ACTION OF PROSTAGLANDIN $F_{2\alpha}$ ON THE COURSE OF PREGNANCY AND PLASMA 17β-ESTRADIOL CONCENTRATION IN MICE

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UDC 612.63.014.46:577.175.859

The effect of prostaglandin $F_{2\alpha}$ (2 mg/kg at each stage of development) on the preimplantation development of mice and on their plasma 17β-estradiol concentration was investigated. Prostaglandin $F_{2\alpha}$ inhibited mitotic division in the embryo and reduced the percentage of embryos shedding the zona pellucida. Meanwhile the 17β-estradiol concentration in the peripheral blood plasma fell. Under physiological conditions there was a significant increase in the 17β-estradiol concentration at the blastocyst stage after shedding of the zona pellucida.

KEY WORDS: zona pellucida; blastocyst; 17β-estradiol; prostaglandin $F_{2\alpha}$; mitosis.

It has recently been shown that ovarian steroid hormones and, in particular, 17β-estradiol, are essential for normal development of the preimplantation embryo [2, 4, 8]. Prostaglandins are known to inhibit steroid formation in vivo during pregnancy in rats [7], guinea pigs [2], rabbits [5, 7], sheep [10], and man [12]. However, there is no general agreement as yet regarding the effect of prostaglandins and, in particular, the $F_{2\alpha}$ type, on 17β-estradiol synthesis by pregnant animals in the preimplantation period of development.

The study of the effect of prostaglandin $F_{2\alpha}$ on preimplantation development of the embryo and also on the 17β-estradiol concentration in peripheral blood plasma is of great interest for the possible elucidation of the mechanism of action of prostaglandin $F_{2\alpha}$.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino mice weighing 18-20 g. Females were mated with males in the ratio of 5 : 1; the first day of pregnancy was identified by the presence of a vaginal plug. The animals were given subcutaneous injections of prostaglandin $F_{2\alpha}$, so that one injection of 40 μg (2 mg/kg body weight) coincided with each stage of development (zygote, 2, 4, and 8 blastomeres, morula, blastocyst). The time for the injections was chosen on the basis of data in the literature [13] for the duration of each stage.

TABLE 1. Effect of Prostaglandin $F_{2\alpha}$ on Preimplantation Development of Embryo (4th day of pregnancy)

Experimental conditions	Time of investigation					
	8 a.m.		10 a.m.		5 p.m.	
	number of cells in embryo (M ± m)	percent of embryos without zona pellucida	number of cells in embryo (M ± m)	percent of embryos without zona pellucida	number of cells in embryo (M ± m)	percent of embryos without zona pellucida
Physiological saline	23,27±0,61 (26)	0	29,29±1,97 (34)	8,82	57,28±2,1 (25)	76,0
Prostaglandin $F_{2\alpha}$	19,95±0,47 (22)	0	24,91±0,7 (35)	0	44,67±1,4 (33)	57,58

Legend. Number of embryos studied given in parentheses.

Department of Histology and Embryology, Pediatric Faculty, N. I. Pirogov Second Moscow Medical Institute. Laboratory of Endocrinology, Moscow University. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kupriyanov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 88, No. 10, pp. 468-470, October, 1979. Original article submitted March 28, 1979.

 0007-4888/79/8810-1194$07.50

TABLE 2. Effect of Prostaglandin $F_{2\alpha}$ on 17β-Estradiol Concentration in Peripheral Blood Plasma on 4th Day of Pregnancy

Experimental conditions	Time of investigation			
	10 A.M.		5 P.M.	
	number of animals	estradiol concentration, pg (M ± m)	No. of animals	estradiol concentration, pg (M ± m)
Physiological saline	15	144,75±7,01	15	192,6±18,61
Prostaglandin $F_{2\alpha}$	15	132,2±5,47	15	151,75±7,5

TABLE 3. Effect of Prostaglandin $F_{2\alpha}$ on Pregnancy in Mice

Experimental conditions	number of animals	percent of pregnant animals	Mean number of resorbed embryos on 18th day of pregnancy per mouse	Mean number of embryos surviving to 18th day of pregnancy per mouse
			M±m	
Physiological saline	26	80,77	0,43±0,14	8,52±0,44
Prostaglandin $F_{2\alpha}$	25	64,00	1,80±0,49	6,06±0,64

In the experiments of series I, the ova were flushed out of the uterine cornua on the 4th day of pregnancy at 8 and 10 a.m. and 5 p.m. and treated by Tarkowski's method [15]. The number of cells in the embryo was then counted and the presence of a zona pellucida was noted.

In the experiments of series II, prostaglandin $F_{2\alpha}$ and 0.9% physiological saline were injected during the first 4 days of pregnancy according to the scheme described above. The animals were killed on the 18th day of pregnancy and the number of normal fetuses and number of fetuses undergoing resorption were counted.

In the experiments of series I, blood was taken from the animals on the 4th day of pregnancy at 10 a.m. and 5 p.m. The blood was then centrifuged at 3000 rpm; the resulting plasma was kept at −20°C. 17β-Estradiol was determined in two parallel samples by radioimmunological analysis, using kits from CIS (France). The 17β-estradiol was extracted with 2 volumes of double-distilled methylene chloride from 0.3 ml blood plasma. The blanks did not exceed 2 pg/mo. Antiserum against 17β-estradiol did not bind closely related compounds. Radioactivity was counted in Bray's liquid scintillator with an efficiency of 28-29% on a Nuclear Chicago Mark II counter.

EXPERIMENTAL RESULTS

In the experiments of series I, after subcutaneous injection of prostaglandin $F_{2\alpha}$ in a dose of 2 mg/kg body weight at each stage of preimplantation development during the first 4 days of pregnancy the number of blastocysts still in the zona pellucida (delay in shedding of the zona pellucida) was greater than the number of such blastocysts in the control groups (Table 1). Investigation of the cellular composition of the embryo on the 4th day of pregnancy in mice receiving prostaglandin $F_{2\alpha}$ showed that this dose of the biologically active substance inhibits mitotic activity of the embryo (Table 1).

The results of determination of the 17β-estradiol concentration in the peripheral blood plasma show that under the influence of prostaglandin $F_{2\alpha}$ there was a tendency for the 17β-estradiol level to fall compared with the control at 10 a.m. At 5 p.m. the plasma 17β-estradiol concentration was lower in mice receiving prostaglandin $F_{2\alpha}$ than in the control animals. The difference was statistically significant ($P < 0.05$) (Table 2).

The experiments of series II showed that prostaglandin $F_{2\alpha}$, if injected into the animals during the first 4 days of pregnancy, led to a significant increase in the number of surviving embryos and an increase in the number of resorbed embryos at the end of pregnancy. The number of pregnant animals was greater than in the control (Table 3).

The results showing the inhibitory action of prostaglandin $F_{2\alpha}$ on mitotic activity of the embryos in the preimplantation period of development confirm earlier observations [1]. The same is also true of the effect of prostaglandin $F_{2\alpha}$ on shedding of the zona pellucida.

The experiments of series II showed that injection of prostaglandin $F_{2\alpha}$ during the first 4 days of pregnancy can give rise to irreversible changes in the embryo. This subsequently leads to a decrease in the mean number of fetuses in the mothers before birth and to an increase in the number of resorbed embryos. It can accordingly be postulated that prostaglandin $F_{2\alpha}$, in the dose specified above, has an embryotoxic action. These findings agree with data in the literature [11], when prostaglandin $F_{2\alpha}$ in a dose of 2 mg/kg body weight was injected into the mice during the first 5 days of pregnancy.

Since it is known that shedding of the zona pellucida depends on the 17β-estradiol level [3], this suggests that delay in shedding of the zona pellucida under these experimental conditions under the influence of exogenous prostaglandin $F_{2\alpha}$ is possibly due to a decrease in the concentration of this hormone in the animal's

blood plasma: a tendency for the plasma 17β-estradiol concentration to fall was observed at 10 a.m., and at 5 p.m. the decrease in its level was significant ($P < 0.05$). Other workers also have observed a similar decrease in the 17β-estradiol level [12].

The results of these experiments suggest that normal development of the preimplantation embryo is closely connected with the blood 17β-estradiol concentration on the day before implantation has also been observed in hamsters [9], rats [14], and rabbits [6].

Consequently, besides the possible direct effect of prostaglandin $F_{2\alpha}$ on the embryos, an important role in the mechanism of action of prostaglandins may perhaps also be played by their effect on synthesis of estrogens, changes in whose level may, in turn, affect somehow or other the preimplantation development of the embryo and implantation and subsequent development of the fetus in utero.

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ULTRASTRUCTURE AND DIFFERENTIAL ANTIGENS OF HUMAN T-LYMPHOCYTES DURING EMBRYOGENESIS

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UDC 611.438-013

Thymocytes of human embryos at the 7-8-week stage have irregularly shaped nuclei with one to three distinct nucleoli, characterized by absence of compact chromatin or heterochromatin. The electron-dense cytoplasm of these cells contains polysomes and a few mitochondria. Receptors for sheep's red blood cells and T-antigen are absent on the surface of the cells. In 11-12-week human embryos, the T-lymphocytes are reduced in size, clumps of heterochromatin appear in their nuclei and receptors for sheep's red blood cells (79%) and T-antigen (60%) appear on the surface of the cells. Later, the quantity of compact chromatin in the nuclei of the thermocytes increases and the cells acquire their definitive properties and structure.

KEY WORDS: human embryogenesis; antigens of T-lymphocytes; ultrastructure of T-lymphocytes.

The properties and kinetics of the T-lymphocyte population of human embryos and fetuses have recently been described [1, 3, 6, 11, 12]. Meanwhile the early stages of development of human thymocytes, their fine

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 10, pp. 470-473, October, 1979. Original article submitted January 10, 1979.

0007-4888/79/8810-1196$07.50