Research Article ISSN 2639-9342

Gynecology & Reproductive Health

Pioneering *In Vitro* Fertilization in Nepal: Effectiveness in Different Age-Groups

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Received: 30 March 2020; **Accepted:** 15 April 2020

Citation: Shrivastava U, Amatya R. Pioneering *In Vitro* Fertilization in Nepal: Effectiveness in Different Age-Groups. Gynecol Reprod Health. 2020; 4(2): 1-5.

ABSTRACT

Introduction: *The study evaluates the effectiveness of different protocols used in In-Vitro Fertilization procedures and their role in recruiting follicles in different age groups affecting fertility outcome.*

Methods: *This prospective study was conducted in between August 2002 and September 2003, in which sixty-eight cases were enrolled, who had tubal block, anovulation, male factor infertility, endometriosis, failed IUI /IVF or idiopathic infertility. Women were categorized into three age groups A (24-29 years), B (30-34 years) and C (35-40 years). Their ovarian response using long, short and micro-dose stimulation protocols were monitored via ultrasonic follicular measurements and endometrial thickening. Oocytes were collected by aspiration of follicles under anesthesia, inseminated and incubated at 37°C with humidified 5% CO_2 for fertilization. Two to three fertilized embryos were transferred into uterine cavity.*

Results: *Out of total 190 oocytes, 103 were fertilized and 87 were unfertilized. Conception results showed 17(53%) biochemical pregnancies out of 32 transfers. Of them, 8(25%) had clinical pregnancies, 4(12) abortions, 1(3%) ectopic pregnancy and 4(12%) had live births.*

Conclusion: *The major predictor of In vitro fertilization lays at the qualities of oocytes and sperm. However, age was the determinant of implantation and continuation of pregnancy in this study.*

Keywords

Anovulation, Endometriosis, Follicles, In-Vitro Fertilization (IVF), Oocytes.

Introduction

In Vitro Fertilization (IVF) & Embryo transfer (ET) was proved successful by Edwards & Steptoe in 1978 in the treatment of critical infertility conditions which resulted in the delivery of Louise Brown thirty years ago [1]. Although many types of Assisted Reproductive Techniques (ART) have been introduced such as extracorporal fertilization & cleavage of human egg by Rock & Menkin in 1944 & first Intrauterine Insemination (IUI) of washed human semen by Kaskerelis in 1959 & were proved successful in the past [2].

IVF & ET is indicated mainly for blocked fallopian tubes, male factor & anovulation. Use of testicular sperm aspiration (TESA) & culture of the sperm for maturation followed by IVF or ICSI are providing successful pregnancies in recent years [4,5].

Conception begins after embryo implantation in the endometrium. Implantation depends upon receptivity of endometrium, responsiveness of maternal corpus luteum & viability of embryo for successful pregnancy which is affected by aging over forty [6]. Miscarriage & fetal chromosomal abnormalities occur frequently as these women remain poor responder in IVF cycles [7,8].

Hence, the gold standard governing the successful pregnancy lies on the female age, ovarian response to superovulation, quality of oocytes & sperm, endometrial pattern & quality of embryos transferred.

Methods

A prospective interventional study was conducted on 68 infertile couple who were unable to conceive with other conventional treatment procedures at various clinics and hospitals. Patients were selected randomly on the basis of previous failure to other treatment modalities including IVF at various settings. The study was conducted between August 2002 & September 2003 following all ethical norms with written consent from the patients. The study group consisted of patients with Blocked fallopian tubes, Male factor, Anovulation, Failed previous IVF, Unexplained infertility & Endometriosis. Cases with ovarian failure, over-age, adhesion after uterine, ovarian or tubal surgery were also included. However, this study was carried out with consideration of varying age of patients, rather than the cause of infertility.

These patients were categorized into three groups according to their age: Group I (24-29 yrs.), Group II (30-34 yrs.) & Group III (35-40 yrs.).

In pre-IVF cycle, both partners were tested for blood count, routine urine, X-ray chest, VDRL, HIV, HBsAg, & for male partner semen culture was done routinely. TORCH test was done in selected cases. For female, basal hormone profile, basal ultrasound & skull X-ray were also done in hypergonadotropic cases. High prolactin & TSH were controlled with bromocriptin & thyroxin respectively prior to IVF.

Groups	Group-I	Group-II	Group-III
Age of women (yrs.)	24-29	30-34	35-40
Number of women (N)	15	20	33

Table 1: Total Number of patients studied (N=68).

Hormone Assay

Blood Hormones were measured using commercially available ELISA kits reported previously13. Venous blood of about 5 ml was collected in the morning of D2 from the women for hormone profile of Follicle Stimulating Hormone (FSH), Lutenizing Hormone (LH), Estradiol, Prolactin & Thyroid Stimulating Hormone (TSH). Serum was obtained after centrifugation. 25-100µl of serum was processed for immunological reaction & the results were analyzed using a Computerized Micro-well Elisa Reader (Awareness Technology, USA). In addition, serial Estradiol was assayed from D5 according to the number & size of the follicle till ovulation.

	Gr. I	Gr. II	Gr.III	Total
FSH (>10mIU/ml)	3	4	10	17
LH (>10mIU/ml)	3	10	3	16
FSH&LH (>10mIU/ml)	1	3	11	15
E2 (< 20 pg/ml)	3	5	12	20

Table 2: Hormone levels.

Stimulation protocol

In planned IVF, long, short & microdose stimulation protocols were used. In long protocol, GnRH agonist of 500µg twice daily injected subcutaneously from D21 of the previous cycle until menses. From D1 of IVF cycle, the dose was reduced to half & injected once daily along with intramuscular pure or combined Gonadotropins 150-225IU/daily.

The short protocol consisted of GnRH 500µg daily subcutaneously & Gonadotropin 150-225 from D2 till the day of Human Chorionic Gonadotropin (HCG) when follicles reach the diameter of 18mm.

Microdose protocol was made up of 30µg/day GnRH along with Gonadotropin 150-225 IU/day D2 till the day of HCG as above.

Type of protocol	Gr. I	Gr. II	Gr. III	Total
Long protocol	16	10	3	29
Short protocol	3	9	8	20
Microdose protocol	5	7	7	19

Table 3: Ovarian Stimulation protocol.

Pelvic ultrasound

Transvaginal sonography (TVS) was performed to assess the follicular development & endometrial thickness using (6.5 MHz) transvaginal probe on Medison 5500 (USA) Ultrasonographer. During the IVF cycle, each woman underwent D2 sonography to exclude retention cysts. Along with the injections from D6 onwards, folliculometry & endometrial thickness was measured till the day of HCG. When two or more follicles reached 18-20mm, 10,000 IU HCG was injected intramuscular for triggering ovulation. After 34-36 hours following HCG injection oocyte retrieval was processed.

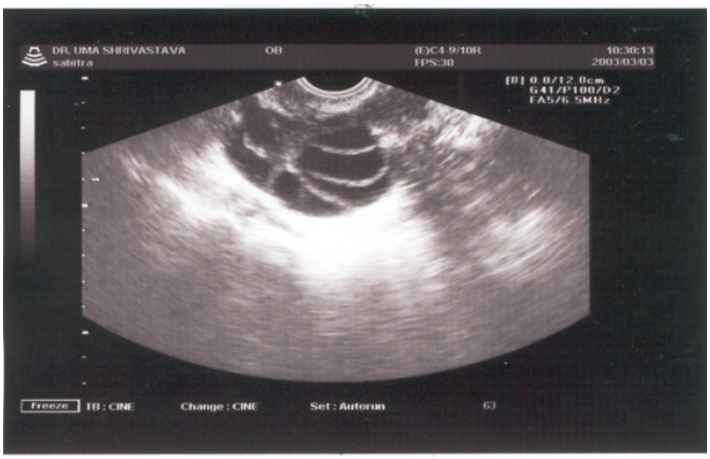

Figure 1: TVS of a stimulated ovary.

Oocyte retrieval

In a minor operation theatre, with prophylactic antibiotics & intravenous sedation, the vulva & vagina of the female were cleaned with sterile phosphate buffered saline. The TVS probe was attached to the guide to hold the oocyte retrieval needle (Cook, Australia). The tubing of the needle was connected to the Craft suction unit (London) & 15 ml test tubes containing flushing medium standing on a dry bath at 37ºC. Using a foot pedal of suction with vacuum pressure of 100-150 mm Hg, oocytes were aspirated in the test tube inserting the oocyte retrieval needle along

with the vaginal probe through the fornices of the vagina. Under ultrasound guidance, each follicle was punctured & the content was collected in the attached test-tubes & immediately transferred to the embryology.

Sperm recovery

For sperm preparation, semen was collected by masturbation in a wide mouthed sterile container & left for liquefaction for about 30 minutes at 37ºC. Swim-up & Density gradient methods were used as methods for sperm recovery.

For swim-up method, the semen was mixed with wash medium & centrifuged for 10 minutes. Decanting the supernatant 2 ml of the medium was layered on the pellet & re-centrifuged for 10 minutes. Supernatant removed & the pellet was layered with 1 ml of media & incubated at 37ºC with 5% CO_2 in the CO_2 incubator (Binder, Germany & Forma Scientific, USA).

Poor quality sperm were processed for density gradient method, Semen was layered over 5ml of the gradient & centrifuged for 10 minutes. Then the supernatant was carefully removed & mixed the pellet with gradient. Adding 2ml of medium on the pellet were re-centrifuged for 7 minutes. The supernatant was decanted & over-layered 1ml of medium over the pellet & incubated at 37ºC with 5% CO_2 till further use.

In Vitro Fertilization & Embryology

The working station in the Embryology Laboratory was the Vertical Laminar flow cabinet with heated base at 37ºC. Under the Laminar hood, the content of the aspirated follicles was poured in a culture disc & scanned under Sterio-microscope (Olympus, Japan) fitted with a stage warmer 37ºC & digital CCD camera with monitor. The oocyte-cumulus complex is picked up with a pasture pipette & washed in flushing medium to remove the cumulus & transferred in a centre hole culture disc. The cleaned oocytes were transferred to another disc containing culture droplets & kept in CO_2 incubator at 37ºC with 5% CO_2.

After 4-5hours the oocytes & sperm were transferred in a single culture droplet under oil. Under careful Sterio-microscopic visualization & video guide after 20-24 hours, the eggs were denuded with flexipet & fertilization was assessed. The presence of two pronuclei & two polar bodies indicated fertilization with the formation of zygote. The embryos were graded as good, fair & poor according to their fragmentation & the shape of blastomeres. The 2PN embryos were incubated further. The next day these embryos were checked for cleavage, division & fragmentation. Two-cell or four-cell stage good quality embryos were selected for transfer.

Embryo Transfer

On the day of transfer, the best embryos were selected. 2-3 embryos were loaded in the Embryo transfer catheter (Cook, Australia). In minor theatre, the patient was kept in Trendelenberg position. Vulva, vagina & cervix were cleaned with saline & draped. With the help of duck-billed speculum, vagina was exposed. The embryos loaded catheter was carefully inserted into the cervix & further into the uterine cavity. The embryos were carefully pushed into the cavity under the guidance of ultrasound. The patient was send home after 4-24hours for bed rest at least two week. Every patient was tested for blood HCG after two weeks of ET. Positive HCG patients were asked for further bed rest & enrolled in special Ante-natal group for follow up.

Results

The primary outcome of the study was the number of oocytes retrieved. It was found that all the follicles did not contain the oocytes. The study showed that follicle development & their numbers cannot predict good oocyte recovery. However, some degree of technical error during OPU which might have lost some oocytes during pick-up cannot be excluded.

	Group I (N=14)	Group II (N= 18)	Group III (N=32)	Total (N=64)
No. of Follicles	112	107	79	298
Mean Endometrial thickness	8 (mm)	9 (mm)	6(mm)	-
No. of Oocytes	61	54	23	138
No. of Fertilized embryos	21	34	22	77
No. of Unfertilized oocytes	19	19	21	59
Deformed embryos	-	-	2	2
No. of patients for ET	14	18	25	57
Cancellation of ET	0	0	7	7

Table 4: Embryological outcome.

Figure 2: Oocyte with cumulus.

Figure 3: Fertilized oocyte with 2 Pronuclei.

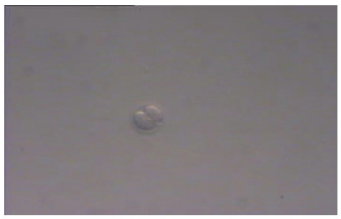

Figure 4: 2-celled embryo.

Figure 5: 4-celled embryo.

Figure 6: Damaged embryo.

Figure 7: Unfertilized oocyte.

Our secondary outcome measures were implantation & patterns of pregnancy.

Pregnancy outcome	Gr. I	Gr. II	Gr. III	Total (%)
No. of (chem.) pregnancies	7	11	3	21 (36.8%)
No. of (clin.) pregnancies	5	7	2	14 (24.5%)
No. of abortions	3	3	2	8 (14%)
No of ectopic pregnancy	1	1	-	2 (3.5%)
No. of multiple pregnancy	1	1	-	Twins/Triplet (3.5%)
No. of deliveries	2	1	1	4 (7%)

Table 5: Pregnancy outcome of Infertility centre.

Data analysis & statistical comparisons were done using chi-square test at p=0.05. The age group & the ovulation stimulation protocol are dependant, which is significant of the study.

However, the results show that the age group of sample population & pregnancy outcome are independent. This was probably due to fact that the principal component of the analysis depended on the chemical pregnancy i.e. analysis of pregnancy outcome was derived from number of chemical pregnancies. Elective Caeserian section under general anaesthesia was the mode of delivery.

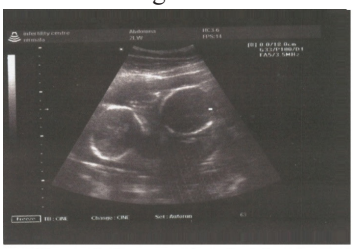

Figure 8: Sonography of triplet.

Figure 9: Triplet.

Discussion

IVF as an ART has crossed more than three decades. There have been a lot of technical modifications in these years. The recent advances of diagnostic ultrasonography including transvaginal route to map the pelvic pathology, testing tubal patency, details of endometrium & follicular tracking are essential procedures while diagnosing the etiology of infertility.

TVS has allowed us to differentiate the patterns of endometrium affecting the conception. Hence, several types of thickening of endometrium or the embryonic bed has been classified. The best among all seems the homogenously hypoechoic endometrium without a central echogenic line as a predictor of normal conception [9]. We observed in many of our cases irregular, heterogenous endometrium, probably due to past Koch's infection, which might have obstructed implantation.

Pioneers of IVF retrieved oocytes through trans-abdominal route under the guidance of laparoscope & it was proved to be very difficult procedure. Trans-vesical, ultrasound guided follicular punctures dominated in 1985 and transvaginal ultrasound-guided punctures were freely used only after 1987 [10].

Trans-vaginal route has been found very easy & less traumatic procedure. In addition, the serial follicular study helps to save patients from ovarian hyper-stimulation syndrome (OHSS) while using ovulation stimulating protocols for IVF. In the stimulation protocol, long protocol has been very popular [11]. We used long protocol, newly introduced micro-dose protocol & the usual short protocol.

Our result showed a better response & result to long protocol even though it was a long painful procedure with daily injections along with physical & mental stress. The protocol selection criteria based upon the levels of hormones on D2 of the pre-IVF cycle. Our short protocol result was not worse than the compared previous studies of short protocol [12].

In our study, the micro dose protocol showed worst result. This protocol did not recover good oocytes neither good embryos. In most cases long protocol produced more follicles but majority of them did not develop good oocytes. There were high prolactin & high LH:FSH ratio in most of these cases. It must also be noted that there might have been some degree of technical error during OPU which might have lost some oocytes during pick-up.

Profound ovulatory disorder with low estrogen levels, primary or secondary ovarian failure with diminished ovarian reserve were cancelled for OPU.

The metabolic requirements of human embryo change day by day. Early embryos need minimal glucose & amino acid while blastocysts need more of them in quantity. It was a trend to use the same media (Ham- F-10) for the whole embryo culture period [13]. Ready-made supplied or locally freshly made culture media available now–a–days are sequential media with the change in the quantity & quality of nutrients to meet the need of embryo at different stages. The disadvantage of these media is their short shelf life but fertilization & growth of embryos are found quite good [14].

Sequential media (Medicult, Denmark) was used in this study with regular checking of pH. Quite a number of oocytes did not fertilize & some of them were damaged. However, contamination of the media also could not be excluded. In addition, it was interesting to note that in few cases the cleavage potential of all embryos from the same woman was not the same. On the day of transfer, among two embryos, one was found at 2-cell stage, another 3-cell stage & the third one almost at 4- cell stage. In IVF, multiple-gestation is highly expected. This study used two to three embryos for transfer. American Society of Reproductive Medicine (ASRM) & International Council on Infertility Information Dissemination (INCIID) have recommended not more than two embryos for transfer per cycle.

Atraumatic embryo transfer has a great role in the implantation & continuation of pregnancy. There were two types of transfer catheters used, soft without guide & comparatively hard with guide. However, most of the cases had no difficulty in transfer.

Moke transfer was routine on D5 of the IVF cycle. Our data on pregnancy outcome was satisfying for a single cycle IVF. However, take home baby rate was low & abortion rate was high. Since

this was the first treatment program in the country, the chemical pregnancy rate with serum HCG levels above 20mIU/ml were also included in the study data.

Evidence shows in normal conception 38% pregnancy loss before implantation & 29% loss after implantation [15]. In Assisted reproduction report shows 36.8% miscarriages, including 7.7% ectopic pregnancies [16]. This work also included several conditions even with least possibility of conception. These were cases with serum Estradiol <100 pg/ml on D8 after 5 days gonadotropin stimulation, cases with serum Estradiol <500 pg/ml with 4 follicles of preovulatory size on the day of HCG. Such E2 levels produce poor quality oocytes due to reduced ovarian reserve with low probability of successful pregnancy. In addition, high abortion rate might have also related to uncontrolled hyperprolactinemia during induction [18].

The early abortion in our study probably had two things to relate, they could have been poor uterine receptivity due to age factor & or poor embryo viability.

Conclusion

This is only a survey of the IVF treatment held for the first time in this climatic condition. Any step in our IVF-ET process might have been complicated by unforeseen events, such as hazards, high altitude, equipment failure, laboratory conditions, infection, human error etc. More work needs to be done on each as trouble-shooting during IVF.

With multi-factorial cases & having majority patients above thirty our result was well comparable to the previous studies. However, none of the compared studies have provided single cycle IVF result in each case. Unlike most studies, which have declared several cycle treatments results, our results are based on single cycle IVF treatment.

The three stimulation protocols used in single cycle IVF showed best results with long type protocol in younger women.

References

1. Edwards RG, Broody SA. Principles & Practices of Assisted Human Reproduction. Saunders company. UK. 1995.
2. Allen NC, Herbert CM, Maxson WS, et al. Intrauterine insemination: a critical review. Fertility & Sterility. 1985; 44: 569-580.
3. Edwards RG, Steptoe PC. Current status of *in vitro* fertilization & implantation of human embryos. Lancet. 1983; 2: 1265.
4. Palmero GH, Joris H, Devroey P, et al. Pregnancies after intracytoplasmic sperm injection of single spermatozoa into an oocyte. Lancet. 1992; 340: 17-21.
5. Avery SM, In Brindsen PR. 1st Indian eds. A textbook of *In Vitro* Fertilization & Assisted Reproduction. Parthenon (UK) Jaypee brothers Press, India. 2001; 203-209.
6. Lenton EA, Woodward AJ. The endocrinology of conception cycles & implantation in women. J of Reprod Fert. 1988; 36: 1-15.
7. Smith KE, Buyalos RP. The profound effect of patient age on pregnancy outcome after early detection of fetal cardiac activity. Fertil Steril. 1996; 65: 3540.
8. Scott RT, Hofmann GE, Oehninger S, et al. Intercycle variability of day 3 follicle stimulating hormone levels and its effect on stimulation quality in *in vitro* fertilization. Fertil Steril. 1990; 54: 297-302.
9. Puerto B, Creus M, Carmona F, et al. Ultrasonography as a predictor of embryo implantation after *In Vitro* Fertilization: a controlled study. Fertility & Sterility. 2003; 79: 1015-1022.
10. Avery SM. In Brindsen PR1st Indian eds. A textbook of *In Vitro* Fertilization & Assisted Reproduction. Parthenon (UK) Jaypee brothers Press, India. 2001; 203-209.
11. Weissman A Farhi J, Royburt M, Nahum H, et al. Prospective evaluation of two stimulation protocols for low responders who were undergoing IVF -ET. Fertility & Sterility. 2003; 79: 886-892.
12. Surrey ES, Schoolcraft WB. Evaluating strategies for improving ovarian response of the poor responder undergoing assisted reproductive techniques. 1: Fertil Steril. 2000; 73: 667-676.
13. Shrivastava U. Pioneering IUI in Nepal. Proceeding of III National Conference on Science & Technology. 1999; 692-698.
14. Bongso A. Handbook of Blastocyst culture. Sydney press. Singapore. 1999; 55-65.
15. Cooke ID. Failure of implantation & its relevance to subfertility. J Reprod Fert. 1988; 36: 155-159.
16. Scott L. Embryological strategies for overcoming recurrent assisted reproductive technology treatment failure. Human Fertility. 2002; 5: 206-214.
17. Avery SM. In Brindsen PR 1st Indian eds. A textbook of *In Vitro* Fertilization & Assisted Reproduction. Parthenon (UK) Jaypee brothers Press, India. 2001; 203-209.
18. Rossi AM, Vilska S, Heinonen PK. Outcome of pregnancies in women with treated or untreated hyperprolactinemia. Eur J Obstet Gynacol Reprod Biol. 1995; 63: 143-146.