LIPID PATTERN IN FEMALE REPRODUCTIVE TISSUES DURING DIFFERENT PHASES OF ESTROUS CYCLE

S. MANIMEKALAI, E. UMAPATHY AND P. GOVINDARAJULU

Department of Endocrinology,
P. G. Institute of Basic Medical Sciences, Taramani, Madras-600 042

Summary: The normal pattern of lipids during various phases of estrous cycle in different reproductive tissues of female rats was analysed. The various lipid classes undergo striking cyclical changes in harmony with the rhythm of endogenous gonadal and gonadotrophic hormones. A comparison of lipid pattern was made between various phases of estrous cycle.

Ovary exhibited marked alterations in the distribution of lipids in different phases of the cycle compared to other tissues. Total lipids were maximum in diestrus phospholipids in estrus with a concomittant fall in triglycerides in all the tissues. The fluctuation in oviductal and vaginal lipids was much less compared to other tissues. Hormone-lipid interrelationship and tissue specificity may be attributed to these changes.

Key words: estrus, metestrus, diestrus, proestrus, ovary, oviduct, uterus, vagina, lipids

INTRODUCTION

The normal endogenous rhythm of hormones maintain the integrity, growth and physiological responses of the reproductive tissues (15). The profound influence of hormones on the metabolic activities, has been well elucidated (10). A good deal of attention has already been focused on the uterine lipids during the various phases of estrus cycle, which suggest that the rhythmic pattern of hormones may play a role in the distribution of lipids in different reproductive tissues (5). The present study is mainly an analysis of distribution of various classes of lipids in different reproductive tissues during the various phases of the estrous cycle, to delineate the role of hormone-lipid interrelationship and tissue specificity.

MATERIALS AND METHODS

Female albino rats of Wistar strain weighing 120-150 *g* obtained from Cancer Research Institute, Bombay, India were used. The animals were allowed food and water *ad libitum*.

The characteristic cell types found during the different stages of estrous cycle were made out by vaginal smear technique as follows :

(a) Proestrous : Nucleated cuboidal epithelial cells
(b) Estrous : Cornified epithelial cells
(c) Metestrous : Cornified epithelial cells plus leukocytes
(d) Diestrous : Leukocytes plus a few epithelial cells

Animals which were showing a regular four days cycle were sacrificed by cervical dislocation. The ovary, oviduct, uterus and vagina were immediately dissected out, freed from the adhering connective tissues and weighed, accurately to the nearest milligrams on a torsion balance. The tissues were immediately processed for the extraction of lipids. All the extraction processes were carried out under nitrogen. Extraction procedures and estimation techniques have already been published (11).

RESULTS

A comparison is drawn between proestrus and estrus (proliferative phase), metestrus and diestrus (secretory phase) and estrus and diestrus phases to demarcate the interphase and intraphase variations.

Table I depicts the lipid pattern of ovarian tissues during various phases of the estrus cycle. The concentration of total phospholipids was more in estrus phase than in proestrus phase ($P<0.01$) due to phosphatidic acid ($P<0.001$), sphingomyelin ($P<0.01$) and phosphatidyl inositol plus-serine ($P<0.05$). However, the di-and triglycerides were less in estrus phase. Free cholesterol was higher in estrus phase ($P<0.05$).

The concentration of total lipids during diestrus phase was less than that of metestrus, due to a marked fall in glycerides ($P<0.001$), especially due to mono - ($P<0.001$) and diglycerides ($P<0.01$). Free and esterified cholesterol were not much altered. However, the increase in total phospholipids during diestrus was contributed by almost all classes of phospholipids ($P<0.001$), and to a certain extent by phosphatidic acid ($P<0.02$). But lysophosphatidyl choline was not much altered.

The concentration of total lipids during diestrus phase was comparatively more than the estrus phase ($P<0.05$) due to glycerides ($P<0.001$). Mono- ($P<0.001$) tri-($P<0.001$) and diglycerides ($P<0.02$) as well as free cholesterol ($P<0.05$) contributed for the rise. However, a significant rise in phosphatidyl choline ($P<0.01$) and a marked fall in phosphatidic acid ($P<0.001$) were observed in diestrus phase.

Table II shows the lipid pattern of oviduct during estrus cycle.

TABLE I : Normal lipid pattern in various phases of estrus cycle - OVARY
(Values are means ± S.E.M. of 10 animals per group).

Parameters	*Proestrus* (I)	*Estrus* (II)	*Metestrus* (III)	*Dietrus* (IV)	*P value* (I–II)	(III–IV)	(II–IV)
Total Lipid	196.0 ± 7.3	157.8 ± 6.6	180.3 ± 6.8	168.3 ± 4.2	N.S.	N.S.	0.05
Total Phospolipid	25.4 ± 2.8	31.9 ± 1.5	21.7 ± 2.8	30.8 ± 4.0	0.01	N.S.	N.S.
Total Neutral Lipid	170.6 ± 8.8	125.9 ± 7.7	168.6 ± 5.7	137.5 ± 5.1	N.S.	0.001	0.05
Total Cholesterol	27.2 ± 2.5	30.8 ± 3.8	27.3 ± 4.6	28.8 ± 2.2	N.S.	N.S.	N.S.
Total Glycerides	143.4 ± 6.5	95.1 ± 3.3	141.3 ± 4.7	110.7 ± 5.0	0.001	0.001	0.001
Free Cholesterol	6.2 ± 0.4	7.5 ± 0.8	6.7 ± 0.4	6.3 ± 0.4	0.05	N.S.	0.05
Ester Cholesterol	21.0 ± 1.7	23.3 ± 2.3	20.6 ± 1.8	20.5 ± 2.6	N.S.	N.S.	N.S.
Mono Glycerides	31.3 ± 6.4	32.1 ± 2.2	36.3 ± 3.1	22.4 ± 1.9	N.S.	0.001	0.001
Diglycerides	63.7 ± 5.7	44.7 ± 3.9	47.9 ± 4.2	35.7 ± 3.8	0.001	0.01	0.02
Triglycerides	48.4 ± 2.8	18.3 ± 1.7	57.1 ± 2.9	52.6 ± 3.7	0.001	N.S.	0.001
Phosphatidyl Inosital	5.1 ± 0.3	6.2 ± 0.6	4.3 ± 0.4	5.2 ± 0.4	0.05	0.001	N.S.
Lysophosphatidyl Choline	0 6 ± 0.1	0.9 ± 0.2	0.7 ± 0.2	1.0 ± 0.2	N.S.	N.S.	N.S.
Sphingomyelin	2.5 ± 0.3	3.7 ± 0.4	2.0 ± 0.3	3.9 ± 0.4	0.01	0.001	N.S.
Phosphatidyl Choline	10.2 ± 0.7	11.5 ± 0.9	8.7 ± 0.4	13.4 ± 0.5	N.S.	0.001	0.01
Phosphatidyl Ethanolamine	6.0 ± 0.7	7.0 ± 0.7	5.3 ± 0.5	6.1 ± 0.7	N.S.	0.001	N.S.
Phosphatidic Acid	1.0 ± 0.3	2.6± 0.3	0.7 ± 0.1	1.2 ± 0.2	0.001	0.02	0.001

TABLE II : Normal lipid pattern in various phases of estrus cycle - OVIDUCT
(Values are means ± S.E.M. of 10 animals per group).

Parameters	*Proestrus* (I)	*Estrus* (II)	*Metestrus* (III)	*Diestrus* (IV)	*P value* (I–II)	(III–IV)	(II–IV)
Total Lipid	104.3 ± 9.8	100.3 ±11.0	121.6 ±31.4	122.9 ±11.5	N.S.	N.S.	0.05
Total Phospholipid	21.6 ± 5.7	24.4 ± 2.9	23.3 ± 1.5	25.1 ± 2.8	N.S.	N.S.	N.S.
Total Neutral Lipid	82.7 ± 5.5	75.8 ± 4.6	105.3 ± 5.6	97.8 ± 6.3	N.S.	N.S.	0.001
Total Cholesterol	6.1 ± 1.6	6.9 ± 0.8	5.8 ± 0.6	6.9 ± 1.3	N.S.	N.S.	N.S.
Total Glycerides	76.6 ± 4.0	68.8 ± 5.7	99.5 ± 5.9	90.9 ± 4.6	N.S.	N.S.	0.001
Free Cholesterol	2.5 ± 0.4	3.0 ± 0.4	2.2 ± 0.3	3.1 ± 0.5	N.S.	0.02	N.S.
Easter Cholesterol	3.6 ± 0.7	3.9 ± 0.3	3.5 ± 0.2	3.8 ± 1.4	N.S.	N.S.	N.S.
Mono Glycerides	17.3 ± 1.6	18.8 ± 2.9	20.5 ± 1.8	18.2 ± 2.8	N.S.	N.S.	N.S.
Diglycerides	25.3 ± 3.0	28.9 ± 4.0	39.6 ± 3.5	31.8 ± 3.3	N.S.	N.S.	N.S.
Triglycerides	33.9 ± 1.6	21.0 ±10.6	39.3 ± 5.9	40.9 ± 4.7	0.001	N.S.	N.S.
Phosphatidyl Inositol	4.3 ± 0.6	4.9 ± 0.4	3.7 ± 0.4	3.7 ± 0.4	N.S.	N.S.	N.S.
Lysophosphatidyl Choline	0.6 ± 0.6	0.7 ± 0.2	0.6 ± 0.0	0.8 ± 0.3	N.S.	N.S.	N.S.
Sphingomyelin	1.5 ± 0.3	2.2 ± 0.9	1.1 ± 0.5	2.3 ± 0.9	N.S.	N.S.	N.S.
Phosphatidyl Choline	10.5 ± 1.8	11.5 ± 1.3	12.8 ± 3.2	14.0 ± 2.2	NS.	N.S.	N.S.
Phosphatidyl Ethanolamine	3.2 ± 0.5	3.4 ± 0.4	3.6 ± 0.3	3.1 ± 0.3	N.S.	N.S.	N.S.
Phosphatidic Acid	1.4 ± 0.4	1.7 ± 0.6	1.3 ± 0.6	1.1 ± 0.3	N.S.	N.S.	N.S.

TABLE III : Normal lipid pattern in various phases of estrus cycle - UTERUS
(Values are means ± S.E.M. of 10 animals per group).

Parameters	*Proestrus* (I)	*Estrus* (II)	*Metestrus* (III)	*Diestrus* (IV)	*P value* (I–II)	(III–IV)	(II-IV)
Total Lipids	21.9 ± 2.9	23.4 ± 2.9	29.1 ± 2.4	32.0 ± 1.9	N.S.	N.S.	0.01
Total Phospholipid	8.7 ± 0.5	13.9 ± 1.7	11.6 ± 1.7	12.8 ± 1.1	0.001	N.S.	N.S.
Total Neutral Lipid	12.3 ± 3.2	9.5 ± 2.3	17.5 ± 1.1	19.2 ± 2.6	N.S.	N.S.	0.001
Total Cholesterol	4.0 ± 0.7	5.5 ± 1.0	5.1 ± 0.9	5.2 ± 0.5	N.S.	N.S.	N.S.
Total Glycerides	8.3 ± 3.4	4.0 ± 1.7	12.4 ± 1.3	14.0 ± 2.6	N.S.	N.S.	0.001
Free Cholesterol	1.7 ± 0.3	1.8 ± 0.4	1.8 ± 0.3	1.5 ± 0.7	N.S.	N.S.	N.S.
Ester Cholesterol	3.5 ± 2.3	3.7 ± 1.4	3.2 ± 1.0	3.7 ± 0.9	N.S.	N.S.	N.S.
Mono Glycerides	2.1 ± 0.6	1.0 ± 0.2	2.4 ± 0.5	2.3 ± 0.3	N.S.	N.S.	0.001
Diglycrides	2.3 ± 0.4	0.8 ± 0.2	1.7 ± 0.6	2.4 ± 0.3	N.S.	N.S.	N.S.
Triglycerides	3.9 ± 0.5	2.3 ± 1.8	8.3 ± 1.2	9.3 ± 2.7	N.S.	N.S.	0.01
Phosphatidyl Inosital	1.1 ± 0.4	2.0 ± 0.5	1.7 ± 0.3	1.3 ± 0.5	0.05	N.S.	N.S.
Lysophosphatidyl Choline	0.2 ± 0.0	0.3 ± 0.2	0.3 ± 0.1	0.2 ± 0.1	N.S.	N.S.	N.S.
Sphingomyelin	0.2 ± 0.2	0.3 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	N.S.	N.S.	N.S.
Phosphatidyl Choline	5.0 ± 1.1	8.7 ± 1.2	7.0 ± 0.5	9.0 ± 0.5	0.01	0.001	N.S.
Phosphatidyl Ethanolamine	1.4 ± 0.6	1.5 ± 0.4	1.3 ± 0.3	1.1 ± 0.3	N.S.	N.S.	N.S.
Phosphatidic Acid	0.9 ± 0.8	1.1 ± 0.3	1.2 ± 0.3	1.0 ± 0.6	N.S.	N.S.	N.S.

TABLE IV : Normal lipid pattern in various phases of estrus cycle - VAGINA
(Values are means ± S.E.M. of 10 animals per group).

Parameters	*Proestrus*	*Estrus*	*Metestrus*	*Diestrus*	*P value*		
	(I)	*(II)*	*(III)*	*(IV)*	*(I–II)*	*(III–IV)*	*(II–IV)*
Total Lipid	30.8 ± 4.6	33.2 ± 2.3	32.1 ± 4.6	35.5 ± 3.5	N.S.	N.S.	N.S.
Total Phospholipid	15.1 ± 2.5	16.8 ± 1.4	13.4 ± 1.5	10.3 ± 3.2	N.S.	N.S.	0.01
Total Neutral Lipid	15.7 ± 1.1	16.3 ± 0.9	18.6 ± 2.0	25.2 ± 6.4	N.S.	N.S.	0.05
Total Cholesterol	8.0 ± 0.6	8.4 ± 1.0	9.6 ± 0.8	9.4 ± 0.4	N.S.	N.S.	N.S.
Total Glycerides	7.7 ± 0.5	7.9 ± 0.5	9.0 ± 0.8	15.7 ± 2.6	N.S.	0.01	0.001
Free Cholesterol	2.2 ± 0.4	2.6 ± 0.5	2.9 ± 0.8	2.4 ± 0.5	N.S.	N.S.	N.S.
Ester Cholesterol	5.8 ± 0.4	5.8 ± 0.9	6.7 ± 0.5	7.0 ± 0.6	N.S.	N.S.	N.S.
Mono Glycerides	1.4 ± 0.3	1.3 ± 0.5	1.4 ± 0.3	5.2 ± 1.7	N.S.	0.01	0.01
Diglycerides	1.6 ± 0.8	1.5 ± 0.9	1.0 ± 0.4	3.5 ± 0.6	N.S.	N.S.	0.02
Triglycerides	4.6 ± 0.4	5.1 ± 2.1	6.6 ± 0.7	7.0 ± 2.1	N.S.	N.S.	N.S.
Phosphotidyl Inosital	2.4 ± 1.2	2.1 ± 0.7	1.5 ± 0.4	1.1 ± 0.3	N.S.	N.S.	0.05
Lysophosphatidyl Choline	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.5 ± 0.3	N.S.	0.05	N.S.
Sphingomyelin	0.9 ± 0.4	0.9 ± 0.5	0.3 ± 0.2	1.1 ± 0.3	N.S.	0.01	N.S.
Phosphatidyl Choline	8.5 ± 2.3	10.5 ± 2.0	8.5 ± 0.9	6.0 ± 2.3	N.S.	N.S.	0.02
Phosphatidyl Ethanolamine	2.5 ± 0.4	2.6 ± 0.3	1.6 ± 0.4	1.2 ± 0.7	N.S.	N.S.	0.01
Phosphatidiic Acid	0.45 ± 0.2	0.4 ± 0.1	1.1 ± 1.1	0.2 ± 0.1	N.S.	N.S.	N.S.

The concentration of total lipids as well as their classes of lipids in proestrus and estrus phases did not show marked variations except triglycerides, which showed a decrease in estrus phase to that of proestrus phase ($P<0.001$).

In the same way, the concentration of lipids in metestrus and diestrus phases did not show much alteration except a rise in free cholesterol during diestrus phase to that of metestrus ($P<0.01$). But a slight increase in total lipids during diestrus was observed compared to estrus phase ($P<0.05$) due to total glycerides ($P<0.001$).

Table III presents the normal pattern of uterine lipids during various phases of the estrus cycle. A marked rise in total phospholipid in estrus phase to that of proestrus phase ($P<0.001$) was contributed by phosphatidyl choline ($P<0.01$) and phosphatidyl inositol plus-serine ($P<0.05$). Other classes of lipids did not show marked alterations. Phosphatidyl choline was more in diestrus phase than in metestrus phase ($P<0.001$). Mono- and triglycerides in diestrus phase were comparatively more than in estrus phase ($P<0.001$) due to glycerides ($P<0.01$).

Table IV depicts the vaginal lipid pattern in estrous cycle.

Though the concentration of total lipids in estrous phase was more, compared to proestrus phase, there was no significant change in their classes of lipids.

The concentration of total lipids during diestrus phase was more due to glycerides ($P<0.01$), especially due to monoglycerides ($P<0.01$). But, a decrease in total phospholipids in diestrus phase was contributed by sphingomyelin ($P<0.01$) and lysophosphatidyl choline ($P<0.05$).

Compared to the concentrations of lipids in diestrus to that of estrous phase, a marked decrease in total phospholipids in diestrus phase was due to phosphotidyl ethanolamine ($P<0.01$), phosphatidyl inositol plus serine ($P<0.05$) and phosphatidyl choline ($P<0.02$). However, a rise in total glycrides in diestrus phase was due to mono - ($P<0.01$) and diglycerides ($P<0.02$). Free and esterified cholesterol were not significantly altered throughout the cycle.

DISCUSSION

In normal cycling rate, the secretion of follicle stimulating hormone (FSH), luteunizing hormone (LH) and prolactin (PRL) was found to be higher in proestrus phase than in other phases of the estrous cycle (3,7), which in turn affects the secretion of estrogen and progesterone (12,13). The proliferation and the metabolic activity of the ovarian cells depend upon the secretion of estrogen to a greater extent and progesterone to a lesser

extent (10). However, the secretory activity and the luteunization of the ovarian cells depend very much upon the secretion of progesterone and luteunizing hormone (4). Increased cellular proliferation and metabolic activity may be the reason for an accumulation of glycerides and depletion of phospholipids in proestrus phase. The ovarian lipid pattern in estrous phase was suggestive of a direct action of LH and indirect action of oestrogen as reported by previous workers (10). Excepting FSH, other gonadal and gonadotrophic hormones have been found at a lower ebb in metestrus (7). This may be the reason for the picture that is obtained in the lipid pattern of ovarian tissues at this stage. Ovarian lipids during diestrus phase were more or less similar to that of estrous phase which could be due to the presence of a second peak of estrogen and progesterone in this phase (10).

Histochemical and biochemical studies show the concentration of uterine lipids to be maximum during diestrus phase and minimum during estrous phase (5). It has been demonstrated that administration of progesterone increased the total lipids while estrogen depleted the same in ovariectromized animals (9,14). The accumulation of lipids during diestrus may be due to the high potency of progesterone. In the same way, the lower concentration of lipids during estrous phase may be due to the high potency of estrogen. Estrogen administration has been shown to stimulate the phospholipid synthesis (1,14). Phospholipids were proportionately related to glycerides (6). A rise in phospholipids with a fall in glycerides during estrous phase and vice versa during diestrus phase may be due to the sequencial secretion of estrogen during estrous and progesterone during diestrus phases. Uterine lipid probably serves as an energy source and is utilised during early pregnancy to meet the metabolic requirements of the blastocyst and nidation (5). Therefore, the rise in glycerides during secretary phase may be due to the increased synthesis of fatty acids to esterify them to form diglycerides and triglycerides to provide energy for future need (17).

The fluctuation of lipids in oviduct and vagina, as observed by earlier workers (8) though minimal, was found to be similar to that of uterus. This may be because, uterus, vagina and oviduct are under the control of gonadal hormones. The slightly changed pattern seen in ovaries may be because they are under the dual control of gonadotrophins and gonadal hormones (12). The minimal amount of response observed in oviduct and vagina may be due to less number of receptor sites available in these tissues (2,16). Nevertheless, the role played by tissue specificity can not be ignored.

The present study points out that not only the uterine lipids but the ovarian, oviductal and vaginal lipids show alteration in their pattern as a consequence of fluctuation in gonadal and gonadotrophic hormones.

REFERENCES

1. Aizawa, Y. and Hereshi-Koga. Action of estradiol on the mechanism of phospholipid synthesis in rat uterus. *A. Rep. Tokyo Cell. Pharm. Tohaku.*, **17** : 151-156, 1969.
2. Alger, M., E.E. Banlien and E. Milgrom. An investigation of progesterone receptors in guineapig vagina uterine cervix, mammary glands pituitary and hypothalamus. *Endocrinology.*, **94** : 161-167, 1974.
3. Amenomori, Y., C. L. Chen and J. Maites. Serum prolactin levels in rats during different reproductive states. *Endocrinology*, **86** : 506-510, 1970.
4. Armstrong, D. T. III Hormones and Reproduction. *Rec. Pro. Hor. Res.*, **24** : 255-319, 1968.
5. Beall, J. R. Uterine lipid metabolism. A review of the literature. *Comp. Biochem. Physiol.*, **42B** : 175-195, 1972.
6. Biswas, R. and A. M. Mukherjee. Tissue lipid pattern during estrous cycle of rat. *Indian J. Med. Res.*, **61** : 1835-1840, 1973.
7. Butcher R. L., W. E. Collins and N. W. Fugo. Plasma concentration of LH, FSH, prolactin, progesterone estradiol through the 4-day cycle of the rat. *Endocrinology*, **94** : 1704-1710, 1974.
8. Chiari, D.J. and M.A. Manguella-Dicoun. Cyclic modifications of lipids in vaginal cytology in the pubertal rat *Bull. Assoc. Anat.*, **58** (163) : 823-832, 1975.
9. Goswami, A., A. B. Kar and S. R. Chowdhury. Uterine lipid metabolism in mice during the estrous cycle: effect of ovariectomy and replacement therapy. *J. Reprod. Fert.*, **6** : 287, 1963.
10. Guraya, S.S. Histochemical observations on the lipid changes in rat corpora lutea during various reproductive states after treatment with exogenous hormones. *J. Reprod. Fertil.*, **43** : 67-75, 1975.
11. Manimekalai, S., E. Umapathy and P. Govindarajulu. Hormonal influence on uterine lipids. *Hormone Res.*, **10** : 213-221, 1979.
12. Marsh, J.M.Z., G. Telegdy and K. Savard. Effects of gonadotropins on steroidogenesis in rat ovaries at diestrus. *Nature (Lond.)*, **212** : 950-952, 1966.
13. Motta, M., F. Piva and L. Martini. The hypothalamus as the center of endocrine feedback mechanisms. In "The Hypothalamus" by Martini, L., M. Motta and F. Fraschini. New York Academic Press Inc., 1970
14. Mukherjea, M. and R. Biswas. Effect of ovariectomy and replacement therapy on the tissue lipid pattern in rats. *Endocrinologie*, **66**(2) : 113-121, 1975.
15. Page, E. W., C.A. Ville and D.B. Ville. In "Human Reproduction" W. V. Saunders Company., Philadelphia, London, Toronto p. 54, 1972.
16. Panerstein, C. J. In "The Fallopian Tube", A reappraisal Lea and Febiger, Philadelphia, 1974.
17. Spooner, P. M. and J. Gorski. Early oestrogen effects on lipid metabolism in the rat uterus. *Endocrinology*, **91**(5) : 1273-1284, 1972.