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Does high polyunsaturated free fatty acid level at the feto-maternal interface alter steroid hormone message during pregnancy?

C. Benassayag,[1] V. Rigourd,[1] T. M. Mignot,[1] J. Hassid,[2] M. J. Leroy,[1] B. Robert,[1] C. Civel,[1] G. Grangé,[1] E. Dallot,[1] J. Tanguy,[1] E. A. Nunez,[2] F. Ferré[1]

[1]INSERM U361 Maternité Baudelocque, Université René Descartes, 75014 Paris, France
[2]Laboratoire de biochimie endocrinienne, Faculté de Médecine Xavier Bichat, Université Denis Diderot, 75870 Paris, France

Summary Polyunsaturated fatty acids (PUFA) are important in pregnancy, fetal development and parturition. We measured free fatty acids (FFA), albumin and alpha-fetoprotein (AFP) in the maternal and fetal circulations of women undergoing elective Caesarean section at term. We also studied the impact of PUFAs on estrogen (ER) and progesterone receptors (PR) binding properties in vitro in the myometria of pregnant women and ex vivo in human myometrial cells in culture.

FFA in intervillous blood (I) (feto-maternal interface) and maternal peripheral blood (M) were similar, while those in the umbilical vein (V) and arteries (A) were 2–4 fold lower ($P<0.001$). PUFA levels were low in M and 3 fold higher in I, A and V ($P<0.001$); consequently C20:4 and C22:6 were most abundant in intervillous space.

Albumin was uniformly distributed throughout the maternal-fetal unit, but there was a transplacental gradient in AFP. The AFP in the intervillous space had a special conformation (less immuno-reactive, more anionic), suggesting loading with PUFA.

Physiological concentrations of C20:4 stimulated estradiol binding, but inhibited progestin binding. C20:4 inhibited progesterone binding by decreasing the number of binding sites, with no change in apparent affinity, in vitro in myometrial tissue and ex vivo in myometrial cells. Thus PUFA may modulate the steroid hormone message, so that the high C20:4 concentration at the maternal-fetal interface at term may help amplify the estrogen signal and inhibit the progesterone signal.

INTRODUCTION

The relationship between lipid metabolism of the mother, the placenta and the fetus is vital for controlling many aspects of normal gestation, fetal development and parturition.[1,2] There is no clear correlation between the changes in the human maternal peripheral plasma concentrations of effectors of uterine activity and the onset of labor. This has led to a suggestion that human parturition is initiated by local paracrine/autocrine interactions between the various tissues of the fetal-maternal interface. The hemomonochorial nature of the human placenta means that the chorial villi are bathed in maternal blood in the intervillous space, at term. This space is delineated by the fetal epithelium, the syncytiotrophoblast and is in direct contact with the maternal decidua. Almost all transfers between the maternal and fetal blood streams takes place at this point in the placenta.[3]

Many factors are released at the fetal-maternal interface, including steroid hormones and essential fatty acids. The demand of the fetus for free fatty acids is particularly high in the last few weeks of pregnancy. Fatty acids, especially the polyunsaturated free fatty acids (PUFAs) act as cell signalling molecules as well as a source of energy and as structural elements in cells, and as such, they are crucial for mediating immunological, metabolic and endocrine signals within the uterine-fetal-placental unit. PUFAs have pleiotropic effects: these factors act as immunomodulators in the local regulation of maternal cell-mediated immunity.[4–7] They may also influence the

Correspondence to: C. Benassayag. Tel.: +33 1 43 26 28 26; Fax: +33 1 43 26 44 08; E-mail: u361@cochin.inserm.fr.

proliferation of smooth muscle cells by acting as growth factors.[8] Lastly, they are precursors of cell signal molecules like the eicosanoids. And there is now good evidence that they regulate membrane signal transduction pathways[9] and are involved in the action of steroid hormones. Thus, PUFAs act as endogenous modulators of the activities of the key enzymes of steroid metabolism[10] and modify the function of certain plasma steroid binding proteins.[11–14] It also seems likely that PUFAs are involved in the feedback control of steroid action by modulating the binding of estrogen, progesterone and glucorticoid to their intracellular receptors.[14–19]

Although these data illustrate the considerable impact of essential fatty acids on the multifactorial processes involved in pregnancy and parturition, little information is available on the essential fatty acid status of the maternal intervillous blood and the potential of PUFAs to influence the steroid receptor sensitivities in the adjacent myometrium of pregnant women. The first goal of this study was to evaluate the quantity and spectrum of free fatty acids (FFA), and that of their plasma carrier proteins, albumin and alpha-feto-protein (AFP) at the fetal-maternal interface, and to compare these profiles with those in the peripheral maternal and fetal circulations.[20] We have also assessed the impact of physiological concentrations of PUFAs on the binding properties of estrogen (ER) and progesterone receptors (PR) in the myometrium of pregnant women under two conditions: first in vitro in the myometria of pregnant women who were near to term and underwent elective Caesarean section, and ex vivo in human myometrial cells in culture.

METHODS

Blood and tissue samples

This study was approved by the Consultative Committee for the Protection of Persons in Biomedical Research, Cochin, Paris (France).

Blood samples were collected during elective Caesarean section from 26 women (17 Europeans, 9 Africans) with uncomplicated pregnancies (38–40 weeks pregnancy), in good health, normotensive and on no medication. Cord blood samples were taken during delivery before clamping of the cord with the placenta still attached to the uterus. Maternal peripheral blood at the time of delivery was taken from the antecubital vein, and the blood in the intervillous space was collected from the basal plate of the placenta. Blood samples were collected into tubes containing ethylene diamine tetraacetic acid, in an ice bath. Plasma was separated from the red blood cells by centrifugation (2000 rpm/20 min at 4°C) within 30 min of sample collection, and stored at –80°C.

Tissue samples and cytosol preparation: myometrial biopsies were obtained from pregnant women (n=8) with normal uncomplicated pregnancies who were delivered by elective Cesarean section. The Cesarean section was done for previously diagnosed cephalopelvic disproportion before the onset of labor between weeks 38–40 of pregnancy. Normal non-pregnant myometrial specimens were obtained from cyclic women (n=10) aged 39–51 years who underwent hysterectomy for benign gynecological indications. Tissue samples were excized from normal muscle in areas free of macroscopically visible abnormalities (n=10). The biopsies were immediately frozen in liquid nitrogen and stored at –80°C. Cytosol was prepared as previously described.[14–16]

Binding properties of estrogen (ER) and progesterone (PR) receptors

The cytosol samples were treated with a dextran-coated charcoal (DCC 10% NoritA and 0.1% dextran T70) for one hour at 4°C to remove endogenous steroids. PR binding properties in the absence or presence of physiological concentrations of arachidonic acid (1–30 μM) were measured by incubating an aliquot of charcoal-treated cytosol (500 μg proteins) with 0.4–20 nM (17α-methyl ^{3}H) R5020 (Promegestone) (85 Ci/mmol NEN, UK) for 16 h at 4°C, with or without excess unlabeled progesterone. Similar experiments were performed to measure myometrial ER binding properties with 0.25–12 nM 17β-(2,4,6,7 ^{3}H) Estradiol (85–110 Ci/mmol Amersham, France), with or without excess unlabeled of diethylstilbestrol. Bound and free hormone fractions were measured by the DCC method.[14–16] The affinity constant (Ka) and binding capacity (equivalent to total number of binding sites: fmoles/mg protein) were estimated by Scatchard graphical analysis after subtraction of the non-specific binding.

Qualitative and quantitative analysis of free fatty acid

This was performed by gas chromatography as previously described.[20]

Immunoassay of human albumin, alpha-fetoprotein (AFP)

Both proteins were measured by the Laurell rocket electroimmunodiffusion technique[21] using polyclonal anti-human albumin or anti-human AFP antibodies (DAKO, Denmark). Crossed-immunoelectrophoresis was performed as previously described.[22–23]

Myometrial cell cultures

Biopsies of myometrium from pregnant women were placed in Dulbelcco's modified Eagle's medium (DMEM)

supplemented with 1000 U/ml penicillin and 100 μg/ml streptomycin and transported immediately to the laboratory. Myometrial cells were prepared by the explant method and cells were cultured in DMEM supplemented with antibiotics and 10% fetal calf serum.[24] The experiments described in this report were performed during subcultures.[4–5] The confluent cells were placed in a serum-free medium for 72 h to allow expression of smooth muscle markers: α-smooth muscle actin, myosin heavy chain isoforms (SM1, SM2) and desmin.[24] The effect of arachidonic acid (C20:4) on progesterone receptor (PR) status of the myometrial cells was examined by incubating cells for 24 h or 48 h in serum-free medium with or without C20:4 (1 μM). The cells exposed to C20:4 were harvested with trypsin-EDTA (0.05–0.02%), collected by centrifugation and sonicated in homogenisation buffer (TRIS-glycine 10% glycerol pH 7.4). The homogenates were centrifugated at 105 000 g for one hour to obtain cytosolic PR recentors. Cell viability determined by the trypan blue exclusion method was always greater than 95%.

Statistical analysis

Results are expressed as means±S.E.M. Data were analyzed by analysis of variance (ANOVA) for repeated measures. Dunnett's test was used for multiple comparisons of means.

RESULTS AND DISCUSSION

Quantity and spectrum of FFA

Figure 1A shows that the plasma FFA concentrations in intervillous blood are similar to those in the maternal peripheral blood, but they are 2–4 fold higher than those of the FFA in fetal blood. Qualitative analyses of the FFA in each of the compartments of the maternal-fetal unit indicated that there are major differences in the distribution of FFA near to term. The most striking differences were in the polyunsaturated free fatty acids (PUFA). These were low in the maternal peripheral blood and 3 fold higher in intervillous blood and in cord blood (Fig. 1B). Consequently, the concentrations of arachidonic acid (C20:4) and docosahexaenoic acid (C22:6) in the fetal blood were the same as those in the maternal peripheral blood (Fig. 1C). But the concentrations of these two polyunsaturated fatty acids in the intervillous space were 3 times higher than in the maternal peripheral plasma or in the cord blood. Thus, the PUFA status of the maternal intervillous blood does not seem to be an average of the

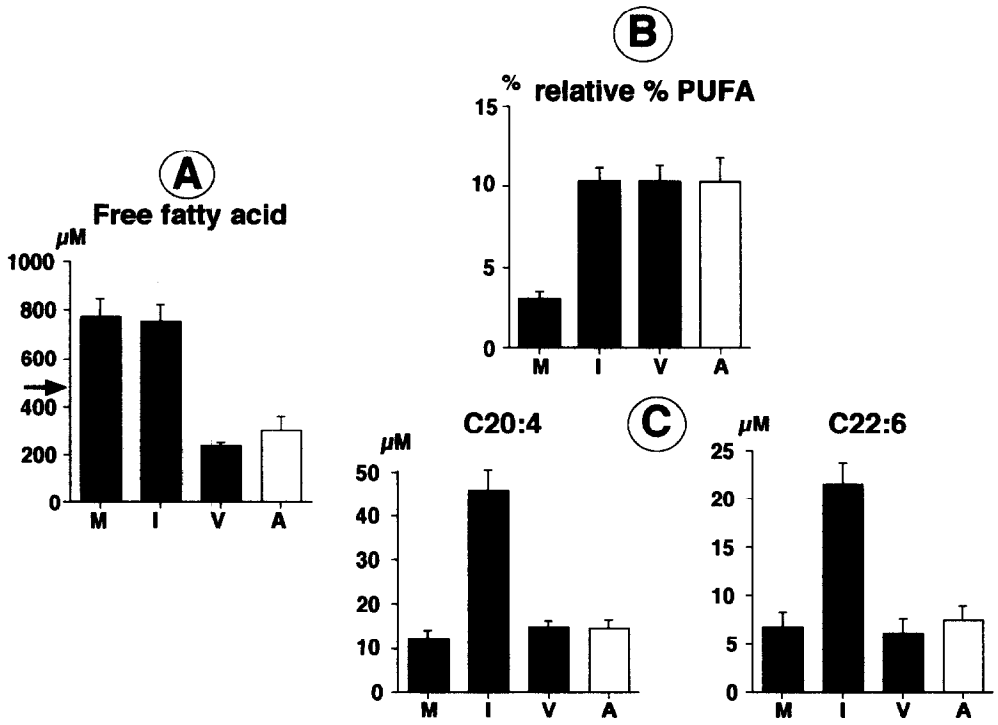

Fig. 1 Plasma free fatty acid concentrations (A), polyunsaturated FFA levels (B) and concentrations (C) in the maternal vein (M), intervillous space (I), umbilical vein (V) and umbilical arteries (A) were measured by gas chromatography. Arrow indicates the FFA concentration of plasma from non-pregnant women. Values are means±S.E.M. from 26 pregnant women at term.

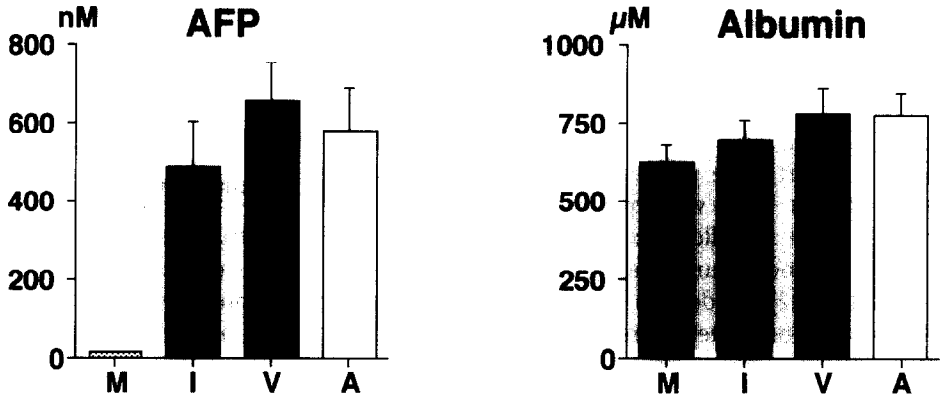

Fig. 2 Immunoassays of albumin and alpha-fetoprotein in the maternal vein (M), intervillous space (I), umbilical vein (V) and arteries (A). The concentrations of albumin and AFP were measured by rocket immunoelectrophoresis using anti-human albumin or anti-human AFP antibodies (0.5%).

concentrations in the maternal peripheral and fetal circulations, but has its own specific characteristics.[20]

Free fatty acid plasma carrier proteins, albumin and AFP

The distribution of fatty acids and their uptake into the diffusion spaces may be mediated by plasma carrier proteins, such as albumin and AFP. When both albumin and AFP are present in the plasma as they are in pregnancy, the albumin tends to bind saturated FFA, while AFP binds PUFA with high affinity. Figure 2 shows that albumin was uniformly distributed in all the various compartments of the maternal-fetal unit, but there was a transplacental gradient in the concentration of AFP. Thus, the AFP concentration in the maternal peripheral blood is very low, but it is 500–1000 fold higher in cord blood. These data are not surprising, because AFP is mainly produced by the fetal liver. The most striking feature is the high concentration of AFP in the maternal intervillous blood.[20] The immuno-electrophoretic behavior of AFP in intervillous blood is also rather informative (Fig. 3). The AFP from the intervillous space was more anionic than the AFP from the umbilical fetal vein and arteries, and was considerably less immunoreactive towards specific anti-AFP antibodies. This is more clearly seen by superimposing the figures one on the other (Fig. 3). Our earlier in vitro and in vivo studies on purified AFP from the rat and human provided reference parameters that indicate that the AFP in the intervillous space has a specific conformation, probably because the fetal protein is highly loaded with PUFA at term.[13,22]

Fig. 3 Crossed immunoelectrophoresis of AFP from umbilical arteries (A), umbilical vein (V) and intervillous space (I). The method involves: First dimension electrophoresis of AFP from V (175 ng), A (185 ng) and I (18 ng) in 1% agarose gel. Second dimension: on 1% agarose gel containing 0.5% anti-human AFP antibody (Dako). The gels were dried and stained with Coomassie blue. Superimposed patterns of human AFP, one from the intervillous blood space, and the others from cord blood (V,A), are also shown (I/V/A).

Impact of PUFAs on the binding properties of estrogen (ER) and progesterone (PR) receptors in myometrium of pregnant women

There is compelling evidence that estrogens and progestin, hormones produced in large amounts by the placenta during pregnancy, play a central role in modulating the growth, differentiation and function of target tissues like the myometrium.[25] During pregnancy, the myometrium gradually changes from being dominated by progesterone, which leads to hypocontractility, to being influenced by estrogen, and this state is much more favorable for contraction (Fig. 6)[26,27] As there is no clear correlation between the changes in steroid concentration in the maternal peripheral plasma and the onset of labor, it was suggested that the altered steroid hormone message resulted from a change in the myometrial steroid receptor status. We wondered whether the large amounts of polyunsaturated FFA at the maternal-fetal interface in term

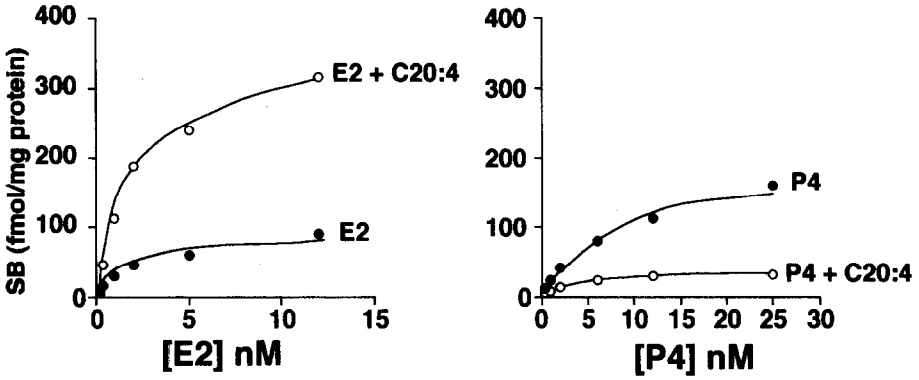

Fig. 4 Opposite effect of arachidonic acid (C20:4) on the ER and receptors in pregnant myometria. The saturation kinetics for ER and PR receptors in the absence or presence of C20:4 were analyzed by incubating 500 μg cytosol proteins overnight at 4°C with increasing concentrations of ^{3}H 17β-estradiol or ^{3}H Promegestone without or with C20:4 (30 μM). Free steroid was removed by the DCC method. Specific binding was calculated after subtraction of non-specific steroid binding. These data are representative of three experiments. Each point is the mean of triplicate assays.

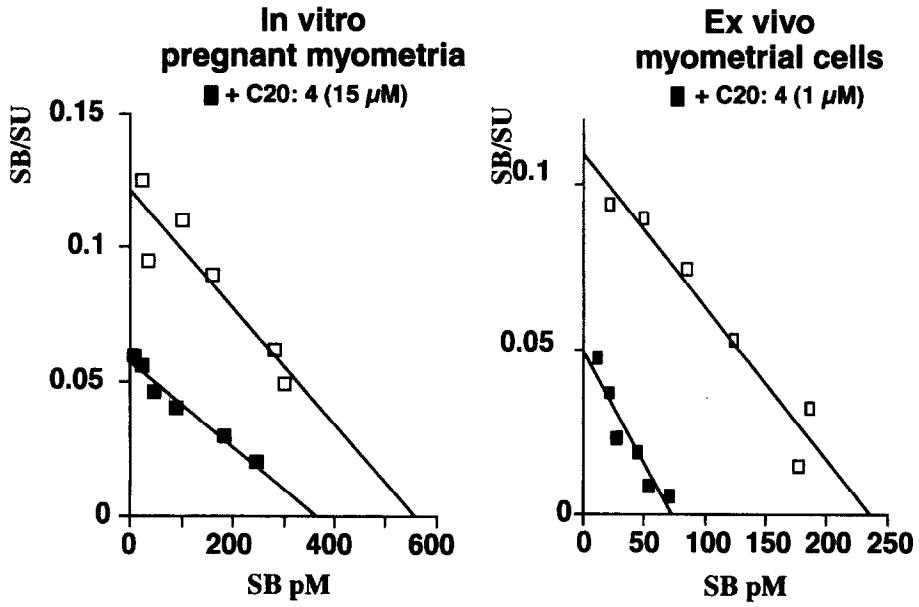

Fig. 5 Effects of C20:4 on PR binding parameters in vitro in the myometria of pregnant women and ex vivo in human pregnant myometrial cells in culture. In vitro experiments (left): the incubation conditions were as shown in Figure 4. The effect of 15 μM C20:4 is shown. Ex vivo experiments (right): cells were cultured as described in Methods. The effect of C20:4 on the PR status of myometrial cells was examined by incubating cells for 48 h in serum-free medium with or without 1 μM C20:4. These data are representative of two cell culture experiments. Each point is the mean of triplicate assays.

pregnancy changed the sensitivity of the adjacent myometrium to steroid hormones.

There appears to be no real consensus about the steroid receptor status of the myometrium during human gestation.[28–30] Studies from our laboratory indicate that PR and ER receptors are present in the full-term myometrium, but there may be less receptor mRNA and steroid receptor binding protein, indicating fewer PR and ER in the pregnant myometrium than in the non-pregnant myometria ($P < 0.01$).

The impact of physiological concentrations of arachidonic acid (C20:4) on specific binding of 17β-estradiol and progesterone to the ER and PR receptors in these myometria are shown in Figure 4. C20:4 has opposite effects on the receptivities of the ER and PR receptors in pregnant myometria. It stimulates estradiol binding and

inhibits progestin binding. These data are in agreement with data obtained for other target tissues.[15–17,19]

The effect of C20:4 on the binding parameters of progesterone receptors was then tested in vitro the myometrium of pregnant women (Fig. 5 left) and ex vivo in human pregnant myometrial cells in culture (Fig. 5 right). These myometrial cells keep their smooth muscle contractile protein phenotype under our culture conditions. These myometrial cells in culture also bear both ER (Ka= 0.2±0.05 10^9 M^{-1}, n= 203±50 fmoles/mg proteins) and PR receptors (Ka= 0.3±0.1 $10^9/M^{-1}$, n= 276±60 fmoles/mg proteins). The effects of C20:4 in vitro in the myometrial tissue and ex vivo in myometrial cell in culture were similar. Thus, Scatchard analysis indicated that C20:4 (15 μM) inhibited progesterone binding to its PR in myometrial tissue, leading to fewer binding sites, with no apparent change in the affinity constant, suggesting that the inhibition was non-competitive. Similarly, myometrial cells from pregnant women incubated with 1 μM C20:4 had fewer progesterone binding sites than cell controls, without any change in the apparent affinity constant after 24 h (data not shown) or 48 h (Fig. 5). These decreases in the apparent number of PR do not seem to be due to the production of prostaglandin or peroxide, since neither indomethacin, nor the antioxidant BHT restored the binding capacities of the PR, and the MDA concentration did no vary significantly in our culture conditions (data not shown).

CONCLUSION

Our overall interpretation of these results is that PUFA may modulate the steroid hormone message, so that the high concentration of C20:4 at the maternal-fetal interface at term may help to amplify the estrogen signal and reduce or block the progesterone signal (Fig. 6). Thus, local variations in the concentrations of PUFA may be particularly important for increasing the contractile action of 17β-estradiol, while reducing the relaxing influence of progesterone on the myometrium, so providing conditions favoring parturition. Further studies on the influence of PUFA on the profiles of the ER and PR subtypes could also shed light on several long standing questions about the uterotropic response to steroid hormones that are mediated via steroid-regulated genes.

In conclusion, given the known effects of PUFA, AFP and receptor-steroid interactions on cell growth and immunomodulation,[14–18,31–32] we suggest that subtle changes in the concentrations of these factors, influence immune reactions at the feto-maternal interface. These changes enable the fetal allograft to survive. But they may also help to trigger parturition by their action on the adjacent maternal myometrium, thereby participating in the chain of events by which a new human being enters the world.

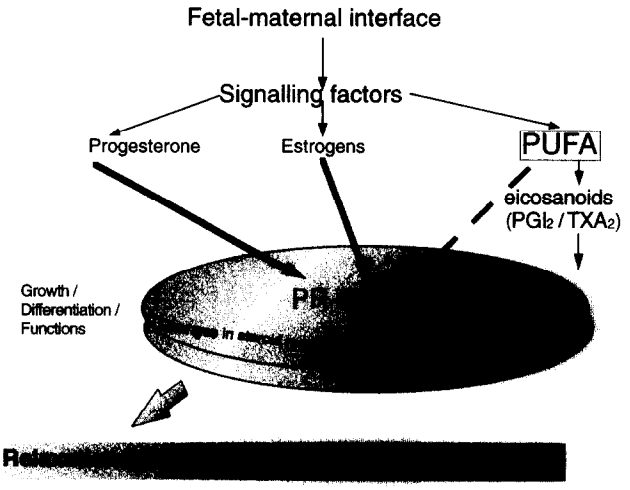

Fig. 6

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REFERENCES

1. Coleman R. A. The role of the placenta in lipid metabolism and transport. *Semin Perinatol* 1989; **13**: 180–191.
2. Crawford M. A., Doyle W., Williams G., Drury P. J. The role of fats and EFAs for energy and cell structures in the growth of fetus and neonate. In: The Role of Fats in the Human Nutrition. Vergroesen A. J., Crawford M. A. (Eds). New York: Academic Press 1989; 81–115.
3. Kaufman P., Burton G. Anatomy and genesis of the placenta. In: The Physiology of Reproduction. Knobil E. K., Neill J. D. (Eds). New York: Raven Press Ltd, 1994; 441–484.
4. Yaqoob P., Calder P. C. The effects of fatty acids on lymphocyte functions. *Int J Biochem* 1993; **25**: 1705–1714.
5. Khair-El-Din T. A., Sicher S. C., Vazquez M. C., Wright W. J., Lu C. Docoshexaenoic acid, a major constituent of fetal serum and fish oil diets, inhibits INFY-induced Ia-expression by murine macrophage in vitro. *J Immunol* 1995; **154**: 1296–1306.
6. Lalmanach Girard A. C., Guillaumin M. et al. Inhibition of induced lymphocyte proliferation by lipid and protein components of the syncytiotrophoblast plasma membrane. *AJRI* 1995; **33**: 182–189.
7. Siiteri P. K., Stites D. Immunologic and endocrine interrelationship in pregnancy. *Biol Reprod* 1982; **26**: 1–14.
8. Tessier C., Fayard J. M., Cohen H., Pageaux J. F., Lagarde M., Laugier C. Docosahexaenoic acid is a potent inhibitor of rat uterine stromal cell proliferation. *Biochem Biophys Res Commun* 1995; **207**: 1015–1021.
9. Graber R., Sumida C., Nunez E. A. Fatty acids and cell signal transduction. *J Lipid Mediators Cell Signalling* 1994; **9**: 91–116.
10. Nunez E. A., Haourigui M., Martin M. E., Benassayag C. Fatty acids and steroids hormone action. *Prostaglandins Leukot Essent Fatty Acids* 1995; **52**: 185–190.

11. Martin M. E., Benassayag C., Nunez E. A. Selective changes in binding and immunological properties of human corticosteroid binding globulin by free fatty acids. *Endocrinology* 1988; **123**: 1178–1186.
12. Haourigui M., Sakr S., Martin M. E. et al. Postprandial free fatty acids stimulate activity of human corticosteroid binding globulin. *Am J Physiol* 1995; **269**, 32: 1067–1075.
13. Haourigui M., Thobie N., Martin M. E., Benassayag C., Nunez E. A. In vivo transient rise in plasma free fatty acids alters the functional properties of alpha-fetoprotein. *Biochim Biophys Acta* 1992; **1125**: 157–165.
14. Haourigui M., Vallette G., Martin M. E., Sumida C., Benassayag C., Nunez E. A. In vivo effect of free fatty acids on the specific binding of glucocorticosteroids to Corticosteroid binding globulin and liver receptors in immature rats. *Steroids* 1994; **59**: 46–54.
15. Benassayag C., Vallette G., Hassid J., Raymond J. P., Nunez E. A. Potentiation of estradiol binding to human tissue proteins by unsaturated nonesterified fatty acids. *Endocrinology* 1986; **118**: 1–7.
16. Vallette G., Christeff N., Bogard C., Benassayag C., Nunez E. A. Dynamic pattern of estradiol binding to uterine receptors of the rat. *J Biol Chem* 1988; **263**: 3639–3645.
17. Kato J. Arachidonic acid as a possible modulator of estrogen, progestin, androgen and glucocorticoid receptors in the central and peripheral tissues. *J Steroid Biochem* 1989; **34**: 219–227.
18. Vallette G., Vannet A., Sumida C., Nunez E. A. Modulatory effects of unsaturated fatty acids on the binding of glucocorticoids to liver glucocorticoid receptors. *Endocrinology* 1991; **129**: 1363–1369.
19. Benassayag C., Vitte C., Hassid J. et al. Phytoestrogens and free fatty acids nutrional factors, as modulators of the estrogen action in target cells. Proceedings of the XVI International cancer congress. (Eds) Monduzzi. *New Delhi* 1994; **1**: 609–613.
20. Benassayag C., Mignot T. M., Haourigui M. et al. High polyunsaturated fatty acid, thromboxane A2 and alphafetoprotein concentrations at the human feto-maternal interface. *J Lipid Res* 1997; **38**: 71–81.
21. Laurell C. B. Electroimmunoassay. *Scand J Clin Lab Invest* 1972; **29**: 21–23.
22. Vallette G., Vranckx R., Martin M. E., Benassayag C., Nunez E. A. Conformational changes in rodent and human alpha-fetoprotein: influence of fatty acids. *Biochim Biophys Acta* 1989; **997**: 302–312.
23. Magne D., Seta N., Lebrun D., Durand G., Durand D. Factors influencing the reaction of alpha-fetoprotein with Concanavalin A and Lens culinaris Agglutin in crossed affino-immuno-electrophoresis. *Clin Chem* 1992; **38**: 1418–1424.
24. Cavaillé F., Fournier T., Dallot E., Dhellemes C., Ferré F. Myosin heavy chain isoform expression in human myometrium: presence of an embryonic nonmuscle isoform in leiomyomas and in cultured cells. *Cell Mol Cytoskel* 1995; **30**: 183–193.
25. Clark J. H., Mani S. K. Actions of ovarian steroid htormones. In: Knobil E., Neill J. D., Ewing L. L., Greenwald G. S., Marke C. L., Pfaff D. W. (Eds). The physiology of Reproduction. New York: Raven Press, 1994; 1011–1059.
26. Ferré F., Janssens Y., Tangy G., Breuiller M., De Pariente D., Cedard L. Steroid concentration in human myometrial and placental tissues at the 39th week of pregnancy. *Am J Obstet Gynecol* 1978; **131**: 500–502.
27. Doualla-Bell Kotto Maka F., Ferré F. Regulation of myometrial contractility in human pregnancy. In: Care, Concern and Cure in perinatal medicine. Koppe J. G., Eskes T. K. A. B., Van Geijn H. P., Wiesenhaan P. S., Ruys J. H. (Eds). New York: The Partenon, 1992; 131–146.
28. Padayachi T., Pegooraro R. J., Rom L., Joubert S. M. Enzyme immunoassay of estrogen and progesterone receptors in uterine and intrauterine tissue during human pregnancy and labour. *J Steroid Biochem Mol Biol* 1990; **37**: 509–511.
29. Perrot-Applanat M., Deng M., Fernandez H., Lelaidier C., Meduri G., Bouchard P. Immunohistochemical localization of estradiol and progesterone receptors in human uterus throughout pregnancy: expression in endometrial blood vessels. *J Clin Endocrinol Metab* 1994; **78**: 216–224.
30. How H., Huang Z. H., Zuo J., Lei Z. M., Spinnato J. A., Rao Ch. V. Myometrial estradiol and progesterone receptor changes in preterm and term pregnancies. *Obstet Gynecol* 1995; **86**: 936–940.
31. Toder V., Blank M., Gold-Gefter L., Nebel L. The effect of alpha-fetoprotein on the growth of placental cells in vitro. *Placenta* 1983; **4**: 79–86.
32. Yamashita T., Nakane A., Watanabe T., Miyoshi I., Kasai N. Evidence that alpha-fetoprotein suppresses the immunological function in transgenic mice. *Biochem Biophys Res Commun* 1994; **201**: 1154–1159.