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LOW COST INVITRO FERTILIZATION USING MICROCULTURE TECHNIQUE

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ABSTRACT

Objective: To describe Low cost in vitro fertilization in the third world country.

Design: Retrospective review of lVF clinical data.

Setting: Assisted Reproductive Technology, Medical Center, Ikeja, Nigeria.

Patient(s): Seven couple that underwent assisted reproductive technology (ART) program.

Intervention (s): Semen Analysis (SA), Oocyte retrieval, in vitro fertilization (IVF), Embryo Culture, Embryo Transfer (ET).

Main Outcome Measurements: Oocyte quantity and quality, fertilization rate, embryo
quantity and quality, implantation rate and pregnancy rate.

Rcsult(s): Seven (7) patients that underwent IVF treatment five (5) patients were positive
for pregnancy two(2) were negative.

Conclusion: Low volume culture media save the cost of IVF

Keywords: In vitro fertilization^ embryo transfer, preovulation and semen analysis.

INTRODUCTION

The advances in the field of assisted reproductive technologies during the past two decades have led to improved pregnancy rate. There are few invasive predictors of successful IVF outcome to clinicians before starting an IVF treatment such prcclicator are helpful to clinician regarding the potential success expected when pursing these financially and emotionally taxing treatment. Accurate methods of predicting IVF success allow for appropriate stimulation protocol and culture method.

The objective of this study is to describe a low cost method for establishing pregnancy in an infertile woman or man by .means of in vitro fertilization, resulting embryo in tissue culture media and subsequent transfer into the uterine cavity. As several groups have commenced work in this field, and our methods are presented in a form, which will enable their detailed appraisal in the hope, that further improvement of the procedures will result in a clinical acceptable low cost IVF treatment.

IVF initially presented as a treatment of severe female mechanical factor infertility but was quickly utilized in other'areas in the field of infertility, such as unexplained infertility, male factor (1) infertility, immunological infertility, endomctriosis (2,3), cervical factor infertility, polycystic ovarian diseases, preimplantation genetic diagnosis and ovarian failure.

MATERIALS AND METHODS

Patients

This study was performed between January 2005 and July 2005 at the Assisted Reproductive Technology Medical Center Ikeja, Nigeria. Seven couples who had several years of unprotected intercourse without conception, and enrolled forWF-ET treatment. This data reported was validated independently by medical records review and the invitro fertilization procedure is as follows.

Stimulation Protocol

GnRH- was given daily at a dose of Img until a serum E2 level was obtained to assess whether adequate suppression had been achieved.

When serum E2 and FSH levels observed ovarian suppression on the third day of the menstrual cycle, GnRH-a was given daily at a dose of 0.5mg in the morning and one or two amupules of 75IU of hMG and FSH were given IM in the evening, depending on the follicular development.

Aspiration Technique ;

Oocyte retrieval by transvaginal ultrasonographic guidance was performed, placing the patients in the dorsal lithotomy position and the cervix is washed with equilibrated suffered saline solution. A seminged Carthter is used aspirate approximately 36hours after the hCG administration. Follicular aspirates were transferred into 60-mm tissues culture dishes (Falcon 3002; Becton Dickinson,. Lincoln Park, NJ). Oocyte-cunjulus cell complexes were isolated under a dissecting microscope (SZH, Olympus, Tokyo, Japan).

The maturity and quality each Oocytes-cumulus cell complex was grated under inverted phase contrast microscope (Olympus 1X70, Olympus) by spreading each oocyte-cumulus cell complex on one line of the surface of 60-mm culture dishes containing Qocytes with first polar body and extensive cumulus cells were regarded as mature.

Culture Technique

Four of five Oocytes were placed into each culture dish (Falcon 3037; Becton

Dickson) contain 50ul HEPES media. Highly motile spermatozoa were collected by a discontinuous three-layered percoll gradient (100%-50%) and suspended in glucose-containing HTF medium'with 10% FF.

Insemination

Then they were inseminated 4-12 hours after oocytc retrieval, depending on the oocyte maturity. Fertilization was confirmed 16-20hours after insemination by visualization of two pronuclei. Fertilized oocytes were transferred to fresh and then cultured for 24hours or more. After culture, embryos were graded based on the size of the blastomere and the presence of fragmentation, using Bolton's definition (4), just before ET. The ET was performed with Embryo transfer catheter. The patient is kept recumbent in mild trendelenburgs position for 3hrs to allow the transfer fluid to absorb 3 days after oocyte recovery in fresh cycles and 3 days after ovulation in frozen thawed cycles.

Semen Collection and Assessment of Semen Variable

Semen specimens were collected by masturbation after 48-72hrs of abstinence. After liquefaction at 37°C for 20 minutes, 5ul of each specimen was loaded on a counting chamber (Conception Technologies, San Diego CA) and analyzed for sperm concentration and motility for morphological evaluation, and assessed according to World Health Organization (WHO) guideline (8).

An aliquot (ImL) of the liquefied semen was loaded on to a 47% and 9% discontinuous Isolate gradient and centrifuged at 500 X g for 20 minutes at room temperature. The resulting pellet was washed with (Ferticult) media, by centrifugation at 500 x g for 7 minutes. The supernatant was aspirated, and the pellet was resuspended.

Semen Analysis

After liquefaction, 5ul of semen was loaded on a counting chamber, the total sperm count (X 10 /mL) and percentage motiisty were measured manually, according to WHO guidclines(8).

RESULTS

Result of seven in vitro fertilization tests was shown in Table 1 . Pregnancy test confirmed positive in (71.49%) while negative in 2 patients (28.57%).

TABLE 1 : RESULT OF INTRAUTERINE IN VITRO FERTILIZATION

Serial Number	Number of Eggs collected	Number of Embryo transfer	Day 14 Pregnancy test outcome
1	4	4	+ 7/52
2	2	2	-
3	2	1	+ 7/52
4	3	2	+ 6/52
5	2	1	+ 6/52
6	1	1	+ 6/52
7	2	1	-

DISCUSSION

This study shows no significant differences in low volume of 5Qul of culture media compared with lOOul. This is the first study to date establishing culture medi'a of 50ul, as the lowest volume adequate for IVF outcomes. In all parameters efficiency at the lower volume of 50ul was comparable to the standard volume of lOOul at most IVF centers. The successful pregnancy and delivery (5) following IVF was probably the most spectacular development in female infertility management in the second half of the 20^{lh} century. The groundwork for the breakthrough had been laid by work in animal models several years before human IVF(7). Stcptoe and Edwards(6) reported pregnancy and subsequent delivery of a baby through in-vitro fertilization and embryo transfer (IVF-ET) in 1978. Following the pioneering report by Ashiru and Giwa-Osagie(lQ) of similar success in Nigeria in 1986, the quest for ART has increased in Nigeria and the West African sub-region. Currently, there are very few programs that offer ART in Nigeria. The techniques employed today arc very different to those first pioneered by Steptoe and Edwards in the late 1970s, but they aJI employ the same underlying.principles. The period from 1990 to 2000 witnessed significant developments in the field and the emergence of cutting edge technologies that have advanced the practice of assisted conception (supported by Medical Art Center Research and Training Program Ikeja, Nigeria).

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