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Immunohistochemical demonstration of prostaglandin E-2 in preimplantation mouse embryos

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Summary. An antiserum to prostaglandin (PG) E-2 and indirect immunofluorescence were used to demonstrate immunohistochemically the presence of PGE-2 in preimplantation mouse embryos. Fluorescence was observed in the cytoplasm of unfertilized 1-cell embryos to the blastocyst stage. The strongest fluorescence was detected at the 8-cell and morula stages. In embryos cultured from the 2-cell stage on, the fluorescence was observed in the cytoplasm of 4-cell embryos to the blastocyst stage. No differences were observed in the intensity and the distribution of the fluorescence between embryos *in vivo* and those *in vitro*. However, when blastocysts were cultured in a medium containing 100 μM-indomethacin, the fluorescence was diminished markedly. We therefore suggest that preimplanted mouse embryos contain PGE-2 during their early developmental stages and that the embryos synthesize the PGE-2.

Introduction

The detection of prostaglandins (PGs) in mammalian embryos was first reported by Dickmann & Spilman (1975). They detected, by radioimmunoassay (RIA), PGE-A and PGF in rabbit blastocysts 144 h *post coitum* and suggested that the embryos had the ability to synthesize the PGs. PGs have since been detected by RIA in blastocysts of rabbits (Dey *et al.*, 1980; Pakrasi & Dey, 1982; Harper *et al.*, 1983), cows (Shemesh *et al.*, 1979; Lewis *et al.*, 1982), ewes (Lacroix & Kann, 1982; Hyland *et al.*, 1982) and sows (Davis *et al.*, 1983).

While the exact function of the embryonic PGs is unknown, PGs in blastocysts have been considered to participate in the process of implantation (Pakrasi & Dey, 1982). It has also been reported that PGE-2 has a major role in the hatching of mouse blastocysts (Baskar *et al.*, 1981) and is able to induce implantation of mouse blastocysts (Holmes & Gordashko, 1980). However, the detection of PGs was only possible for blastocysts which are of a size suitable for RIA, and PGs have not been measured in unfertilized eggs and embryos at cleavage stages.

This investigation was designed to demonstrate immunohistochemically whether PGE-2 is present in mouse embryos at various developmental stages, and to explore the possibility of PGE-2 synthesis in the embryos.

Materials and Methods

Animals and collection of embryos. ICR female mice, 8 weeks old, were superovulated with 5 i.u. PMSG (Teikokuzoki, Tokyo, Japan), and with 5 i.u. hCG (Teikokuzoki) injected 48 h later. They were mated with ICR males of proven fertility. The embryos were collected at the following times by flushing oviducts or uteri with culture medium (Whittingham, 1971); 24 h after hCG injection to obtain pronuclear embryos; 48 h for 2-cell embryos; 60 h for 4 cell embryos; 67 h for 8-cell embryos; 80 h for morulae and 96 h for blastocysts. Unfertilized eggs were also collected from the oviducts of unmated females at 24 h after hCG injection.

Culture of embryos. Two-cell embryos from superovulated mice were collected from the oviducts into culture medium (Whittingham, 1971) containing 0·4% bovine serum albumin (Sigma, St Louis, MO, U.S.A.). The embryos

were cultured for 48 h in the medium mentioned above in a humidified atmosphere of 5% CO_2 and 95% air at 37°C, and were examined at the 4-cell, 8-cell, morula and blastocyst stages. Blastocysts cultured from the 2-cell stage in medium containing 100 μM-indomethacin were also examined. A stock solution of 10 mM-indomethacin was prepared by dissolving indomethacin (Wako, Osaka, Japan) in 100% ethanol. This solution was diluted with the culture medium to 100 μM. As a control, blastocysts cultured from the 2-cell stage in an indomethacin-free medium with ethanol were observed.

Indirect immunofluorescence assay. Embryos developed *in vivo* and *in vitro* were washed twice with phosphate-buffered saline (PBS) (Dulbecco & Vogt, 1954), slightly modified and the zonae pellucidae were removed in 0·2% pronase (Sigma) in PBS. The composition of the PBS was 8·0 g NaCl, 0·2 g KCl, 1·15 g Na_2HPO_4, 0·2 g KH_2PO_4 and 1000 ml water. The naked embryos were washed again in PBS and fixed in 10% formalin–PBS for 60 min at room temperature. They were further washed 3 times in PBS for 60 min in all and incubated in the rabbit anti-PGE-2 serum (Polysciences, Warrington, PA, U.S.A.) for 60 min. The anti-PGE-2 serum was diluted with PBS to 100 times. All these procedures were carried out in a moist chamber at room temperature. The antiserum to PGE-2 reacts with PGE-2 (100%), 15-keto-PGE-2 (13·2%), PGE-1 (6·5%), 13,14-dihydro-PGE-2 (2·1%), 13,14-dihydro-15-keto-PGE-2 (0·6%) and <0·3% with the A, B, D or F series of PGs. The embryos were washed again in PBS for 60 min and then incubated in the goat anti-rabbit IgG conjugated with fluorescein (Cappel, West Chester, PA, U.S.A.) for 60 min. The goat anti-rabbit IgG conjugated with fluorescein was diluted with PBS to 64 times. The control embryos were incubated in normal rabbit serum (1:100) before the fluorescein treatment, or in the fluorescein conjugate only. The same procedures of assay were applied 3 times to 30–45 embryos at every stage. The embryos were placed in a small amount of PBS on glass slides to be observed, and were photographed under a reflected-light fluorescing microscope (Olympus, Tokyo, Japan) equipped with HBO-200 (Osram, München, West Germany) as an ultraviolet light source (exposures of 2 min).

Results

Embryos in vivo

Fluorescence was observed in the cytoplasm of all these embryos from the unfertilized 1-cell through to the blastocyst stage (Fig. 1). The fluorescence was not observed in the embryos incubated in normal rabbit serum or in fluorescein conjugate only. When positive, the fluorescence was spread evenly throughout the cytoplasm, although its intensity was not even in the blastomeres. In blastocysts, while the intensity of the fluorescence varied amongst the cells in both the inner cell mass and the trophoblast, there was no difference in the distribution of the fluorescence in the two cell types. The intensities of the fluorescence in the embryos at various developmental stages are shown in Table 1. The intensity of fluorescence was weak in the embryos at the 1-cell stage, a bit stronger in those at the 2-cell and 4-cell stages, strongest at the 8-cell and morula stages, and slightly weaker again at the blastocyst stage. No differences were observed in the intensity of fluorescence between unfertilized eggs and pronuclear embryos at the 1-cell stage.

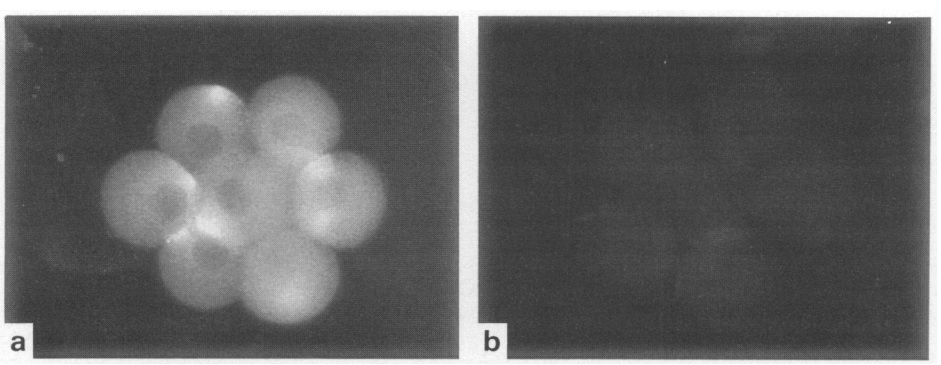

Fig. 1. Fluorescence showing the presence of PGE-2 in each blastomere of an 8-cell mouse embryo (a) and its absence in a control 8-cell mouse embryo treated only with the FITC-conjugated anti-rabbit IgG (b). × 400.

Table 1. Intensity of fluorescence in mouse embryos

Embryos	Developmental stages						
	Unfertilized	Pronuclear	2-Cell	4-Cell	8-Cell	Morula	Blastocyst
In vivo	+	+	++	++	+++	+++	++
In vitro				++	+++	+++	++

The symbols +, ++ and +++ represent weak, moderate and strong fluorescence, respectively.

Table 2. Intensity of fluorescence in mouse blastocysts cultured from the 2-cell stage in a medium with 100 μM-indomethacin

Medium	No. of blastocysts observed	Intensity of fluorescence		
		Moderate	Weak	None
Indomethacin-free	34	30*	4	0
Indomethacin-containing	35	9*	22	4

*$P < 0.001$ by χ^2 test.

Embryos in vitro

Fluorescence was observed in the cytoplasm of embryos *in vitro* at the 4-cell through to blastocyst stages. Its intensity and distribution were similar to those of embryos *in vivo* (Table 1).

The intensities of fluorescence in blastocysts cultured from the 2-cell stage in the medium containing indomethacin are shown in Table 2. In blastocysts cultured in an indomethacin-free medium, 30 out of 34 embryos showed a moderate intensity of fluorescence, while the rest exhibited only weak fluorescence. Significantly fewer blastocysts cultured in the indomethacin-containing medium exhibited moderate fluorescence.

Discussion

The results from the present experiments prove for the first time the presence of PGE-2 in mouse embryos, not only at the blastocyst stage but at all the stages from the 1-cell to the morula. Because the PGE-2 was detected in the embryos developed *in vitro* and because that in blastocysts was markedly reduced by indomethacin, an inhibitor of PG synthesis, we conclude that preimplantation mouse embryos have synthesized the PGE-2 they contain. Racowsky & Biggers (1983), however, failed to demonstrate PGs or PG synthesis from arachidonic acid in mouse blastocysts *in vitro*. The discrepancy may be due to the sensitivity of the methods used to detect the PGs.

From the fluctuations in the fluorescence intensities in the present experiments, it is considered that the PGE-2 content in mouse embryos is greatest at the 8-cell and morula stages. This agrees with the results obtained by Niimura & Ishida (1978) who detected histochemically the presence of 15-hydroxy PG dehydrogenase, a major enzyme metabolizing PGs to less active metabolites, in hamster embryos up to the 8-cell stage only. Large amounts of PGE-2 in 8-cell embryos and morulae may play roles in the hatching (Baskar *et al.*,1981) and implantation (Holmes & Gordashko, 1980) of blastocysts.

It has been reported that Δ^5-3β-hydroxysteroid dehydrogenase, a key enzyme of steroid biosynthesis, can be detected histochemically in preimplantation mouse embryos (Dickmann *et al.*, 1976; Niimura & Ishida, 1980) and biosynthesis of some steroid hormones in preimplantation mouse embryos can therefore be presumed. We conclude that the PGE-2 demonstrated in the present experiments may act as a mediator in mouse embryo steroidogenesis.

References

Baskar, J.F., Torchiana, D.F., Biggers, J.D., Corey, E.J., Andersen, N.H. & Subramanian, N. (1981) Inhibition of hatching of mouse blastocysts *in vitro* by various prostaglandin antagonists. *J. Reprod. Fert.* **63,** 359–363.

Davis, D.L., Pakrasi, P.L. & Dey, S.K. (1983) Prostaglandins in swine blastocysts. *Biol. Reprod.* **28,** 1114–1118.

Dey, S.K., Chien, S.M., Cox, C.L. & Crist, R.D. (1980) Prostaglandin synthesis in the rabbit blastocyst. *Prostaglandins* **19,** 449–453.

Dickmann, Z. & Spilman, C.H. (1975). Prostaglandins in rabbit blastocysts. *Science, N.Y.* **190,** 997–998.

Dickmann, Z., Dey, S.K. & Gupta, J.S. (1976) A new concept: control of early pregnancy by steroid hormones originating in the preimplantation embryo. *Vitams Horm.* **34,** 215–242.

Dulbecco, R. & Vogt, M. (1954) Plaque formation and isolation of pure lines with poliomyelitis viruses. *J. exp. Med.* **99,** 167–182.

Harper, M.J.K., Norris, C.J. & Rajkumar, K. (1983) Prostaglandin release by zygotes and endometria of pregnant rabbits. *Biol. Reprod.* **28,** 350–362.

Holmes, P.V. & Gordashko, B.J. (1980) Evidence of prostaglandin involvement in blastocyst implantation. *J. Embryol. exp. Morph.* **55,** 109–122.

Hyland, J.H., Manns, J.G. & Humphrey, W.D. (1982) Prostaglandin production by ovine embryos and endometrium *in vitro*. *J. Reprod. Fert.* **65,** 299–304.

Lacroix, M.C. & Kann, G. (1982) Comparative studies of prostaglandins $F_{2\alpha}$ and E_2 in late cycle and early pregnant sheep: in vitro synthesis by endometrium and conceptus effects of in vivo indomethacin treatment on establishment of pregnancy. *Prostaglandins* **23,** 507–526.

Lewis, G.S., Thatcher, W.W., Bazer, F.W. & Curl, J.S. (1982) Metabolism of arachidonic acid in vitro by bovine blastocysts and endometrium. *Biol. Reprod.* **27,** 431–439.

Niimura, S. & Ishida, K. (1978) Histochemical studies of prostaglandin dehydrogenase in hamster eggs. *Jap. J. Anim. Reprod.* **24,** 16–18.

Niimura, S. & Ishida, K. (1980) Histochemical studies of hydroxysteroid dehydrogenases in tubal eggs of mice, rats and guinea pigs. *Jap. J. Anim. Reprod.* **26,** 89–93.

Pakrasi, P.L. & Dey, S.K. (1982) Blastocyst is the source of prostaglandins in the implantation site in the rabbit. *Prostaglandins* **24,** 73–77.

Racowsky, C. & Biggers, J.D. (1983) Are blastocyst prostaglandins produced endogenously? *Biol. Reprod.* **29,** 379–388.

Shemesh, M., Milaguir, F., Ayalon, N. & Hansel, W. (1979) Steroidogenesis and prostaglandin synthesis by cultured bovine blastocysts. *J. Reprod. Fert.* **56,** 181–185.

Whittingham, D.G. (1971) Culture of mouse ova. *J. Reprod. Fert., Suppl.* **14,** 7–21.

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