BIRTH AFTER TRANSFER OF HUMAN PREEMBRYOS WASHED WITH HIGH CONCENTRATION OF ANTIBIOTIC TO ELIMINATE BACTERIAL CONTAMINATION

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SUMMARY

In an IVF programme, all 17 oocytes of a patient were contaminated with E coli during fertilization check, Fourteen of these which had fertilized normally were briefly washed with a concentrated solution of the antibiotic, Cefuroxime Sodium. Only 3 of these oocytes subsequently cleaved. No contamination was observed at this stage, hence the 3 preembryos were transferred. The patient became pregnant and delivered a normal female baby (karyotype 46,XX) at 37 weeks gestation. This study not only provided a novel opportunity to demonstrate the resistance of early hyman preembryos to the suspected teratogenic effects of high dose antibiotic, but also proved useful in salvaging contaminated preembryos.

INTRODUCTION

Presence of bacterial infection or endotoxins have been reported to result in inhibition of, or poor fertilization of human oocytes in vitro, with poor quality fragmented embryos. While there are two reports on poor IVF pregnancy rates due to contamination of culture medium with endotoxin (Snyman and Van der Merwe, 1986; Fishel et al. 1988), bacterial contamination of human oocytes during IVF has been reported by Ng et al. (1987). Hewitt et al (1985) observed a reduction

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in the proportion of mature oocytes fertilized in vitro in the presence of bacterial pathogens derived from semen due to male genital tract infection or by unsatisfactory collection methods.

In order to avoid microbial contamination, in vitro culture of gametes is performed under strict aseptic conditions. Semen samples of husbands are screened twice or thrice prior to and during the IVF treatment cycle. If contamination is obseved semen cultures are performed and appropriate antibiotics administered. Checking of serum of husbands for Hepatitis B surface antigen as well as HIV I and II is mandatory. Since oocyte pickup is carried out transvaginally, the vagina is thoroughly cleaned before follicular aspiration with povidone-iodine, followed by several washes with autoclaved MilliQ water. Any vaginal infection diagnosed prior to or during the treatment cycle is treated appropriately with antibiotics, antifungal agents etc. Prior to follicular aspiration the patients are administered 1 gram of Cefuroxime sodium intravenously.

Ham's F-10 medium used for in vitro culture is supplemented with 75 milligrams per litre of Streptomycin sulphate and Penicillin G respectively. Culture medium is supplemented with 10% heat inactivated maternal serum. Serum and medium are filtered through 0.2 micron sterile disposable Millipore filters. Autoclaved reverse osmosis water is used for maintaining humidity in the carbon dioxide incubators. Incubator shelves are washed and autoclaved prior to each batch of IVF undertaken; Laminar air flow hoods and the interior of the incubators are first cleaned with 1% Sodium Dodecyl Sulphate followed by autoclaved R.O. (reverse osmosis) water. The Milli RO and Milli Q system are regularly sanitized in view of high purity water being of critical importance. Tests for pyrogens and bacterial contamination are carried out regularly.

Despite these stringent measures, we observed a case of contamination of oocytes while checking the oocytes for fertilization, 18 hours after insemination.

MATERIALS AND METHODS

A normal, cycling patient aged 28 years was recruited for IVF, since she had failed to conceive following several cycles of Intrauterine insemination. The protocol used was long term pituitary downregulation with the GnRH analog, Buserelin, followed by pure FSH injections (Metrodin) once downregulation was achieved. 10000 IU hCG (Profasi) was given on the tenth day of stimulation when several follicles of diameter 1.7 cm were observed on ultrasound. Oocyte pickup was done transvaginally 36 hours later. Seventeen oocytes were obtained, of which 10 were preovulatory.

OBSERVATIONS

When observed 18 hours after insemination, all the dishes were found to be heavily contaminated with bacilli. Samples of culture medium with and without serum as well as follicular aspirates placed loosely capped in the incubator were devoid of contamination. The source was traced down to the sperm samples which also had the same gram negative bacilli. The probable cause was improper col-

lection since no bacteria were observed in the initial ejaculate and the "swim up" used for insemination. The microoraganism was identified as E. coli.

On washing the oocytes 14 were found to be fertilized normally. These were given a brief wash (1 minute) with culture medium containing the antibiotic, Cefuroxime Sodium, (Supacef, Glaxo) at a concentration of 62.3 mg/ml, rinsed several times with fresh medium and reincubated. When observed 22 hours later, only three of the fourteen oocytes had cleaved; one to 2 cell and two to 4 cell. The preembryos appeared healthy with even blastomeres and absence of fragmentation. Since there were no visible traces of bacilli present, these three preembryos were replaced to the patient's uterus.

The luteal phase was supported with intramuscular injections of pure progesterone in oil (Uniprogestin). Pregnancy was detected 12 days later and confirmed by rising titres of hCG. The antenatal period was uneventful except for gestational diabetes mellitus and mild Intra uterine growth retardation (IUGR) which developed in the third trimester. The patient delivered a female baby weighing 1.95 kg by caesarean section at 37 weeks gestaion.On clinical examination the baby appeared normal. The baby's blood was karyotyped and found to be 46,XX. No structural or numerical chromosomal aberrations or mosaicism were apparent.

DISCUSSION

This study shows that early human preembryos similar to other mammalian preembryos are highly resistant to suspected teratogens during the preimplantation stage, unlike the later critical period of organogenesis which is susceptible to malformations (Wilson, 1977). The exposure of preembryos of rodents and other non human species to various drugs did not increase the incidence of malformations in fetuses. Cleaving preembryos have been exposed to X rays, toxins, viruses etc. with virtually no confirmed reports of increase in anomalies (Edwards, 1980).

Although there is no experimental evidence of embryopathic or teratogenic effects attributable to Cefuroxome, the response to the high and probably harmful dose of Cefuroxime was an all or none phenomenon, involving the normal survival of three of the fertilized oocytes and death of the remaining eleven. The latter failed to cleave and gradually degenerated. This probably accounts for the absence of malformations in the surviving fetus. Embryo transfer was carried out in this study only since the bacterial contamination had been eliminated by the antibiotic.

The use of high dose antibiotic, in addition to being a good procedure for salvaging contaminated preembryos, also provide a novel opportunity to study the resistance of human preembryos at prounclear stage to suspected teratogens, which would otherwise have been considered unethical.

REFERENCES

1. *Edwares R.G. In Conception in the Human Female, 1980, PP 668, Academic Press, London*
2. *Fishel S., Jackson P., Webster J., Faratian B., Fertil. Steril. 49, 108, 1988*
3. *Hewitt J., Cohen J., Fehilly C., Rowland G., Steptoe P., Webster J., Edwards R., Fishel S., J. In. Vitro. Fert. Embryo Transfer 2, 105, 1985*
4. *Ng S.C., Edirisinghe W.R., Sathananthan A.H., Ratnam S.S. Int. J. Fertil. 32, 298, 1987*
5. *Snyman E., Van der Merwe J. V., Fertil. Steril. 46: 273, 1986*
6. *Wilson J.G. : Wilson J.G. and Fraser F.C., Eds: Handbook of Teratology, 1977, Vol. 1, p 47, Plenum Press, New York.*