

INCIDENCE OF FERTILISATION IN INFERTILE WOMEN USING EARLY PREGNANCY FACTOR (EPF) AS A MARKER

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ABSTRACT

Early pregnancy factor (EPF) is known to be detectable in sera of pregnant women within 24 to 48 hours after conception. To examine the frequency of early embryo loss in infertile women either with proven ovulation (Unexplained Infertility) or during induction/restoration of ovulation, and in those having repeated pregnancy wastage we screened the sera of our patients for EPF by means of Rossette inhibition Test (RIT). Our results showed that in 33.3% (11/33) cycles of unexplained infertility, fertilization was detectable using EPF as a marker thereby indicating nidatory failure or impaired development of embryo as the probable cause of infertility. In the group where ovulation is induced or restored, EPF was detected in 47.3% (18/38) of women and only one third of these continued the pregnancy. EPF correlated with the pregnancy outcome in 83.7% of women with repeated pregnancy wastage. Infertility because of impaired development of embryo or nidatory failure may be due to chromosomal defects, endocrinological, immunological or hormonal deficiencies. Finally pregnancy can be salvaged once fertilization is detected in cases where ovulation is induced or restored and in some BOH cases EPF may be a better marker than hCG for determining the outcome of pregnancy.

INTRODUCTION

Early pregnancy factor (EPF) first described by Morton et al., in 1974 is a pregnancy associated serum constituent having immunosuppressive properties. It is known to be detectable in sera of pregnant women within 24

to 48 hours after fertilization. It is produced in the human as well as in other mammals as soon as oocyte has been fertilized. (Mesroglu and Maes 1985).

In the management of infertility, either unexplained or otherwise, it is often not known whether fertilization fails or implantation and further development of the embryo is impaired.

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To evaluate our results of infertility treatment we screened sera of women undergoing successful ovulation induction/restoration and of those having unexplained infertility or repeated pregnancy wastage for fertilization using early pregnancy factor as a parameter for conception.

MATERIALS AND METHOD

Forty women undergoing induction of ovulation by clomiphene citrate, gonadotropins and restoration of ovulation by bromoergocryptin, 25 women with unexplained infertility and 16 women with repeated pregnancy wastage, attending the Endocrine Infertility clinic of the Institute were screened for the presence of EPF during their monitored cycles.

Blood samples were obtained by venepuncture 3-10 days after ovulation or as soon as the period was missed. Serum was separated and stored at - 20C until assayed. EPF activity in the serum was detected by the rosette inhibition test, (RIT) adopting the method of Morton et al (1982). A known positive (Pregnancy serum) and a known negative (nonpregnant serum) as control were included in each assay. If the control did not give the expected RIT value the test was repeated.

Ovulation was induced by administration of clomiphene citrate 50- 100 mg per day for 5 days beginning from 5th day of spontaneous or induced period or by intramuscular administration of gonadotropins 75-150 iu/day, the dose

TABLE I

Sr.No.	Total No. of subject	Blood collected days after rupture	No.of Cycles studied/subject	Result of EPF		Outcome
				+ve	-ve	
1	11	4-7	1	-	11	All except one menstruated. This women had a missed abortion later
2	7	3-14	2	3	11	Only one with a positive EPF continued pregnancy. Remaining did not retain the embryo
3	2	4-8	3	1	5	Did not retain the embryo after fertilization.
4	2	5-9	4	1	7	One in the positive cycle did not retain the embryo after fertilization. The other in the negative cycle had a missed abortion once and continued pregnancy in the next cycle.
5	2	4-11	5	4	6	None of the positive cycles retained the embryo. In two cycles short luteal phase was identified by hormonal assays (urinary pdg)
6	1	4-14	6	2	4	Did not retain the embryo after fertilization

being increased according to individual endogenous estrogen response. bromoergocryptin in the dose of 2.5 mgm to 7.5 mg/day was given to suppress elevated prolactin levels and restore ovulation.

RESULTS

A single blind study was carried out and on decoding the blood samples the results were analysed.

served from BBT or day of follicular rupture and by hormone assays. One individual in whom EPF was not detected had a missed abortion subsequently. None of the remaining retained the embryo in the positive cycle.

DISTRIBUTION OF EPF POSITIVE AND NEGATIVE CYCLES IN INFERTILE PATIENTS WHERE OVULATION WAS INDUCED OR RESTORED

TABLE II

DISTRIBUTION OF EPF POSITIVE AND NEGATIVE CYCLES WHERE OVULATION IS INDUCED OR RESTORED

	No	EPF Positive Cycles	EPF Negative Cycles
Clomiphene Citrare Therapy	32	15 (5)*(1)**	17 (2)*(1)**
Gonadotrophin Therapy	4	3(1)*	1
Bromoergocryptin Therapy	2	0	2(1)*
Total	38	18	20

*Continuing Pregnancy

**Abortion

DETECTION OF EPF IN WOMEN WITH UNEXPLAINED INFERTILITY

Twenty five women were studied in this group (Table I) of which 11 were screened for 1 cycle only. The results revealed that in all these 11 women there was no detectable EPF when the blood was collected 4-7 days after the follicular rupture (as determined by BBT and/or ultrasonography) and they menstruated in that particular cycle.

In the remaining 14 women more than 1 cycle was studied. Fertilization was detected in 11 of 33 cycles studied and only one individual continued pregnancy. The earliest EPF activity in this group was detected 3 days after ovulation. Two of these women who were positive for EPF had an inadequate or short luteal phase as ob-

Forty women were screened in this group. In 38 women EPF was studied in 1 cycle only (Table II). It was detected in 18 of them, 6 of whom continued pregnancy whilst one aborted. There were 20 cycles where EPF activity was not detectable. However, in 2 of these conception did occur and pregnancy continued while 1 aborted. Repeated cycles were studied in the remaining 2 women (Table III). In one individual (S.S) blood was collected 8 days after period since the flow was scanty and BBT remained elevated it was positive for EPF, hence the samples was repeated every 4 days. It was interesting to observe that EPF became negative 11 days after the first blood collection and the patient menstruated in that particular cycle. In the other individual, fertilization was detected when blood was collected 7 days after ovulation but when repeated 5 days

TABLE III

SUBJECT RESULTS AND DATES ON WHICH EPF WAS ESTIMATED AFTER THE FOLLICULAR RUPTURE

SS	10.8.87 +ve(ET)	14.8.87 +ve	18.8.87 +ve	21.8.87 -ve(M.P)
SK	13.7.87 +ve	18.7.87 +ve(M.P)		

ET = Elevated Temperature

MP = Menstrual Period

later showed a + type reaction and she soon menstruated.

was positive. Both of them continued pregnancy and one of them has delivered. This patient had high hCG level.

EPF IN WOMEN WITH REPEATED PREGNANCY WASTAGE AND INFERTILITY

In this group 16 subjects were studied. Since blood was collected after missed period, urinary hCG was also estimated in most of these patients.

The results indicated a positive correlation between presence of hCG and EPF. The outcome of pregnancy correlated with detection of EPF in 13 of the 16 subjects studied i.e. 83.7% (Table IV). In two subjects EPF was negative but hCG

DISCUSSION

It is well known that even in fertile women not every fertilized oocyte develops into an intact pregnancy. The accepted dogma is that 15 percent of all conceptus end in recognizable foetal loss, while 31% survive till viable birth and the remaining 54% are lost at the earliest stages without being noticed.

The rosette inhibition test inspite of its inherent technical problems is a valid and reliable test for the detection of EPF activity present in

TABLE IV

DETECTION IN EPF IN BOTH CASES - PAST HISTORY OF PREGNANCY WASTAGE AND INFERTILITY

Sr.No.	No.of Subjects	EPF Estimation	Intact hCG estimation	Outcome
1	4	+ve	+ve	All pregnant
2	2	-ve	-ve	All of them menstruated in that particular cycle.
3	5	-ve	+ve	Two pregnant; 2 missed abortion, 1 loss to follow up
4	3	-ve	-	Two menstruated; 1 delivered normal boy
5	2	Low titre	-	Both missed abortion

the maternal serum during pregnancy (Smart et al 1982). It is found to be high in the early pregnancy, gradually decreasing with the continuation of gestation and disappear 8 weeks before delivery (Qin-Zhihai and Zheug-Qnn 1987). The validation of EPF as possible correlate of early fertilization has thus made it possible to detect fertilization episodes in both normal fertile women and in women with unexplained infertility where fertility is being evaluated. The observation of 33% of EPF positive cycles in the unexplained group has suggested early embryo loss or nidatory failure to be a factor responsible for their infertility.

In the group where ovulation is induced we found the fertilization rate to be 47.32% with only one third of these fertilized oocytes developing into an intact pregnancy. The false negative results observed in few individuals could be due to the parabolic dose response curve observed for EPF by rosette inhibition test. Hence both low and high concentrations can give negative results. We are able to detect EPF on diluting the serum samples where hCG levels are observed to be high. The measurement of EPF in several subsequent cycles is of high value for the diagnosis as well as of therapeutic procedure. Women who show several EPF positive cycles but fail to

retain the embryo are likely to have fertility problem due to chromosomal, endocrinological, immunological or hormonal deficiencies. When EPF is not found to be positive and other factors are normal a disorder of conception is very likely. In this case IVF should be treatment of choice.

Thus the measurement of EPF offers new aspect in infertility treatment as it has now become possible to distinguish whether fertilization of the oocyte or early embryonic development is impaired. Pregnancy can be salvaged once fertilization is detected. It has also proved to be a better marker than hCG in determining the outcome of pregnancy as seen in some BOH cases who aborted subsequently when the titre was low or absent.

REFERENCES

1. Mesrogl M, Maes DHA: EPF. In Ellendorf F, Koch E(eds) *Early pregnancy Factors Perinatology Press. Ithaca, New York 1985, p.233.*
2. Morton H, Hegh V, Cluine GJA: *Nature London* 249:459, 1974.
3. Morton H, Tinneberg R, Rolfe B, Wolf M, Mettler L: *J Reprod Immuno* 4:251, 1982.
4. Qin Zhihai and Zheng-Qun: *Amer J. of Reprod Immuno & Microbiol* 13:15, 1987.
5. Smart YC, Roberts TK, Fraser IS, Cripps A, Glancy BL: *Fertile Steril* 37:779, 1982.