatty acids like palmitic, stearic, oleic and linoleic acid comprised about 80% (73.74 to 88.00%) of the total fatty acids in both pig and cow reproductive samples (table 1 & 2).

DISCUSSION

The complete report on fatty acid composition of all reproductive samples from pig and cow are presented here. The fatty acid composition of pig serum and follicular fluid as reported by Yao et al. (1980) was in close relation with our findigs, that the oleic, palmitic and stearic acies were observed in descending order of abundance. It was reported that the combination of palmitic and oleic acid had a synergetic effect in mouse embryo development (Quinn and Whittingham, 1982). This study also showed a higher composition of palmitic and oleic acids in all reproductive samples of both pig and cow which may serve as a storage pool of metabolic precursors, as both these fatty acids are required as substrates for fatty acid elongation and desaturation in embryo development (Jeffcoat, 1979). The common constituents of fatty acid in follicular, oviductal and uterine fluids indicated that the fatty acids may be originated from the same source, the blood (Bishop, 1956 and Oliphant et al., 1984) and these results confirmed this statement. There was a difference in the percentage of fatty acids content but the number of fatty acid remain constant in all these four samples in both animals, may be due to the similar fatty acid requirement for oocytes maturation, fertilization and subsequent embryo development. The difference in compositions of fatty acids also indicated that the secretion, as well as transudation process may be different (Perkins et al., 1965; Edwards, 1974 and Leese, 1988) in both animals. We found linoleic, linolenic and arachidonic acids were the three major poly-unsaturated fatty acid in both pig and cow reproductive fluid samples, which corroborate well with the results of Yao et al.

(1980).

These three fatty acids nutritionally reffered to as essential (McDonald et al., 1973) and specially the content of arachidonic acid in both pig and cow reproductive type samples is interesting since it is the precursor for the biosynthesis of prostaglandin, which have been implicated in ovarian function (Tsafriri et al., 1972; Amstrong et al., 1973 and LeMaire et al., 1973). The absence or lower amount of myristic and palmitoleic acids in oviductal, uterine and follicular fluids of both pig and cow was in concurrence with the studies of Yao et al. (1980). This studies revealed less amount of myristic and palmitoleic acid (1.03 and 2.56%) in pig follicular fluid. This may be due to the less importance of those fatty acids for the congenial environment of reproductive organ. Fatty acid contents in these two animals were similar but the compositions were different, which suggest that the fatty acid distribution in mammalian sample is species-specific. Moreover, palmitic, stearic, oleic and linoleic acids comprised about 80% (73.74 to 88.00%) of the total fatty acid in the different samples analyzed in both pig and cow. Spector (1971) stated that those type of fatty acid comprise approximately 80% of the total fatty acids fraction in human and most animal sera. The rate of accumulation and composition of tubal fluid depends on the reproductive state of the animal (Hafez, 1993) but in present experiment due to sampling difficulties it was not possible to discriminate this factor.

In conclusion, it can be stated that the presence of different fatty acids for oocyte maturation, fertilization and subsequent embryo development and therefore systematic investigation of their role requires in vitro studies. Experiments are now in progress to determine the direct effect of fatty acids on spontaneous maturation of oocyte, fertilization and subsequent embryo development.

ACKNOWLEDGEMENTS

We thank Fumihide Inoue and Akihiko Hatanaka for preparation of gas chromatograph equipment and helpful discussion. M. A. M. Yahia Khandoker also gratefully acknowledges the Ministry of Education, Science, and Culture of Japan for financial assistance by a scholarship during the course of this study.

REFERENCES

Amstrong, D. T., Y. L Moon and D. L. Grimwich. 1973. Possible role of prostaglandins in ovulation. In: Advances in the Biosciences. 9:709:715.

Bavister B. D. 1995. Culture of preimplantation embryos: facts and artifacts. Humn Reprod Update; 1:91:148.

Bligh E. G. and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911:917.

Bishop D. W. 1956. Active secretion in the rabbit oviduct. Am. J. Physiol. 187:347:352.

Biswas S. K, S. Mukherjee, B. Chakrabarti, P. Nandy and P. Chakrabarti. 1986. Lipid composition, fluidity and lectin-reactivity of membranes from normal human uterus and benign and malignant uterine tumours. Indian. J. Biochem. Biophs. 23:13:18.

Edwards R. G. 1974. Follicular fluid. J. Reprod. Fert. 37:189:219.

Flynn T. J. and N. Hillman. 1980. The metabolism of exogenous fatty acids by preimplantation mouse embryos in vitro. J. Embryol. Exp. Morphol. 56:157:168.

Galo M. G, L. E. Unates and R. N. Farias. 1981. Effect of membrane fatty acid composition on the action of thyroid hormones on (Ca^{2+} + Mg^{2+}) adenosine triphosphate from rat erythrocyte. J. Biol. Chem. 256:7113:7114.

Hafez E. S. E. 1993. Anatomy of female reproduction. In: Reproduction in farm animals. Philadelphia Lea & Febiger. 20:55.

Hamilton R. P. and R. F. Seamark. 1978. Lipid content of sheep ovarian follicles in culture. Aust. J. Biol. Sci. 31:621:628.

Hirata F, W. J. Strittmatter and R. Axelrod. 1979. β-adrenergic receptor agonists increase phospholipid methylation, membrane fluidity and β-adrenegic receptor-adenylate cyclase coupling. Proc. Natn. Acad. Sci. USA. 76:368:372.

Holman R. T. and H. H. Hofstetter. The fatty acid composition of the lipids from bovine and porcine reproductive tissues. J. Am. Oil Chem. Soci. 1965; 42:540:544.

Homa S. T, C. Racowsky and R. W. McGaughey. 1986. Lipid analysis of immature pig oocytes. J. Reprod. Fert. 77:425:434.

Ishida K. 1960. Histochemical Studies of Lipids in the ovaries of Domestic Animals. Arch. Hist. jap. 19:547:555.

Jeffcoat R. 1979. The biosynthesis of unsaturated fatty acids and its control in mammalian liver. Essays Biochem. 15:1:36.

Kane M. T. 1979. Fatty acids as energy sources for culture of one-cell rabbit ova to viable morulae. Biol. Reprod. 20:323:333.

Kane M. T. and D. R. Headon. 1980. The role of commercial bovine albumin preparations in the culture of one-cell rabbit embryos to blastocysts. J. Reprod. Fert. 60:469:475.

Khandoker M. A. M. Y., M. Nishioka and H. Tsujii. 1995. Effect of BSA binding fatty acids on mouse and rat embryo development. J. Mamm. Ova. Res. Jpn. 12:113:118.

Khandoker M. A. M. Y., H. Tsujii and D. Karasawa. 1996. Fatty Acid Analysis of Oocytes, Oviductal and Uterine Fluids of Rabbit. Anim. Sci. Technol. Jpn. 67(6):549:553.

Leese H. J. 1988. The formation and function of oviduct fluid. J. Reprod. Fert. 82:843:856.

LeMaire W. J., N. S. T. Young, H. H. Behrman and J. M. Marsh. 1973. Preovulatory changes in the concentration of prostaglandins in rabbit Graafian follicles. Prostaglandins. 3:367:376.

Loewenstein J. E. and A. I. Cohen. 1964. Dry mass, lipid content and protein content of the intact and zona free mouse ovum. J. Embryol. Exp. Morph. 12:113:121.

McDonald P, R. A. Edwards, J. F. D. Greenhalgh. 1973. Lipids. In: Animal Nutrition. 2nd ed. Oliver & Boyd. Edinburgh. 25:41.

Menezo Y., J. P. Renard, B. Delobel and J. F. Pageaux. 1982. Kinetic study of fatty acid composition of day 7 to 14 cow embryos. Biol. Reprod. 26:787:790.

Ohboshi S., K. Hanada, J. Zhao, M. Hattori, N. Fujihara, R. Umetsu, T. Yoshida and H. Tomogane. 1996. *In vitro* development of bovine one-cell embryos fertilized *in vitro* in serum- and feeder cell-free culture systems. Asian-Australasian J. Anim. Sci. 9(5):583:590.

Oliphant G., A. B. Reynolds, R. F. Smith, P. R. Ross and J. S. Marta. 1984. Immuno-cytochemical localization and determination of hormone-induced synthesis of the sulfated glycoproteins. Biol. Reprod. 31:165:174.

Perkins J. L., W. A. Goode, J. R. Wilder and D. B. Henson. 1965. Collection and secretions from the oviduct and uterus of the ewe. J. Anim. Sci. 24:383:38.

Pratt H. R. M. 1978. Lipids and transitions in embryos. In: Johnson MH (ed). Development in mammals (3). Amsterdam: North Holland Publishing Co. 83:129.

Quinn P. and D. G. Whittingham. 1982. Effect of fatty acids on fertilization and development of mouse embryos *in vitro*. J. Androl. 3:440:444.

Quinn P., J. F. Kerin and G. M. Warnes. 1985. Improved pregnancy rate in human *in vitro* fertilization with the use of a medium based on the composition of human tubal fluid. Fertil. Steril. 44:493:498.

Slover H. T. and E. Lanza. 1979. Quantitative analysis of food fatty acids by capillary gas chromatography. J. Am. Oil Chem. Soc. 56:933:943.

Spector A. A. 1971. Metabolism of free fatty acids. Rrog. Biochem. Pharmacol. 6:130:176.

Stoffel W. and F. Chu, E. H. Ahrens. 1959. Analysis of long chain fatty acids by Gas-liquid Chromato-graphy. Anal. Chem. 31:307:308.

Stubbs C. D. and A. D. Smith 1984. The modification of mammalian membrane poly-unsaturated fatty acid composition in relation to membrane fluidity and function. Biochem. Biophs. Acta. 779:89:137.

Tsafriri A, H. R. Linder, U. Zor and S. A. Lamprecht. 1972. *In vitro* induction of meiotic division in follicle-enclosed rat oocytes by LH, cyclic AMP and prostaglandin E_2. J. Reprod. Fert. 31:39:50.

Yao J. K, J. R. Ryan and P. J. Dyck. 1980. The porcine ovarian follicle. VI. Comparison of fatty acid. Composition of serum and follicular fluid at different developmental stages. Biol Reprod. 22:141:147.