OUR INSTITUTE
IVF can be defined as fertilization of a human egg by the spermatozoa in the laboratory and the transfer of the resulting embryo back to the expectant mother’s uterus to induce pregnancy.


Two main varieties of IVF technology:

1) IVF

2) ICSI
1978  1st IVF baby : ‘Louise Brown’
      - Edwards and Steptoe, England
1984  1st Baby from Frozen Embryo
      - Zeilmaker, Netherlands
1985  1st GIFT Baby
      - Ricardo Asch, U.S.A.
1992  Intra-cytoplasmic Sperm Injection (ICSI)
      - Palermo, Belgium.
1995  Testicular Sperm & ICSI (Azoospermia)
      - S. J. Silber / P. DeVroey
Chronology of IVF-ICSI Research in INDIA

• 1978 October : Dr. Subhas Mukherjee & his team. Birth of Durga.

• 1982-1986 : Kolkata-Dr. B. N. Chakravorty, Dr. S. Ghosh Dastidar
Birth of Imran.

• 1983-1986 : Bombay- Dr. I. Hinduja, Dr. Anandkumar
Birth of Harsha (July 1986)

• 1992 : Kolkata- 1st GIFT Baby
Dr. S. Ghosh Dastidar ; Dr. K Ghosh Dastidar

• 1995 : Kolkata- 1st ICSI ZIFT Baby
Dr. S. Ghosh Dastidar ; Dr. K Ghosh Dastidar

• 1995 : Mumbai- ICSI Baby
Dr. Fируza Parikh
Dr. Subhas Mukherjee
The pioneer of IVF Research in India.
Responsible for inducting Dr. Sudarsan Ghosh Dastidar into the emerging field of
In-Vitro Fertilization and related science.
Dr. S. Ghosh Dastidar seen with pioneer IVF Scientist Dr. Edwards & others at 3rd World congress on IVF, Helsinki, 1984
Presentation of our IVF data at 3rd World Congress on IVF at Helsinki, Finland (1984)
Seen with Prof. R. G. Edwards at Helsinki, Finland (1984) and at Istanbul, Turkey (2005)

World Congress on IVF, Helsinki, Finland, 1984

World Congress on IVF, Istanbul, 2005

Congratulation to Prof. Edwards for winning Nobel prize in medicine this year (2010)
With international stalwarts

Cornell Medical Center, USA
Dr. Bedford and Dr. Sudarsan Ghosh Dastidar, 1987

Prof. Struart Campell, Dr. Sudarsan Ghosh Dastidar and Dr. Kakoli Ghosh Dastidar
The story of how we developed IVF in India (Kolkata) is one of Herculean struggle. It is necessary to narrate many personal details which are vital to illustrate how breakthroughs in creative science really happen, specially in a country like ours.

Our challenges

- **Non existent infrastructure for IVF related cell culture** in India. Hence we had to prepare tissue culture media from scratch, using the bare minimum resources available in Kolkata.

- **Frequent power cuts** in 1981-82; standby generator was must.

- There was **no computer and obviously no internet**; thus there was almost no literature/publication/journals available in Kolkata.

- The most challenging job was to develop a tissue culture system which would produce **5% CO₂ in air and 95% relative humidity** atmosphere since we did not have the modern computerized CO₂ incubator.

- Though it may sound extraordinary to researchers today, one basic challenge was to first **identify how the human egg looked** under the microscope, **BECAUSE THERE WAS NO PHOTOGRAPH/PICTURE** available in any book/journal/internet.
Major challenges in the early part of our research

Prof. B. N. Chakraborty was involved in developing the clinical aspects like patient selection, ovarian stimulation and most importantly, oocyte retrieval from ovarian follicles under laparoscopic guidance – ultrasound was not available at that time and finally embryo transfer (ET).

Since Dr. S Ghosh Dastidar was entrusted with the development of laboratory protocol, major challenges were –

1. To identify human oocyte from follicular fluid aspirate.

2. To prevent bacterial contamination in the culture media – since that time we didn’t have a laminar airflow hood.

3. To device some system to produce 5% CO₂ in air atmosphere for IVF and embryo culture – since we did not have modern CO₂ incubator.

4. To capacitate spermatozoa – an absolute necessary step to achieve successful fertilization of oocyte.

5. To standardise the culture media preparation; PH monitoring and control of osmolarity of media. Prof. Subir Dutta extended logistic and technical support. Initially we used his laboratory for media preparation and sterilization.

6. To learn manipulation of human oocyte quickly but gently to prevent oocyte damage due to exposure in-vitro.
1982-83

A typical day

- Morning 5 – 6 a.m. – cleaning of laboratory, final preparation of media and other laboratory quality control protocols.
- 7-9 a.m. – laboratory reparation for egg recovery.
- 10:30 a.m. to late evening – Dr. Dastidar used to carry the test tube containing follicular fluid in a thermos flask to a laboratory in far away Jadavpur University and later, to Indian Statistical Institute, where he was desperately trying to identify the oocytes under stereo microscope (no one in Calcutta then even knew their appearance!).
- However, soon we realized that in order to achieve pregnancy by IVF we needed a laboratory close to the clinic where oocyte retrieval was being done. Otherwise the temperature and pH of the egg-containing follicular fluid was being altered in this long transit.
- Thus, Dr. Dastidar set up Calcutta’s first IVF laboratory (very primitive though!) in his study room at 79/28, A.J.C. Bose Road, Kolkata - 14.
- Some days Dr. Dastidar used to finish work at 12 night or even later.
India’s first indigenous IVF lab at the residence of Dr. Sudarsan Ghosh Dastidar
... and, the modern IVF lab at GDIFR!
The improvised IVF embryo culture system devised by Dr. Sudarsan Ghosh Dastidar in 1983

- A pre-requisite for IVF is perfectly controlled environment for cell culture, currently provided by state-of-art incubators as shown in the previous slide.

- During 1982-86 we did not have modern CO₂ incubator which is an absolutely essential equipment for human IVF.

- Thus Dr. Dastidar tried to improvise several alternative simple systems with locally available materials.

- Finally Dr. Dastidar was able to develop a system to produce 5% CO₂ in air atmosphere and approximately 85% relative humidity.
Improvised embryo culture system using 5% CO$_2$ in air environment (1982)

Modern CO$_2$ Incubators used in our center currently
Microphotographic Documents of oocytes taken during 1982-1983
Different stages of fertilized egg and embryo following IVF/ICSI documented in our Lab

PN Stage

4 cell stage

8 cell stage
After many failures, Dr. Dastidar achieved fertilization of oocyte and its subsequent cleavage to 6-8 cell stage in June/July`83.

Following embryo transfer, a pregnancy occurred. (September`83). Unfortunately, this pregnancy ended in abortion at around 10 weeks.

It was a major break through indicating the feasibility of IVF as a clinical method to treat tubal factor infertility.

Dr. B. N. Chakraborty and Dr. Dastidar jointly presented* this data in 3rd World Congress on IVF, Helsinki, Finland, May 1984.

This was the 1st report of successful IVF and embryo transfer resulting in pregnancy in human from India in any World Congress. (May`84)

*Ref. Our Experience of IVF in India; Chakraborty B.N. and Ghosh Dastidar S.; Congress book; 3rd World Congress on IVF, Helsinki, Finland, May 1984
The respective responsibilities of Dr. B.N. Chakraborty and Dr. Sudarsan Ghosh Dastidar during early phase of our IVF program in 1982 onwards

**Clinical**
- Ovarian Stimulation Protocol
- Monitoring of Ovarian Response
- Timing of hCG Administration
- Laparoscopic Oocyte Retrieval

**Laboratory**
- Oocyte identification under microscope
- To set up basic embryology laboratory
- Preparation of culture media and osmolarity control
- In-Vitro Fertilization & embryo-culture
- Quality-control

Dr. Dastidar was responsible for developing the laboratory aspect
I devised a glass jar with premixed 5% CO2 in air to undertake IVF and embryo culture.

Major Problem
- Modern CO2 incubator was not available with us.
- Essential for achieving 5% CO2 in air, 98% RH and 37°C temp

Dr. Dastidar’s Improvised Alternative
- I devised a glass jar with premixed 5% CO2 in air to undertake IVF and embryo culture.
**Third Phase - Initial Break Through in our IVF Research**

<table>
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<tr>
<th>Major Achievement</th>
<th>Dr. Dastidar documented fertilization of oocyte resulting in cleaving (2-8 cell) embryos</th>
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<td>July, 83</td>
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<th>1st Pregnancy following E.T.</th>
<th>One Mrs. Devi conceived after E.T. (in the same lab. at Dr. GhoshDastidar’s residence)</th>
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<td>Sept, 83</td>
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<th>IVF World Congress 1984</th>
<th>We presented our data in 3rd World Congress on IVF, Helsinki, May, 1984</th>
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<td>: Ref. Chakravorty, B.N. &amp; Ghosh Dastidar, S.</td>
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<td>3rd World Cong. Scientific Abstract Book</td>
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**1st Report from India at the International Level**
End of Our Struggle - Birth of IVF Baby

Re-organisation of IVF Laboratory

- Initial success in IVF & pregnancy stimulated us.
- Relatively improved lab. developed in C.I.T. Rd.
- More number of IVF attempts done

Our Final Achievement November, 1986

- A healthy male baby was born following IVF (Still using improvised tissue culture chamber devised by Dr. Ghosh Dastidar)
- In the same year, the Bombay group also succeeded in IVF just 3 months before us (Dr. Hinduja & Anandkumar)
The technology of sperm preparation in IVF, prompted Dr. Dastidar to venture introducing 100% motile sperm in uterine cavity in patients with unexplained infertility.

Dr. Dastidar was surprised to achieve series of pregnancies following such method during 1982-84.

These were among the first few IUI pregnancies in the world.
IN-VIVO AND IN-VITRO INSEMINATION WITH CAPACITATED SPERMATOZOA - A NEW THERAPEUTIC APPROACH TO HUMAN INFERTILITY.

By S. Ghosh Dasidar, S. Chatterjee, S. Goswami, B.N. Chakravarty, N.R.S. Medical College & Hospital, Calcutta, India.

Based on improved knowledge and technique of in-vitro sperm capacitation and ovulation control 40 infertile couples with cervical hostility and varying grades of seminal defects were treated with artificial insemination using capacitated husband's semen. Insemination was performed through two different routes (a) intrauterine (b) intratubal - under laparoscopic view.

Timed intrauterine insemination was carried out in 30 couples, indication being normospermic or mild to moderate oligo-asthenospermic husbands with persistently negative post coital test. Laparoscopic intratubal insemination performed on 10 women was carried out in two ways: (i) in 5, timed insemination into the ampulla of the tube (ii) in the remaining 5, morphologically mature oocyte aspirated by laparoscopy were inseminated in-vitro and then immediately transferred to tubal ampulla.

Following intrauterine insemination 60% term pregnancy was achieved with normospermic semen while with oligo-asthenospermic samples, the incidence of term pregnancy was 26%. In intratubal insemination however, no term pregnancy has yet been recorded, though two cases conceived and ultimately aborted following ampullary placement of in-vitro inseminated egg.

It appears from our study that in grossly oligo-asthenospermic subjects homologous pregnancy may be possible either with timed intratubal insemination or intratubal transfer of invitro inseminated egg using isolated capacitated sperms of high velocity.
No write with D. hijacker

AJMER. July the third and last yesterday, the Indian court set up court, there was no meeting between the girls and India for seven hijackers.

The hijackers special court, the airlines Bodigore to D. August, 1984.

During cross defence, the knowledge is being sent to any stage. The Dubai police including the hijackers in the Indira against a receipt was passed court.

An official, joint secretary ministry, said the hijackers from ambassador in
আনন্দবাজার পত্রিকা

ভারতের প্রথম ক্রিকেট টেস্ট টিউব ছেলেদের জন্ম কলকাতায়

কং মিছিলে সি পি এসের হামলা, শান্তি রয় আহত

বিমানের জকরি অবতরণ | কল্যাণীতে আতঙ্ক
Immaculate Conception


Editorial story on the first indigenously developed Indian embryology laboratory by Dr. S. Ghosh Dastidar. Photograph showing the two collaborators, Dr. S. Ghosh Dastidar and Dr. B. N. Chakraborty in the IVF laboratory, July '86.
Publication in lay press - 1986

Dr. B.K. Chakrabarti (right) and Dr. S. Ghoshdastider (left) with the baby.

Man's F1 medium with as many as 14 ingredients, in addition to serum albumin of the prospective mother to simulate a perfect "inside-the-womb" condition. For this, a bacteria free atmosphere, a temperature of 37 deg. C and a relative humidity of 90% per cent are the minimal prerequisites.

"The culture medium, in the specialised incubating system will count in direct chamber and a "womb" that is sterile, so that for implantation in the uterus..."
Our IVF baby “Imran” on his first birthday 1987
Our first IVF Lab set up at (1991-2008)

CO₂ incubator

Micro manipulator

Laminar airflow hood
Our ultrasound unit with color doppler -3-D equipment

Dr. Kakoli Ghosh Dastidar performing ultrasound studies in our center
Blastocyst stage embryo in IVF Program

Morula stage

Blastocyst stage
Major steps of ART

- Controlled ovarian stimulation and monitoring
- Oocyte retrieval (earlier laparoscopic, since ’87 - USG guided)
- IVF embryology laboratory -
  1. Identification of oocyte
  2. Tissue culture media & sperm washing techniques
  3. In vitro fertilization of oocytes using worst capacitated spermatozoa (10000-20000 sperm per oocyte).... IVF
  4. Fertilization of oocyte by a single spermatozoa using a micromanipulation system .... ICSI
- Documentation of fertilization (2-PN examination)
- Embryo culture for 2-4 days and finally embryo transfer (Day 3 or Blastocyst)
**ART Flow Chart**

- **GnRH agonist** down regulation of Pituitary (long protocol) D20

- **GnRH antagonist** D6/D7/D8/D9

**Stimulation Regime**

- hMG / FSH or Rec. FSH or Combination
- Dose - 100 - 400 I.U.

- E2 - 1500-3000 pg
- USG- 10-15 follicles

- hCG Injection 5000-10,000 I.U.

- Completes Final Maturation of Oocytes

- OPU USG guided 34 - 36 later

**Options**

- IVF
- ICSI
Till 1993 there was no treatment available for severe male factor infertility with very low sperm count and motility, not even by IVF.

This scenario changed with the advent of Intra Cytoplasmic Sperm Injection (ICSI) which means fertilizing an egg by micro injection with a single viable spermatozoa in the laboratory.


Dr. Dastidar initiated one of India’s first two ICSI programs in Kolkata in January 1994, and delivered first ICSI-ZIFT baby in India in March 1995.
Basic requirement

• sperm
• Micro-manipulated Metaphase II Oocytes
• Motile / Viable on System with Remote Controlled Injection Device (Nikon-Narishegie)
• Rest Lab. Facilities Same as IVF
Intra Cytoplasmic Sperm Injection (ICSI)
Method of ICSI

- MII Oocyte is denuded from surrounding cumulus cells
- Single spermatozoa is immobilized and aspirated in micro-injecting needle
- Sperm injected directly into ooplasm using a micro needle and a micro manipulator under direct vision of inverted microscope with 3D image optics
- Rest of the procedure like confirmation of fertilization by Pronucleus (PN) examination, embryo culture are same as IVF
Position of Polar body & ICSI needle Entry

PB at 12 clock position

12 Clock

Injection needle

3’ O clock
Oolemma rupture & entry tunnel characteristics following ICSI

I. NO TUNNEL (SUDDEN RUPTURE)

II. ENTRY TUNNEL IS FORMED DURING ICSI
A. PB and two Proneuclii in alignment

B. Non-alignment of PB and proneuclii
Establishment of Successful ICSI in Our Center, 1993-1995: India’s 1st ICSI program

Our first ICSI baby in 1995
Risk Factors

1. Less number of M II oocytes(<3).

2. Immotile (dead) /round headed (globozoospermia) spermatozoa.

3. Failure of oocyte activation.
   - Sperm factor. Lack of oscillatory Ca 2+ waves throughout the oocytes.--failure of activation.
   - Oocyte factor.
* Severe oligozoospermic males may produce semen without any sperm cell on the day of ICSI.

**Solution - Current Approach**

1. Ask for a second ejaculated specimen--Spermatozoa will be found in most cases.

2. Emergency Testicular Biopsy (TESE).

3. Recently, workers from Muenster, Germany have reported a novel method using oxytocin for pharmacological enhancement of emptying of spermatozoa reserve.
1. In a recent meeting at Arusha, Tanzania – ESHRE proposed to develop strategies to offer low cost IVF to poor resource countries.

2. Dr. Dastidar was invited to present his experience in early years of IVF using simple improvised equipments.

3. Both World Bank and UNFPA are interested to fund definitive research project to develop low cost IVF program

4. Thus, a meaningful collaboration between India’s premiere technological institute like IIT and our centre could achieve this goal of offering specialized infertility treatments like IUI, IVF or ICSI at an affordable cost by producing many of these disposable materials, culture medium or innovative low cost equipments/procedures.
ESHRE Special Task Force on 'Developing Countries and Infertility'

July 2008

www.eshremonographs.oupjournals.org

ISSN 0268-1161
Dr. S. Ghosh Dastidar with other Members, first meeting of ESHRE Special Task Force on ‘Developing Countries and Infertility’ Arusha, Tanzania, 2007- to develop low cost IVF
Dr. Sudarsan Ghosh Dastidar speaking at ASRM 2010, Denver on Mild approaches in ART and cost reduction
The ESHRE published selected comments on Robert G. Edwards winning the Nobel Prize in 2010 for Physiology/Medicine:

“This is a well deserved honour. IVF has opened new avenues of hope for millions of couples throughout the world. It has also ............ We congratulate him on his award” - Professor Basil Tarlatzis, President, International Federation of Fertility Societies (IFFS)

"I am extremely happy with the announcement that Prof Edwards has been awarded the Nobel Prize for Medicine this year. I am privileged to know him personally since the 3rd World Congress on IVF in Helsinki, Finland. He was very kind to advise me to visit his center at Bourne Hall Clinic, which I visited in 1985 and several times after. I learnt a lot from Bob Edwards during so many scientific meetings worldwide and also during informal discussions. I would like to convey my best regards and wishes to Prof. Edwards. I pray for his long life." Dr. Sudarsan Ghosh Dastidar, Congress President, ISMAAR World Congress, India, 2011
Dr. Sudarsan Ghosh Dstidar on ESHRE meeting