Bulletin of Institute of Reproductive Medicine

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Contents

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Institute of Reproductive Medicine

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Founder President Director: **Late Prof. B.N. Chakravarty**
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Dr. Ratna Chattopadhyay, Dr. Sanghamitra Ghosh & Dr. Sunita Sharma

1928-2022

Our Heartfelt Tribute to Sir

আমাদের বিনম্র শ্রদ্ধাঞ্জলি

Editorial

Prof. Dr. Gita Ganguly Mukherjee

Dr B N Chakravarty was a brilliant student, excellent teacher, bright clinician and distinguished surgeon. His colleagues used to see his surgery for genital cancer and VVF. He became the pioneer of reconstructive surgery in cervico-vaginal atresia and got International fame for his work on congenital genital defect. The delivery of a baby in 1998 following correction of cervical vaginal atresia has been ranked as the fifth viable baby recorded so far in the world literature.

His interest in infertility and ART- the untimely and sad death of Dr Subhas Mukherjee who ultimately was recognized as pioneer the first test tube baby in India and second in the world, acted as a trigger to devote the life of Dr Chakravarty for the treatment of infertile couple. He has witnessed the beginning of ART and the ART revolution in India. He made all of us proud by the birth of his first IVF baby in 1986. I still remember the overwhelming felicitation given to him with his team by BOGS - that time President of the organization was Dr Amiya Kumar Mukherjee and Secretary Dr Gita Ganguly Mukherjee. Huge numbers of BOGS members and also non BOGS people attended the program to show their respect to Dr. Chakravarty.

Setting up IRM– after birth of the first IVF baby, Dr Chakravarty realized the importance of setting up a separate institute for treating, teaching and expanding knowledge in reproductive medicine along with collaborative research activity. Ultimately the Institute of Reproductive Medicine (IRM) was constructed at Salt lake in the year 1989 and the second IRM in 1999. Under the leadership of Dr. B N Chakravarty Clinicians, Embryologists and Scientists worked together to make ART safer and effective. There has been significant achievement in the clinical, scientific and academic performance of the institute. Really IRM is the pioneer institute of reproductive medicine.

Dr Chakravarty is an eminent teacher in Gynaecology, Reproductive medicine and ART in the country. He always desires to integrate clinical medicine with basic science. Since many of the teachers in our country and abroad, have been his students, he is most appropriately called the "Teacher of the Teachers"

Medical Bulletin published quarterly is the mirror of activities of IRM. This also contains important academic articles from the in-house consultants and also from outside stalwarts. During covid situation most of the activities came to a grinding halt except the work of Dr. Chakravarty who continued writing and completed the volume 4 of his book "Clinics in Reproductive Medicine and Assisted Reproductive Technology. After covid situation gradually I.R.M. was back to normal again. Dr. B N Chakravarty asked me to be the editor of the bulletin. I edited the 1st issue after about 2 years of Covid situation. In that issue Dr Chakravarty wrote an article "My journey through IVF". It is very important document of infertility management from the begging till the recent years. I requested him to present interesting article that is Life-time experience of the clinician from Stethoscope to Microscope. This article is published in this issue. Other contributors are Dr Ratna Chattopadhayay Subject- "Role of Cumulus Oocyte Complex Metabolism in Oocyte Competence",

Dr Sunita Sharma Subject- "ART Lab Work: A clinician perspective".

Dr Pratip Chakraborty Subject- "Rethinking Endometriosis pathophysiology: judging the oxidative balance" and Dr Sourav Roy Choudhury Subject- "The Emergence of Artificial Intelligence and Deep Learning Algorithms for Automated Detection of Polycystic Ovary Syndrome"

From the work statement the present operational structure of I.R.M. will be reflected.

Hope the deliberations of this bulletin will be beneficial to the readers and to the readers and if necessary will provide with ample thought for modifying treatment and further research.

Dr Chakravarty was pioneer in the field of ART in India and his devotion, dedication and research was appreciated not only nationally but internationally. Above all he was a great soul being --- calm & quite, always with smiling face, never got angry and had helping attitude to all who approached him for any help.

Dr Chakravarty really was a living legend.

He never stoped work even with illness and during covid times. Till 16th March, 2022 the day of his hospitalization, he was busy with clinical checkup of the patients and writing the scientific article.

The living legend has left for his heavenly abode on 14th April, 2022. But he will remain immortal in the hearts of many and will always be remembered by his students, patients, colleagues, friends, and in the academic world.

My pronam to this superb human being.

From the Desk of Secretary

Dr. Ratna Chattopadhyay

Wishing you all a very Happy New Year 2023.

I have been entrusted with the responsibility of compiling and publishing Bimonthly Bulletin from I.R.M. The task is rather difficult for me particularly in absence of our Sir, because the Bulletin was the Brainchild of late Dr B N Chakravarty who had passion, interests and every care for its continuous development by contributing and projecting valuable chapters. He was very careful about the standard of the Bulletin all through. We remain completely and irrevocably committed to carry on Sir's thoughts and ideas to select the topics and write ups with utmost care and importance and will make sure his legacy lives on forever.

Sir had written so many articles on different aspects of Assisted Reproductive Technologies and Reproductive Medicine. We will try to include each one of these in the forthcoming bulletins.

We have other experienced doctors and associated Scientists with excellent writing skills. No doubt, their write-ups will enrich the future bulletin.

On behalf of all the staff of I.R.M. I would like to convey our heartfelt thanks to Prof. Gita Ganguly Mukherjee for her continuous support to publish and enrich our Bulletin.

Our 'Pranam' to Sir who taught us how to quest after truth and knowledge.

Research module in Reproductive Medicine Life-time experience of the clinician from Stethoscope to Microscope

Dr. Baidyanath Chakravarty

Introduction

Interpreted in common man's language, the term 'Stethoscope' in this chapter indicates a physician who is involved only in clinical diagnosis of the disease and its management.

Whereas '**Microscope**' signifies a physician who is also involved in the understanding of the process behind the disease. The procedure includes,- the genesis of the disease preventable or curable, and to find out ways of prevention and treatment.

But the journey from disease research to disease cure is not generally a smooth one. "Clinician – scientist" or "researcher" is the person who is the bridge between the laboratory and the clinic. He/she is a doctor who can correlate the disease process both in the patient and in the laboratory.

But in practical terms, it is difficult to integrate these two practices in '**One set up or in One individual**'.

In other countries,- In all teaching hospitals, there is an experimental wing attached to clinical unit. The gap between the clinical and basic (research) medicine is bridged by these experimental units.

In India,- such facilities rarely exist. Busy clinicians rarely find time to refresh and update their knowledge through periodical and journals. Only source of refreshing memories and addition of recent knowledge is through pharmaceutical representatives. But further research is essential to improve clinical medicine. Because application of effective clinical medicine entirely depends on a sound, oriented theoretical background.

Let us talk about Robert Edwards and Patrick Steptoe- the pioneers of in-vitro fertilization. Both of them started their medical career with 'Stethoscope' but finally one (Bob Edwards) entirely switched over to microscope and the other (Steptoe) to laparoscope.

Figure-1: Patrick Steptoe and Bob Edwards

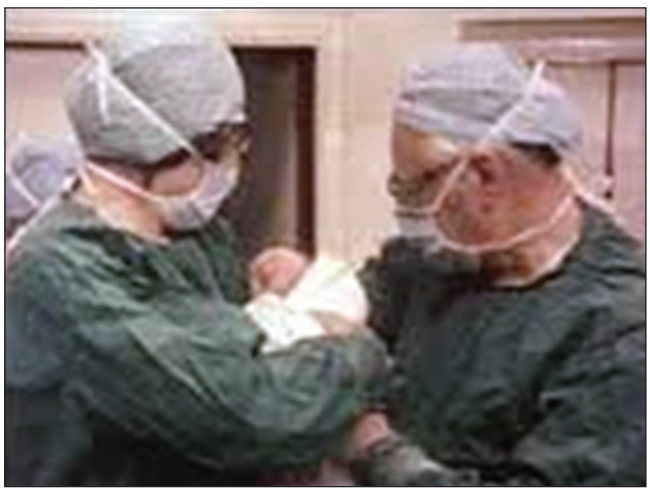


Figure-2: First Test Tube baby

Figure-3: Cloning

Edwards totally diverted to the reproductive physiology. Stepotoe initially limited himself to visualization of pelvic organs with laparoscope and finally switched over to 'follicular' physiology and tried to find out the exact time of oocyte retrieval in IVF (for collection of 'mature' egg. – size, colour, punctum).

But every prospective physician at the time of entry to medical curriculum or thereafter has this rare instinct of deviating from '**Stethoscope to 'Microscope**'???

Or

'Is it desirable or good' for everyone and for the society??? ------

Certainly – not

For example it was once presumable that by cloning celebrities could be duplicated

But subsequently this idea had to be abandoned, because cloning can duplicate 'phenotypic' appearance of the individual but not 'functional potentiality'.

Of course this is apart from ethical/legal issues involved in cloning.

Why is it so?

We all know that cells of all organs and tissues have three functions

- Division – daughter cells divide by mitotic division but maintain the morphological appearance of the mother cell.
- Differentiation – daughter cells deviate from the basic appearance of the mother cell.
- Specialization – daughter cells deviate also from the functional behavior of the mother cell along with the morphological appearance.

Upto specialization, the cells of the fertilized egg undergo and behave more or less in the same way, continuing multiplication in the similar manner. These changes in the cells will induce the potentiality of the specialization for blastocyst stage, and further **specialization and differentiation** will convert these cells into two areas known as peripheral Trophectoderm and Inner cell mass. The function of trophectoderm is to form placenta and membranes whereas Inner cell mass forms the **embryonic plate** (original source of all organs and tissues of human

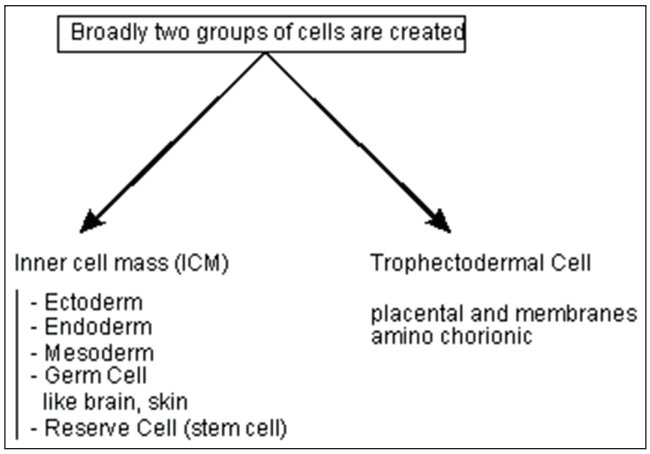


Diagram-1: Differentiation of Blastocyst

body, e.g- heart, lungs, liver, spleen, haemopoitic system, neural tissues etc) and few reserve cells (**stem cells**) which remain undifferentiated. Embryonic plate will be stimulated to form specific areas of human body namely,- **Ectoderm** (brain, skin etc.), **Endoderm** (Gut, liver, lungs etc),

Mesoderm, (muscle, blood, bone, cartilage etc) Germ line cells (sperm, egg) and few **reserve cells** (stem cell) which remains undifferentiated (See Digram 1 and 2).

These specific lines have been identified with types of cells which are functionally differentiated

How functionality is different?

They are differentiated on following characteristic

Totipotent cells from early embryo (1-3 days) can form all types of cells including placenta.

Unipotent – only one type of cells in the body in which they are existing (liver-hepatocytes, spleen-spleenic cell, heart- cardiomyocyte)

Multipotent (Fetal tissue, cord blood and other stem cells) – i.e haemopoitic cells example WBC, RBC, Platelet, bone marrow etc.

Neural cell line,- brain, spinal cord, nerve, ganglia etc

Pluripotent (Some cells of blastocyst-5-14 days)- all tissue, organs of the body except placenta. (See figure-2)

In adult somatic stage,- these cells of different organs are completely different, cannot form adult cells of other organs of the body. Individual specific

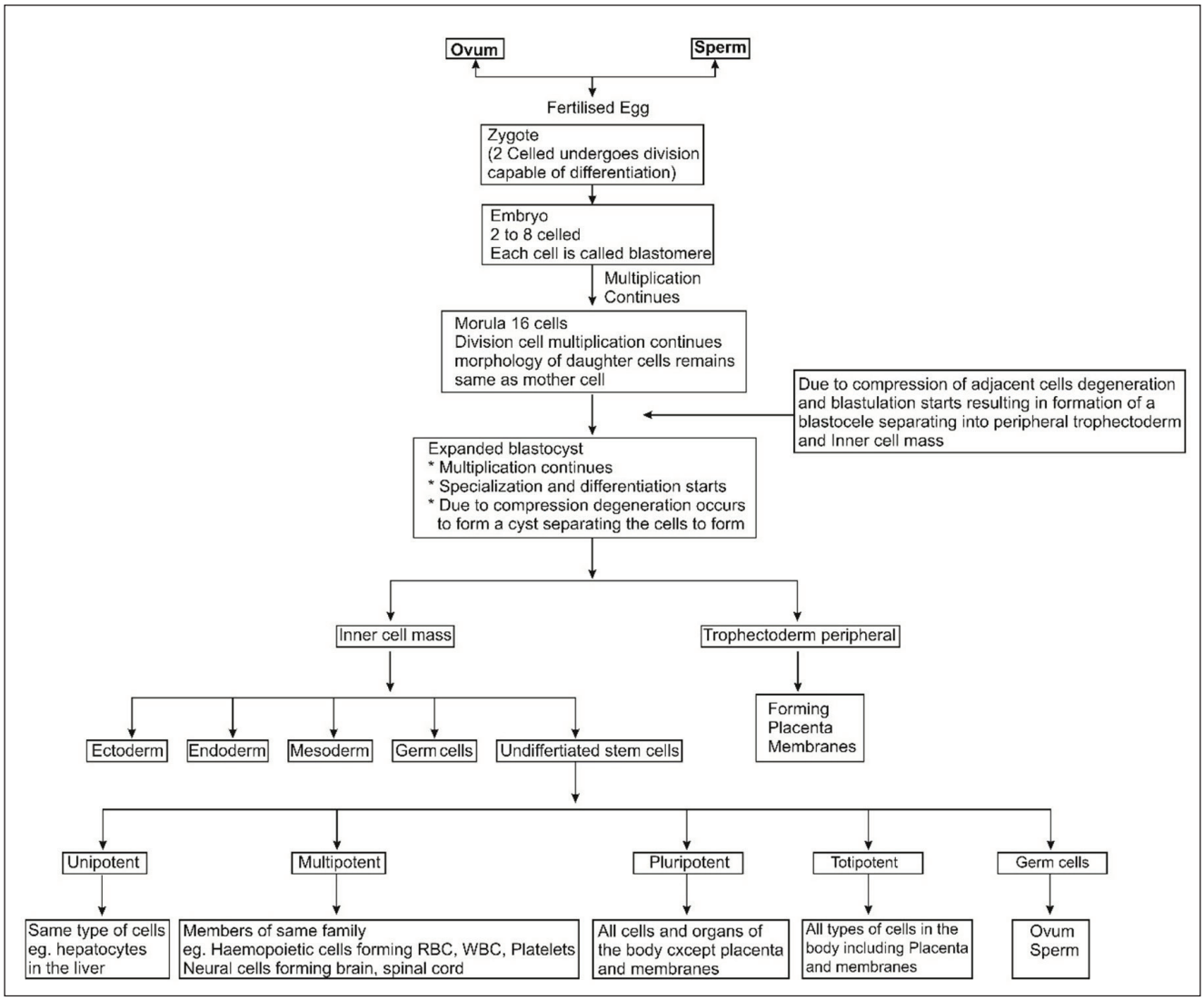


Diagram-2: Flow chart of embryonic cell division

differentiating stimulus is necessary, which is still unknown (deficiency factor in stem cell research). This deficiency in scientific knowledge is responsible for delay in stem cell research.

The following flow chart (diagram-2) illustrates the individual stages of development from zygote to different adult organs of human body.

Origin of Embryonic stem cells

As mentioned earlier, the ability of acquiring specialized function of differentiating cells of blastocyst revealed a few reserve cells in the undifferentiated cells of morula as stem cells. This was also the origin of idea of repairing or replacing human damaged parts in the laboratory mimicking the process occurring In-Vivo by experiment with these cells. This initiated the spark in the scientist, the idea to create a **complete human body** extracorporeally, later known as In-vitro fertilization (IVF). The procedure was known as Assisted Reproductive Technology (ART) and subsequently it developed into complete discipline known as **Reproductive Medicine**.

Uptill now from zygote to blastocyst, all cells of the body were functionally interchangeable. The only exception was blastocyst which could change its function, and form organs under specific signals.

Some of the cells of the inner cell mass after forming all the organs, have the potentiality to change their growth and development into any organs for direct differentiation to perform the directed functionality for repair or replacement. They do not further multiply; and retain their functionality as neutral. These cells are known as **Human embryonic stem cells** or **reserve cells**. They remain in adult organ also

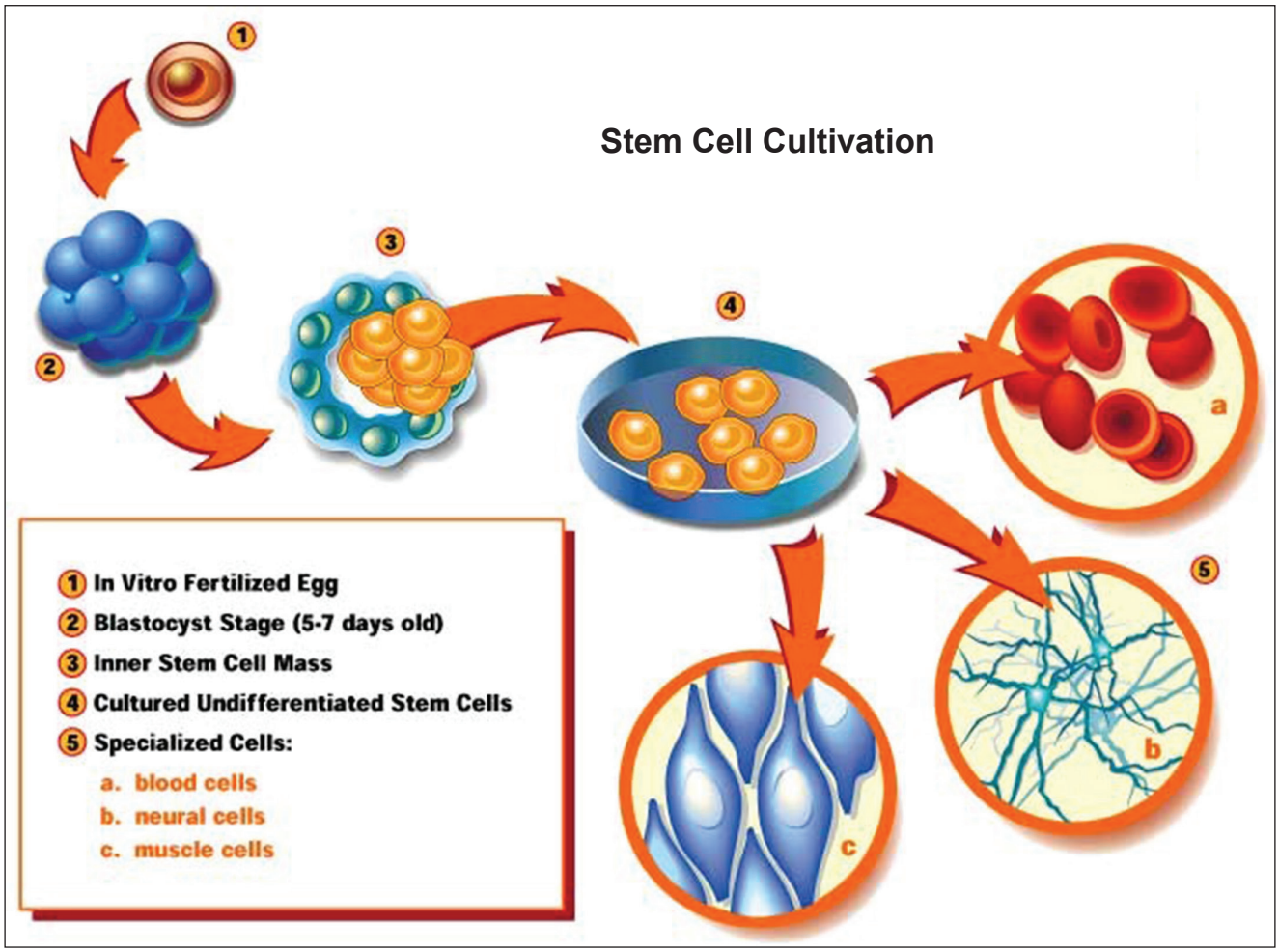


Figure-4: Stem cell origin and cultivation

and are not changeable unless a specific stimulator in the form of growth factor or (any other signal in the media), for the development of any particular organ is known. This has remained unknown till now and therefore further process of stem cell research has not progressed subsequently. (See Fig. 2)

How this individual functionality is acquired?

This individual functionality is brought about by surrounding cells in the organ for example (liver or heart) and not controlled by genetic signaling. Therefore even if the cells look like liver cells, they will not work like liver cells until they stay in the liver for a long time (environmental factor).

In summary, there are two stimuli after formation of all organs and tissues of human body a) Genetic and b) Environmental.

Alternatively, they may work in some other way,- atypical functionality leading to devastating consequences (cancer).

Therefore, though all physician are genetically similar, their attitude towards profession depends on the environment (more academic or more clinical) in which individual has been reared up (environmental). For example the baby speaks English, Hindi, Bengali etc according to the environment of its up-bringing. Similarly the doctor also becomes clinician, basic medical scientist or a clinical scientist according to his or her scientific environment.

In ART

In ART,- clinician treats infertile couples. Previously there was no detail knowledge or understanding of the process of oogenesis, spermatogenesis and fertilization. And the clinician who decided to go into the depth of science will be known as 'clinician-scientist'. Before trying in-vitro process all clinical scientists are supposed to understand the in-vivo thoroughly.

Clinician-scientist should be able to understand the physiological process behind every step of

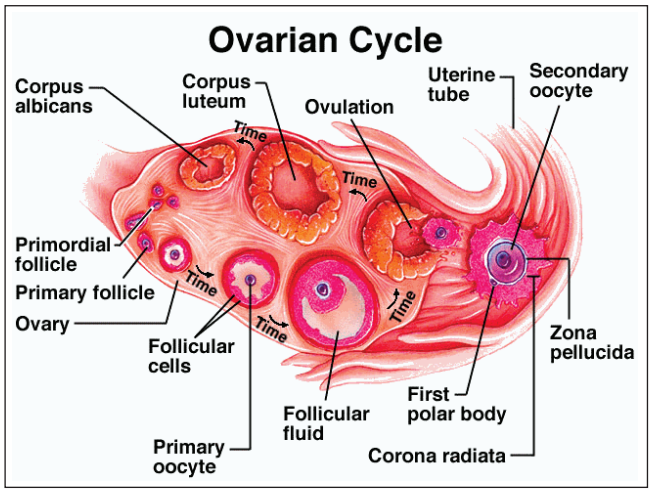


Figure-5: Oogenesis and fertilization In-Vivo

human embryologic development in-vivo. Hence the process of IVF and ART evolved.

For e.g. in ART, clinician treats infertile couples. There was no understanding of the process of "oogenesis" as "fertilisation",- till the "clinician decided to turn into a clinician – scientist".

The process of In-Vivo, oogenesis and fertilization is demonstrated in following photographs

"Clinician Scientist" was able to understand the process behind, hence this process of IVF and ART evolved.

The procedure of different aspects of research in IVF developed because of evolution of inquisitive frame of thought (Clinician) and practical approach of these ideas into areas of application (environmental).

Had the clinician not decided to turn into a clinician scientist IV? Would not have evolved till

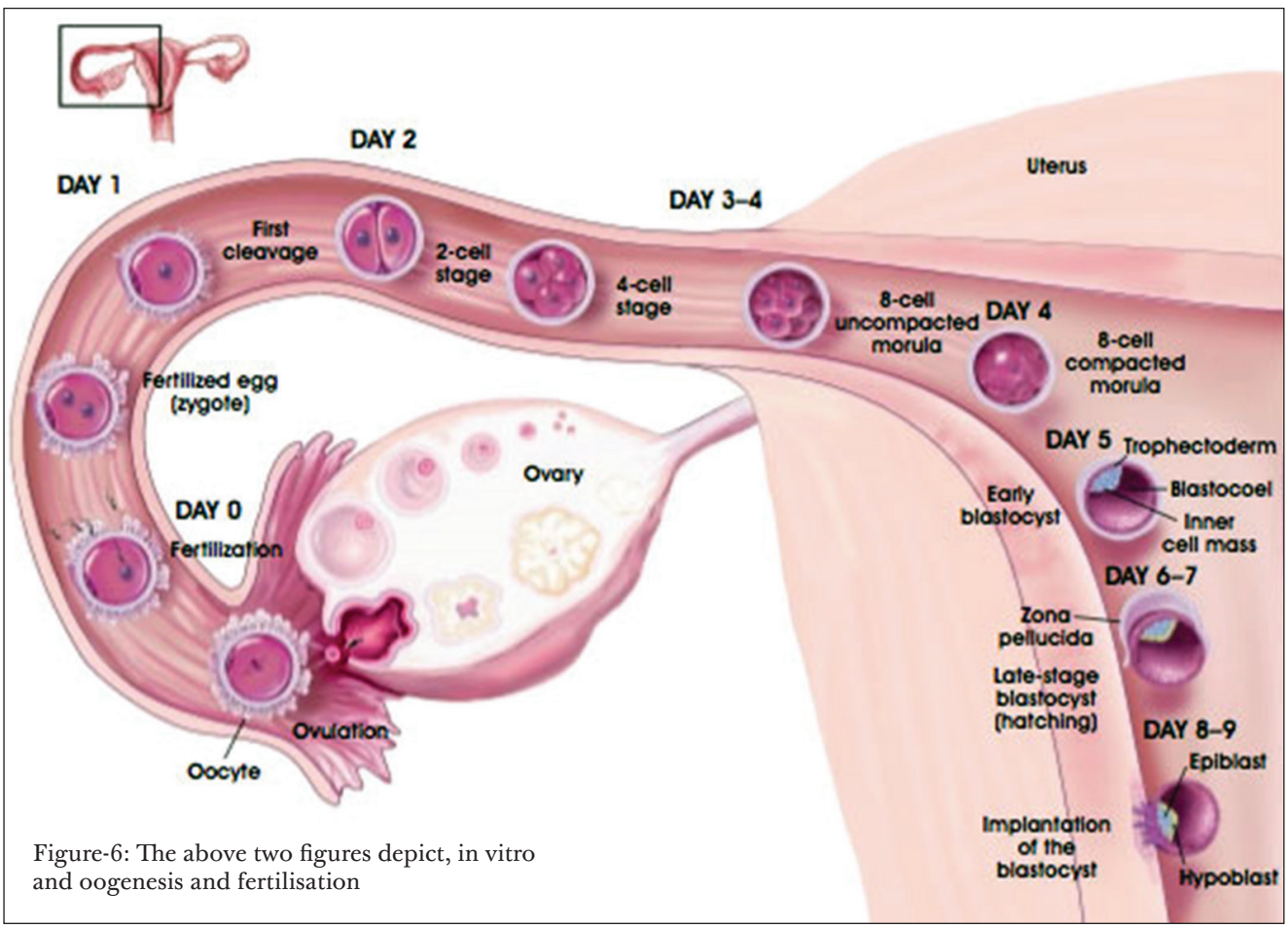


Figure-6: The above two figures depict, in vitro and oogenesis and fertilisation

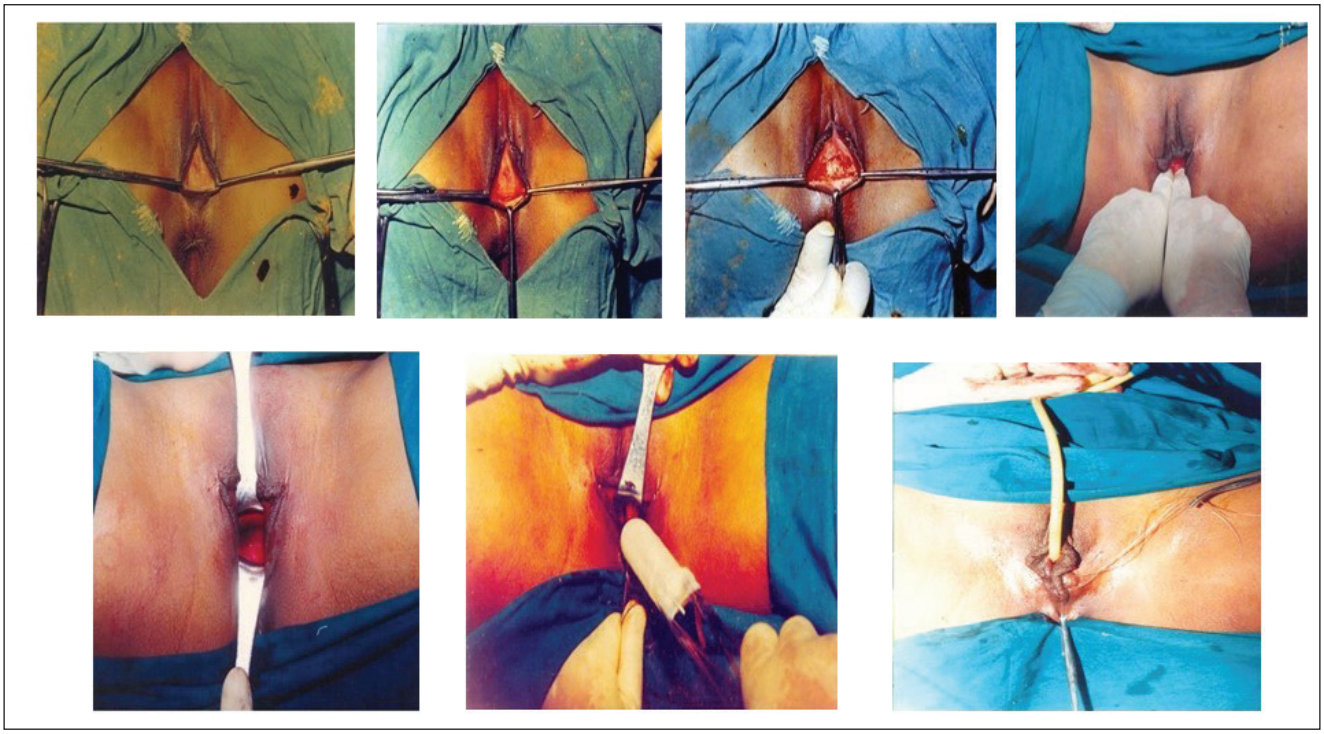


Figure 7: Steps of vaginoplasty

date. Continuous research in this field helps us in improving the success rate by simulating the 'in Vivo' process as close as possible.

My career and long association with NRS Medical College

It is my long cherished feeling that though I was 'born' in Calcutta Medical College I was 'brought up' by NRS Medical College and whatever I have achieved: 'big' or 'small' has been possible entirely because of my long association – may be around 20 years – with this prestigious Institute.

My gradual transition from 'crude' clinician to 'modified' clinician scientist

The 'active phase' of my professional career has covered more than five decades at NRS Medical College.

PHASE – I (1952-1962)

- Graduation (MBBS upto MO & MRCOG)
- Ambition was to become a good doctor and good clinician

PHASE – II (1962-1968)

- I had earned reputation as a skilled surgeon and a good teacher
- But some of my senior professor remarked "Only good batting is not a criteria of a good cricketer, you must learn fielding as well'.
- It means that should be collected on new projects and papers published.

PHASE – III (1968-1975)

I had a special craze for dealing with uncommon patients with ill-defined treatment modalities and obviously with unfavourable outcome

I worked extensively on surgical repair of vesico-vaginal fistula, Pelvic exenteration for advanced carcinoma of cervix and finally landed on 'finer' surgeries like Tuboplasty and surgical repair for congenital absence of vagina and uterus – resulting in delivery of viable babies

I published many papers and attracted visitors from abroad too who came to see vaginoplasty and hysteroplasty at NRS Medical College.

See the steps below: (see my book vol. 4)

PHASE – IV (1975-1982)

I started work in collaboration with Dr. Subhas Mukherjee – a brilliant scientist – Dept. of Physiology, NRS Medical College (environment to become a clinician scientist). Worked extensively on Infertility, endocrinological genetic and developmental disorders

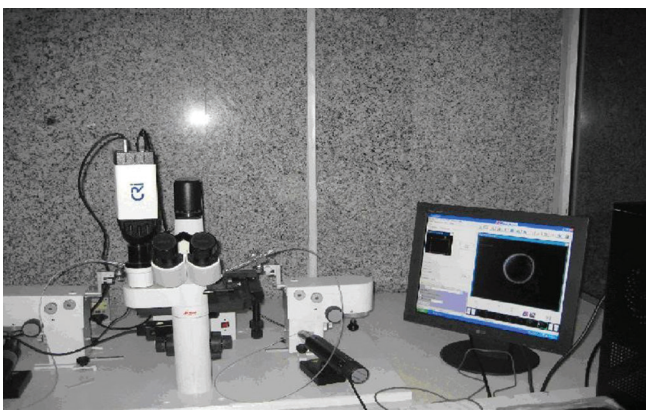
Published papers on Testicular feminizing syndrome, 46XY sex reversed females, 47XXY female Klinefeleter syndrome (Published in Lancet)

PHASE – V (1983 → continuing)

ART work – started in 1983

With Incentive from Dr. Subhas Mukherjee – Pioneer of IVF work in India, we started working on Assisted Reproductive Technology

Experimental work are still being conducted in many areas of human reproduction – work being conducted in collaboration with



- IIT – Kharagpur
- CCMB – Hydrabad
- IICB – Jadavpur, Kolkata
- Ballygunge Science College, Calcutta

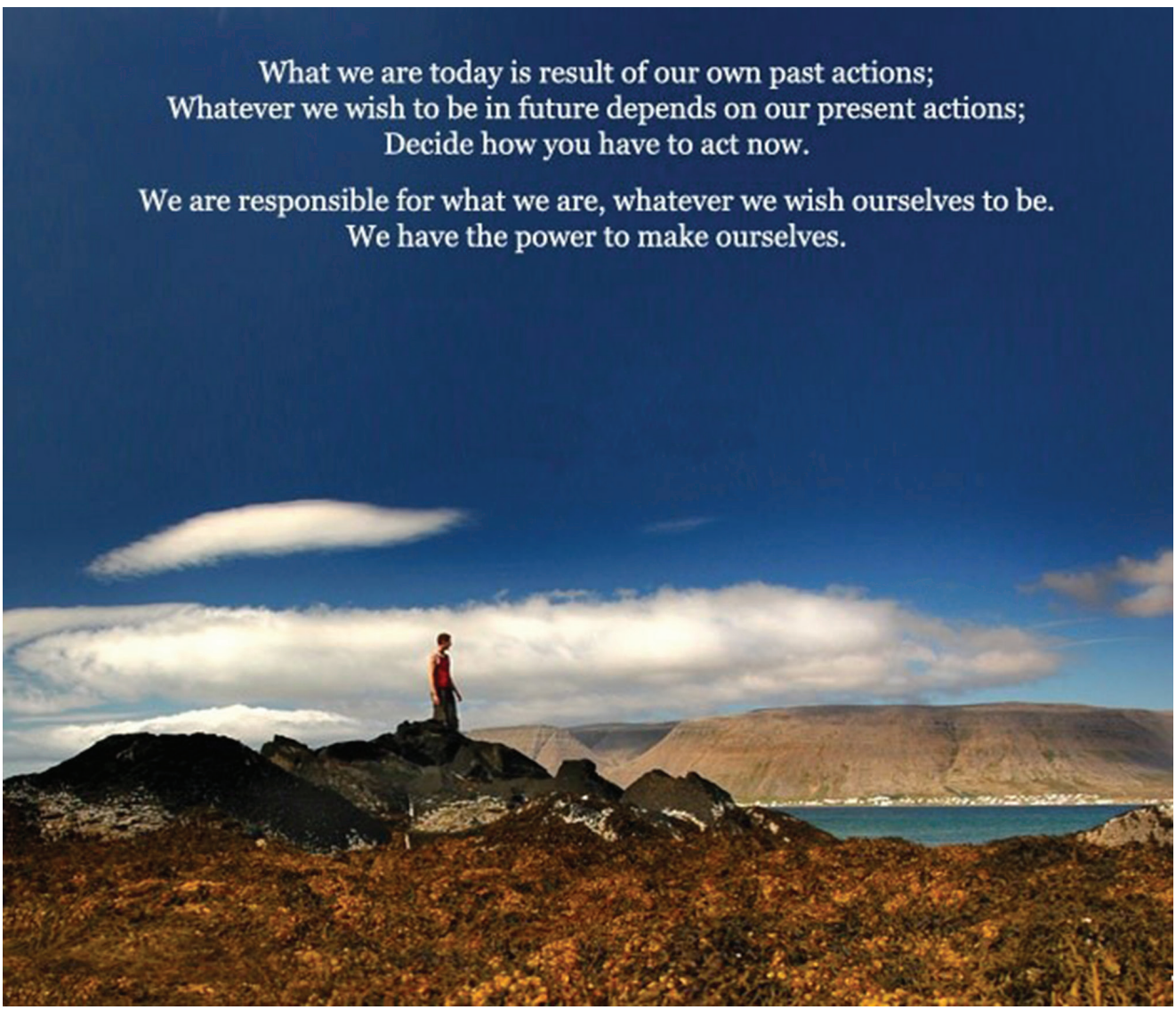
Summary

A physician's primary duty is to work with a stethoscope. But this work pattern is to be improvised by the information gathered with the help of a microscope. This is possible only through bridging the gap between clinical medicine and basic science

Conclusion

What we are today, - is the result of our own past actions;- what we wish to be in future depend on our present action:- decide how we have to act now.

We are responsible for what we are, wish ourselves to be, we have the power to make ourselves.



Role of Oocyte Cumulus Cell Metabolism in Oocyte Competence

Ratna Chattopadhyay, Sunita Sharma, Gunja Bose, Sanghamitra Ghosh

'Oocyte competence' refers to the ability of an oocyte to mature and be ready for fertilization, resulting in the potential to develop into a healthy embryo with a higher likelihood of implantation and live birth. This capability is determined by molecular and biochemical processes within the oocyte, as well as the supportive environment provided by the surrounding cells. In other words, oocyte competence reflects the intrinsic developmental potential of the oocyte, and its ability to successfully progress through the stages of embryonic development.

Understanding the molecular, cellular, and biochemical processes that control oocyte quality is one of the greatest challenges in the field of reproductive medicine. the ovarian follicular microenvironment and maternal signals play important roles in the maturation and development of oocytes, and these processes are mediated through granulosa and cumulus cells. These are responsible for the gradual acquisition of oocyte competence. The oocyte in turn secretes paracrine factors to regulate the biological function of its neighboring granulose and cumulus cells (Su *et al.*, n.d.; Gilchrist *et al.*, 2008). Therefore, oocyte and cumulus cells grow and mature in a mutually dependent and symbiotic relationship. Oocyte and cumulus cell are connected by trans-zonal projection forming a structure known as cumulus-oocyte complex (COC).

This highly specialized trans-zonal, projection form gap junction at their tip with the surface

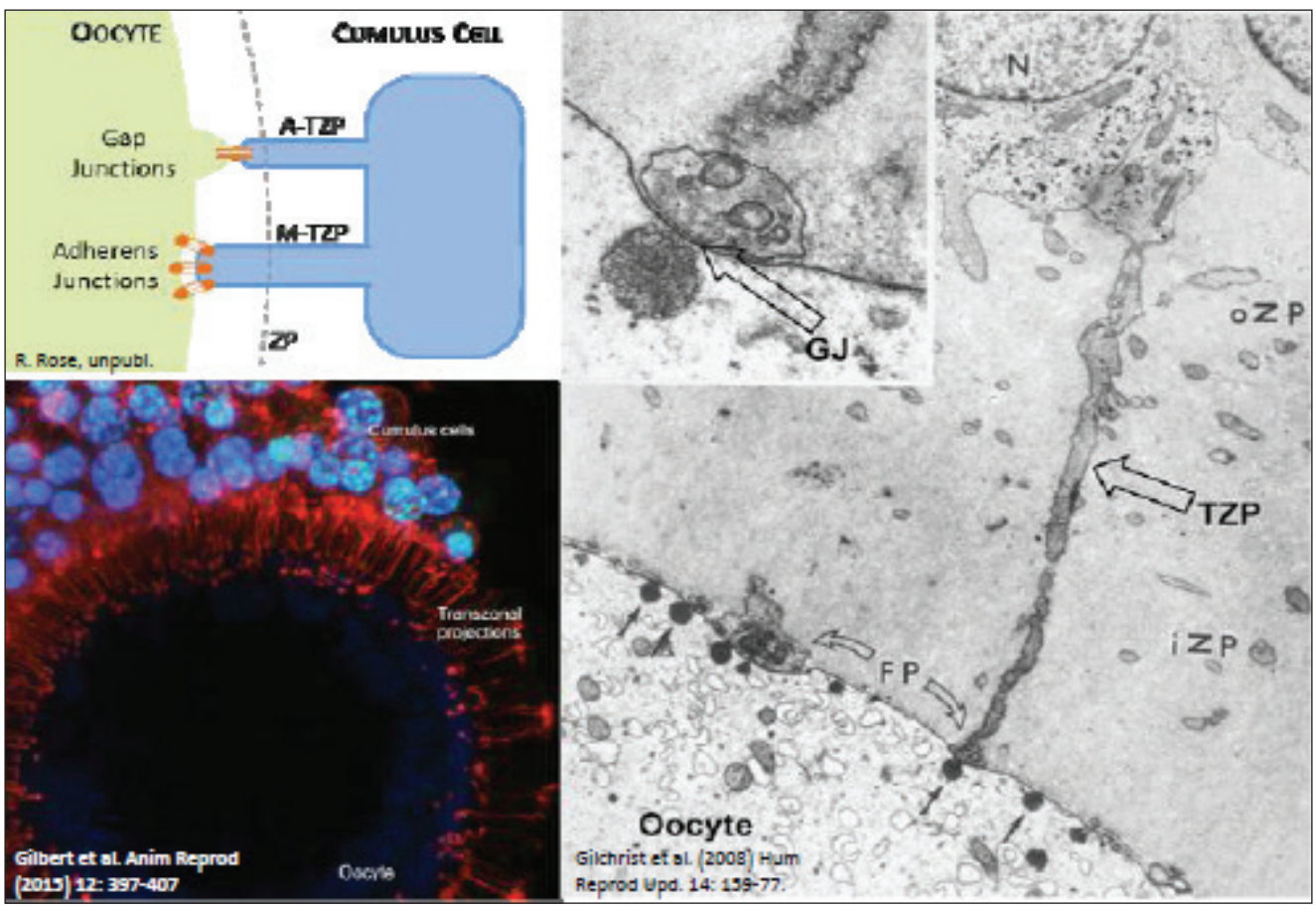


Figure-1: Schematic and electron microscopic picture of Gap Junction. Cumulus Cell — Oocyte Communication Gap Junctions are Key

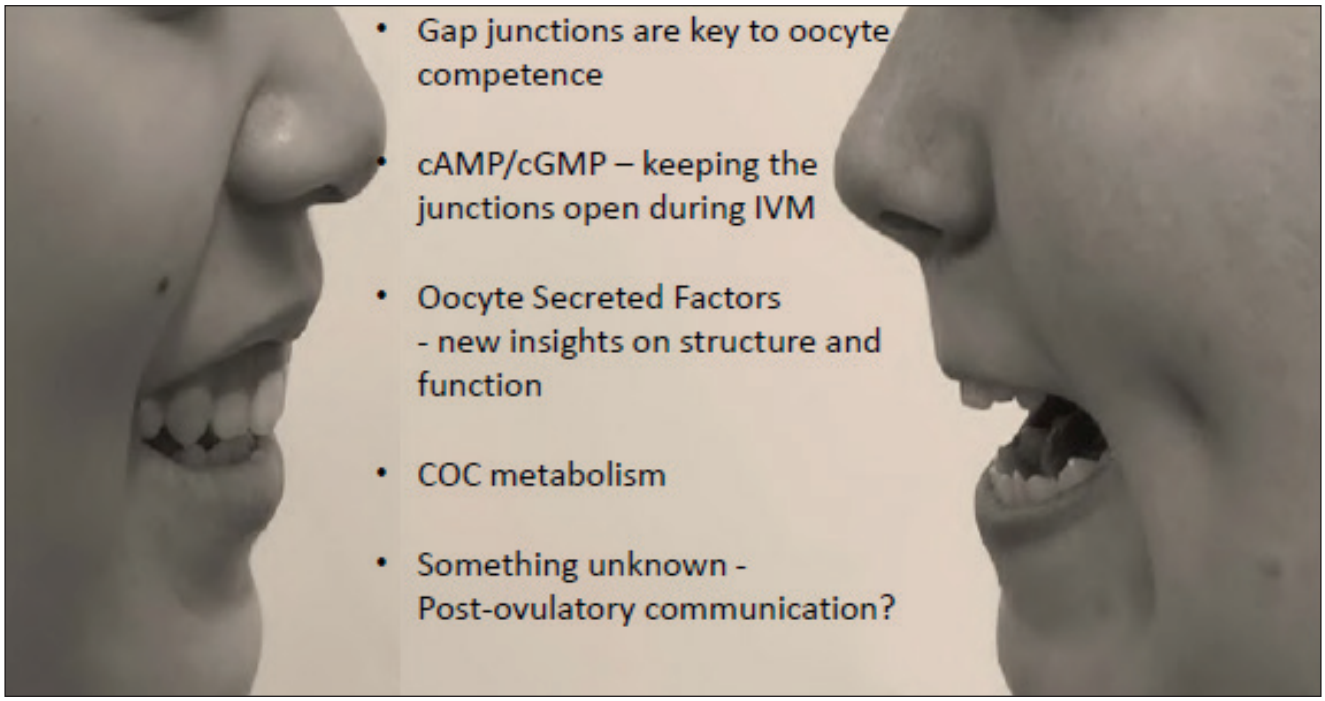


Figure-2: Cumulus — Oocyte Communication. It's a conversation … and both sides benefit!

of the oolema which allows passage of different molecules and signals between them for the viability and functioning of both the structure (Kidder and Mhawi, 2002).

Gap junction creates a conversion by which both partners get benefited. One of the important functions of gap junction is oocyte-cumulus metabolism. Metabolism is one of the major determinants of oocyte quality. The oocyte and cumulus complex have a mutually dependent bilateral relationship that involves the exchange of metabolic substances between them.

Glucose Metabolism:

Glucose is the main source of energy for the COC, either directly or indirectly. Although the oocyte has limited capacity to consume glucose from the surrounding follicular fluid or culture media for its own metabolism, cumulus cells play a key role in consuming and metabolizing glucose. In fact, almost all the glucose consumed by the COC is metabolized in the cumulus cells. This highlights the important role that cumulus cells play in the metabolism of the COC and the exchange of metabolic substances between the oocyte and cumulus cells.

Glycolysis:

A significant portion of the glucose consumed by the cumulus cells within the COC is metabolized through glycolysis, a process that converts glucose into ATP, pyruvate, and lactate. Pyruvate, which is produced by glycolysis, is then further metabolized in both the oocyte and cumulus cells through the tricarboxylic acid (TCA) cycle and oxidative phosphorylation, resulting in the production of additional ATP. Pyruvate is a key source of energy for the oocyte and zygote, and is necessary for the oocyte to mature to the M-II stage during meiosis (Downs, 2002; Johnson *et al*., 2007)..

The oocyte is limited in its capacity to acquire glucose, cholesterol and certain amino acids due to a lack of expression of transporters for their uptake as the oocyte's impaired ability to consume glucose is due to lack of expression of high-affinity insulin-regulated glucose transporter (SLC2A4 or GLUT4) (Sutton-McDowall *et al*., 2010). Oocytes' ability to metabolize glucose via glycolysis is limited due to the low activity of the enzyme phosphofructokinase (PFK) (Cetica *et al*., 2002). Lower glycolytic activity is associated with poor oocyte development competence (Herrick *et al*., 2006).

Pentose Phosphate Pathway:

A small portion of the glucose consumed by cumulus cells (CCs) within the COC is metabolized through the pentose phosphate pathway (PPP), which has both oxidative and non-oxidative phases and generates NADPH and ribose-5 phosphate. The

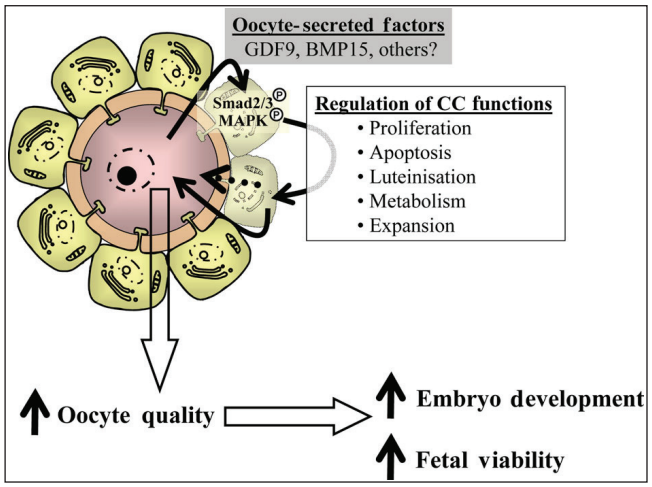


Figure-3: Bidirectional relationship between oocyte and cumulus cells.

PPP is important for maintaining the redox status, nuclear maturation, and antioxidant defense of the oocyte. In addition, the PPP runs parallel to glycolysis and is considered a predictor of oocyte quality. Studies have shown that chemical inhibition of enzymes in the PPP significantly decreases the rate of oocyte maturation to the M-II stage in porcine COCs cultured *in vitro*. In summary, the PPP is a minor pathway of glucose metabolism in CCs within the COC, but it plays important roles in the function and development of the oocyte (Herrick *et al.*, 2006; Sato *et al.*, 2007).

Hexosamine Biosynthesis Pathway (HBP):

During oocyte maturation large amount of hyaluronic acid is secreted from CCs forming the structural backbone of their elastic matrix which entraps and disperses CCs known as cumulus expansion. It helps in ovulation and facilitates oocyte extrusion, transportation to the site of fertilization, and sperm penetration. Disruptions in the hexosamine biosynthesis pathway (HB pathway) during *in vitro* maturation of bovine COCs have been shown to significantly decrease the likelihood of subsequent fertilization (Zhou *et al.*, 2019). This suggests that the HB pathway plays a crucial role in the proper development and function of the oocyte and the COC as a whole.

Over or under-stimulation of the HB pathway in COCs may lower oocyte developmental competence such as seen in maternal hyperglycemia. Therefore, glucose metabolism through various pathways is essential for meiotic maturation as it is used to generate purine and nucleic acids for DNA synthesis, NADPH for redox homeostasis, and hyaluronic acid for cumulus expansion (Johnson *et al.*, 2007; Sutton-McDowall *et al.*, 2010).

As oocytes lack the machinery to take up and metabolize different metabolites such as glucose, cholesterol, and certain amino acids it must depend on CCs for fundamental metabolic procedures. Oocytes instruct cumulus cells to perform their metabolic processes under the direction of oocyte-secreted factors (OSF) like growth differentiation factor 9 (GDF9), bone morphogenetic protein 15 (BMP15), and fibroblast growth factor (FGF8), etc.

Lipid metabolism:

Fatty acids have the ability to produce 3-5 times more energy than glucose and the process of β-oxidation, which involves the breakdown of fatty acids, appears to be a crucial metabolic pathway during oocyte maturation. The inclusion of exogenous fatty acid supplements in *in vitro* maturation (IVM) media can significantly impact the developmental competence of the oocyte. Research has shown that the inclusion of polyunsaturated fatty acids in the IVM media improves oocyte quality, while the inclusion of saturated fatty acids reduces oocyte quality. These findings suggest that the type and quantity of fatty acids present in the IVM media can significantly influence the quality and potential for development of the oocyte.

Role of Oocyte Mitochondria in Metabolism:

Mitochondria play a key role in ATP production by oxidative phosphorylation. Mitochondrial function and reserve in oocyte influence fertilization and early embryo development. An extraordinarily large number of mitochondria in antral follicular oocytes can meet ATP demand. Mitochondrial damage in oocytes decreases ATP production and increases ROS generation leading to impaired spindle formation and chromosomal segregation resulting in disrupted M-II oocyte and embryo formation. Even the distribution of mitochondria in ooplasm helps in the equal distribution of mitochondria within the blastomere resulting in the development of a competent embryo. There is evidence to suggest that an age-related decline in oocyte quality is linked to damage to the mitochondrial DNA, abnormal gene expression in the mitochondria, and a decrease in the number of copies of the mitochondrial DNA. These

factors may contribute to a decrease in the quality and potential for development of the oocyte as a woman ages.

ATP and Oocyte:

CCs have been shown to influence the levels of ATP within the oocyte. Oocytes matured *in vitro* with their surrounding CCs have higher levels of ATP throughout the maturation process compared to oocytes matured without their CCs. However, it has been observed that there is a positive relationship between maternal age and the ATP content in mature bovine oocytes, meaning that as a woman gets older, the ATP content of her oocytes increases. This suggests that while higher ATP levels may be beneficial to oocyte maturation in some cases, they may not always be advantageous and may even be detrimental in certain circumstances. Excess ATP production by mitochondria is likely to be associated with increased mitochondrial reactive oxygen species (ROS) production. This excess ROS can damage mtDNA and impair a range of molecules, which is likely to contribute to poor oocyte developmental competence.

Predicting oocyte outcome using oocyte-cumulus metabolism:

There has been significant interest in reproductive medicine to find metabolic biomarkers to predict treatment outcomes; however, no reliable oocyte metabolic biomarkers that predict ART outcomes are identified so far. Metabolomics or low molecular weight metabolites analysis of oocyte conditioned media, as well as follicular fluid, has been attempted to identify predictors for oocyte quality (Bracewell-Milnes *et al.*, 2017). The follicular fluid of oocytes that failed to cleave after fertilization have higher glucose and lower lactate and choline/phosphocholine level (Wallace *et al.*, 2012). Women who become pregnant have been reported with a lower level of glucose and higher proline, lactate, leucine, and isoleucine in their follicular fluid (Wallace *et al.*, 2012).

Fluorescence lifetime imaging microscopy (FLIM) is a highly advanced methodology that allows for the non-invasive assessment of the mitochondrial functions of human CCs. This technique holds promise for predicting the clinical outcome of the corresponding oocyte, as the mitochondrial functions of the CCs are closely associated with the maturation and potential for development of the oocyte. FLIM may provide valuable insights into the health and quality of oocytes by analyzing the mitochondria of the surrounding CCs. (Venturas *et al.*, 2020).

Take Home Message:

- Metabolism plays an essential role in healthy oocyte development and during the final stage of maturation before ovulation
- The oocyte and its surrounding cumulus are mutually dependent and regulate each-others metabolic functions for the growth, maturation, and competence of oocytes
- Perturbations in oocyte metabolic function whether *in vitro* or *in vivo* impact oocyte developmental competence and reproductive potential
- Metabolic imaging of COC is likely to be exploited using machine learning algorithms providing a highly predictive tool for oocyte developmental competence

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ART Lab Work: A Clinician Perspective

Sunita Sharma, Padmaja Bhattacharya, Ratna Chattopadhyay

Introduction:

The IVF lab essentially takes over the role of a mother's womb in terms of being the environment for the growing embryo. It is the place where gametes and embryos are nurtured. The live birth rate following Assisted Reproductive Technology (ART) has steadily improved from less than 1% to roughly 40% in 40 years. This has been possible due to a better understanding of the biology of gametes and embryos which has guided the designing of better stimulation protocols, optimum culture conditions, and methods of cryopreservation. ART is a complex procedure with multiple factors affecting its outcome. Standardizations of ART practices can decrease the variability in IVF outcomes. The clinician in an ART clinic decides the best protocol for that patient based on the clinical parameters of the patient. Apart from clinical practices, issues pertaining both to the ART lab and to the personnel involved in ART treatment are critical in determining IVF success. Proper coordination of the clinician, embryologist, ART nurses and paramedical staff is needed. This article focuses mainly on the role of the clinician in the ART laboratory and how to provide clinical decisions about difficult situations during the ART procedure.

Standardizing the work process can reduce variation in IVF outcomes and improve overall success. This requires a coordinated functioning clinician and embryologist and additionally supported by other staff in the ART team. The primary commitments of clinicians are patient profiling, individualization of stimulation protocol and ovulation trigger, oocyte retrieval, determining endometrial receptivity, embryo transfer and designing luteal phase support. Whereas the embryologist is responsible for the maintenance of the ART lab, embryo culture, and selection, and gamete cryopreservation.

Responsibility of Clinician

An ART clinician is a gynecologist who has an interest in infertility and reproductive endocrinology and has knowledge in the hormonal control of the menstrual cycle and use of ovulation-inducing agents. The responsibilities of the clinician would include the following:

- Initial history taking of the infertile couple
- Physical examination of the couple and advice of appropriate investigations
- Interpretation of results and categorizing them for procedures like IUI, IVF and ICSI
- Pretreatment procedures like surgical intervention before planning assisted reproduction technology ART (For conditions like Hydrosalpinx, polyp, fibroid, septum, and endometrioma)
- Proper record keeping

Additional involvement of the Clinician:

Clinician in the IVF laboratory has involvement in activities beyond the realm of clinical ART procedures. To name a few such activities which include consultation with the clinical embryologist and staff about the patient's treatment plan, ensuring that appropriate procedures are done, and proper identification and labeling of the patient/patient's specimens.

Apart from the preparation of the patient for IVF, the clinician should also have an overall understanding of the laboratory equipment, culture medium, disposables, sterile culture conditions, and quality control of the laboratory equipment. A few such important information is given below:

a) As the gametes and embryos are exposed to plastic wares in culture media within the incubator for most of the time, pH and temperature may affect embryo quality and thus the pregnancy rate. The clinician should be aware of the basic working principles of the incubator. Knowledge of CO_2 and temperature calibration, the pressure setting of CO_2 Gas cylinders, the need for frequent cleaning of the incubator, and the change of

water are important aspects to know. He should also make sure that in cases of power failure, the CO_2 incubator should have a backup power supply. The knowledge of the pressure settings for the flow, frequency for change of HEPA filter, and frequency for servicing of the unit is also mandatory.

b) The basic operations of the micro-manipulator along with the frequency of service, and changing of the lamp in case it burns out are important points to know. The clinician should also know the method to focus the microscope and set up of pipettes.

c) The clinician should have an idea of the different components of the culture media and the factors on which selection of proper media is done for each procedure like oocyte collection, sperm preparation, micromanipulation, embryo Culture, blastocyst culture, freezing of sperm, oocyte and embryo freezing. It is also important to store the culture media properly by monitoring the temperature using thermometers in refrigerators and freezers.

Central point of Assisted Reproductive Technology: ART Lab

ART laboratory is one of the most important components in the treatment of an infertile couple seeking IVF. It comprises of various clinical treatments and laboratory procedures;- including handling and manipulation of gametes and embryos. ART procedures include *in vitro* fertilization (IVF), Intracytoplasmic sperm injection (ICSI), preimplantation genetic diagnosis (PGD), gametes, and embryo cryopreservation. In human in vitro fertilization (IVF), gametes and embryos are very fastidious in their requirement for their growth and development. There is considerable awareness that the environment of the laboratory itself can alter the quality of the embryos and requires a multidisciplinary approach to optimize ART results. Therefore the ART program should focus on the generation of top-quality embryos by optimizing the embryo development milieu, selecting of best quality embryos and development optimum methods for gamete and embryo cryopreservation.

An embryologist may or may not be a medical doctor, but certainly should have training in embryology from a recognized academic body or institution and should possess reasonable knowledge and experience in biochemistry, biology, reproductive medicine, and genetics.

Services of ART Lab:

- Semen analysis and sperm functional tests, semen preparation, and cryopreservation. It also provides donor semen and cryopreservation facilities for oncology patients before undergoing oncotherapy. Surgical sperm retrieval procedures (epididymal and testicular sperm aspirations or testicular sperm extraction) are done and samples are assessed by an embryologist for ICSI and/ or cryopreservation
- During the oocyte retrieval, the aspirated follicular fluid is handed over to the embryology team from which oocytes are separated and cultured. Depending upon the quality of semen sample, the oocytes are either inseminated or injected and incubated.
- Manipulation of oocytes, sperms, assessment of quality of oocytes, zygotes, and embryo (day 2/ day 3 and blastocysts) is done to prepare them for transfer or cryopreservation.
- Good quality embryos are selected and loaded into transfer catheters for embryo transfer and clinician does the transfer.
- In the presence of thick zona pellucida or in couples with repeated implantation failures assisted hatching may be considered.
- Preimplantation genetic diagnosis (PGD) may be offered to prevent serious inherited diseases and preimplantation genetic screening (PGS) for aneuploidy screening in recurrent implantation failures.
- Documentation and maintenance of records and results of all laboratory procedures is mandatory.

Embryologist's responsibility

a) ***Assessment of oocyte and embryo:*** Of the Oocytes retrieved for ART following ovarian stimulation, only 5% resulted in a live birth. Embryologist can select oocyte with good development competence by observing the expansion of cumulus in relation to COCs and excluding

cytoplasmic/extracytoplasmic anomalies such as increased cytoplasmic granularity, vacuolization, abnormality of first polar body, perivitelline space or zona pellucida. The presence of meiotic spindle and its position, observed with polarized light can also predict a functionally superior oocyte, Although, these criteria are not enough to select an oocyte with higher developmental competence, knowledge of such findings help the clinician to modify stimulation protocol in the subsequent cycle in cases of an unexpected increase in the abnormal oocyte. Therefore, the assessment of oocyte maturity is the combined work of both clinicians dealing with an ovarian reserve and stimulation protocol and the embryologist doing the morphological assessment of the oocyte.

In routine practice, morphological assessment of pronuclear and embryo stages is considered the gold standard to select embryos with higher implantation potential. Combination of several morphological criteria of embryo-like symmetry of blastomeres, extent of fragmentation, and multinucleation along with morphogenetic assessment by time-lapse technology and embryo metabolism help in embryo selection more accurately and may optimize the result of IVF cycles.

b) ***Embryo biopsy:*** Embryo biopsy can be done for embryo selection to identify affected embryos in couples susceptible to transmit specific genetic disorders (PGD - preimplantation genetic diagnosis) or to screen for aneuploid embryos (Preimplantation genetic screening PGS). Clinicians can suggest PGD to couples at risk of transmission of a genetic disorder and thereby avoid the mental and physical trauma a woman has to undergo for termination of an affected pregnancy. Blastocyst stage biopsy has been found to be more useful than polar body or cleavage stage biopsy. PGS with blastomere biopsy and FISH though reported encouraging results initially, failed to demonstrate promising outcome in further RCTs. Use of trophectoderm biopsy with array-based newer technologies which allows all chromosome testing can be a better option than the usage of 9 chromosome FISH to improve IVF results.

c) ***Fertilization failure***

There is no treatment in the same cycle. It may provide as a marker for a problem in gamete quality sperm function, oocyte activation, gamete receptors, sperm processing, or in the number of spermatozoa used for insemination. In IVF cycles, failed fertilization rate should be <5% for stimulated cycles. Observed values above this rate should be reported and investigated. Successful fertilization depends on the inherent quality and cytoplasmic maturity.

If there is a history of failed fertilization in the past it is advisable to follow a few steps--

- ICSI for previous fertilization failure.
- Pre-IVF semen functional tests to be done - if high DFI>30% antioxidant treatment and then ICSI
- Proper stimulation protocols
- Ca ionophore for Assisted oocyte activation (AOA)
- Selective utilization of relatively undamaged sperms (Intracytoplasmic Morphologically Selected Sperm Injection-IMSI, Selection of Hyaluron-bound spermatozoa- PICSI, birefringence – selection of sperms by polarized microscope)

Clinician and Embryologist cross talk:

It is important for the embryologist/clinician to pay attention to minor details related to IVF Lab cleanliness as detailed below:

a) The need for cleaning the IVF lab daily with a proper disinfectant that is non-toxic and odorless

b) CO_2 incubator cleanliness-regular cleaning

c) Disinfection and asepsis while handling the gametes and embryos

d) Washing of hands properly before handling the dishes containing embryos and

e) Wearing gloves while preparing the dishes and handling the culture medium

f) The clinician/embryologist/student should change cloth and slippers cover hair and put mask before entering the IVF laboratory

Each ART laboratory should develop standard operating procedure (SOP) manuals in writing which should have a detailed description of the various procedures carried out in the laboratory step-by-step. While designing SOPs, the clinician should make sure that it addresses different clinical conditions and whether the conditions documented can provide solutions in crisis situations or not.

How can ovarian stimulation influence lab performance?

a) A clinician should know that a good stimulation with follicles > 14mm is expected to yield a well-expanded cumulus oophorus complex (COC) with higher number of MII oocytes whereas an inappropriate stimulation because of excess or sub-optimal doses of gonadotropins or timing of trigger may result in abnormal COC morphology and fertilization and increased rate of aneuploidy. Therefore, ideally COS (controlled ovarian stimulation) should be individualized. Age negatively affects the pregnancy rates in ART cycles, but as it is a non-modifiable factor, we should pay more attention while designing our management strategies. Alpha and ESHRE surveys have shown that oocyte retrieval rate and oocyte maturation rate are important markers for quality of oocyte. The optimum trigger to oocyte retrieval interval is 34- 38hrs. Higher oocyte maturation rate was observed >36 hours compared to <36 hours (trigger-retrieval interval). So the response to ovarian stimulation is closely linked to the maturity and competence of the oocyte.

   Number of MII oocytes retrieved depend on the quality of stimulation (KPI) and presence of other pathologies like PCOS and endometriosis. When we are dealing with women having endometrioma, there is a chance of mixing of endometrioma fluid with the oocyte which is detrimental to the oocyte quality. Embryologist should hence be careful while washing these oocytes.

b) The clinician and embryologist should have information regarding the clinical (dose of gonadotrophin used, number of follicles developed, IVF/ICSI) and embryology records (number of MII oocytes retrieved, number of good quality embryos, cleavage stage or blastocyst transfer, fresh or frozen transfer) of previous IVF cycles. So good communication about cycle planning and review of records between the ART lab and the clinician is crucial for successful IVF programme.

c) **Lab procedures and sperm parameters**

   There should be no specific cut-offs for semen characteristics to decide the procedure for the couple- IUI, IVF, ICSI. A pre-treatment 'trial wash' will decide the definite procedure. The clinic should develop their own semen cut-off levels for deciding the treatment plan.

   Retrograde ejaculation: This is a condition in which semen is released into the bladder during ejaculation. Medical management, urinary sperm retrieval and surgical sperm retrieval (PESA, TESA) exist for the treatment of retrograde ejaculation. Post orgasmic urine sample is collected either after voiding or after catheterisation following alkalinization of the urine with oral sodium bicarbonate or increasing fluid intake. This specimen is then resuspended, centrifuged, checked for count and motility and accordingly used for the selected procedure either IUI, IVF, ICSI.

d) ***Non-obstructive azoospermia*** (NOA): There are reports of presence of ejaculated sperm after letrozole in NOA patient, For this group of patients, if semen analysis is done on the day of oocyte pickup, we can avoid unnecessary sperm retrieval procedure. In cases of primary testicular failure with NOA, medical treatment (Clomiphene, hCG/HMG) in men with NOA can improve surgical sperm retrieval.

e) ***Embryo transfers (ET):*** Embryo transfer technique is simple but also a crucial step in ART programme. ET done under ultrasound guidance gives better results than done blindly. In difficult ET, pregnancy rate is reduced because of the trauma caused by the ET catheter or due to uterine contractions. Clinician decides the number of embryos (one to three) to be transferred depending on age of women, number and quality of available embryos, IVF cycles done earlier and medical indication of the women.

If difficulty is experienced during transfer, physicians have the option either to go ahead with the ET with the additional maneuvers like -

- After loading ET technique if preloading was done earlier. Outer sheath of ET catheter to be introduced first till the level of cervical internal os and then embryologist negotiates the inner catheter through the outer sheath into uterine cavity.
- If still resistance in negotiation through internal os, still persists outer sheath along with stylet can be tried with or without tenaculum traction

  Clinician may proceed with embryo freezing, do the mock transfer reattempting before the actual ET.
- If needed ET can be done under anesthesia.
- When a difficult Embryo transfer is anticipated, trial is done prior to the index cycle and embryologist should be informed about anticipated difficulty and additional measure to be taken during ET

Trouble-shooting in ART laboratory

ART lab and clinician should have more frequent communication while dealing with few panic situation mentioned below:-

a) **OHSS:** If there is risk of ovarian hyperstimulation, lab should know about
   - Type of trigger used GnRH agonist/hCG
   - prophylactic use of IV albumin / macromolecules/ bromocriptin
   - According to severity of symptoms of OHSS consideration of freezing all embryos & transfer next cycle
   - Decision for blastocyst culture

In case, OHSS has developed just before or after oocyte retrieval, - transfer should be delayed by day 1 or day 2 as during these period OHSS might settle down. Otherwise the embryo should be cryopreserved for transfer in the next cycle.

b) **Poor response:**

   If there is no oocyte in follicular fluid,
   - Flushing of the follicle
   - Checking aspiration niddle and suction machine/pressure/flow rate
   - Stopping the procedure mid-way and checking the ampoule of hCG

c) **If no sperm has been retrieved in case of non-obstructive azoospermia next step is**
   - Abandon the procedure
   - Use of donor sperm (need for a consent form duly signed by couple prior to start of the procedure)

d) **If all sperms are nonmotile-hypo-osmotic swelling (HOS) test is to be done to check vitality and ICSI can be performed.**

e) **If patients fails to ejaculate**
   - o Trial for collection of semen again – in the same cycle after 6 hours
   - o TESA – with consent
   - o Cryopreservation of semen in advance – next cycle

These points are to be discussed with the couple prior to start of the actual procedure of ICSI.

Take home message:

- IVF success depends on proper coordination of the clinician, embryologist, ART nurses and paramedical staff.
- Clinician should also have a thorough understanding of the laboratory environment, equipment, culture medium and its quality control..
- Clinician should also have knowledge about laboratory SOPs
- Good communication between clinician and embryologist will be deciding the best management strategy of the patient.
- For planning IVF cycle, the clinician and embryologist should be aware of previous records of infertility management.

Successful IVF workup depends not only on the commitments of clinician and embryologists but involves collaborative devotion and dedication of multidisciplinary experts like biochemists, ultrasonologist and geneticist etc.

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Rethinking Endometriosis Pathophysiology: Judging the Oxidative Balance

Pratip Chakraborty, Ratna Chattopadhyay, Shovandeb Kalapahar

Endometriosis is a chronic, debilitating disease associated with pelvic pain and infertility having a prevalence of about 10%.[1] It comprises three well- recognized phenotypes: superficial peritoneal lesions (SUP), ovarian endometriomas (OMA) and deep infiltrating endometriosis (DIE).[2] Symptom/s for endometriosis starts with chronic pain during adolescence with pathophysiological steps of initiation slowly develops thereafter. Although pathogenetic mechanisms responsible of endometriosis have not been definitively elucidated, during 'endometriosis life', inflammation, angiogenesis, neurogenesis, oxidative stress, progesterone resistance and disrupted uterotubal motility constitute some of the proposed mechanisms.[3] During each menstrual cycle, endometriotic lesions are responsible for repeated tissue injury and repair, with presence of local inflammation due to variety of factor/s. Inflammatory process induces oxidative stress (OS) and reduces cellular antioxidant capacity. OS thereafter destruct peritoneal mesothelium, producing adhesions for ectopic endometrial cells. However, the effect of OS on developmental dynamics of the reproductive tissue has been little showcased.

ROS drives the way to a vicious circle of inflammation

Pro-inflammatory cytokines and growth factors were found to be elevated in the peritoneal fluid and serum of women with endometriosis and could contribute to the proliferation of endometriotic implants and neo-angiogenesis.[4,5,6] Incidentally, erythrocytes, apoptotic endometrial tissue and cell debris in the peritoneal cavity by menstrual reflux and macrophages are potential inducers of OS. Conversely, pro-inflammatory cytokines may impact the recruitment of macrophages, which are one of the main producers of reactive oxygen species.[7] Presence of oxidative stress in the peritoneal cavity of women with endometriosis, stimulates non-scavenging properties of macrophages that are non-adherent, "switching on" the synergistic interaction between macrophages, oxidative stress, and the endometrial cells.[8] The pro-oxidants exacerbate the growth of endometriosis by inducing chemo-attractants such as monocyte chemoattractant protein 1 (MCP-1) or interleukin-8 (IL-8) and endometrial cell growth-promoting activity by selectins, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1).[9] At the site of inflammation the activated inflammatory cells release many enzymes (neutral proteases, elastase, collagenase, acid hydrolases, phosphatases, lipases, etc.), reactive species (superoxide, hydrogen peroxide, hydroxyl radical, hypochlorous acid, etc.), and chemical mediators (eicosanoids, complement components, cytokines, chemokines, nitric oxide, etc.) ultimately inducing tissue damage and oxidative stress.[10] Increased inflammatory mediators in the peritoneum, endometrium, and serum of endometriosis cases have been recently observed in several studies.[11,12] Interleukin-1β (IL-1β) enhances the proliferation of endometriotic cells.[13] IL-1β also triggers the production of IL-6 and IL-8 in endometriotic tissues, leading to more proliferation[12,13] and decreased apoptotic rate.[12] In endometriotic cells, TNF-α is associated with pluripotency mediation and the production of inflammatory cytokines, especially IL-8.[14] NF-κB, an important regulator of various inflammatory and pathologic responses induces cell proliferation and inflammation and inhibits apoptotic process in endometriosis.[15] Recently, it has been revealed that NF-κB–IκB complex creation increased the inflammatory mediator, such as IL-6 and IL-8 in endometriosis.[14] High ROS concentrations in endometriosis increase DNA fragmentation, modulate NF-κB expression, and stimulate numerous genes encoding angiogenic growth, and pro-inflammatory factors, cyclooxygenases, and adhesion molecule (Figure 1) helping endometriosis maintenance. Furthermore, the inflammatory cytokine IL-6 has been found to produce ROS through increased expression of NADPH oxidase 4 (NOX4) in non-small cell lung cancer.[16] The NOX4 overexpression has also been found to enhance IL-6 production, and a positive reciprocal feedback loop

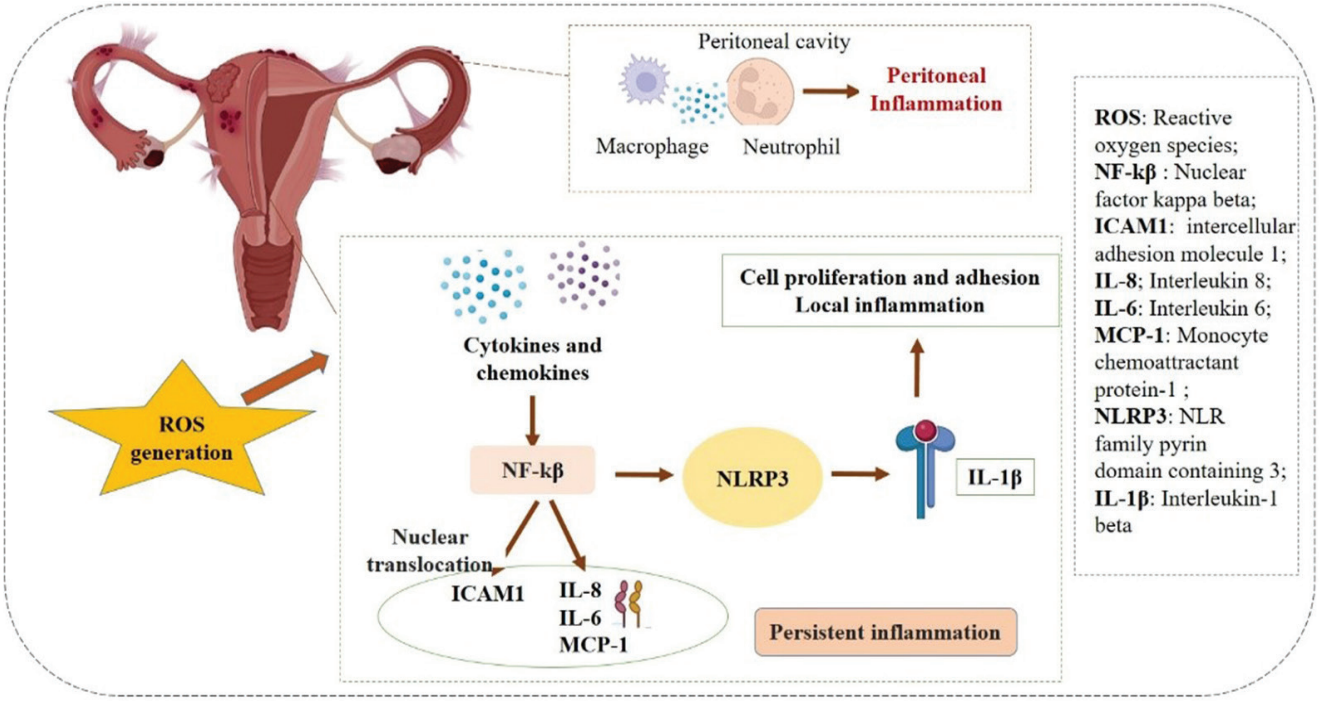


Figure 1. The reason behind persistent inflammation in endometriosis: the generation of reactive oxygen species at the hindsight.

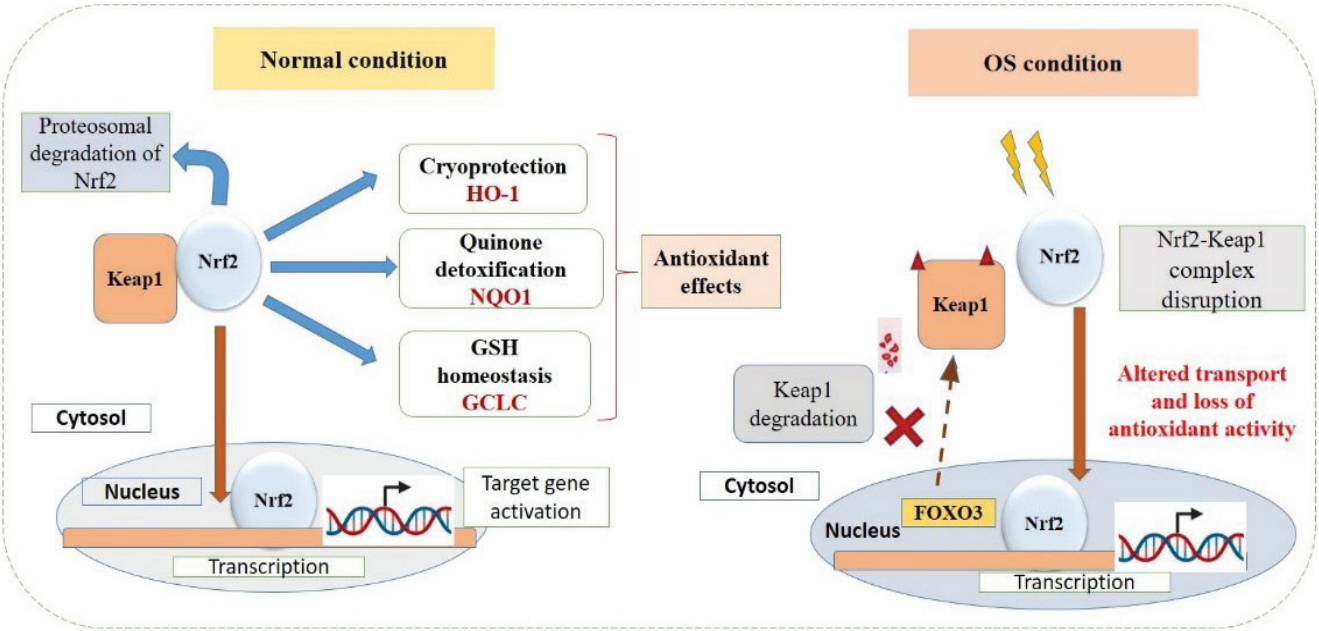
has been found between IL-6 and NOX4, the two mediators of inflammation and oxidative stress, respectively.[16]

Body, and its signalling system in respect to oxidative stress in endometriosis

The body's signalling pathway will manifest a series of changes following to exposure to the dual impact of OS and inflammation. Excessive ROS cause an imbalance between oxidation and anti-oxidation, leading to OS involving p38 MAPK pathway, the Kelch-like ECH-associated protein 1 (Keap1)-Nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, the Jun N-terminal kinase (JNK) pathway, the fork-head transcription factors of the O class (FOXO) family, and apoptosis.[17] Nrf2 is a key molecule activated in response to OS, which regulates antioxidant response to protect cell function. Primarily, Nrf2 binds to Keap1; then sequestered in cytoplasm, and subsequently degraded by a proteasome pathway. Post activation, it is transferred to the nucleus to activate a large number of antioxidant defense genes and restoration of vascular redox homeostasis as and when OS erupt/s. It is implied that Nrf2 deficiency cause fetal DNA damage and neurological deficits, to underlie inflammation-induced trophoblastic apoptosis. Studies have highlighted the role of Nrf2 in antioxidant defense in vascular dysfunction and oxidative damage in endometriosis.[18] Moreover, suitable OS increase Nrf2 and the expression of downstream targets, such as heme oxygenase 1 (HO-1), NAD(P)H: quinone-oxidoreductase (NQO1), and glutamate-cysteine ligase subunit catalysis (GCLC), etc., to resist OS.[19] Related studies have shown that FOXO3 participates in the interaction between Keap1 and Nrf2[20] and loss of FOXO3 cues to inactivation of Keap1; hence, failing to activate Nrf2 due to presence of OS. Henceforth, if OS induces inflammation the changes in FOXO3 affect the interaction between Keap1 and Nrf2, which could be a marker of damaging OS[17] (Figure 2).

Conclusion

A double-edged sword of ROS in health and pathology is being increasingly appreciated, although the underlying mechanisms are ill understood. While ROS production is up-regulated in endometriotic cells, the origin of this dysregulation is unclear. Impaired detoxification process lead to excess ROS and OS, and may be involved in increased cellular proliferation and inhibition of apoptosis in endometriotic cells. Interestingly, published data suggest that their detrimental effect does not always correlate with severity of endometriosis. Considering the aforementioned, and in the era of precision and personalized medicine, it appears that future studies



OS: Oxidative stress; Nrf2: nuclear factor erythroid 2–related factor 2; Keap1:Kelch-like ECH-associated protein 1; HO-1: heme oxygenase 1, NQO1 : NAD(P)H: quinone-oxidoreductase, GCLC: glutamate-cysteine ligase subunit; FOXO3: Forkhead box O3

Figure 2: Body, and its signalling system in respect to oxidative stress in endometriosis

should be focused on discovering sensitive-novel tools reporting on both oxidative stress status as well as on the inflammation system status.

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The Emergence of Artificial Intelligence and Deep Learning Algorithms for Automated Detection of Polycystic Ovary Syndrome

Sourav Roy Choudhury

Polycystic ovary syndrome (PCOS) is a common endocrine disorder that affects women of reproductive age. The diagnosis of PCOS is often made based on clinical symptoms and laboratory test results, but it can be challenging to accurately diagnose due to the complex and variable nature of the condition. The emergence of artificial intelligence (AI) and deep learning algorithms has led to the development of automated systems for the detection of PCOS. These systems use machine learning techniques to analyse medical images, such as ultrasound (USG) or magnetic resonance imaging scans, to identify the presence of ovarian cysts and other characteristics associated with PCOS. Researchers have made efforts to use AI techniques to detect and diagnose PCOS, distinguishing it from non-PCOS cases, using both USG and non-USG image datasets as well as non-image clinical datasets. These studies have employed feature extraction and classification methods for predictions, and some have demonstrated good accuracy in automated PCOS identification. Although the technology is still in the early stages, there is strong potential for it to be applied in regular clinical practice. AI has the ability to significantly reduce the risk of human error, improve the patient experience, and speed up decision-making processes. It is likely that in the future, clinical settings will utilize AI-enabled technologies to augment the capabilities of human professionals and provide more efficient and effective healthcare services, particularly in countries with large populations such as India.

Background:

Polycystic ovary syndrome (PCOS) is the most common endocrine-metabolic disorder among women of reproductive age. PCOS can have negative impacts on psychological well-being and quality of life due to the symptoms it can cause. It is a major disorder that still goes unrecognized and is often termed a "silent epidemic" and "hidden epidemic".[1] PCOS is a leading cause of infertility, but several other symptoms unrelated to fertility include obesity, abnormal hair growth, skin problems, abnormal menstrual periods, and longer-term health consequences such as diabetes and cardiovascular diseases. It is a major public health issue that affects 5–15% of women of reproductive age worldwide,[2] but the numbers seem to be underestimated. The prevalence of PCOS in India ranges from 3.7 to 22.5 % depending on the population and diagnostic criteria. The diagnosis of PCOS is often made based on clinical symptoms and laboratory test results, but it can be challenging to accurately diagnose due to the complex and variable nature of the condition. The problem with the underdiagnosis of the disease has led to the establishment of different diagnostic criteria since 1990 and the Rotterdam criteria of 2003 are the most well-accepted. However, diagnosing PCOS can still be difficult, particularly in adolescents and in the early stages of the condition.

Technology, including machine learning, is playing a significant role in shaping the future of healthcare. With the ability to analyze large datasets and provide clinical insights, machine learning is being used to assist in the diagnosis of various diseases and the clinical decision-making process. These advances are helping to transform the way we approach healthcare and medical treatment. Artificial intelligence (AI) is

a field of computer science that involves the creation of intelligent machines that can perform tasks that typically require human-like intelligence, such as learning, problem-solving, perception, and natural language processing. One application of AI in healthcare is the use of deep learning, a subfield of machine learning, to improve diagnostic performance through the analysis of electronic medical data and images. AI has the potential to significantly improve healthcare using big data and focused problem-solving techniques.[3] When it comes to patient care and smart health systems, AI can help clinical decision-makers. AI methods, including machine learning and deep learning, are being used for a range of purposes, including patient risk assessment, disease diagnosis, and drug discovery.[4] AI-based disease detection requires the use of various medical data sources, such as ultrasound (USG), magnetic resonance imaging, mammography, genomics, and computed tomography scans. Additionally, new sources of medical data are continually emerging.

AI has the potential to improve the speed and accuracy of clinical decision-making and reduce the workload of healthcare professionals. It has been used to identify a range of diseases and health conditions, including Alzheimer's, cancer, diabetes, heart disease, tuberculosis, stroke, and skin, liver, and hypertension conditions. Several techniques, including the support vector machine (SVM), Boltzmann machine, K closest neighbor (k-NN), decision tree, logistic regression, fuzzy logic, and artificial neural network, have been used to diagnose diseases and their accuracy has been tested.

Artificial Intelligence-Based Detection of PCOS:

There has been growing interest in using AI and automation for detecting PCOS within the AI research community, using various clinical data sources. Some studies have used a sequential process of classification and feature extraction or stereology to detect follicles in PCOS. A summary of some of these studies can be found in Table 1. Adiwijaya and colleagues used a simple stereology method to count and measure the size of follicles in a PCO ovary, using Euclidean distance to measure their diameters.[5] Whereas, in two other studies, Gabor Wavelet is used for feature extraction.[6,7] Additionally in these studies, Lavenberg-Marquardt Optimization and Conjugate Gradient- Fletcher Reeves were used as a variation of backpropagation to classify PCOS. In a study by Setiawati and colleagues, particle swarm optimization (PSO) was used to segment follicles by making modifications to the fitness function.[8] Whereas, Purnama et al. used a combination of three classifying approaches, including support vector machine (SVM) with RBF kernel, k nearest neighbors (K-NN) with Euclidean distance, and neural network with learning vector quantization (LVQ), to detect PCOS.[9] In another study by Setiawati and colleagues, a region-growing scheme was used to segment follicles by testing whether the neighbors of the initial seeds should be added to the segmentation region.[10] In studies that use USG images to detect PCOS, the segmentation process involves edge detection. One study used a median filter to remove noise from PCOS images by replacing the center window with the median value of the window in a specific picture element window.[11] In contrast, Rihana et al. used the Otsu global threshold of the image, which is a way to find the pixel similarity to its neighbors.[12] Otsu's thresholding method can be used to detect follicles in ultrasound images for PCOS detection by iterating through different threshold values and calculating a measure of the spread for the pixel levels on each side of the threshold. Another edge detection method called Canny edge detection can also be used for this purpose. Bharati et al. used multiple machine-learning techniques, including gradient boosting, random forest, logistic regression, and a hybrid approach combining random forest and logistic regression (RFLR), to screen for PCOS based on clinical parameters. They found that the RFLR method had the highest testing accuracy.[13] In a study, FSH and LH were identified as the most important features among the 10 highest-ranked attributes for classifying and detecting PCOS. Soni et al. used histogram equalized grayscale USG images of PCO ovaries, separating the background and object classes using the global basic threshold and Otsu threshold. The region-based and watershed methods were then used to binarize the foreground and background, and a cellular neural network (CNN) was applied to differentiate between PCOS and non-PCOS ovaries.[14] A study by Lv et al. proposed an automated deep-learning method for detecting PCOS by analyzing scleral abnormalities in the eyes.[15] The study used full-eye images of women with PCOS as the training dataset and applied an

Table 1: Studies using AI and deep learning-based detection of PCOS

Sl No	Aim	Machine learning processes	Studies
1.	Determination of PCOS	Euclidean distance to measure the diameter of the follicle	Adiwijaya et al., (2015)[5]
2.	USG images-based detection of PCOS	Modified backpropagation algorithm	Wisesty et al., (2017)[7]
3.	Digital image compression	Wavelet-SVD	Adiwijaya et al., (2013)[6]
4.	Detection of PCOS	Particle swarm optimization	Setiawati et al., (2015)[8]
5.	Follicle detection of PCOS using ultrasound images	• SVM – RBF kernel, • KNN – Euclidean distance • Neural network - learning vector quantization (LVQ)	Purnama et al., (2015)[9]
6.	PCOS classification using USG images	Particle Swarm Optimization and Supervised Learning	Setiawati et al., (2016)[10]
7.	PCOS image processing	Automated ovarian follicle recognition	Mehrotra et al., (2011)[11]
8.	Ovarian cysts detection	Automated algorithm	Rihana et al., (2013)[12]
9.	Data-driven diagnosis of PCOS (hormonal attributes)	• Gradient boosting • Random forest, Logistic regression • Hybrid random forest and logistic regression (RFLR)	Bharati et al., (2020)[13]
10.	PCOS identification using USG images	Automated segmentation before Convolutional Neural Network (CNN)	Soni et al., (2019)[14]
11.	Detection of PCOS using changes in scleral images	• Improved U-Net, and Resnet model • Multi-instance model	Lv et al (2022)[15]
12.	Differences in endometrial immune cells between cycle phases and between samples from PCOS women	Convolutional neural network	Kangasniemi et al.[16]

enhanced U-Net to segment scleral images from the full-eye images. A Resnet model was then used to extract deep features from the segmented scleral images, and a multi-instance model was used for image classification with high accuracy in identifying PCOS. Kangasniemi and colleagues used an AI and deep learning model to assess leukocyte counts and proliferation in immune-stained tissue sections of the endometrium in women with and without PCOS.[16] Supervised training of a CNN was used to create an endometrial AI model. The first layer of the model was trained with semantic segmentation to classify the endometrium into the epithelium and stroma, and the second layer was trained with an object detector to classify positive and negative nuclei within the epithelium and stroma.

Conclusion:

AI and machine learning techniques, particularly deep learning, have the potential to improve the detection and management of PCOS by analyzing large amounts of data quickly and accurately, reducing the time and resources required for manual analysis. In addition, these algorithms can identify patterns and trends that may not be immediately apparent to human analysts, increasing the accuracy and reliability of the diagnosis. AI algorithms can analyze electronic health records, imaging studies, and other data sources to predict the likelihood of a PCOS diagnosis and facilitate personalized treatment recommendations. Studies have shown that AI-based methods can accurately detect PCOS using various clinical data sources and AI tools, including machine learning algorithms, deep learning algorithms, and artificial neural networks. In the future, clinical settings will probably use AI technology to improve the efficiency of healthcare services and patient experience. While AI and automation may be able to aid in the detection of PCOS and augment the capabilities of human healthcare professionals, it is ultimately the responsibility of trained healthcare providers to diagnose and treat the condition. However, further research is needed to fully understand the potential of these technologies in the management of PCOS and to assess their accuracy and reliability in clinical practice.

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A New Approach towards Pain and Infertility Management in Women Suffering from Endometriosis and Adenomyosis

Dr Sunita Sharma & Dr Sourav Roy Choudhury

The 2nd Patient Awareness Programme was organized at Gaytri Mandir, Tegharia, Kishanganj, Bihar. 25 patients were trained in that program.

The 3rd Patient Awareness Programme was organized on 18th September at the distant rural Sundarban forest area at the village Gopalgauj, Kultali, West Bengal. More than 50 women were participated in this program.

Project 1

Dr. Pratip Chakraborty

Institute of Reproductive Medicine boasts a rich heritage of academic interest/s from decades. The overall theme of my lab is to understand the role of metabolic disturbances in the crossroads of pro- and anti-inflammatory context/s in physiology of pregnancy. We are inspired by the overt presence of hyperhomocysteinemia in our country which envisaged hypoxic elements in the placental template.

Recently, we got two grants from Department of Science and Technology, Govt. of India on "Exploration of molecular cross-talk involved in the roadmap to hyperhomocysteinemia-induced pregnancy loss" and from Department of Biotechnology, Govt. of India on "role of TSLP in polarization of macrophage to M2 phenotype for a potential beneficial outcome in recurrent implantation failure". Below are the excerpts from the project/s.

Exploration of molecular cross-talk involved in the roadmap to hyperhomocysteinemia-induced pregnancy loss

Impaired placental function due to overt oxidative stress is one of the key mechanism in development of pregnancy loss; hyperhomocysteinemia being aggravator of the situation. Spectrum of miRNAs documented till date in embryonic–endometrial communication differs between normal and pathologic conditions. Paucity of pathway–based microarray approach warrants elucidation of distinct profile of miRNAs in hyperhomocysteinemia-associated pregnancy loss (HAPL). Hyperhomocysteinemia was induced at a dose of 100mg/kg bodyweight/day for D1-D18 of pregnancy. Placental histology was done by haematoxylin-eosin staining (Fig 1); Histological evidence of clotting and haemorrhage was documented at the fetal–maternal interface in Hcy treated group (B) compared to saline treated control (A) on GD 18.5. The presence of a clot in the lumen of a maternal vessel located in the region of the mesometrial triangle was observed (Fig. 1B). Notably, placentas were dark in the experimental set on GD 18.5 but not in control (Fig. 1C & D). mRNA

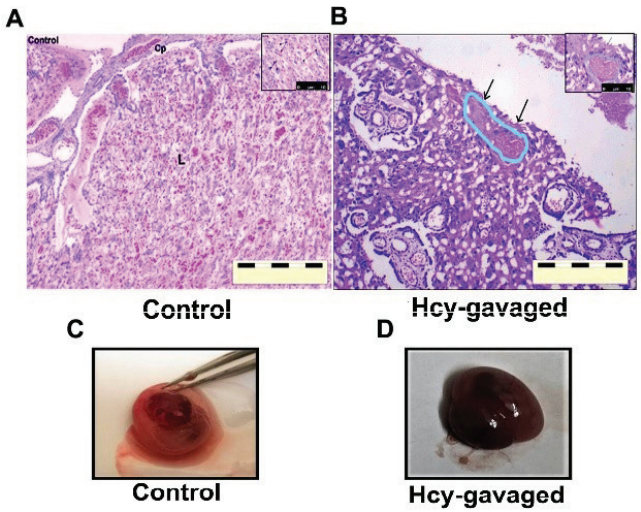


Figure 1

was subjected to microarray analysis followed by pathway-analysis using Ingenuity Pathway Analysis (IPA). The heat map shows correlation analysis of samples and 248 selected differentially expressed genes (Fig. 2).

Genes with absolute fold change differences greater than 3.0 and FDR ≤ 10% (in SAM analysis) when comparing HHcy-induced pregnancy loss samples and controls (Fig. 3) constitute list of differentially expressed genes Recursive feature elimination (RFE) was applied to evaluate the predictive ability. Expression/s was reconfirmed by quantitative real-time PCR (qRT-PCR), western blot and flow cytometric analysis (FACS). Genes involved in reproductive physiology, inflammatory pathways, immune responses, homocysteine metabolism, glucose metabolism, and oxidative stress were differentially expressed in hyperhomocysteinemia associated pregnancy loss (HAPL) (Fig. 4).

21 pathways documented by IPA, were skewed to 10 by RFE. Nine priori molecules (PKM2, AKT, PI3K, NF-κB, COX-2, sflt-1, HIF-1α, bax, caspase 9) were specifically modulated in HAPL as demonstrated by protein and mRNA (Fig.5) expression. highlighted molecules show parallel increase in insulin signaling (PI3K+,AKT+) inflammation (COX2+,NF-κB+),

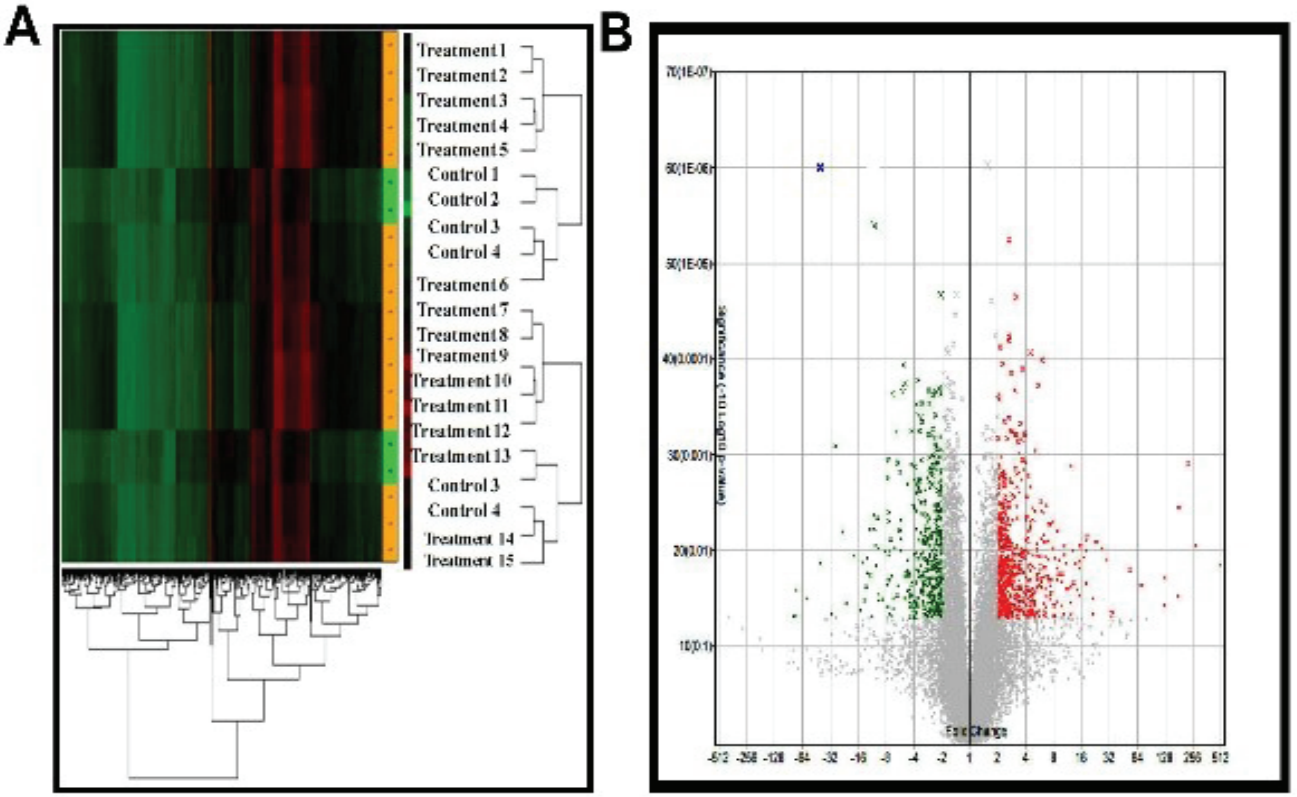
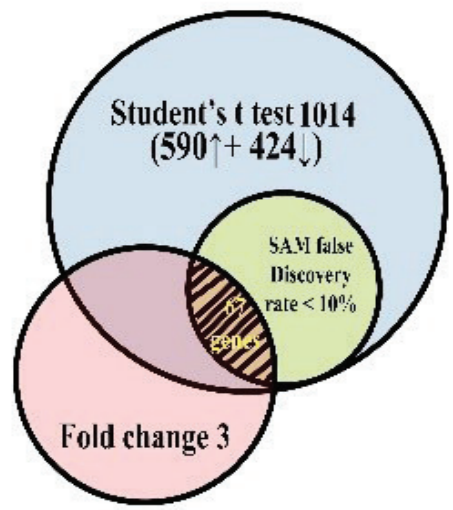


Figure 2



B

Gene Symbol	Gene Name	Location	Fold Change
PKM2	Pyruvate Kinase Isoenzyme M2	15q23	8.87
FLT-1	Fms Related Tyrosine Kinase-1	13q12	2.12
Akt	Protein Kinase B	14q32.32	3.01
PI3K	Phosphoinositide 3-kinase	3q26.32	3.15
HIF-1a	Hypoxia Inducible Factor 1a	14q61.75	3.05
COX-2	Cyclooxygenase 2	1q186.67	2.74
NF-kb	Nuclear factor kappa/beta	4q24	2.91
bax	Bcl2 associated X protein	19q48.95	3.01
Casp 9	Caspase 9	1q15.49	6.28

Figure 3

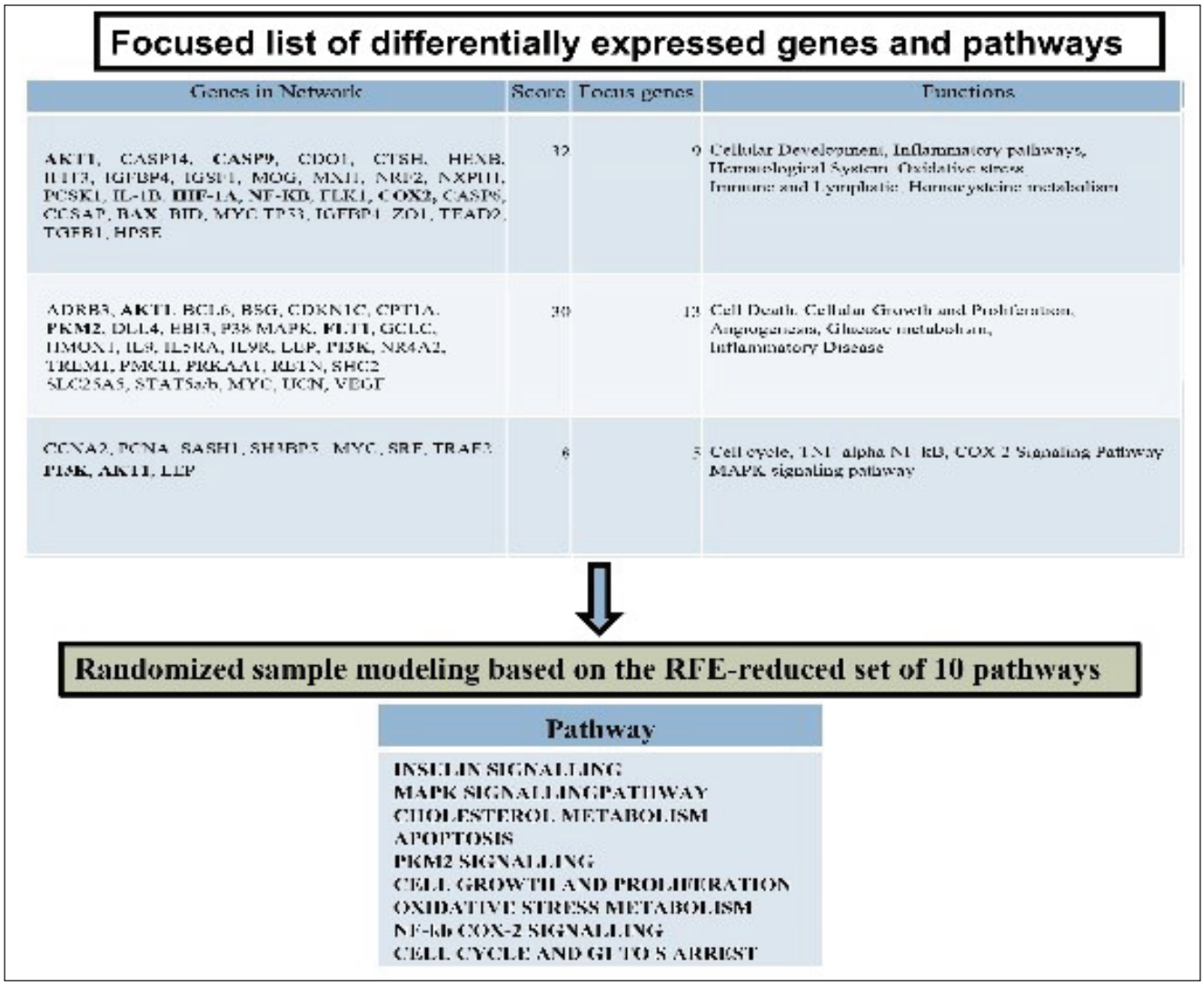


Figure 4

hypoxia(sflt-1+,HIF-1α+),apoptosis(bax+,caspase9+) with concomitant decrease in pyruvate kinase M2 in hyperhomocysteinemic placental cells by FACS with CD56, a marker for pregnancy loss (Fig.6). One major limitation of the present study is absence of any other experimentally-induced threatened abortion group without involving HHcy. The results, therefore, provide little evidence that attenuated PKM2 pathways were a direct attribute of HHcy, rather than a consequence of placental abruption/ pregnancy loss. In addition, molecule/s other than the priori set can be responsible for HHcy-mediated miscarriage which should be taken care of in future studies.

Nevertheless, these findings provide evidence that an oxidative stress-mediated placental damage perhaps represents the pathogenesis of HHcy-associated pregnancy loss, which may pave the path towards development of target-based therapeutic options categorizing to cell growth and proliferation, inflammation, placental structure (angiogenesis), and hypoxia-induced metabolic reprogramming for unexplained miscarriage.

Role of TSLP in polarization of macrophage to M2 phenotype for a potential beneficial outcome in recurrent implantation failure

The behaviour of endometrium in Recurrent Implantation Failure is less well understood. The molecular complexities associated with this disorder needs to be unravelled. Macrophages constitute the second largest group of leukocytes at the feto-maternal interface. Macrophages have been associated with normal and pathological pregnancy. However, the role of macrophages remains unclear in RIF. Hence, the project aims to delineate the role

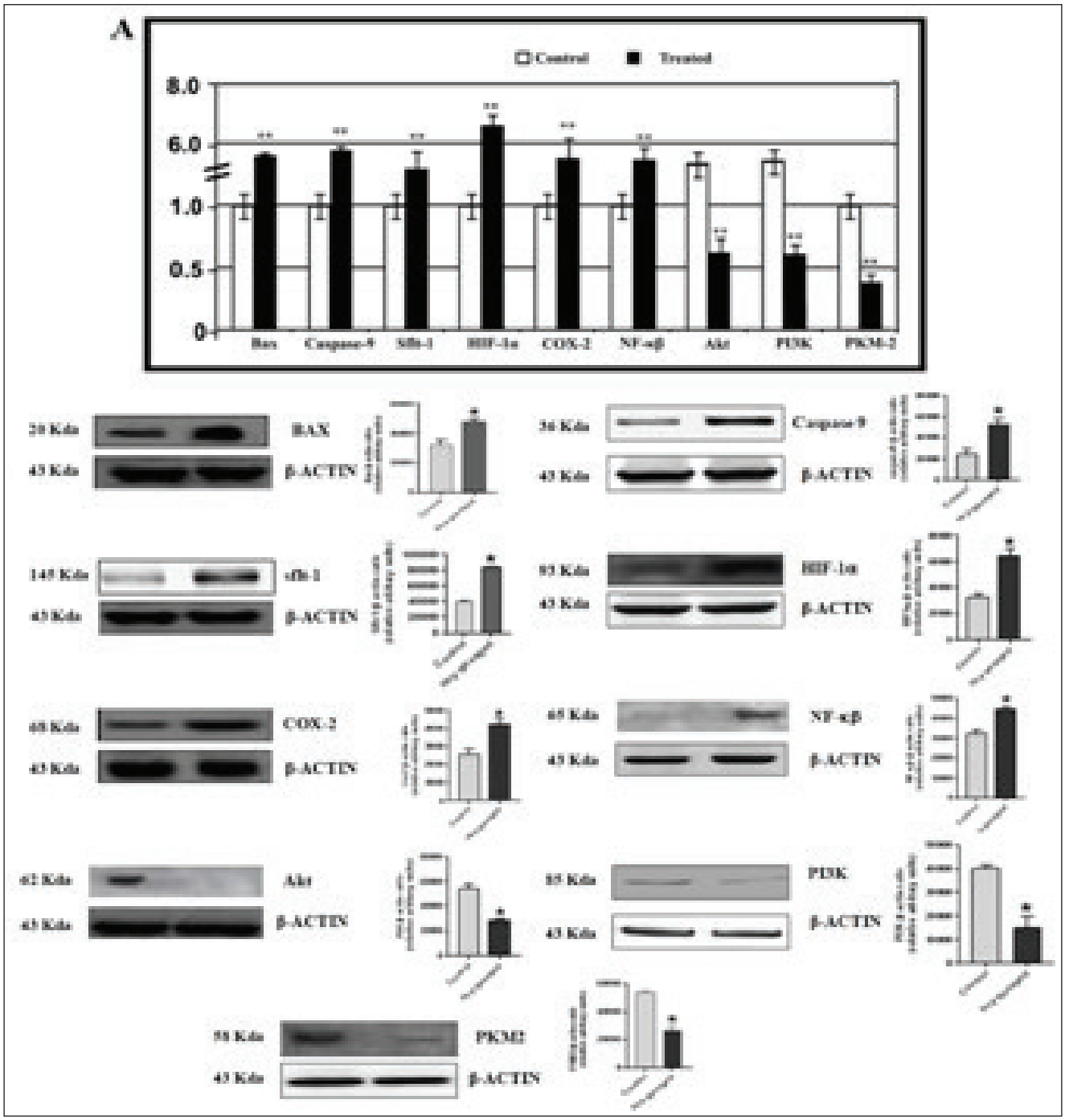


Figure 5

of macrophages in RIF. The cytokine TSLP is known to regulate the differentiation of macrophages to the anti-inflammatory M2-type. In normal pregnancy TSLP promotes the invasion of trophoblasts. An increased level of TSLP can lead to the development of endometriosis. Therefore, in this proposal we aim to determine how TSLP-primed macrophages can regulate RIF.

E2 plays a physiological role in embryonic development and implantation, as demonstrated by a number of in vitro studies. Recently, Chang et al. 2021 have suggested that high serum E2 concentrations in clinical ART not only affect the endometrium, but also affect blastocysts directly during the period of implantation. Letrozole is generally used to treat the normal responders during IVF stimulation in order to improve response to FSH by increasing the intrafollicular androgen concentration, and to reduce circulating estrogen concentrations. In this context, we hypothesized management of RIF with a combination of letrozole

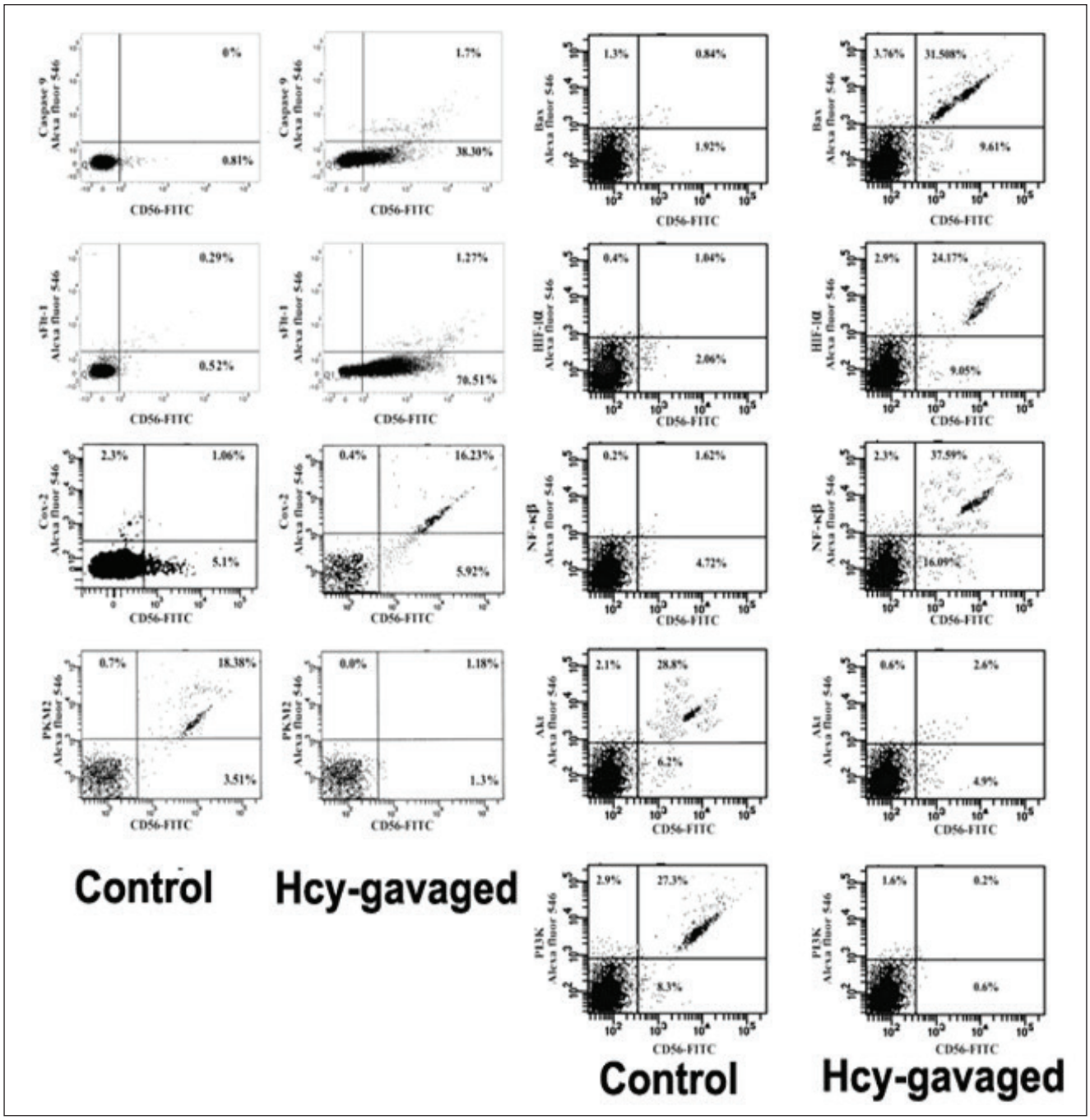


Figure 6

with TSLP, as the former could positively influence the implantation process. We hypothesize that this combinatorial effect will help improve the receptive function of the endometrium leading to enhanced pregnancy salvage in women with RIF.

Our key questions from this proposal are i) Does macrophage polarization play an important immunomodulatory role in RIF? ii) If so, how the importance of macrophages can be teased out? iii) Can TSLP-primed macrophages provide a better functional outcome in RIF? iv) In that case, what will be the combinatorial effect of letrozole and TSLP in improving the receptive status of the endometrium?

"Implantation efficiency" is a sum of embryo quality (EQ), endometrial receptivity (ER) and transfer efficiency (TE); (EQ + ER + TE). Therefore, improving the WOI and in turn ER could pave the way for the clinicians to manage the disease better. Apart from deciphering the molecular and cellular mechanisms, the proposed work has the potential to tease the therapeutic potential of TSLP intervention in RIF management.

Work Statement from August to October 2022

1. GYANECOLOGICAL AND OBSTETRICS CASE

Total no. of Patient's attended: ***532***

- Infertility 441
  - — Primary 326
  - — Secondary 115
- History of Recurrent Spont Miscarriage (≥ 3) 18
- History of Unexplained Spont Miscarriage (≥ 1) 11
- Primary Amenorrhoea 04
- Secondary Amenorrhoea (Without infertility) 10
- Other Gynaecological case 21

Infertility

Female Factor: 188 (42.54 %)
Male Factor: 160 (36.19 %)
Unexplained: 64 (14.60 %)
Combined Factor: 29 (6.67%)

Female Factor **188 (42.54%)**

- PCO 46 (24.62 %)
- Endometriosis 32 (17.16 %)
  - Early: 14
  - Advanced: 18
  - Advanced with Adenomyosis 12
- Tubal Block 70 (37.23 %)
- POF 28 (14.89 %)
- Uterine Anomalies 08 (4.48%)
- Fibroid 10 (5.32 %)

Male Factor **160 (36.19 %)**

- Azoospermia 65 (40.35%)
- Asthenospermia 30 (18.42%)
- Oligospermia 24 (14.91%)
- Oligoasthenospermia 23 (14.03%)
- Oligoteratozooasthenospermia 09 (6.14%)
- Aspermia 02 (1.25%)
- Pyospermia 04 (2.63%)
- Sexual Dysfunction 05 (3.12%)
- Retrograde Ejaculation 03 (1.75%)

Age Factor of Female Partner: (n=441)

- Less than 25 Yrs: 55 (12.38%)
- 25-35 Yrs: 301 (68.25%)
- 36-40 Yrs: 64 (14.60%)
- More than 40 Yrs: 21 (4.76%)

Duration of Infertility: (n=441)

- Less than 5Yrs: 186 (42.22%)
- 5-10 Yrs: 190 (43.17%)
- More than 10 Yrs: 65 (14.60%)

Residential Status: (n=441)

- Urban: 363 (82.22%)
- Rural: 78 (17.77%)

2 OBSTETRIC CASES (Pregnancy following investigation & treatment excluding IVF)

A. Confirmation of Pregnancy 105

- Following treatment of Infertility: 91
- Following previous foetal wastage (Spont): 14

Treatment modalities for achieving these pregnancies

- Following medical treatment (Induction Ovulation): 15
- Following Surgical Treatment: 03
- Following Hydrotubation: 06
- Following HSG & Laparoscopy: 07
- During investigation: 18
- Intrauterine Insemination: 27

B. Complication in Ongoing Pregnancy

- Threatened Abortion: 05
- Hypertension: 14
- Diabetes Mellitus: 12
- Sub Clinical Hypothyroidism: 09
- RH Negative: 02
- Pulmonary Hypertension 01
- Psoriasis 01

3 IVF UNIT

OCR: 73

Embryo Transfer 122

- Fresh 11
- FET 111

Pregnancy 54

- Fresh 02 (18.18%)
- FET 52 (46.84%)

Cancellation due to Failed Stimulation: (Poor Responder) 05

Discontinued other than poor responder 02

Fresh ET cancelled due to 62

- Due to OHSS 8
- Endometrium abnormality: 17
  - Thin endometrium (<7 mm) 10
  - Thick endometrium (>14 mm) 5
  - Endometrium Polyp 2
- Endometriosis/ Adenomyosis 37

Protocol

- Long 43
- Antagonist 30

IVF Cycles

- Conventional IVF 63
- PESA/ICSI Cycles: 02
- Ejaculated ICSI Cycles: 08

4 USG UNIT

- Folliculometry: 460
- Antenatal Monitoring: 528
- Pelvic Scan: 188

5 IUI UNIT

Total No. of Patients Treated: 311

	N	Preg
• AIH:	262	32 (12.21%)
• AID:	49	10 (20.40%)

6 SURGICAL UNIT

- Laparoscope: ovarian drilling 2
- Hysteroscope: 20
- Laparo + Hystero: 25
- Laparoscopic ectopic pregnancy 2
- Bilateral tubal recanalization 2
- Laparoscopic tubal clipping 3
- D+E+C: 11
- Mc Donald/Shirodkar: 04
- LUCS 41

Achievements:

Publication in IFS Year Book 2022 –

i) Mullerian anomalies;- a new clinical classification– review and report of few rare anomalies - by Dr. B N Chakravarty.
ii) Ovum Pick Up by Dr. Ratna Chattopadhyay

Papers accepted in ESHRE 2022:

1. Chakraborty P, Mitra I, Dattachaudhuri R, Sharma S, Kalapahar S, Ghosh S, Chattopadhyay R, Sarkar S, Chaudhury K, Chakravarty B Mapping the uterine pathologies in endometrium: differential genetic interactions set the algorithm for improved diagnosis in women with adenomyosis, Hum.Reprod. Volume 37, (2022) deac107.293.

2. Sharma S, RoyChoudhury S, Bhattacharya M.P, Mitra I, Hazra S, Chakraborty P, Chaudhury K, Majhi A.K, Chakravarty B Effectiveness of letrozole in symptomatic women with adenomyosis undergoing IVF: An overview of clinical features, sonographic characteristics, and reproductive outcome, Hum. Reprod.Volume 37, (2022) deac107.308.

3. Chattopadhyay R, Mitra I, Ghosh S, Kalapahar S, Sharma S, Chakraborty P, Chaudhury K, Chakravarty B, Gene expression profiling of cumulus cells from women with adenomyosis disclose differential pattern to endometriosis: striking the chord for evaluation of a biomarker, Hum.Reprod. Volume 37, (2022) deac107.671.

4. Kalapahar S, Chakraborty P, Shama S, Mitra I, Chattopadhyay R, Ghosh S, Chakraborty M, Chakravarty B, Serum homocysteine and uterine artery Doppler ultrasound in combination has better

predictive accuracy in women with preeclampsia: useful tool for early screening in everyday practice, Hum.Reprod.Volume 37, (2022) deac107.367.

5. Chakravarty B, Kalapahar S, Sharma S, Chattopadhyay R, Ghosh S, Chakraborty M, Chakraborty R, Bose U, Chakraborty P Activation of NLRP3 inflammasome enhances neutrophil extracellular traps in women with stage III/IV endometriosis: convergence of multiple signaling portray association between genetics and lesion type, Hum.Reprod.Volume 37, (2022) deac104.031

Publications in peered reviewed Journal

1. Kalapahar S, Gupta N, Sharma S, Chattopadhyay, R, Konar H, Chakravarty BN, Chakraborty P. Consequence of Diabetic Environment and Significance of Glycemic Control in Early Pregnancy: A Case Report (2022), 4 (11). Impact Factor: 1.014

2. Chakraborty, I., Chattopadhyay, R., Chakraborty, P., & Chakravarty, B. (2022). Hyperhomocysteinemia, the player behind the curtain: a new insight into polycystic ovary syndrome. Indian journal of physiology and allied sciences, 74(3). Impact Factor: NA

3. Chakraborty P., Mitra I, Kalapahar S, Sharma S, Chattopadhyay R, Chakravarty BN. Significance of Hyperhomocysteinemia in Immediate As Well As Long-Term Health Risk/s in Women with Polycystic Ovary Syndrome: a Probabilistic Model Using Dynamic Bayesian Network Analysis (2022) Reprod. Sci. doi: 10.1007/s43032-022-01028-4. Impact Factor: 4.315

On-going Projects

1. A new approach towards pain and infertility management in women suffering from endometriosis and adenomyosis (PI Dr. Sunita Sharma, Co-PI: Dr. Sourav Roy Choudhury)

2. TSLP modulates polarization of macrophage to M2 phenotype: A potential beneficial outcome during recurrent implantation failure (PC Dr. Ritobrata Goswami, Asst. Prof. Indian Institute of Technology, Kharagpur, PI Dr Pratip Chakraborty, Co-PI: Dr. Koel Chowdhury Prof., Indian Institute of Technology-Kharagpur. Dr. Sunita Sharma, Consultant, Institute of Reproductive Medicine, Kolkata).