Fatty Acid Analysis of Oocytes, Oviductal and Uterine Fluids of Rabbit

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Abstract The fatty acid compositions of oocytes, oviductal and uterine fluids of rabbit were analyzed by gas chromatography and were compared with those of blood serum and body fat. Myristic (C 14 : 0), palmitic (C 16 : 0), palmitoleic (C 16 : 1), stearic (C 18 : 0), oleic (C 18 : 1), linoleic (C 18 : 2) and linolenic (C 18 : 3) acids were identified as the common fatty acid constituents in the objects. The fatty acid constituents of reproductive tracts fluids seemed to be quite similar to those of blood serum and body fat. In oocytes and different reproductive fluids, oleic acid composition was the highest (26.59 to 32.79%), followed by palmitic (21.83 to 25.8%), and linoleic (18.33 to 23.55%) acid. Myristic, palmitoleic, stearic and linolenic acid compositions were always low in oviductal (2.12 to 7.91%) and uterine (2.05 to 10.48%) fluids. On the other hand, myristic and stearic acid were moderate (16.66 and 14.17%, respectively) but palmitoleic and linolenic acid were not detected in oocytes. Palmitic, stearic, oleic and linoleic acids comprised more than 80% (82.24 to 86.43%) of the total fatty acid fraction in all samples analyzed.

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Our previous work indicated that reproductive fluids of pig and cow contained several saturated (myristic, palmitic and stearic) and unsaturated (palmitoleic, oleic and linoleic) fatty acids. Quantitatively, the most abundant fatty acids were palmitic, stearic, oleic and linoleic acids. The fatty acid constituents and compositions of different reproductive fluids were more or less same and they seemed to be quite similar to those of body fat of the same animal⁸⁾. We observed that BSA binding fatty acids had considerable effect on mouse and rat embryo development⁷⁾. Moreo-

ver, it was reported that exogenously supplied fatty acids are beneficial for growth and continued development of rabbit embryo^{4,5)}. The culture media, usually devised based on the composition of reproductive fluids^{15,19)}. There is no report about fatty acid composition of oocytes, oviductal and uterine fluids of rabbit to date. In this situation, the analysis of fatty acid composition of reproductive fluids of rabbit bears importance, because they provide environment to the oocytes and preimplantation embryo *in vivo*. So, the present study was undertaken to determine the fatty acid compo-

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sition of oocytes, oviductal and uterine fluids of rabbit and for comparison we also analyzed blood serum and body fat of the same animal.

Materials and Methods

Sample collection : Reproductive ducts were collected from superovulated New Zealand White does which were maintained singly in metal cage. Superovulation was performed by marginal ear vein injection of 50IU pregnant mare serum gonadotrophin (PMSG) followed 48 h later by 50IU human chorionic gonadotrophin (hCG). Reproductive ducts were collected from 30 does, 8-10 h after hCG injection. Ovary, oviduct and uterus were separated. They were dissected free of other tissues, rinsed thoroughly in 0.9% (w/v) NaCl, blotted dry on paper towel. Oocytes were collected by scratching of ovary in phosphate buffer saline (PBS) solution and cumulus cells were removed by using hyaluronidase (1 mg/ml PBS). The oocytes were then washed three times in PBS and 240 cumulus cell free oocytes were collected. To collect the oviductal and uterine fluids they were flushed with chloroform-methanol mixture (2:1) and precautions were taken to avoid contamination. The oviductal and uterine fluids were then filtered, using 75 μ m filter paper (Toyo Roshi Kaisha, Tokyo, Japan) and most of the solvent were evaporated. Body fat and blood serum (from blood) were also collected to analyze fatty acid. All the samples were stored at -40°C until further preparation for analysis.

Sample preparation : All the samples were extracted separately with diethyl ether, the ether layers were collected and dried with anhydrous Na₂SO₄ overnight at 4°C. Total lipid extracts were evaporated to dryness using evaporator and the weight was measured. In order to identify the unknown acids, the methylation was performed according to the method described by Stoffel *et al.*¹⁸⁾. Briefly, 1 ml benzene and 1 ml 0.5N sodium methoxide (GL Science, Japan) were added for each 10-30

mg extract in a centrifuge tube, a conical bottom with stopper. The tube was heated to 80°C and maintained for 15-20 min in a water bath for complete methylation. It was then allowed to cool in 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 Na₂SO₄ at 4°C. Final evaporation was performed and stored at -40°C until analysis.

Gas Chromatography : The methyl ester of fatty acids were dissolved in 10-20 μ l ethyl acetate. Composition of fatty acid methyl esters were determined by gas chromatograph (Shimadzu GC-6 A, Japan) with a flame ionization detector. The chromatograms were recorded on a thermal-pen recorder (Shimadzu, C-R 1 A, Chromatopac). A diethylene glycol succinate (DEGS) column was used for analysis : 2 m long and 3 mm i.d. packed with Chromosorb W 60-80 mesh. The column temperature was, initial 120°C and final 190°C ; carrier gas was helium (30 ml/min) and the maximum sample size was 1 μ l. The sequence of methylated fatty acid peaks were identified by comparison with authentic standards (Lipid standard, Sigma Chemical Co., USA) and the results were reported as area percent. All samples analyzed were properly separated within 1 hour.

Results and Discussion

The fatty acid contents and compositions 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) and linolenic (C 18 : 3) acids were the common fatty acid constituents. The fatty acid composition of oocytes and different reproductive fluids seemed to be quite similar to that of blood serum and body fat. In oocytes and different reproductive fluids, oleic acid constituents was the highest (26.59 to 32.79%), followed by pal-

Fatty Acids of Rabbit Reproductive Sample

Table 1. Fatty acid constituents and composition (%) in rabbit sample

Fatty acid C	Sample (ether extract)				
	Body fat (80.6) ^a	Blood serum (61.8)	Oocytes (5.1)	Oviductal fluid (44.8)	Uterine fluid (60.2)
14:0	2.15	3.06	16.66	2.12	2.05
16:0	25.56	24.23	22.08	25.81	21.83
16:1	3.67	3.18	ND	7.91	6.70
18:0	5.95	6.25	14.17	7.01	10.48
18:1	20.10	21.52	28.75	26.59	32.79
18:2	30.63	30.41	18.33	23.55	21.33
18:3	11.94	11.34	ND	6.99	4.82

^a Average amount of ether extract (mg) per pool are shown in parentheses. ND, Not detected.

mitic (21.83 to 25.81%), and linoleic (18.33 to 23.55%) acids. Myristic, palmitoleic, stearic and linolenic acid composition were determined to lower in oviductal (2.12 to 7.91%) and uterine (2.05 to 10.48%) fluids. On the other hand, myristic and stearic acids were moderate (16.66 and 14.17% respectively) but palmitoleic and linolenic acid were not detected in oocytes. Linoleic and linolenic acids were the two poly-unsaturated fatty acid of oviductal and uterine fluids.

These results were supported by our previous work and we obtained the same types of fatty acids by analyzing different reproductive fluids of pig and cow⁸. Oleic, palmitic and linoleic acids were observed in descending order of abundance. This result was also supported by other researchers, and the same trend of fatty acid composition was observed by analyzing pig depot fats, serum and follicular fluid^{6,8,20}. The higher composition of oleic and palmitic acids found in all reproductive fluids may serve as a storage pool of metabolic precursors, as these fatty acids are required as substrates for fatty acid elongation and desaturation in embryo development³. On the other hand, the combination of palmitic and oleic acid was more effective in mouse embryo development¹⁴. The common constituents of fatty acid in different reproductive fluids indicated that the origin of those fatty

acids is same, the blood^{1,12}. Similarly, the different composition of those fatty acids also indicated that the secretion, as well as transudation process may be different^{2,9,13}.

We found linoleic and linolenic acid as the two poly-unsaturated fatty acids in oviductal and uterine fluids. This result differs from our previous observation⁸, and might be due to species difference. Nutritionally, linoleic, linolenic and arachidonic acids are usually referred to as essential fatty acids, although neither linolenic nor arachidonic acid is truly essential, since linoleic acid is able to transform itself to other two fatty acids when required¹⁰. Therefore, the high concentration of linoleic acid found in oviductal fluid (23.55%) and uterine fluid (21.33%) might fulfill the essentiality of these three fatty acids. The high concentration of linoleic acid in rabbit reproductive fluids may be indicative of the capacity of those samples to synthesize prostaglandin, as arachidonic acid, which is derived from linoleic acid, and is the primary substrate for cyclooxygenase and lipoxygenase¹⁶. The lower amount of myristic and palmitoleic acids in all samples analyzed, except myristic acid in oocytes, was also supported by Yao *et al.*²⁰. They found only 1.0 and 2.8% myristic and palmitoleic acid, respectively, in pig follicular fluid. The lower amount of myristic, palmitoleic, stearic and linolenic acids in oviductal

and uterine fluids may imply the less importance of those fatty acids for the maintenance of congenial environment in the oviduct and uterus. Palmitic, stearic, oleic and linoleic acids comprised more than 80% (82.24 to 86.43%) of the total fatty acid in the different samples analyzed. Spector¹⁷⁾, stated that those type of fatty acids comprise approximately 80% of the total fatty acids fraction in human and most animal sera.

Kane⁴⁾ reported that myristic, palmitic, stearic, oleic and to a lesser extent, the short chain acids, i.e. acetic and propionic, can support growth of 1-cell rabbit ova to viable morulae in a simple salt solution containing defatted BSA. The information in the literature indicated that BSA contain 0.17 mol fatty acids¹⁾. On the other hand, the chromatographic results of BSA indicate that it contains several saturated and unsaturated fatty acids^{7,11)}. So, it can be assumed that BSA in culture media and reproductive tract fluid may be supplies different fatty acids *in vitro* and *in vivo*, respectively, in the case of rabbit embryo development.

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