Fatty Acid Composition of Total Lipids in Bovine Oocytes from Ovaries Different Morphological and Functional State after Maturation in Vitro

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Introduction

The processes during animal oocyte maturation are one of the key problems in developmental biology. Until recently, most attention of investigators had been focussed on the changes in nucleus components during the process of meiosis. In studies of biochemical alterations in maturing oocytes interest was being shown generally in changes in protein profiles. Thus, it was established that the process of nuclear maturation in bovine oocytes both in vivo and in vitro was related to the changes in various protein fractions. Meanwhile, the lipids are essential constituents of animal cells. However, their role and in paticular of fatty acids one in mammalian oocyte maturation, fertilization and development is still not clearly understood. Although it has been found that the fatty acid composition in preimplantation embryos in vivo may vary significantly with developmental stage on cattle. The role of lipids during meiosis remains also unclear. It should be to noted on practical importance of investigation in this area, since lipids are responsible for resistance of oocytes under cryopreservation.

We have shown that the ability of morphologically normal oocytes to reach the metaphase II in vitro does not depend on MFS of the ovary. It is not known, however, whether or not the MFS of the ovary influences the biochemical composition of oocytes, in particular, the fatty acid composition and wthether or not this composition varies with nuclear maturation. Taking this into account, in the present study an attempt was made to investigate fatty acid composition of total lipids in bovine oocytes collected from ovaries of various MFS after their nuclear maturation in vitro.

MATERIALS AND METHODS

- **Ovaries of Black and- White bovine were collected immediately after animals were killed at local abattoirs and were stored at 30^{0}C in Dulbecco's PBS during transport to the laboratory. On arrival at the laboratory, ovaries were rinsed in PBS at 30^{0}C and were classified in conformity with MFS: 1)ovaries with luteal body from last cycle, without dominant follicle, with follicles different diameter (< 10мм); 2) ovaries with luteal body from last cycle, with dominant follicle (d > 10мм) and with follicles different diameter (<10mm); 3) ovaries with large function luteal body and with follicles different diameter (< 10мм); 4) ovaries with follicular cyst (d > 25 mm) ; 5) ovaries with luteal body from last cycle and small (1-2mm) follicles - supposedly with weak hormonal function.**

Cumulus-oocyte complexes(COC) were retrieved by surface slicing in 90-mm plastic Petri dishes (Medpolymer, Sankt-Petersburg, Russia) within 4h after the animals were killed. The COCs were retrieved from follicles of 2-6mm in diameter. The COCs were washed 3-4times in oocytes collection medium - Dulbecco's PBS supplemented 5% estrous cow serum (ECS) and then were washed 2-3 times in Tissue Culture Medium 199 HEPES (TCM 199 HEPES; Sigma) supplemented 10% ECS.Oocytes with at least three layers of compact cumulus cells and evenly granulated cytoplasm were used in the experiments.

A basal medim for IVM was Tissue Culture Medium 199 (TCM 199; Sigma) which contained 10% ECS, 0.2mM sodium pyruvate (Serva), 1.5mM l-glutamine (Serva). COCs were placed in 100μl droplets of IVM medium (20 COCs per droplet)under paraffin oil (Fluka) in 40-mm plastic Petri dishes (Medpolymer, Sankt-Petersburg, Russia) and were matured for 24h at 38.5^{0}C in a humidified atmosphere of 5% CO_2.

- **After maturation the cumulus cells were removed by 0.5% hyaluronidase (Sigma) and by repeated pipetting. The denuded oocytes were washed three times with Dulbecco's PBS. The denuded oocytes from each variant (five different MFS of ovarie) were distributed into two group : 1) with polar body; 2) without polar body. Oocytes were collected from 4-5 repeated experiments. Samples stored at -20 C^0 until further preparation for analysis. Each of ten variants analysed twice by gas chromatography.**
- **No pleliminary total lipid extraction of samples was carried in connection with limited quantity of oocytes. Each sample is gomogenized with 10ml a mixture of chloroform and methanol (2:1, v/v, Sigma), after 12 h. added 10ml distilled water.The lower aqueous layer was removed. The top layer was decanted in tube. The methylation mixture (MeOH : HCl, 1:1,v/v, Sigma) was added after evaporation of chloroform. Tubes were heated to 80 C^0 and maintained for 4 h. for complete methylation, then methyl esters of fatty acids were extracted with 3ml hexane (Sigma). Hexane was then evaporated to 0.2ml by vacuum aspiration. The residue containing the fatty acid methyl esters were used for gas chromatographic analysis. The fatty acid methyl esters of the total lipids were analysed with «Chrom-5» gas chromatograph (Laboratorni Pristroje, Praha), equipped with a glass column (3m x 3mm ; Borovsk, Russia). The chromatograms were recorded on a data processing recorder CI-100 (Laboratorni Pristroje, Praha). The solid carrier - Chromaton AW HMDS 125-140 mesh (Shimadsu Seisakusho LTD., Kyoto, Japan). The liquid phase - 12% deethyleen glycol succinate (Shimadsu Seisakusho LTD. ,Kyoto, Japan). Individual fatty acids were identified on the basis of their retention times using authentic lipid standards (Sigma).**
- **The data obtained from gas chromatography were statistically analyzed by Student's t - test and expressed as mean percentage ± SEM.**

Results

- **The fatty acid compositions of bovine oocytes from ovaries different MFS after maturation in vitro is shown in Table. A total of 15 different fatty acids were detected in bovine oocyte lipid, but only 10 of these without «trace».After maturation in vitro some fatty acids in bovine oocytes were moderate independent of MFS of ovarie. Palmitic acid are significantly higher in oocytes with polar body independent of MFS of ovarie. There is the tendency to increase of stearic and oleic acids in oocytes with polar body independent of MFS of ovarie. At the same time there is the tendency to decrease of linoleic, linolenic, arachidonic acids in oocytes with polar body independent of MFS of ovaries.**
- **For determination of fatty acid composition of bovine oocytes after maturation in vitro were used data in all MFSs of ovaries.Unsaturated fatty acids were predominant in oocytes with polar body and in oocytes without one (76.45% vs. 82.71%, respectively). In consequence of this, the saturation index of fatty acids was higher for oocytes with polar body. Bovine oocytes with polar body contained significantly more palmitic, stearic, oleic and significantly less linoleic, linolenic, arachidonic acids in comparison with oocytes without polar body.**

FATTY ACID COMPOSITION OF TOTAL LIPID EXTRACTED FROM BOVINE OOCYTES AFTER MATURATION IN VITRO (% , W/W)[A]

[a] Percentage (w/w) of the total fatty acids in oocyte lipid.
** Values differ significantly P<0.01
*** P<0.001
[b] Index saturation of fatty acids.

Formula	Availability of polar body	Availability of polar body
	Yes	No
C8:0	0.31+0.05	0.22+0.02
C10:0	0.87+0.12	0.80+0.06
C12:0	1.15+0.17	0.93+0.06
C14:0	1.29+0.11	1.18+0.05
C16:0	11.96+0.95	7.34+0.51***
C18:0	7.37+0.42	5.81+0.19**
C18:1	5.99+0.25	4.63+0.25**
C18:2	37.10+0.55	39.50+0.26***
C18:3	27.03+0.88	31.03+0.22***
C20:4	6.33+0.33	7.55+0.25**
Others	0.60	1.01
ISFA [b]	0.3	0.2

Conclusions

- **A decrease in the content of linoleic, linolenic and arachidonic acids in oocytes with a polar body may indicate an increase in prostaglandin synthesis. Arachidonic acid is known to be a direct precursor of prostaglandins. The main physiological function of prostaglandins is to modulate the activity of adenylatecyclase. Thus, prostaglandins are known to increase the level of cAMP in platelets, thyroid gland, ovarian corpus luteum, fetal bone tissue, anterior pituitary gland and lungs and reduce the activity of cAMP in renal tubule cells. It is possible that through such modeling prostaglandins are involved in the maturation of animal oocytes. It is possible to assume the existence of a common biochemical mechanism underlying the processes of the first meiotic division.**
- **Although, it has been impossible to exactly evaluate the role of individual fatty acids in the process of bovine oocyte nucleus maturation, but the data from our experiments showed, that of fatty acids metabolism may play a significant role in the early development of preimplantation bovine embryos. The futher investigations of the lipids (and, especially, fatty acids) composition, metabolism and biological role in the mammals oocytes can be promote the profound understanding of oogenez mechanisms. At the same time these investigations may be of practical importance in the development of a defined culture media for bovine oocytes and efficient methods their cryopreservation.**

Thanks for your attention!