My Journey Through IVF

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My interest and journey in infertility started around 1965, 12 years after my medical graduation, through association with Dr Subhash Mukherjee, while both of us were posted in our respective disciplines at NRS Medical College, Kolkata. We liked each other because we were interested in uncommon subjects of ill-understood aetiology and unfavourable outcomes with treatment. For example, while Subhash had a keen interest in endocrinological abnormalities, both in adolescent boys and girls, like Turner's, testicular feminizing syndrome, intersex problems, etc. I was more interested in surgical correction like a congenital absence of vagina, reconstructive surgery of ambiguous external genitalia, and recanalization of blocked tubes, vas, etc.

The evolution of infertility and interest in management started around 1960, immediately after the Second World War. The reasons for the new revolution differed in two different hemispheres. On one side, technologies and interventions required in the ongoing warfare added new information, which helped develop a society with rapidly available methods involving less human resources and physical activity. One example was "information technology, transport and communication system." Western society grabbed the advantage and made life easy. "Easy life and richer society' possibly might have been a background for the genesis of three fundamental causes of 'female infertility,': Obesity, PCOS and 'endometriosis.'

The picture was, however, different in the Eastern hemisphere. Here, the explosive increase in population demanded a situation of "task force" population control administration. Poverty, lack of sanitation, lack of development of sophistication, and lack of nutrition led to transmission of infection and contagious diseases like malaria, kala-azar, STD, etc. Implementation of population control had to be achieved through undesirable procedures like vasectomy and tubectomy, even in unmarried boys and girls, especially in many areas of the poverty-stricken world. India was a glaring example of this unfortunate crisis, which was believed to be one of the causes of the fall of Mrs Indira Gandhi's Government.

Technologies and methods were introduced through different approaches for managing infertility during this period to balance the difference between the two hemispheres. Population control in the developing countries in the Eastern hemisphere and population

stabilization in the West were attempted in two different ways.

In the Western hemisphere, women were treated with human menopausal gonadotropin, which was isolated from menopausal urine. These achievements were credited to two individuals: Gemzell in the USA and Lunenfeld in Israel. Clomiphene citrate, initially believed to be an infertility agent, later proved to be a fertility-enhancing drug. Extensive basic and clinical research on folliculogenesis, preparation of media, *in vitro* growth of human oocytes, and clinical use of pelviscope to diagnose pelvic pathology were introduced during this period.

As mentioned earlier, in the developing world, the unexpected global population explosion, led to unplanned mass vasectomy, tubectomy, MTP, etc. These procedures necessitated the introduction of newer technologies in the treatment (reversal of blocked tubes and vas).

Pioneers in the above field were Victor Gomel in Canada, Robert Winston in Hammersmith, and Green Armytage in London. Shirodkar in Bombay and many others. These are a few names to be mentioned in the introduction and popularization of different procedure techniques. For population control, hormonal contraceptive tablets (OC pills) were a remarkable contribution, which was introduced during this period, though the procedure had certain limitations.

Moreover, in developed areas of the world, changing lifestyles and diet habits led to multiplying incidences of PCO and endometriosis, requiring special attention for fertility stabilization.

MANAGEMENT

The procedures for the management of problems in the two different hemispheres were:

1. Ovulation inductions—based on the concept "two gonadotropin—two cell-theory" (West)—PCOS, endometriosis—more towards medical treatment
2. Microsurgery for correction of tubal/vasal block (East)—pelvic inflammatory diseases—required more surgical attention

This brought in the concept of understanding the physiology of folliculogenesis, ovulation induction and also prevention of conception (both medical and surgical procedures).

KNOWLEDGE ABOUT THE PHYSIOLOGY OF FOLLICULOGENESIS AND OVULATION

The concept of "two gonadotropin—two cell theory" was known in 1941, but practical application was implemented in 1960. The gonadotropins were: FSH (follicle stimulating hormone) for follicular growth and development (oestrogen) and LH for maturation of follicles. The two cells were: granulose cells—for oestrogen production and thecal cells—for progesterone and androgen production. These are basic hormones essential for folliculogenesis and ovulation.

The impact of a few other adjunctive hormones on the reproductive axis (hypothalamic pituitary ovarian axis) was explained. These were initially and still essentially used for induction of ovulation in unsuccessful cases. The indications are:

a. Hypothyroidism—in hypothyroidism, SHBG is low, and therefore androgen is elevated. The addition of drugs like Eltroxin, with primary ovulation-inducing drugs (clomiphene, letrozole, gonadotropin), may benefit in successful induction.
b. Prolactin—elevated prolactin—borderline increase of prolactin will decrease basal gonadotropin level and elevation of adrenal androgen, leading to non-ovulation. Supplementation with bromocriptine might lead to success in ovulation.
c. Hyperactive adrenal (as in a few examples of PCOS) dexamethasone in low dose in early follicular phase, with ovulation inducing drugs, may bring successful ovulation.
d. Insulin—a co-gonadotropin androgen elevator through hepatic suppression of

SHBG or stimulation of IGF-1I in theca cells (not very much known at that time).

Hence, for ovulation induction in these specific situations, in addition to clomiphene citrate (CC) and gonadotropin, eltroxin, bromocriptine, dexamethasone and occasionally metformin were used in appropriate doses in these abnormal situations. Though controversial, estrogen 0.01 mg was added to CC (from d7 to d11) to counteract its anti-estrogenic effect on endometrium and to make cervical mucus more permeable for sperm penetration.

Wedge Resection in Non-ovulation

In intractable cases of anovulation, wedge resection was very popular. If properly performed, the results were encouraging, with minimal side effects. Monitoring of ovulation and timing of ovulation trigger, especially for IUI-3 procedures were followed. Standard procedures for IUI started becoming popular in 1975.

Parameters were:

- Insler's cervical mucus scoring
- Basal body temperature (BBT) chart
- Urinary LH assay (by colour change on paper strip

Insler's Cervical Mucus Scoring

Two Methods were Popular

Cervical mucus is the good biological mirror of follicular development and ovulation.

Daily observation of cervical mucus from the first day after menstruation ceases is a good sign of follicular measurement and estradiol production. A nearly mature follicle (17–18 mm in diameter) will produce about 100–150 pg estradiol.

OBSERVATION OF STRETCH ABILITY OF CERVICAL MUCUS *(Starting from D8 or D9 of Menstrual Cycle)*

Every day the cervical mucus is collected in between two fingers or tips of artery forceps, and maximum stretchability without break should be around 10 cm (Fig. 1.2), which indicates the ovulatory cycle and oestradiol (100 to 150 pg in a stimulated cycle) on the day before ovulation. That is when hCG should trigger ovulation in the stimulated cycle. Ovulation is likely to occur 36 to 42 hrs. after the injection. We have seen a drop at the central point of the stretched-out cervical mucus. In addition, under the microscope, the mucus will appear as branches of a fern tree (first and second-order branches) (Figs 1.2 to 1.5). The exact time of injection will be indicated by:

- The appearance of stretchedout cervical mucus, preferably with a drop at the centre between two fingertips or tips of artery forceps (Fig. 1.2).
- Transparent mucus (Fig. 1.1).
- Gaping cervical external OS (Fig. 1.1).

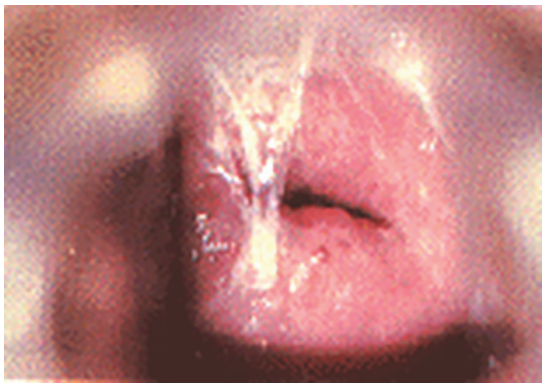

Fig. 1.1: Gaping external OS; thin transparent copious mucous—E2 peak

Fig. 1.2: Stretchable thin mucous with drop formation at the centre—timing of trigger

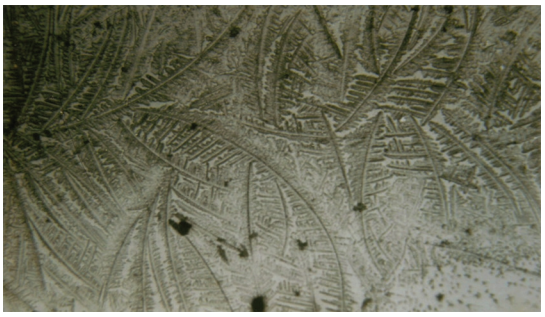

Fig. 1.3: 2nd order ferning also E2 peak and plateau—timing of hCG

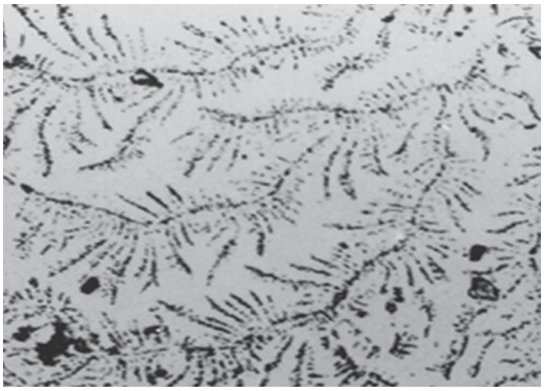

Fig. 1.4: First order branching; clear transparent background—E2 high

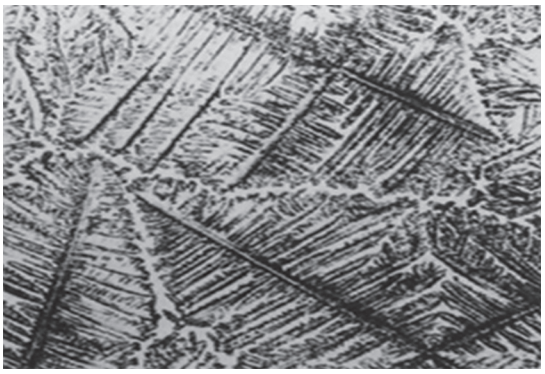

Fig. 1.5: Second order branching—E2 reached peak and plateau—LH surge start

- Second-order branches indicate peak oestradiol level and exact timing of injection (triggering) (Figs 1.3 to 1.6).
- Figure 1.6 demonstrates the microscopic appearance of cervical mucus after ovulation.

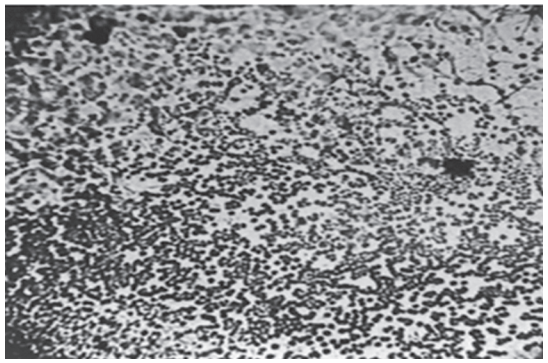

Fig. 1.6: Dark background branches broken—LH surge completed; P effect

- Dark background, branches broken, LH surge completed, the effect of progesterone.

Appearance under microscope: Hazy to the dark background (Fig. 1.6), inflammatory cell migration, and branching addition indicate progesterone appearance and completion of ovulatory phases (extrusion of 1st polar body). Therefore, cervical mucus in the pre-ovulatory period is considered a "biological mirror" of the endocrine status around the period of ovulation.

ANOTHER PARAMETER OF OVULATION MONITORING WAS BBT

What is basal body temperature (BBT)?

We defined BBT as a marker of normal and abnormal endocrine function of a woman undergoing infertility treatment. Because USG, RIA, and EIA were not developed at that time, BBT was considered a mirror of many endocrine functions or dysfunction. For example, we know that progesterone and androgen are thermogenic hormones, and oestrogen is a non-thermogenic hormone (in the follicular phase). In the earlier part of the menstrual cycle, there is a higher level of oestrogen, whereas during the later part, the progesterone level is higher than that of oestrogen. Therefore, the temperature chart will be designated as biphasic; higher temperature in the later part (luteal phase—progesterone), compared to the earlier part

(follicular phase—oestrogen). Considering it is an instance of a consistent phenomenon in an infertility work-up, we marked out different types of BBT in various types of disorders causing infertility (etiologic type).

BBT should be recorded through oral, rectal and axillary routes. Timing of recording is early morning, before any activity starts (thermometer at the bed side and measurement should be accurate as far as possible).

BBT was used as clinical marker of infertility in the following cases. Typically, ideal ovulatory BBT chart should be biphasic.

1. **Typical ovulatory—biphasic:** During the follicular phase, oestrogen is the prominent hormone while progesterone is the controlling hormone in the luteal phase. Therefore, in the earlier period of menstrual cycle the hormonal pattern will be non-thermogenic. During post-ovulation, the temperature will be elevated above the basal level. The cut off has been considered as 97.8°F. This is known as a biphasic pattern. This biphasic pattern has been considered as defining point of ovulatory type of BBT. The biphasic pattern will be of three sub-types depending on the type and function of developing corpus luteum: (a) Sharp rise, (b) gradual rise, and (c) step-ladder like (Fig. 1.7).

Abnormal varieties are as follows:

- Typical anovulatory—flat, non-biphasic
  - Elevated flat—PCO
  - Low flat—POF
  - Discordant—mixture of PCO and normal
- Abnormal luteal—dysovulatory (Fig. 1.12)
  - Short luteal—dysovulatory (Fig. 1.11)
  - Discordant luteal

2. **Anovulatory:** Anovulatory BBT is of three types: Anovulatory high monophasic, anovulatory low monophasic, and discordant (Figs 1.8 to 1.10). Monophasic is of two kinds—elevated monophasic and low monophasic. Elevated monophasic is due to persistent hyperandrogenism-PCO. *Androgen is a thermogenic hormone.* Low monophasic is due to hypoestrogenic (POF or hyperprolactinemia). Discordant here, the temperature is sometimes high

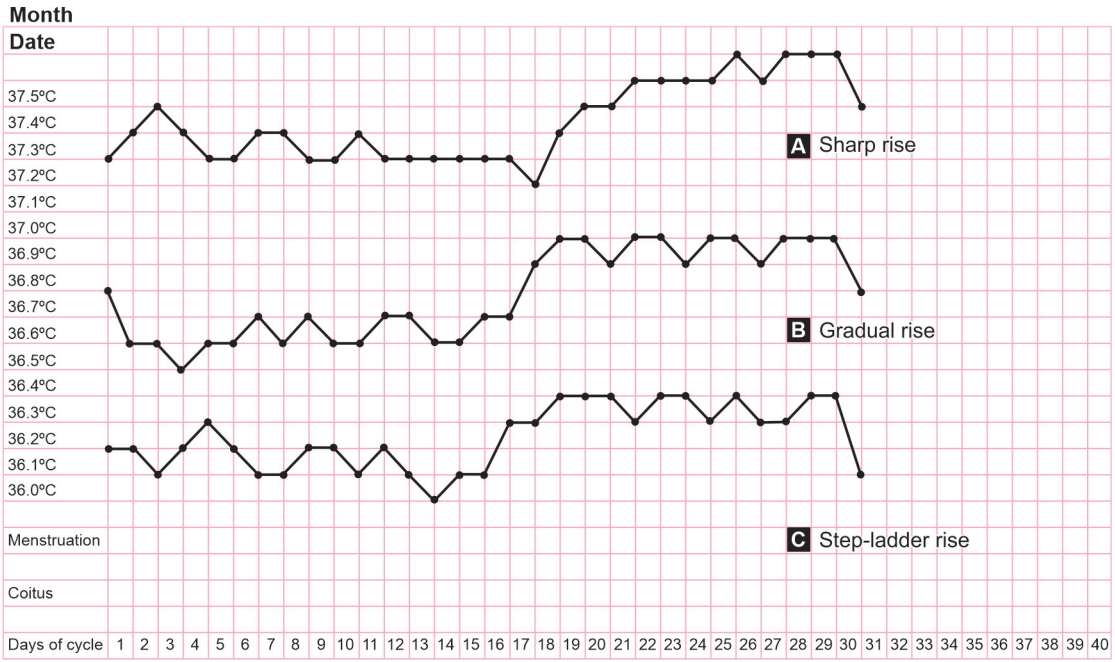

Fig. 1.7: BBT chart indicating ovulation with sharp rise, gradual rise and step-ladder rise

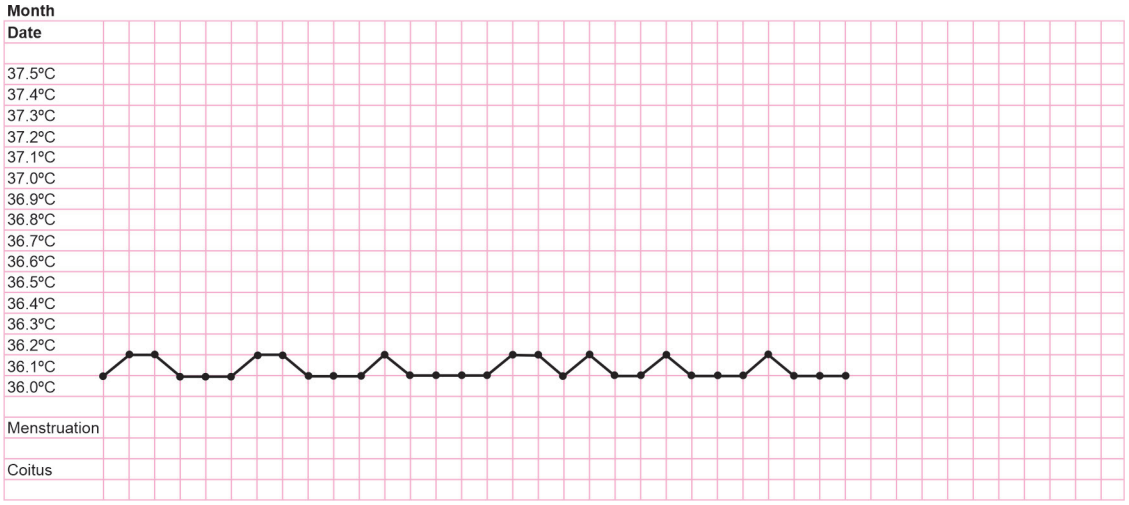

Fig. 1.8: Anovulatory low, flat and monophasic BBT

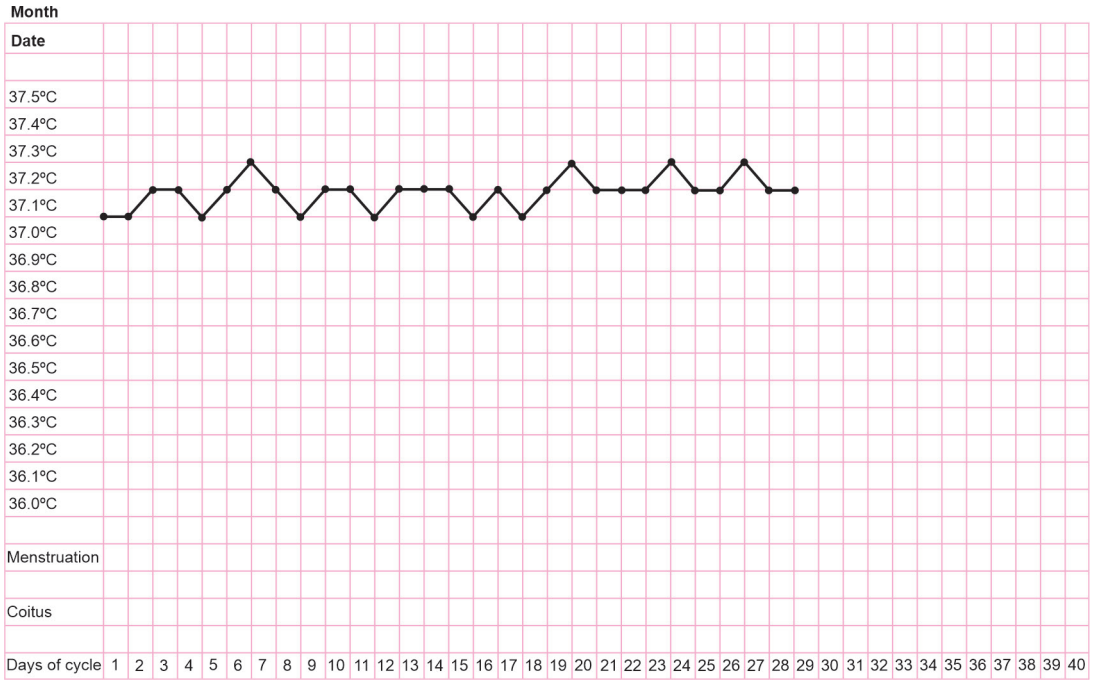

Fig. 1.9: Anovulatory elevated, flat and monophasic BBT

and sometimes low due to an excess level of androgen, progesterone or oestrogen, respectively.

Luteal Phase BBT Defect: Short Luteal and Discordant Luteal

The luteal phase defect is of two types—short luteal and discordant luteal (Figs 1.11 and 1.12).

The normal luteal phase lasts 7 days, and the level of mid-luteal progesterone is 15 ng/ml. A well-balanced follicular phase is a prerequisite for an efficient luteal phase.

Short luteal and discordant luteal phases are inefficient for endometrial preparation and blastocyst implantation.

In summary, many endocrinological defects like PCO and POF could be detected

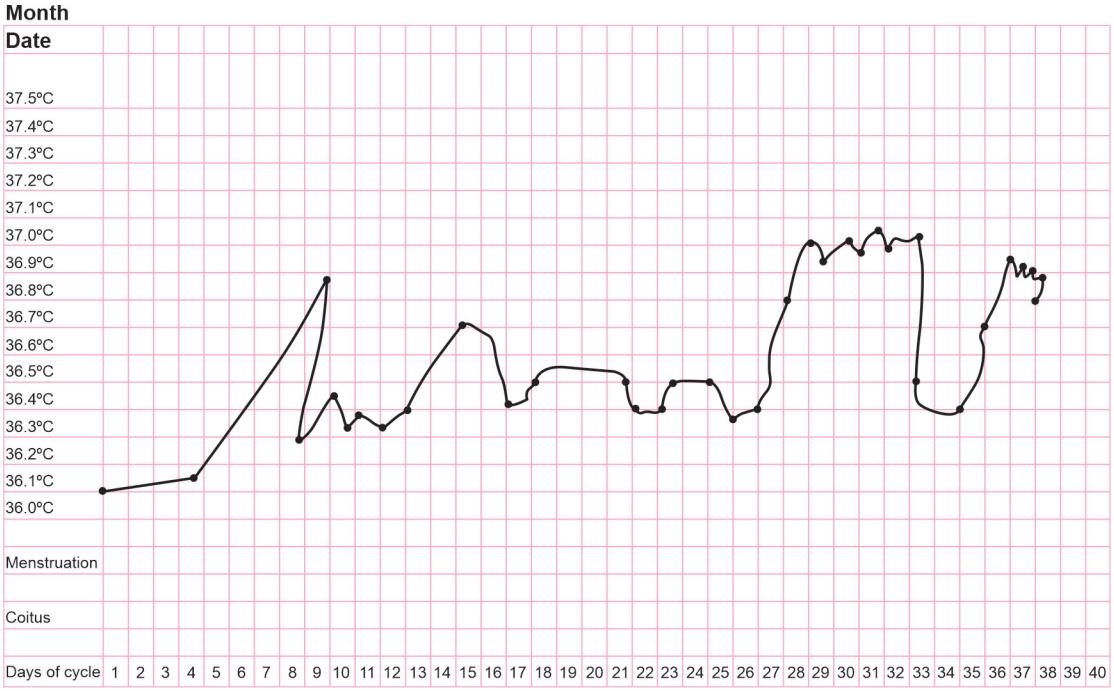

Fig. 1.10: Discordant throughout non-ovulatory—not satisfactory for pregnancy

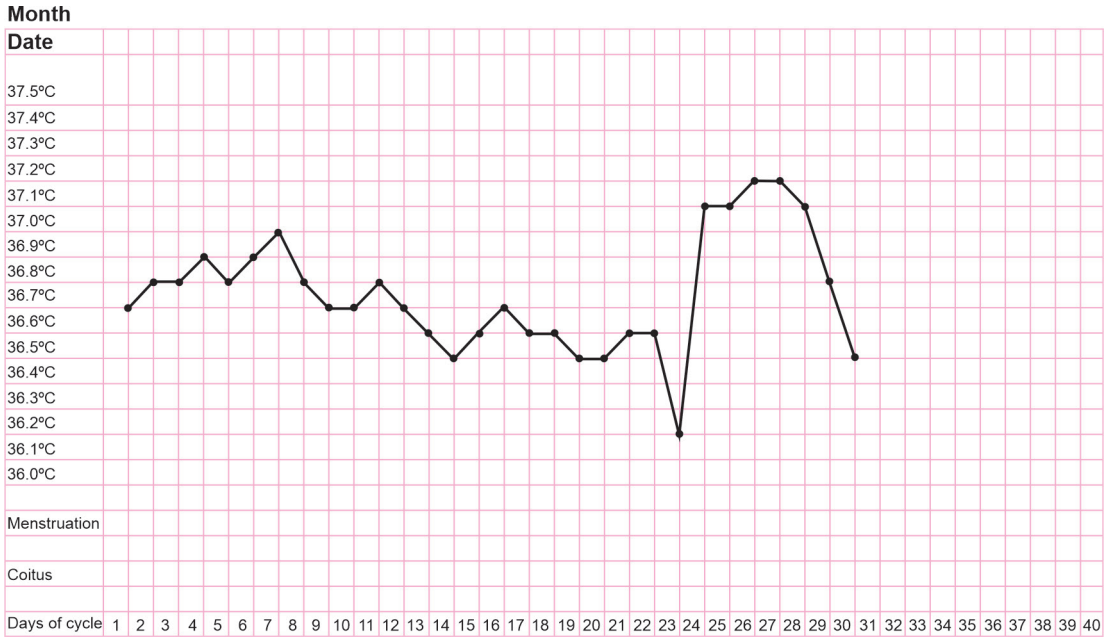

Fig. 1.11: Short luteal

Fig. 1.12: Discordant luteal

in their pattern of BBT, which helped as a fundamental parameter for planning the treatment protocol.

Urinary LH assay (By colour change on the paper strip)

Paper strip LH estimation: Urinary LH assessment from D10.

The presence of LH was indicated on the change of colour from white to orange depending on the rising concentration of LH (indicating occurrence and gradual increase in density of orange colour on the paper strip).

FOCUS ON IVF

Beginning in the 1950s, around 1961, attention was gradually being directed towards the management of infertility and research on different aspects of *in vitro* fertilization and embryo transfer (IVF-ET). The move was initiated in 1959 when babies were delivered with an injection of urinary gonadotropin In individuals in two countries of the world: Gemzell in USA and Lunenfeld in Israel. Though successful, the procedure was laborious, expensive and not safe. Subsequently, sporadic attempts to manufacture human pituitary gonadotropin (HPG) to replace human menopausal gonadotropin (HMG) came into vogue. This procedure also proved to be equally laborious and practical. Because 5 cadavers were required to prepare the requisite amount of human gonadotropin for induction of ovulation of one anovulatory cycle of hypogonadotropic hypogonadism to complete the treatment. In addition, the method was unsafe because of the risk of infection, so the procedure was abandoned.

During the same period, research on hormonal contraceptives was intensified because of the population explosion, and combined contraceptive tablets with the combination of oestrogen and progesterone were initiated. As mentioned earlier, clomiphene citrate (CC) was introduced, presuming it to be an anti-fertility drug. Infertility was indeed a complicated topic, and research started on both technology and hormones related to childbirth. Simultaneously, research went further, and people began trying to fertilize the egg outside the body (extracorporeal fertilization or

in vitro fertilization). The test tube baby Louise Brown was born in Oldham in July 1978. Contemporary international pioneers were Dr Howard Jones of the USA, Dr Carl Wood, Dr Trounson, Dr John Yobich of Australia, and many others.

RESEARCH ON IVF IN CALCUTTA

Dr Subhash Mukherjee started working on infertility in 1965 when both of us were posted at Nil Ratan Sircar Medical College and Hospital. Subhash concentrated on a fundamental aspect of endocrinology: Oocyte maturation, oocyte growth *in vitro*, and extra corporal fertilization. I was working more on clinical aspects, such as tuboplasty, wedge resection, vaginoplasty, hysteroplasty, etc. Finally, we developed a common interest in starting IVF in India, a technology that, at that time, was an ill-understood subject globally. While the research by the Calcutta duo (myself and Subhash) was developing, it was dealt with a blow when we were transferred: Mukherjee to Bankura and myself to Siliguri. Still, we came to Calcutta at weekends to continue the work. Amid this confusion in continuing the work Subhash announced the birth of Durga alias Kanupriya, India's first and world's 2nd test tube baby, on October 3, 1978. However, a few people believed the research would have been possible in a power-cut-prone district without basic facilities. Subhash, however, could not accept the criticism and committed suicide in 1981. His death made me more adamant about carrying the research forward. But I had also to face and share a lot of 'doubters.' As a surgeon by that time, I had been successful in reconstructing the cervix and vagina in a series of women born with cervical vagina atresia. Finally, three women married, conceived and successfully delivered viable babies (1990 to 2000).

Like Subhash, I also shared ignorance when I lectured about my success in Delhi, Vellore, and Bombay. People clapped politely, but their lack of credence in research happening in Calcutta was apparent. The same fate awaited my paper and publications abroad. The papers initially created excitement—which fizzled out learning that the researcher was from India. This made me more challenged, and I continued my work with determination.

Fig. 1.13: My 1st test tube baby

With a team of youngsters that involved Dr Sudarshan Ghosh Dastidar, Dr Siddhartha Chatterjee, Dr Bani Kumar Mitra, Dr Arup Majhi, Dr Partha Goswami, Dr Sanghamitra Ghosh, Dr Ratna Chattopadhaya and young but senior associate late Dr Subir Dutta. I started IVF research in a small garage in my own CIT Road chamber at Moulali. Finally 'Imran' my first test tube baby, was born on November 3 1986 (Fig. 1.13). Around the same period, other Indian pioneers who developed IVF in India were Dr Indira Hinduja, Dr TC Anant Kumar, Dr Mehroo Hansotia, Dr Sadhna Desai, Dr Mohanlal Swarankar, Dr Kamala Selvaraj, Dr Sudarshan Ghosh Dastidar and many others.

FINANCIAL AND LEGAL CONSTRAINTS

This was a time of strict foreign exchange monitoring. Only £5 was allowed for foreign travel. Importing machines and disposables were impossible.

Little things like single-use embryo transfer catheters made of plastic would have an import duty of £300 slapped on each.

Fig. 1.14: IRM old building

Fig. 1.15: IRM new building

This type of turbulence continued till 1992. So, it was not until 1989 that the second test tube baby was born in our unit. But when Dr Manmohan Singh, the then Finance Minister, liberalized foreign exchange policy, I could deliver 4 test tube babies in a month. Finally, people stopped doubting my work.

Initiating IRM for Further Expansion

After my retirement, I put my retirement benefit into acquiring a plot of land in Salt Lake for the research facility. A Deputy Secretary who was my patient helped me acquire 2880 sq ft of land in DD Block, Salt Lake, Kolkata, and to get permission for a five-storied building. The building was constructed over 4 years and was inaugurated in 1989 (Fig. 1.14). The flow of patients suddenly increased, and accommodating research, clinic, and academic activities required some extra space.

Second Building in HB Block (New IRM)

The government, headed by Sri Jyoti Basu, CM, helped me provide a plot of 7200 sq ft in HB Block to construct our second building (Fig. 1.15).

Our activities started attracting students from across the country

Two post-doctoral students come annually for the FNB course through national selection in Delhi.

ADDITIONAL ACADEMIC ACTIVITIES

We are recognized for PhD courses in reproductive medicine by University of Calcutta and the West Bengal University of Health Sciences. In addition, we are also involved in collaborative research with SMST at IIT Kharagpur, IICB, Jadavpur, and Bose Institute, Kolkata.

PhD students from these institutions also work in our laboratory.

Dr Manjushree Chakravarty, an active senior obstetrician, gynaecologist, and infertility specialist is now silent help (due to her present indisposition) in all our clinical, academic, and research activities.

Finally, My current approach in infertility management is to identify 'markers' that could 'predict success' and find measures that may 'prevent' failures.

SUMMARY

I had an 'inspiring, ambitious but challenging journey.'