Decrease of cellular growth potential in «in vitro» culture of amnions with premature rupture of membranes

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Summary: The "in vitro" cellular growth of 8 amniotic membranes from preterm deliveries with premature rupture of membrane (PROM) in absence of risk factors as cervical or vaginal infection (microbiologic negativity), cervical incontinence and other mechanical factors, was compared with cellular growth of 9 amnions from preterm deliveries without PROM. Amniotic membranes were set up in the Eagle basal medium with Earle salts and heat-inactivated fetal bovine serum (10%), gentamicin 50 μg/ml and amphotericin B 0.5 μg/ml. The results suggested that the growth potential of the cells (epithelial cells and fibroblasts) obtained from amnions with PROM was lower than that of cells obtained from amnions without PROM.

We postulated that the premature rupture of membranes in patients without risk factors for PROM, would be conditioned by an intrinsic decrease of cellular growth potential.

Key words: Amnion; PROM; Cellular growth; In-vitro culture.

INTRODUCTION

There have been many reports emphatically attributing the cause of premature rupture of membranes (PROM) to vaginal or cervical infections and latent chorioamnionitis ([1]).

Other Authors investigated structural differences in preterm amnions with PROM. Skinner *et al.* have reported that the collagen content in amnions with PROM is lower than that of normal amnions ([2]), whereas Al-Zaid *et al.* reported that the collagen content does not change with PROM ([3]). Recently, Kanayama *et al.* observed a significant reduction of collagen content, especially type III collagen with a consequent reduction of membrane elasticity ([4]). Up to date, the cause of PROM remains unexplained in most cases ([5]).

In the present study we have tried an evaluation of cellular growth of amnions with PROM. The aim of this study is to detect eventual modifications on the in vitro cellular growth of amnions with PROM in absence of risk factors (infective and mechanical factors), as compared with the cellular growth of amnions without PROM, that may justify an intrinsic disposition to premature rupture of membranes.

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MATERIALS AND METHODS

8 patients with PROM were included, 6 of whom were primigravidae with mean gestational age at delivery of 34.0 weeks (range 28-37) and the other 2 were secondigravidae with gestational age at delivery of 33 and 34 weeks, with previous pregnancy complicated by PROM in the 34th (the first) and 36th week (the second). In all the cases negativity of vaginal and cervical cultures, absence of uterine contractions and of other known risk conditions (cervical incontinence, polyhydramnios, twin pregnancy and other mechanical factors) were proved.

The controls consisteted of 9 pregnancies without PROM with mean gestational age at delivery of 34.9 weeks (range 30-36); the delivery was: in 5 cases PIH, in 2 cases maternal cardiopathy and in 2 cases preterm labor without PROM.

In the two groups (patients and controls) there were no significant differences as to mean age (30.4 years vs 33.2 years), parity (0.25 vs 0.33) and week of gestation at delivery (34.0 weeks vs 34.9 weeks) (Table 1).

At the time of delivery amnion tissue was removed, from extraplacental membranes by blunt dissection and minced into small pieces (1 cmq). Four pieces of membranes were set up in sterile flat-bottom flascks and grown in the Eagle basal medium with Earle salts (EBME) and heat-inactivated fetal bovine serum (10%), gentamicin 50 μg/ml and amphotericin B 0.5 μg/ml (Fungizone). All this was maintained at 37° C.

Part of the medium (50%) was replaced with fresh medium after 3 days. At intervals of 48 hours, the cellular growth was observed with phase-contrast microscope, examining the morphology of the cells and their growth modality in culture until confluence.

For statistical analysis of the result t-test was used.

Table 1. — *Characteristics of patients.*

Patients (n=8)	Years	Parity	Gestational age at PROM (weeks)	Time between PROM and Delivery (days)
1	32	0	28	1
2	31	1	24	2
3	27	0	34	2
4	29	0	32	3
5	34	1	31	2
6	28	0	37	0
7	30	0	36	1
8	32	0	37	4
m.v.	30.4	0.25	33.6	1.9

(m.v.: mean value)

RESULTS

The cultural growth of human amnion cells showed different behaviour in the case of premature rupture of membranes in comparison with the controls. The controls, in fact, presented initial aspects of cellular growth (from the pieces of membranes set up) after a middle period of 7.67 days (range 5-12), with attainment of confluence after 30.7 days (mean value). The cellular elements were: fibroblast and epithelial cells (optic microscopic examination).

In the case of PROM, the in-vitro growth of human amnion cells (fibroblasts and epithelial cells) was different: there were initial aspects of cellular outgrowth from the pieces of membranes after a middle period of 16.37 days (range 8-22) (delayed growth) ($p<0.001$) with signs of cellular suffering (wrinkled cells). The subsequent cellular growth was also less exuberant, without attainment of confluence (Table 2).

DISCUSSION

Although PROM is associated with many risk factors, the underlying basis of these associations has not been determined [5]. There is increasing evidence that maternal cervical and vaginal infections (C. Trachomata, Mycoplasma, Bacterioides) may cause preterm labor and PROM [6]. But the data are not sufficent to establish whether this association between infections and adverse pregnancy outcome is causal or related to some other factors. If causal, the mechanism remains speculative. The effects of inflammation or synthesis and release of prostaglandins may be involved in preterm labor or PROM. Many microrganisms produce phospholi-

Table 2. — *Characteristic of "in vitro" cellular growth.*

Patients (n=8)	Initial aspects of cellular outgrowth (days)	Confluence Yes (days) - No
1	8	-
2	22	-
3	19	-
4	14	-
5	18	-
6	17	-
7	18	-
8	15	-
	16.37+4.17 (m.v.) $p<0.001$	
Controls (n=9)		
1	5	24
2	12	31
3	8	29
4	9	30
5	8	27
6	7	26
7	7	26
8	6	25
9	7	26
	7.67+2.00 (m.v.)	

(m.v.: mean value)

pase A2, which can initiate prostaglandin synthesis by cleaving arachidonic acid (a precursor of prostaglandins) from the phospholipid components of fetal membranes ([7]).

Recently much attention has been paid to the collagen content of membranes from gestations with or without PROM. The content of collagen, particularly type III, of membranes from preterm deliveries with PROM is lower than those without PROM ([8]). Some Authors have attempted to determine the factors responsible for this reduction in membrane collagen.

Bacterial collagenase and other proteolytic enzymes (trypsin) can be held responsible for the loss of membrane collagen ([8, 9]). McGregor *et al.* observed a significant decrease in membrane strength after exposure of fetal membranes to bacterial collagenase or collagenase-producing organisms ([9]). Kanayama *et al.* reported that amniotic fluid trypsin activity was higher and alpha antitrypsin lower in pregnancy with PROM ([8]).

If cervical and vaginal infections and reduced collagen content of amniotic membranes can justify most PROM cases, there are, however, pregnancies with PROM that do not present any of these causes. Following this presupposition, we thought of a possible intrinsic factor pertaining directly to the fetus and involved in the PROM. This hypothesis originated in particular from the study of two cases with PROM that had already had, in previous pregnancies, rupture of membranes at the same gestational age.

Therefore, we performed a dynamic evaluation of the status of membranes through the in vitro culture of pieces of the amniotic membranes of patients with PROM free of any known risk conditions and we compared it with that of patients without PROM.

The two groups so compared did not differ significantly in terms of average age, parity and week of gestation at delivery. Before performing this evaluation, we studied the potential of cellular growth of amnions throughout all the pregnancy to check if differences in cellular growth would occur during this time. Since the development of the fetus during pregnancy goes through different subsequent phases of growth with a considerable increase in weight between the 28th and 36th week, we were inclined to believe that the membrane growth followed these rhythms. On the contrary, we found that the culture of membranes both in precocious gestational age, and in the age of greatest fetal growth (28-36 weeks), and at the end of pregnancy (39 weeks) behaves in a regular way from the point of view of in vitro cellular growth with aspects of initial growth after a middle

period of 7.67 days and attainment of confluence after 30.7 days. In all the 8 cases with PROM we observed a significant decrease in the potential of cellular growth with aspects of initial growth after a middle period of 16.37 days ($p < 0.001$) and signs of cellular suffering (wrinkled cells). Therefore, in all those cases in which we excluded infective or mechanical factors, the intrinsic factor connected to the potential of the membrane cellular growth could be involved. In fact, our data suggest that the cells deriving from amnions with PROM lack the typical exuberant growth capable of giving confluence which is characteristic of the in vitro cultures of pieces of amnions without PROM.

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