# ANTIMICROBIC ACTIVITY OF THE AMNIOTIC FLUID

- M. MEGA (\*), F. L. GIORGINO (\*),
- U. DAL MUTTO (\*), A. STEVANIN (\*\*),
- M. DE NEGRI (\*\*)
- (\*) Institute of Obstetric and Gynecologic Clinic, University of Padua (Italy)

(\*\*) Microbiology Centre, Civil Hospital of Padua (Italy)

#### SUMMARY

The amniotic fluid is commonly, though not unanimously, believed to include among its various functions a defensive one, against infections.

This is due to the presence of immunoglobulins, immunocompetent cells and such factors like lysozyme, transferrin and zinc.

However, evidence of this AF antimicrobic activity had been found only in the second half of the pregnancy period starting from the 20th gestational week.

Our study has demonstrated its presence in a very high percentage (58.7%) of AF samples taken in early amniocenteses.

Clin. Exp. Obst. Gyn. - ISSN: 0390-6663 VIII, n. 4, 1981

### INTRODUCTION

In pregnancy, significant changes occur in the mother's organism and in its capability of responding to infections. They probably stem from the need to keep what can be regarded as an (the fetus and the placenta) inside the uterus  $(^1)$ .

The cell responsiveness  $(^{2})$ , and in particular the IgG  $(^{1})$ , decrease.

The combined effect of these changes is a lowered maternal resistence to infections but an increasingly safe defence mechanism for the fetus. For instance, ascending infections are opposed by vaginal acidity, cervical agents, like lysozyme (100 times more concentrated than in serum) and by IgA.

Undoubtedly, the amniotic fluid (AF) too plays an important role in the defence against infections, although some Authors disagree on this.

Walsh and Coll. (<sup>3</sup>) have pointed out that Escherichia and Streptococci develop in the AF just as well as in trypticase + soy.

Conversely, Schlievert and Coll. (<sup>4</sup>) have observed an AF inhibiting effect on the growth of Escherichia coli.

Rouquet and Coll. (<sup>5</sup>) have recently demonstrated that the AF has a definite bacteriostatic activity which increases alongside with the gestational age. But the bactericidal activity can seldom be proved and occure as from the 31st gestational week. This activity is certainly ascribable to many factors including lysozyme (<sup>6</sup>), transferrin (<sup>6</sup>) and immunoglobulins (<sup>7</sup>).

Recently, attention has been drown on a new bactericid element, zinc (<sup>4</sup>). Zinc probably interferes competitively with metabolic and bacterial processes requiring magnesium, presumably owing to similarities in their electric charges (<sup>4</sup>). The zinc inhibiting action on the bacterial growth is closely related to the concentration of phosphate. A high phosphate concentration produces a zinc-phosphate recipitate which eliminates the inhibiting power.

Therefore, in the AF, the antibacterial action depends on the phosphate/zinc relation.

It is worth noting that most Authors have observed no antibacterial activity in the AF before the 20th gestational week.

# MATERIAL AND METHODS

We examined 92 samples of AF obtained by early amniocentesis on pregnant women who had not used antibiotics during their pregnancy and showed no sign of infection in progress. On these samples we performed an antibacterial activity test following a method similar to the antibiogramme. The amniotic fluid replaced the antibiotic. Each sample was used to soak a standard paper pad. 4 pads were put on each plate. The plates contained a very rich, nonselective medium (Mueller-Hinton Agar) on which a very sensitive bacterium - Micrococcus lutens - was sown.

It grew luxuriantly in that medium and the inhibition halo, when present, was clearly visible, though not very intense. All the samples were collected in three years, between 1978 and 1980, and centrifuged at 2900 r.p.m. for 5'.

The overfloating substance was then taken and stored at -20 °C till the time of its utilization.

#### RESULTS

We studied the antibacterial potential in 92 samples of AF and evaluated its variations according to the maternal and gestational ages.

54 of these samples showed antibacterial activity.

As the risk of intrauterine infections increases alongside with the mother's age, we tried to evaluate the latter's influence on the AF antibacterial potential (table 1).

According to these results and in agreement with the literature, the increase in intrauterine infections alongside with the mother's age increase, is not due to a decrease in the AF antibacterial activity. On the other hand, the literature agrees on the parallel increase in the gestational age and the AF antibacterial activity, but this concerns the last period of pregnancy (<sup>5</sup>).

Table 1. — AF	antimicrobic	activity at	the various
maternal ages.			

Maternal years	No. of cases	%
≤ 34	19 +	46.3
	22 —	53.7
35-39	24 +	70.5
	10 —	29.5
≥ 40	11 +	64.7
	6 —	35.3
	Tot. 54 +	
	38 —	

+: AF inhibiting the growth of Micrococcus lutens.

-: AF not inhibiting the growth of Micrococcus lutens

We examined AF samples taken from patients between the 16th and the 20th gestational week, and in 54 samples we positively observed antibacterial activity.

### CONCLUSION

The most important result of our study is the evidence that, contrary to what was commonly believed, the AF performs an antimicrobic activity before the 20th week of pregnancy too. This activity is aspecific, probably due to many factors, but not to the lack of nourishing substances that were provided, in our case, by a very rich culture medium.

# BIBLIOGRAPHY

- 1) Ledger W. J.: Clin. Obst. Gyn., 21, 455, 1978.
- 2) Jones E., Curzen P.: J. Obst. Gyn. Br. Cwlth., 80, 608, 1973.
- 3) Walsh H., Hildebrandt R., Prystowsky H.: Am. J. Obst. Gyn., 93, 590, 1965.
- Gun. J. Oost. Gyn., 39, 590, 1965.
   Schlievert P., Johnson W., Galask R. P.: Am. J. Obst. Gyn., 125, 899, 1976.
   Rouquet Y., Paul G., Philippon A., Tour naire M., Nevot P., Chavinié J.: J. Gyn. Obst. Biol. Repr., 10, 119, 1981.
   Galask R. P., Snyder I.S.: Am. J. Obst. Gyn., 106, 59, 1970.
- Gyn., 106, 59, 1970.
  7) Zervoudakis I. A., Silverman F., Senterfit L.B.: Obst. Gyn., 55, 165, 1980.

# PROPOSAL FOR PROTOCOL IN THE DIAGNOSIS OF STERILE COUPLES

M. MARCHETTI, G. L. ONNIS

Department of Obstetrics and Gynecology, University of Padua (Italy)

#### SUMMARY

The lack of system in the diagnosis and treatment of sterility in the couples we have met in our clinical practice has made us feel the need for a protocol.

All the tests relating to the two partners should be studied and the couple should be informed as to the nature of treatment and the time it will take.

An outline plan of the therapeutic approach is attatched.

Clin. Exp. Obst. Gyn. - ISSN: 0390-6663 VIII, n. 4, 1981 The formulation of this protocol aims at standardising a diagnostic iter in the case of sterile couples, since in our clinical practice we have often recognised a lack of method in clinical address.

Such lack very often causes the sterile couple's disappointment and lost confidence in regard to the diagnostic and therapeutic methods applied, because although followed over a prolonged period of time, they have only resulted in considerable waste of time and money.

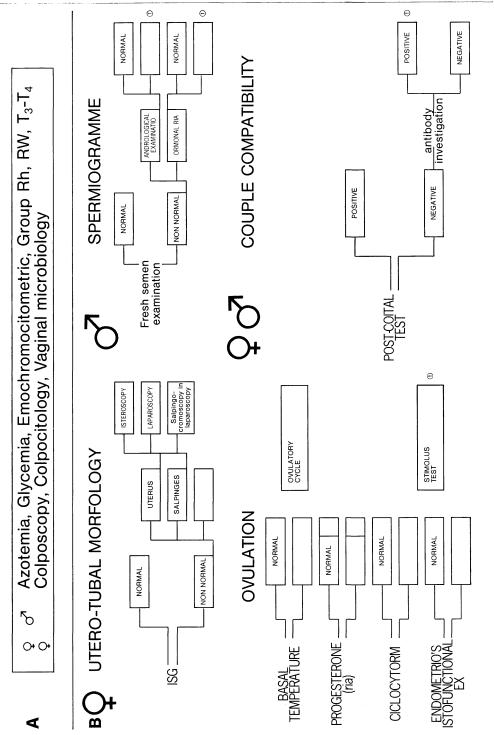
The sterile couple, with some difficulty, but rightly, comes to consider failure to conceive as an illness; the dislike of submitting to tests, often tiresome on account of the time they take, is, at the beginning overcome by enthusiasm at the prospect of results.

Like all protocols, this aims at involving not only the specialist, but also the general practitioner with the couple themselves, who therefore become partecipants in the diagnostic and therapeutic choice.

The protocol has thus a didactic and informative function; both the doctor and the couple co-operate in the diagnostic iter from the beginning to the end, with collaboration and responsability.

We consider it indispensable that all tests are carried out in one cycle, or at the most, two, at least those following a first consultation. The subsequent treatments must be begun only after a definitive diagnosis has been formulated. Unfortunately we have often seen patients (both men and women) who have begun treatment just as one of the tests has turned out badly, without the iter having been completed, thus proving the absurdity of initiating medical or surgical treatment when there still exist concomitant problems in the case, whose nature has not been fully understood.

We have divided the tests into two distinct groups: general examinations and specialised examinations, which, we re-



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peat, must be carried out in a single cycle, and contemporaneously on both partners.

The description of each individual test seems to us superfluous. A definition should be added to the endometrial biopsy which, besides permitting a hysto-functional examination, tells us of eventual alterations: flogosis, fibromas, structural degenerations that concur in determining sterility, impeding the implantation of an eventual fertilised ovum.

## BIBLIOGRAPHY

A. Onnis, M. Marchetti, G. L. Onnis: Gin. Clin., I, 4, 260, 1980.