Clinical Results of the Intra Cytoplasmic Sperm Injection (ICSI) at Baqai Institute of Reproduction and Developmental Sciences (BIRDS)

Shaheen Zafar,Shaheen Panjwani,Mohammad Kouser,Asrna Munir,Serwat Jehan,Zahida Baqai (Baqai Institute of Reproduction and Developmental Sciences, Baqai Medical University, Karachi.)

Abstract

Objective: To analyse the results of the new revolutionary technique of Intra Cytoplasmic Sperm Injection (ICSI) for male infertility. Furthermore, to find out the ways of reducing cost of the expensive methods of Assisted Reproductive Techniques.
Design: This is an analytical study of results of 71 patients (86 Cycles) of ICSI performed in first year of its launching in Baqai Institute of Reproduction and Developmental Sciences (BIRDS) from May 1997 to April 1998. Men with semen reports of parameters less than WHO criteria were treated including Azospermic men.
Results: After ICSI procedure a fertilisation rate of 58.9% was achieved in 86 cycles. A total of 17 clinical pregnancies were had from the transfer of fresh embryos. This includes three pregnancies from Surgical Sperm Collection in azoospermic men. Cost could be reduced by avoiding high dosage of drugs and by cutting out serial serum estrogen levels. Ultrasonic assessment for follicle monitoring is equally satisfactory. **Conclusion:** ICSI proved to he the only successful treatment for men with poor semen quality. It is giving nearly 20% chances of fatherhood in a man who was labeled infertile previously. Results are promising and will be improving with time. The cost could be reduced to an extent by cutting down drug doses and laboratory investigations without compromising the results (JPMA 50:228, 2000).

Introduction

Assisted Reproductive Techniques have changed the whole concept of infertility management since the first In Vitro Fertilisation (IVF) baby in 1978 [1]. Male infertility was still a problem until recentl and has very limited success with IVF. The advent of Intra Cytoplasm ic Sperm Injection (ICSI) has revolutionised the concept of male infertility management. Those men are fathering children who could not even dream of it before the first successful case in 1992 [2]. The incidence of male infertility is increasing all over the world[3] as Well as in Pakistan. The cases which were labeled as unexplained in the past, are now being diagnosed as sperm malfunction. In the Pakistani social set up with a male dominating society, it becomes all the more important to find a solution for male infertility. Sperm donation is not an option to be considered in a Muslim communities it is not permitted in Islamic Ethics [4].
As only one viable sperm per oocyte is required for ICSI, men with occasional sperms somewhere in the genital tract can be offered ICSI programme. In response to the impressive results of ICSI. reported by Van Steirtegham [5,6] and Payne [7], the lCSl programme was started at BIRDS for patients with severe defects of semen quality or who had previously failed fertilisation in vitro. As the success of ICSI became evident, the number of patients entering the ICSI program has increased many folds. At present the unit is doing -more ICSI procedures than the conventional IVF.

This report presents the results of7I patients with 86 ICSI cycles performed in the first year of its introduction at BIRDS from May 97 to April 98.

Material and Methods

Patient Selection

The patients were selected from BIRDS out patients as they attended here for the management of infertility. After initial base line investigation and detail counseling the couples with male factor infertility were selected for Intra Cytoplasmic Sperm Injection. The criteria for normal semen parameters was as explained in WHO manual [8] i.e., Sperm count >20x I 0 ^{6}/ml. with >50% progressive motility and >30% normal morphology. All those men whose semen did not fullfil this criteria were considered as male factor infertility. The selection criteria for ICSI procedure was as follows: Sperm count <20 mill/mI And/or Sperm progressively motile <25% And/or Sperm with normal morphology <15% with TZI (TeratoZoospermic Index) of more than 1.4

The type of different male factors included in this group was as follows:

1.Severe Oligozoospermia: average sperm concentration <2x I 0 ^{6}/ml.
2.Oligoasthenoteratozoospermia: average sperm concentration <20x10 ^{6}/ml. with <25% progressive motility and/or <50% total motility and <15% normal sperm morphology.
3.A sthenotertozoosperm i a: average sperm concentration >20x106/ml. with <25% progressive motility and/or <50% total motility and <15% normal sperm morphology.
4.Teratozospermia; <15% normal sperm morphology.
5.Azoospermia with Epididymal sperm: (7 patients, 7 cycle) Patients requiring either microsurgical Sperm Aspiration (MESA) or Percutaneous Epididymal Sperm Aspiration (PESA) for sperm retrieval due to obstructive Azoosperm ia.
6.Azospermia with Testicular Biopsy, TESE, (8 patients, 8 cycle); in these patients sperm retrieval was performed by open testicular biopsy.
7.Round headed acrosomeless sperm (3 patients, 3 cycles): 100% acrosomeless sperms as assessed by light microscopy.
8.Completely immotile sperm (2 patient, 2 cycle): paEients in which no motile sperm was isolated from a testicular biopsy, sperms were selected for injection by observing tail coiling in hypo-osmotic medium (HOS test).

Methods

After initial assessment the female partner's ovaries were stimulated for super-ovulation.

Super-Ovulation

Pituitary desensitisation was performed by Gonadotrophin releasing hormone agonist. Long protocol of Busereline nasal spray was used for this purpose. After ultrasonic confirmation of inactive ovaries and endometrium, Human Menopausal Gonadotrophin (HMG) was started for ovarian stimulation. Monitoring was performed by serial Trans Vaginal Ultrasound only. Once the follicle reached 1.7-2.0 cm size, Human Chorionic Gonadotrophin (HCG) was given for leutinisation. After 32-34 hours, oocyte retrieval was planned.

Oocyte Retrieval

Ultrasound Directed Follicle Aspiration (UDFA) was performed transvaginally. All the follicles were aspirated. The follicular fluid was shifted to the embiyology lab immediately. The procedure was performed under general anaesthesia or sedation according to patients choice and

depending on the number of follicles to be retrieved.

Ovum Pick Up (OPU)

The ova were picked and washed in the media and were incubated at 37°C in 5% CO^2 environment.

Preparation of Oocytes

All the ova destined for ICSI were incubated for 12 hours prior to cumulus removal. The cumulus masses were removed until only the corona radiata and oocyte was left behind. The final coronal cells were sheared off until the cytoplasm was easily observed. The denuded oocyte was then placed in the fresh culture medium and allowed to further mature for 2-4 hours prior to injection. Oocytes at maturation stage of Metaphase II were selected for injection (Figure 1).

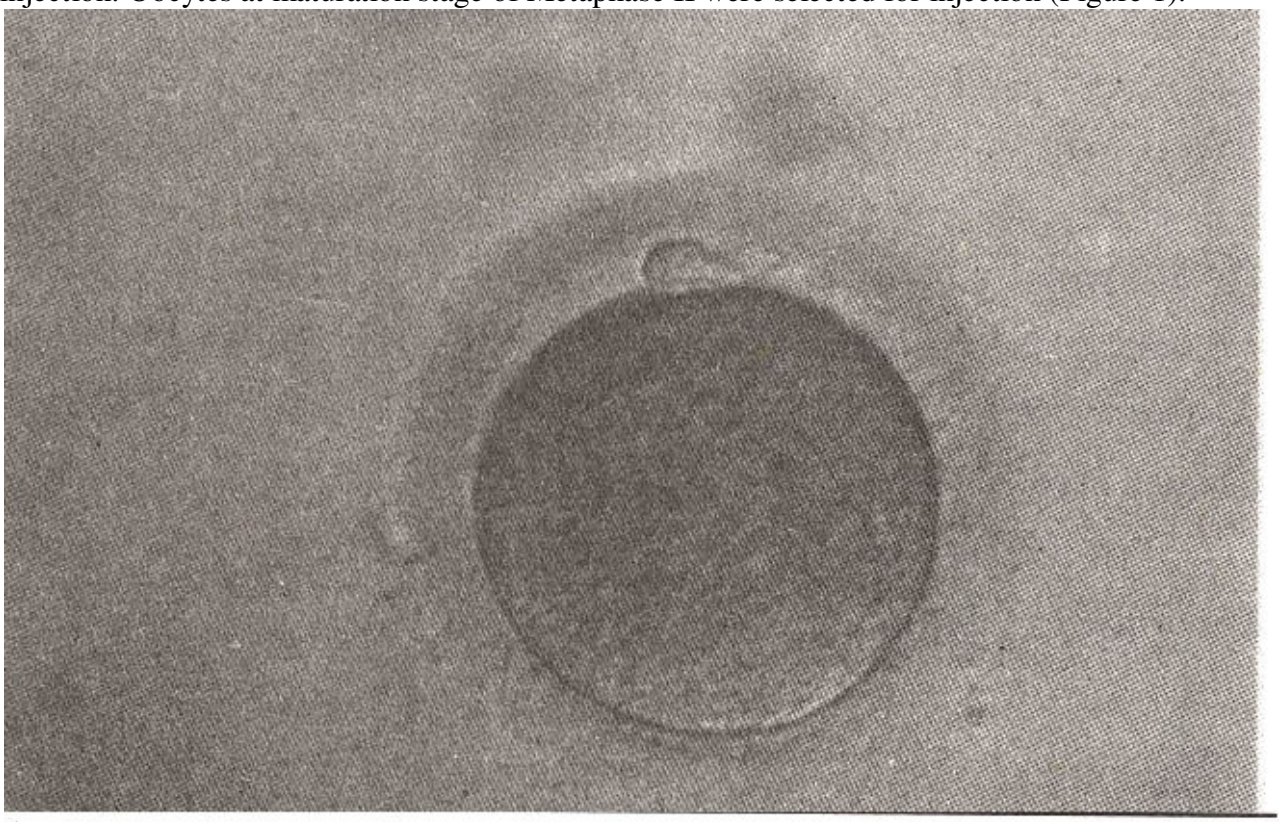

Figure 1. Denuded Oocyte without cumulus cells at metaphase II stage. Note polar body at the top.

58.94% was observed. Fertilisation rate in cases of PESA, MESA or TESE was 64% (Table 3).

Micro Injection Procedure

Micro-injection was based on the methodology described by Palermo et al [2,9]. Four oocytes were injected at any one time. The oocytes were kept in 5 ml drops of media. Final sperm suspension was mixed with a 50% PVP solution to slow down the sperm's forward progression (Figure 2).

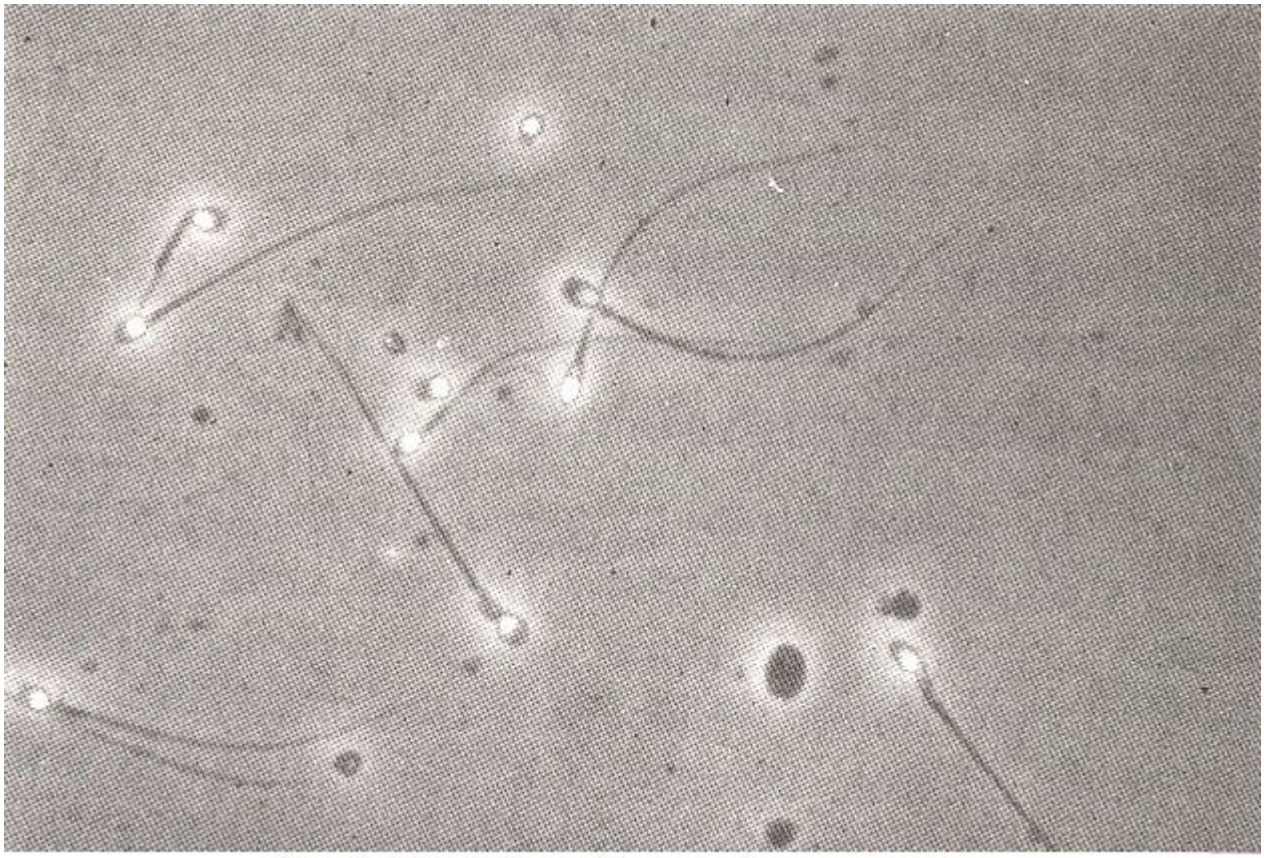

Figure 2. Sperms from Oligospermic semen sample.

A normal looking sperm was rendered immobilised by crushing the tail between the injection pipette and dish. The oocyte was held in a holding pipette in a position so that the polar body was at 6 or 12 O'clock position. The freshly immobilised spermatozoon was aspirated into an injection pipette. The injection pipette was pushed through the zona pcllucida to puncture the oolema (Figure 3).

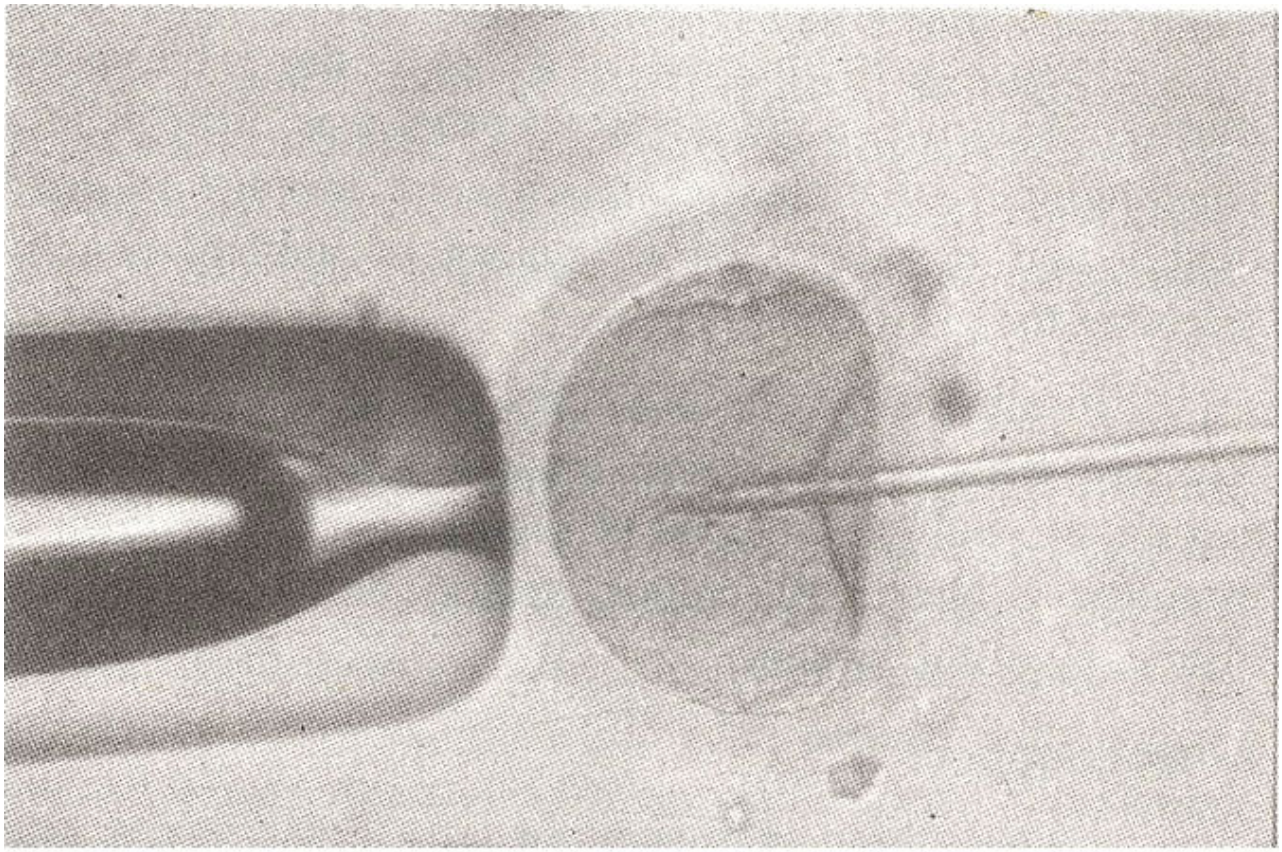

Figure 3. Intra Cytoplasmic Sperm Injection: Injecting pipette loaded with sperm is breaking the oolema to reach the cytoplasm while holding pipette is stabilising the oocyte. Note the 12 O'clock position of polar body. Sperm can be seen in the pipette.

Rupture of the oolema was confirmed by aspiration of a small volume of the oocyte cytoplasm into the injection pipette and then spermatozoon was released into the oocyte cytoplasm (Figure 4).

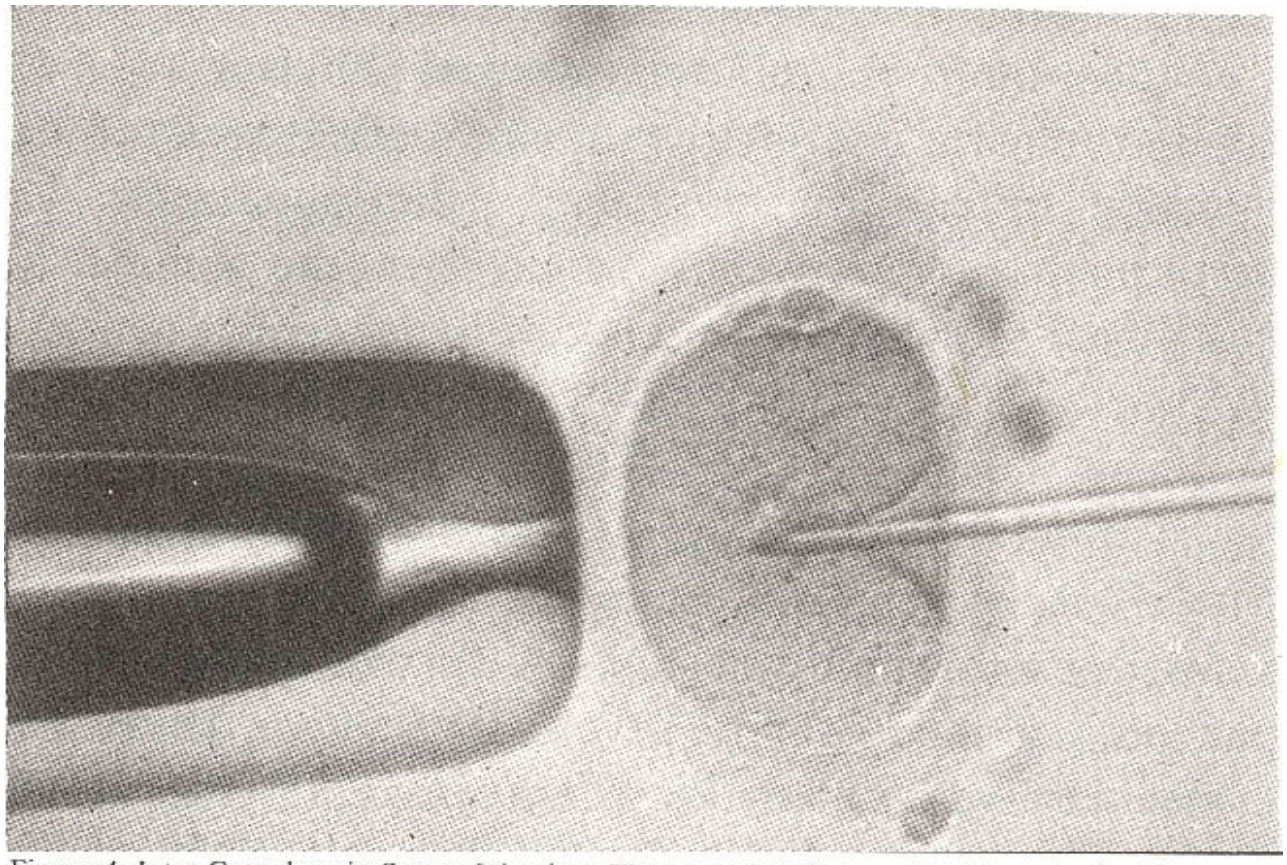

Figure 4. Intra Cytoplasmic Sperm Injection: The procedure is completed by releasing the sperm into the cytoplasm.

Hypo-osmotic medium was used to select viable sperm in patients with completely immotile sperm. One of the droplets was replaced with Hypo-osmotic medium in ICSI injection dish. Immotile sperm were aspirated into the injection pipette from the sperm drop and transferred to this drop, sperm exhibiting tail coiling on contact with hypoosmotic mediumwas used to select viable sperm in patients with completely immotile sperm. One of the droplets was replaced with hypoosmotic medium in ICSI injection dish. Immotile sperms were aspirated into the injection pipette from the sperm drop and transferred to this drop, sperm exhibiting tail coiling on contact with hypoosmotic medium were selected and used for injection.
After injection the oocytes were replaced into the incubator.

Zygote Fertilisation and Cleavage

All zygotes were checked for signs of fertilisation i.e., pronuclei formation, 16-18 hours post injection. The oocytes were rotated to identify the total number of pronuclei present (Figure 5). All two pronucleate zygotes were cultured in 10 ml droplets of medium under oil for 24 hours and were examined for cleavage. Zygotes were co-inhabited with up to five other zygotes so that maximum co-culture conditions could be achieved resulting from the sharing of growth factors.

Embryo Transfer

Most of the embryo transfer were perfbrmed on day two however few patients received a day three transfer. The cleaved embryos were judged for quality and the best 2-3

Table 1. Male factor incidence in the study group.

PN = Pronuclei.

on ovarian stimulation and cycles were abandoned due were selected for transfer (Figure 6).
Selection of embryo for transfer was based on:
The number of cell divisions.
The clarity of the cytoplasm.
The shape and size of blastomeres.
The presence and degree of fragmentation.
The embryos were aspirated in approximately 7-10 ml of culture medium within a Labotect catheter that was aided by a precision Hamilton syringe. Occasionally a more rigid catheter (Bourn Wallace with introducer) was needed for more difficult cervical cannulation. The Hamilton syringes allowed the embryos to be taken up in a minute amount of medium and hence have a greater contact with the endornetrium.
In two cases embryos were kept for day 5, blastocyst transfer. This was done when selection was difficult in a large number of embryos, which were average to good quality and it gave a better chance of implantation by selecting the grown embryos.
In all other cases after day 2 embryo transfer, the lefts over embryos were kept for blastocyst culture using coculture as well as sequential media. This was done to establish a blastocyst culture system and to check the efficiency of the system.

Luteal Support

Luteal phase was supported by Progesterone oral tablets twice a day starting from second day of egg retrieval. After first few cases, Inj. HCG 2000 iu was also given once a week starting on 3rd day of ET.
Serum b I-I.C.G. was checked 20 days after the embryo transfer and at least 10 days after the last HCG injection to confirm pregnancy. Ultrasound scan was performed after 2 weeks to confirm the number of gestational sacs and the presence of foetal cardiac activity.

Results

Total 71 patients were selected for ICSI cycle from May 97-April 98. The female age ranged from 32 to 40 years. The total number of stimulated cycles was 86 as some patients had two or three cycles attempted. Thirty two cycles were run on females of more than 35 years of age (38%). The pattern of male factor is shown in Table 1.

Table 1. Male factor incidence in the study group.

Sperm Specifications	No. of patients
Sperms from ejaculated semen	56
Oligo-zoospermia	1
Teratozoospermia	20
Oligo-astheno-teratozoospermia	35
Sperms from surgical sperm collection	
Azoospermia	15
Per Epididymal Semen Aspiration (PESA)	7
Testicular Sperm Excision (TESE)	8

In 65 males, sperms were collected from ejaculated semen. Fifteen males had azoosperrnia, in whom sperms were collected by PESA (7 cases) or TESE (8 cases).
In the ejaculated semen group there was one case with oligospermia, 20 with teratozoospermia, while 35 semen samples showed multiple problems with sperms.
Eighty cycles responded well went through UDFA while six to poor stimulation response.
The numbers of ova collected were 838, (an average of 10.5 ova per aspiration). They were checked for maturity and 676 of them were found to be at Metaphase II stage (80.6%) which were selected for injection. Fifty seven ovum were immature, 60 were damaged or degenerated and 45 were out of zona or got lysed in hyluronidase (Table 2).

Table 2. Details of oocytes collected.

Oocytes	No.
UDFA procedures performed	80
Oocytes collected	838 (10.3 per UDFA)
Immature oocytes	57 (6.8%)
Oocytes injected (Metaphase II)	676
Intact injected oocytes (survival rate)	601 (88.9%)

UDFA = Ultrasound directed follicle aspiration.

Normal fertilisation rate (2PN) of 56.6% was achieved whereas overall fertilisation rate (2PN + 3PN) of 58.94% was observed. Fertilisation rate in cases of PESA. MESA or TESE was 64% (Table 3).

Table 3. Fertilisation observed.

Fertilisation	No. (rate)
Oocytes Injected	676
Normal Fertilisation	
2 PN (Fertilisation rate)	383 (58.6%)
Abnormal Fertilisation	
3 PN	15 (2.2%)
1 PN	31 (4.5%)

PN = Pronuclei

The embryo cleavage rate was 94.7% whereas failed cleavage was 5.2%; 35 cases were selected for blastocyst growth as they had plenty of spare embryos. Total 213 embryos were cultured and 76 (3 6%) of them reached the blastocyst stage.

Total 64 patients went through Embryo Transfer (ET). Two patients were selected for Day 5 Blastocyst tranfer and 62 had Day 2 embryos tranferred. A total of 175 embryos were transferred, giving an average of 2.73 ET per patient (Table 4).

Table 4. Fertilisation and Pregnancy rates for the Intra-Cytoplasmic Sperm Injection (ICSI) programme at BIRDS in first year.

	No. (Rate)
2 PN Oocytes	383
No. of cleaved embryos	363 (94.7%)
No. of embryo tranfers ET	64
ET at Day 2	61
ET at Blastocyst Day 5	3
No. of embryos transferred	175
Blastocyst Culture	213
Blastocyst Grown	76 (36%)
Total no. of clinical pregnancies	17
Ejaculated semen	14
In SSC cases	3
Blastocyst Pregnancy	1
Pregnancy rate/cycle	19.7%
Pregnancy rate/embryo transfer	26.5%
Implantation rate per embryo	16% (28/175)

PN = Pronuclei, ET = Embryo Transfer, SSC = Surgical Sperm Collection.

Clinical pregnancies were achieved in 17 cycles. Four of them were from Azospermic men and sperms were collected by PESA (one case) and TESE (three cases). One pregnancy was achieved in a patient who was selected for Blastocyst transfer (Table 4). Overall pregnancy rate per cycle was 19.7%. The pregnancy rate per embryo transfer was 26.5%. Three patients developed mild Ovarian Hyperstimulation Syndrome (OHSS) which settled on conservative management. One women had triplet while four had twin pregnancies others were singletons. Eleven women have delivered safely with 13 healthy infants including two sets of twins. Unfortunately six women aborted including two twin and one triplet pregnancies.

Discussion

Assisted Reproductive Techniques are developing every day and giving better results. Even in an overpopulated country like Pakistan childlessness is still a personal tragedy especially with its peculiar social and cultural values. There is actually a need of balanced reproduction. A childless couple should have an access to modern techniques. Unfortunately ART includes expensive methods of treatment and results are always equivocal with no guarantee of success rather more of failed attempts.

There is a need to make it more and more cost effective by cutting down the expenses without compromising on quality.

Since male factor infertility is increasing alarmingly, there is an immense demand for effective treatment. Intra Cytoplasmic Sperm Injection (ICSI) is the most successful mirco-manipulation technique for treating male factor infertility.

The technique was used initially in veterinarian practice. The injection of sperm into an egg was first done on the echinoderm by Harimoto [10] whereas the first mammalian egg injection procedure was reported by Lin [11]. Later Uehara in Yanagimachi[12] described the microinjection of human and golden hamster spermatozoa into a hamster egg. The first pregnancy in human was reported in July, 1992[2] although this procedure was first applied to human gametes in 1988[13].

Before the introduction of ICSI, Partial Zona Dissection (PZD) had become cotroversial and was subsequently abandoned by many workers[14,15] and subzonal insemination (SUZI) only produced low fertilisation rate[14,16,18]. ICSI overcomes the relatively low fertilisation rate (10-40%) and high polyspermy rates (up to 40%) of sub zonal insemination (SUZI) and Is more effective treatment modality[5,7,9,19].

Increasing male factor infertility around the world and the limited effectiveness of IVF in the cases of severe male factor problems, introduction of ICSI has given many couples the chance of biological parenthood which they otherwise would not have had.

It has been introduced successfully for the first time in Pakistan at Baqal Institute of Reproduction and Developmental Sciences (BIRDS) Baqai Medical University, Karachi since 1997. The centre has had encouraging results for IVF in the previous three years.

This report discusses the experience of first year of ICSI in the centre on 71 patients selected of ICSI. Fifteen patients came for a second cycle after failure in first cycle so total 86 ICSI cycles were started on stimulation regimen. Six females did not respond to ovarian stimulation so their cycle had to be abandoned. All of them were more than 37 years of age with higher FSH levels. They were warned about the high risk of poor response. Maximum doses of HMG (according to the BIRD's protocol) were administered. Average number of ovua collected per patient (10.5) were optimal, too many follicles and eggs may cause Ovarian Hyper Stimulation Syndrome (OHSS) which should be avoided.

In this series, only Vaginal Ultrasound Scan was used for follicular monitoring. It cuts down the cost of serial lab for.

The only criteria for a successful attempt of ICSI is the presence of a few motile or live serms somewhere in the male reproductive tract which can be retrieved for injection. Fertilisation rate of 60-70% is obtained with lCSl once the injection procedure has been optimised as reported by pioneer workers [5,7,9]. The fertilisation rate of 58.9% achieved at BIRDS in its first group of patients is quite convincing for a relatively new center. Blastocyst growth rate of 36% gives confidence on the culture system and laboratory function, specially when one of the two blastocyst transfer cases has conceived.

In azoospermic men, Surgical Sperm Collection (SSC) PESA or TESE has also given good results in terms of fertilisation rate and pregnancies achieved. Out of 15 cases in which sperms

were retrieved from PESA or TESE, three women became pregnant. This pregnancy rate is encouraging and is comparable with other reports[20].
These are the first ICSI, SSC and Blastocyst pregnancies achieved in Pakistan. The results achieved are satisfactory as an initial phase of adopting a new technology. It must improve with time as the working team gains more experience.
Triplet pregnancy is alarming especially in this part of world where neonatal care is scarce and very expensive. The number of embryos transferred must be discussed with the couple and one must try to keep it at two only.
Abortion rate was rather high in this group, which needs to be looked into. Multiple pregnancies and higher female age group is probably the answer as in cases after this series the results improved, with strict selection criteria (to be reported later).
Serial serum estradiol levels are checked in other centres to monitor ovarian follicle maturation. This was avoided in this series. Drugs were reduced as the clinical team was aiming for 10-12 oocytes per patient instead of 20-25. These two measures have significant effect on cutting down the cost of treatment without compromising the results.

Conclusion

The results of the report and the data obtained from other published studies clearly show that the lCSl has revolutionised the treatment of male factor infertility. It is the only significant breakthrough in the treatment of male factor infertility since the advent of IVF. A few live sperms from reproductive tract is the only requirement and many
serum esterogen estimation, which is the usual practice in subject including those who were labeled infertile before, other centers now have a very good chance of producing their own biological children. Cost of treatment can be reduced by limiting the investigations and cutting down the dosage of drug ovarian stimulation without compromising the results.

Acknowledgements

We thank all the staff members of BIRDS for their dedication and hard work.

References

1.Steptoe PC, Edwards RC Birth after reimplantation of human embryo. Lancet, 1978;2:366.
2.Palermo 0, joris II, Dcvroey P. et at. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet. 1 992;340: 17-18,
3.Michael DL. What's wrong with our sperms. Times, 1996, March 18, pp.42-43.
4.Gamal I Serour-, Omran AR. Ethical gwdclines for human reproduction research in the Muslim world. The International Islamic Center for Population Studies and Research, Cairo, Al-Azhar University. 1992. pp. 19-40.
5.Van Steirtegheni AC. Liu J. Joris H, et at. Higher success rate by intracytoplasmic injection than lsy subzonal insemination. Report of a second series of 300 consecutive treatment cycles. Hum. Reprod. (0xf.)1993;(a)8: 1055-60.
6.Van Steirtegham. High fertilisalion and implantation rates after intracytoplasmic sperm injection. Hwn. Reprod. (Oxf)1 993:(b)8: 1061—62.
7.Payne E. Successful treatment of severe male factor infertility in 100 consecutive cycles using

intracytoplasmic sperm injection. Human Reproduction (oxf), 1994(b);9:205 1-57.
8.WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical mucus interaction. - World Health Organisation - Cambridge, University Press, 1992, p. 44.
9.Palerino D. Sperm characteristics and outcome of human assisted fertilisation by subzonal insemination and ICSI. Fertility Sterility. 1993;59:826-35.
10.Harimoto V. Microtnjection of the live spermatozoa into sea urchin eggs. Exp. Cell Res., 1962;27:4l6-26.
11.Lin TP. Microinjection of mouse eggs. Science, 1996;151:333-37.
12.Uehara T, Yanagiinachi R. Microsurgical injection of spermatozoa into hamster eggs with subsequent transformatioin of sperm nuclei into male nuclei. Biol. Reprod., 1976:1 5:467-7t).
13.Lanzendorf SE, Maloney MK, Veeck LL. et at. A preclinical evaluation of pronuclear formation by microinlection of' human spermatozoa into human oocytes. Fertil. Sieril., I 988(b);49:835-42.
14.Fishel S Micro-assisted t'ertilisation with human gametes. Human Reprod. (Oxf.);1 993:8:1780-84.
15.Tucker M, Wiker S. Masses J. Rational approach to assisted fertilisation Hum. Reprod. (Oxf.)J, 1993:8:1778.
16.Fishel S. Timson J, Lisi F, et al. Evaluation of 225 patients undergoing subzonal insemiisation for the procurement of' fertilisation in vitro Fertil. Steril., 1992:57:840-49
17.Fishel S Micro-assisted fertilisation in patients who have failed subzonal insemination. Hum. Reprod. (OxtI), 1 994(a):9:50 1-505.
18.Fishel S. Subzonal insemination and zona breaching techniques f'or assisting conception in vitro. Bailliere's Cliii. Obstetric. Gynaecol., l994(b);8:65-84.
19.Payne D, Warnes GM, Flaherty SP, et al. Local experience with zona drilling, zona cutting and sperm microinjection. In "The iiifertile Male: Advanced Assisted Reproductive Technology". Reprod. Fertil. 0ev., I 994(a):6:45-50.
20.Silber E. High fertilisation and pregnancy rate after lCSI with spermatozoa obtained from testicular biopsy. Hum. Reprod. (oxf.), 1995;10:148-52,