ACE Newsletter

The Newsleter of the Academy of Clinical Embryologists of India

Volume 04 Issue 02 April 2016

ACE KOCHI 2015, the 4th International Conference of Academy of Clinical Embryologists (ACE)

A Big Thank You to All

A Big Thank You to ALL, for the overwhelming response and support for the conference and we are truly humbled.

It was indeed a big moment, a huge achievement for all of "US" who are a part of **Team Ace Kochi**.

ACE KOCHI 2015, the **4th International Conference of Academy of Clinical Embryologists (ACE)** was held at Le Meridian & Convention centre, Kochi, Kerala from 18th-20th Sept 2015. The conference started on with four pre-congress workshops on 18th Sept and the main conference on 19th & 20th Sept 2015. The Pre-congress workshops were

1. Embryo Culture Systems - Know your Culture Media and Incubators
2. Andrology – Advances in Sperm Pathology, testing and selection.
3. OPU & ET – The Science Unfolds
4. Heterologous ART: A Grip to Get Something

Embryo Culture System workshop was one of the most popular workshops of ACE 2015. It was a unique workshop with several experts from this field both national and international discussing on the different aspects of Embryo Culture. Topics discussed varied from the historical perspective of culture media, to the importance of temperature and PH of the culture media, types of incubators and quality control in ART lab. The afternoon session had an exclusive round table discussion between delegates and representative from different culture media companies. The workshop concluded with a very interesting panel discussion on trouble shooting in ART.

Andrology Workshop had discussions regarding the advances in Sperm pathology, testing and selection. The workshop had a very informative live demonstration of WHO Standardized Semen Analysis by none other than Dr Lars Bjorndahl. Other topics like Semen processing and semen retrieval techniques were also discussed. There was a live demo and discussion on the new technique of Piezo ICSI by Iwamoto & Team.

OPU & ET Workshop was again a very unique workshop targeted at upcoming infertility specialists. Topics discussed were Anesthesia to trouble shooting and dealing with complication during OPU. Several important aspects about planning an Embryo transfer and embryo loading techniques were discussed and this whole workshop was continuously moderated by a panel of experts which comprised of the senior most experts in this field.

And finally we had a first of its kind workshop on a boathouse discussing one of the hottest and most controversial topic in ART today – Heterologous ART. This workshop was moderated by Dr RS Sharma (ICMR) and discussed on the different guidelines and medico legal aspects involved in third part reproduction. This workshop was attended by several experts and faculty from ART banks, Surrogacy consultants, advocates, press and representatives of INSTAR. The same workshop was very positively covered and supported in the following day news papers.

The main congress was conducted on 19th and 20th Sept in over 3 halls. The main hall was dedicated to Embryology, the second hall to clinical topics and the third hall had few panel discussions and free paper presentations. The main congress had very interesting panel discussion and lectures on ART. The first day had the MSD Sponsored Robert Edward Oration by Professor John Aitken on the topic of "Revelations on the Road to Conception – Why did the Human Race need a Bob Edwards" which was followed by Keynote Lectures by Dr Peter Plateau about "Safety of ART" and by Dr John Aitken again on . The second day had the Subhash Mukherjee oration by Prof Dr Satish Gupta on "Trophoblast Biology – Regulation of invasion and differentiation". This was followed by Key note lectures by Dr Sandros Esteves on "Benefits of TQM in ART" and by Prof Dr Pratap Tharayan on "How to read and interpret a Scientific Publication".

The conference was inaugurated by Prof Dr John Aitken (Pro Vice Chancellor, The University of Newcastle and also the President, International Society of Andrology) on the evening of 19th Sept 2015. The prestigious ACE LIFE TIME Achievement Award was given to Dr Joshua Peter for his contribution to the field of Embryology and Infertility. The inauguration was followed by an exclusive hour long cultural show called "KERALEEYAM", depicting the different art forms of Kerala. This was further followed by Banquet dinner in which the delegates had a splendid time.

The ACE "Embryologist" logo car stickers and badges were launched and released by President Dr Charudutt Joshi.

The conference had been an astounding success with over 600 delegate registration and over 150 company representatives. We had one of the best exhibitions seen in the recent times with participation from over 40 companies. There were over 40 free paper presentation and 100 lectures which were presented during the conference. The conference concluded on 20th Sept 2015 after the Valedictory function where prizes were distributed for the best free papers and posters.

Thank you once again!

Dr Parasuram Gopinath
Org Secretary - ACE KOCHI 2015

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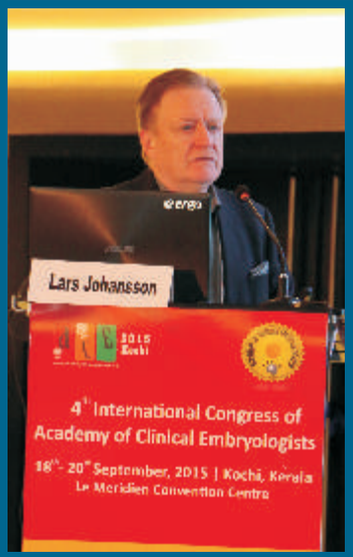


Oocyte assessment forecasts outcome of ICSI

Charulata

Introduction

Ovarian Follicular growth is described as a continuum and encompassing with both intra and extra ovarian signals such as gonadotropins and metabolic hormones. In stimulated cycles of ART, pharmacologic doses of gonadotropins create a supraphysiologic hormonal environment that induces the growth of a cohort of follicles [1], allowing maturation of oocytes [2]. Nuclear and cytoplasmic maturation is essential for fertilization, thus proper evaluation of oocytes are must.

Oocyte morphology assessment is not very crucial in case of conventional IVF as progression of the oocyte through meiosis can continue in vitro and fertilization is likely to occur when the oocytes are mature.

However, In ICSI precise assessment is easy after removal of cumulus by denuding oocytes in Hyluronidase enzyme. After denuding nuclear and cytoplasmic status ambiguously determined. Nuclear maturation in oocytes can be divided into Immature. Metaphase I and Metaphase II oocytes.

During oocyte development, cytoplasmic changes occur that includes mRNA transcription, protein translation, post translational modification of proteins and ultra structural changes [3,4] Successful completion of these events is independent of nuclear maturation and is collectively referred as cytoplasmic maturation. [5]

As ICSI has allowed precise assessment of oocyte morphology, conflicting results regularly arise from different studies, concerning the incidence of oocyte dysmorphism on fertilization, embryo development stage and affecting implantation rate .

In stimulated cycles, 10 – 15 % retrived oocytes showed different cytoplasmic abnormalities.

Intra cytoplasmic abnormalities includes :

- Vacuoles and / or cytoplasmic inclusion,
- Endoplasmic reticulum clustering,
- Cytoplasmic granularity – affecting whole gamete or centrally located

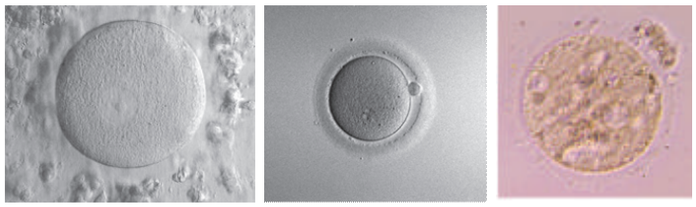
Extra cytoplasmic abnormalities includes;

- Perivitelline space [PVS] – debris in PVS, large PVS
- Polar body – fragmented, abnormal size and shape
- Zona pellucida [ZP] - distorted, multi layered, pigmented, hairy appearance, thin or thick ZP

Abnormal shape oocytes

- Irregular, giant and small oocytes

Intra cytoplasmic abnormalities includes:
Vacuoles and / or cytoplasmic inclusion :



Normal oocyte has smooth shiny cytoplasm. Vacuoles are round shaped clear looking structures observed more if oocytes are overly mature or aged in culture.

Vacoules may be observed with SER clusters [6] or can also be observed with inclusions like refractive bodies, dark incorporations, fragments, spots and dense granules. [7,8]

Results of two different studies showed that fertilization and embryo quality are not altered [9] bur cryo survival was affected. [10] Another study observed that results of ICSI on oocytes with vacuoles increased biochemical pregnancy rate [11] and aneuploidy rate. [12]

Endoplasmic reticulum clustering:

The endoplasmic reticulum is a network of membrane formed throughout the cell and is connected to nucleus. Endoplasmic reticulum functions as a manufacturing and packaging system to make cellular products like hormones and lipids.

There are two types of Endoplasmic reticulum

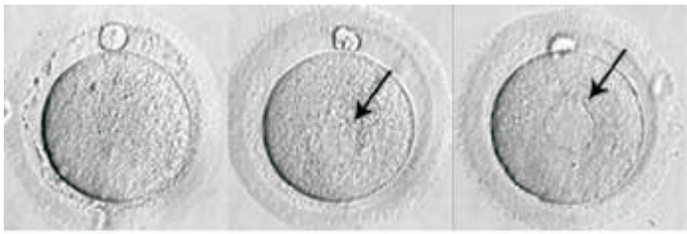
Rough Endoplasmic reticulum – it is involved in some protein production, protein folding, QC, and dispatch. As it is studded with ribosomes it is known as rough Endoplasmic reticulum.

Smooth Endoplasmic reticulum [SER] – it is associated with production and metabolism of fats and steroid hormones. It is also associated with smooth slippery fats.

There are three forms of SER which can be classified by size using light and electron microscopy. The large 18 mm , medium 10±17 mm and small 2±9 mm SERs. Medium size SER when cultured grew to large SER in 18 hours suggesting that the three forms of SER were divided from the same origin.

In oocytes , the localization of mobilizable ca2+ was detected in the small vesicles beneath the plasma membrane of SER. Because ca2+ release from SER plays pivotal roles in oocyte maturation, fertilization and early embryonic development [13]. Ca2+ oscillations in GV oocytes are facilitated by 17 b estradiol. High estradiol concentration on the day of HCG administration and total days of stimulation may be the reason for oocytes having SERs.

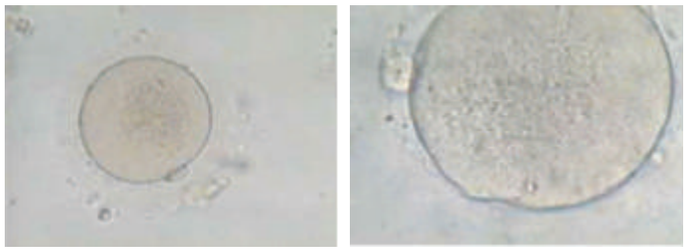
In one study low pregnancy outcome reported in ICSI done on oocytes with SER [14] It has been suggested that presence of SER may interfere with normal calcium stores and calcium oscillations during fertilization and therefore may have a detrimental effect on embryo development and implantation. [15]



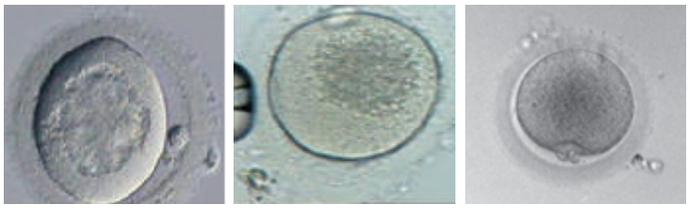
Granulated cytoplasm :

Extensive cytoplasmic granularity may be homogeneous, either affecting the whole gamete, or centrally located. Centrally located granularity also referred centrally granulated cytoplasm [CGC] or dark cytoplasm.

Slight to moderate CGC



Severe CGC



Two different studies contradict the result on quality of embryo development and Implantation rate. [16,17,18]

Another comparivie study says that CGC group oocytes had better fertilization rate than incompletely absent granulation in cytoplasm. [12]

On penetration of injecting pipette into oolemma funnel or channel is formed, which is dissolved immediately after withdrawing the pipette. In CGC group oocytes due to membrane elasticity or cytoplasm viscosity restoration of spherical shape is delayed.[˜ 1- 2 minutes]. This may affect the outcome in IVF cycles. Ebner et al. (2003)

Oocytes with CGC shows late dissolution of channel [1-2 minutes] after ICSI are more prone to degeneration and compromised embryo quality.

Reason for more degeneration in CGC group in comparison to immediate dissolution of channel -injection channel is likely to be responsible for sealing the breach during injection[16,17] The absence of such a protective mechanism in oocytes with sudden breakage (without any injection channel at all) causes an increase in oocyte degeneration. [18]

Pronuclear and embryo stage pattern in CGC group

It is speculated that sub optimal cytoplasmic texture compromises microtubule organized concentration of mitochondria to perinuclear regions and thus impairs cell cycle regulation severely .Optimal pro nuclear pattern results in good quality embryos . [19] Reason for fragmentation in CGC group is change of frequency and periodicity of cytoplasmic waves, which are known to influence to embryo quality.

Extra cytoplasmic abnormalities

Zona Pellucida [Z P]:

ZP is an outer layer of an oocyte.

Dark ZP – Study reveals that darkness of zona did not affect fertilization rate, embryo quality or implantation rate. [20,9] It also did not influence blastocyst rate and cryo survival rate. [21]

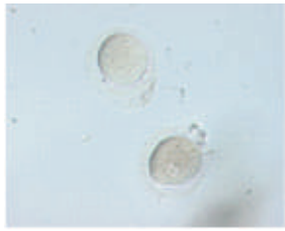
Thick ZP – No correlation was found with thick ZP and fertilization rate [22] ,Infact it is positively correlated with good blastocyst rate [23] and pregnancy rate. [24]

High birefringence of ZP – Conflicting results of two studies found where in one study correlated with increased fertilization rate [25] and other study found no relation with fertilization rate [26]

Perivitelline space [PVS]

PVS is the space between the zona pellucida and the cell membrane of an oocyte or fertilized ovum.

Debris in PVS ; No correlation is found between the presence of debris in PVS and in vitro development .



Granularity in PVS : Presence of coarse granules affects the implantation rate [27]

Size of PVS – large PVS may affect fertilization and pronuclear stage but not the embryo quality [28]

Polar body [PB] :

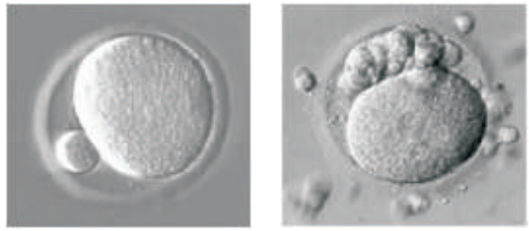
A polar body is a small haploid cell that is formed concomitantly as an egg cell during oogenesis, but which generally does not have the ability to be fertilized.

Shape of PB :

Shape of PB did not influence embryo quality. [29]

Size of PB –

No affect on fertilization rate and pregnancy rate[30], where as another group [31] found lower pregnancy rate with larger PB.



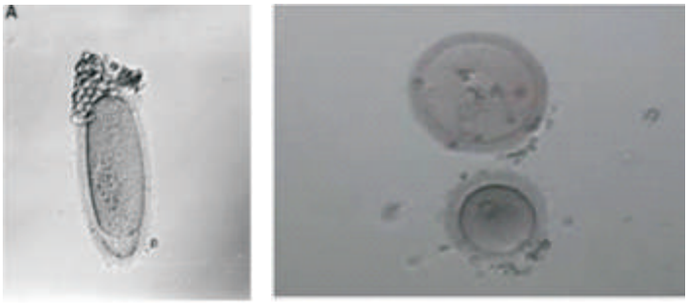
Fragmented PB :

Study reveals no relation between fragmentation of PB and pregnancy rate. [32], yet another group [17,15] reported in decreased fertilization rate in ICSI done on oocytes with fragmented PB.

Abnormal shape oocytes ;

Irregular shape ;

Ovoid shape oocytes resulted in reduced embryo quality [16]



Giant oocyte – embryos developed from giant oocytes have increased chance for digynic triploidy [33]

Conclusion :

Stripping of cumulus to facilitate ICSI procedure ,helps embryologist to look into oocyte cytoplasmic, nuclear and structural morphology. Numerous studies reported conflicting ICSI results with intra or extra cytoplasmic abnormal oocytes.

The cause of this cytoplasmic abnormalities is multifactorial which includes age of lady , follicular environment, ovarian function, ovarian stimulation drugs etc.

Only morphological assessment may not be enough to predict embryo quality and implantation failure. More precise methos like cellular and molecular findings of the oocyte helpsto understand pathophysiology and may help to design lab protocol to improve implantation and pregnancy rate.

Acknowledgement:

I would like to thank Dr Papolu Rama Devi for her constant support and guidance.

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ENDOMETRIAL STEM CELLS: A DOUBLE EDGED SWORD

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Introduction

Stem cells are quite unlike the specialized, or differentiated, cells in our body — such as the nerve cells, muscle cells and blood cells that enable us to function. In contrast, they are the body's silent reserves. At any given moment, many of the stem cells in our body won't be doing very much. They will only spring into action when we need either to produce more stem cells or make more of other, specialized types of cells. A stem cell is an undifferentiated cell that is defined by its ability to both self-renew and to produce mature progeny cells.

Properties of stem cells

- Stem cells differ from other kinds of cells in the body.
- Stem cells are unspecialized.
- Stem cells are capable of dividing and renewing themselves for long periods.
- Stem cells can give rise to specialized cells. **(**Ramirez Del Rio *et al, 2002***)**

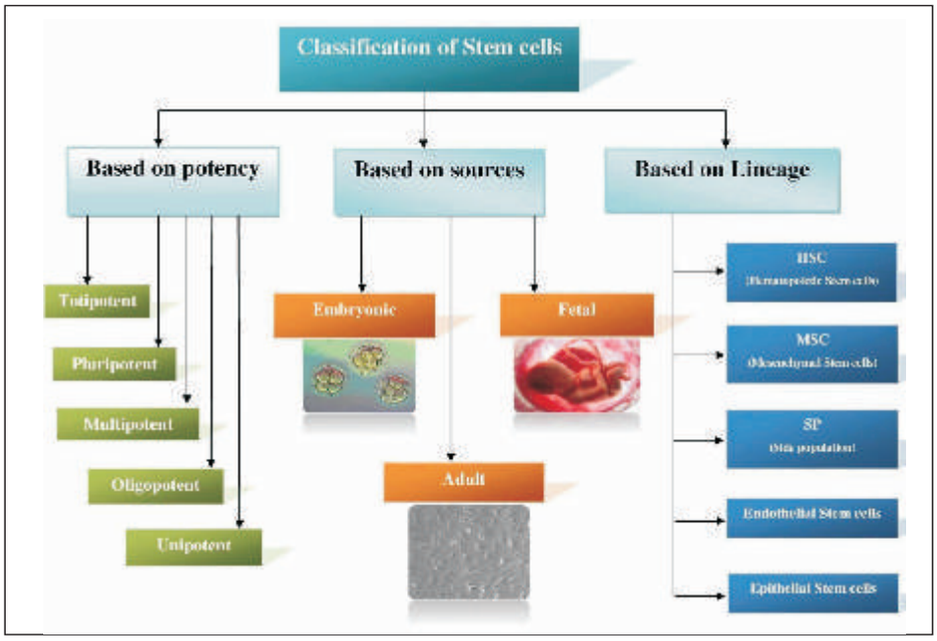


Fig 1: Classification of Stem cells

Embryonic stem cells **(**ES cells**)** are stem cells derived from the inner cell mass of an early stage embryo known as a blastocyst. They are said to be pluripotent. Because of their plasticity and potentially unlimited capacity for self-renewal, ES cell therapies have been proposed for regenerative medicine and tissue replacement after injury or disease. However, to date, no approved medical treatments have been derived from embryonic stem cell research, due to its disadvantageous such as ethical issues, immune rejections and teratoma formation. Hence, adult stem cells and fetal stem cells are gaining attention since many decades and have so far being successful in many clinical interventions**.** Although stem cells lies in every adult tissues, the endometrial stem cells stands far apart from other post natal stem cells due to its dynamic proliferative, remodeling and regenerative ability. However, endometrial started gaining importance only in last decade. Here, in this article, we summarize the key role of endometrial stem cells, its utility as a double edged sword, in cause and cure of diseases. It is concluded with the ways of overcoming the threats and challenges facing endometrial disorders.

Endometrium and its derived stem cells The human endometrium is an extraordinary model of controlled tissue remodeling, unparalleled in other organs, At this rate, there is a very rapid rate of angiogenesis for approximately 400 cycles within a tightly controlled manner in a woman's lifetime. Endometrium is divided into two zones, the inner functionalis which is adjacent to the uterine cavity and a deeper basalis layer which overlies the myometrium**.**

The functionalis layer is shed each month with menstruation, and is then regenerated from the basalis layer which is not shed. The functionalis, comprising the upper two-third of the endometrium is divided into stratum compactum and stratum spongiosum. The stratum compactum is a superficial thin layer nearest to the uterine cavity and contains the lining cells, necks of the uterine gland and relatively dense stroma. The stratum spongiosum is the deeper part of functionalis composed of main portions of the uterine glands and accompanying blood vessels; the stromal cells are more loosely arranged and larger than in the stratum compactum.

The lower basalis contains the basal region of the uterine glands, dense stroma **(**that remains relatively unaltered during the menstrual cycle**)**, large blood vessel remains and lymphoid aggregates. It serves as the germinal compartment for generating new functionalis each month.

It has been postulated that the niche of these adult stem or progenitor cells of the endometrium is the lower basalis. These stem or progenitor cells were also identified to be in the trophic

endometrium of post menopausal women**.** The main cell populations within the functional stratus are epithelial and stromal cells accompanied by a variable number of leukocytes. Epithelial cells are found covering the luminal surface and tubular glands in basal and functional layers.

Endometrial stroma contains reticular connective tissue comprised mainly by uterine fibroblasts that rapidly differentiate into decidualized cells when stimulated by an implanting blastocyst.

The stromal compartment contains also abundant lymphocytes, granulocytes and macrophages during luteal phase of the menstrual cycle. These cells along with epithelial and stromal fibroblasts are source and target of paracrine signals of proliferation and differentiation. During a normal menstrual cycles, human endometrium display unique features for an adult tissue: undergoes cyclic construction and sloughing. The outer layer of the endometrium is lost while the basal layer containing the deep glandular epithelium gets preserved. Later on, stem cells located in this layer will originate the various endometrial cell types in response to the appropriate hormonal stimulus, regenerating the whole endometrium.

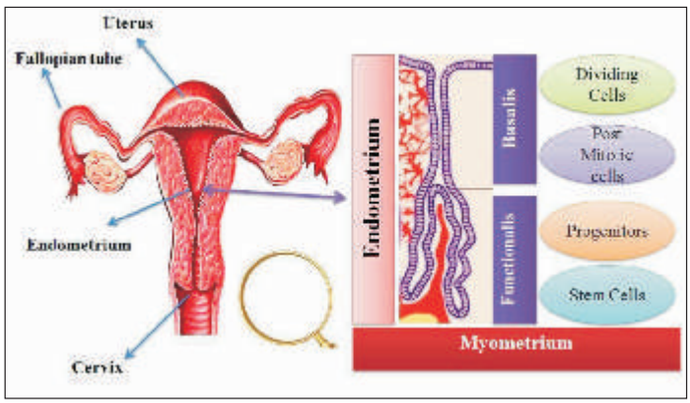
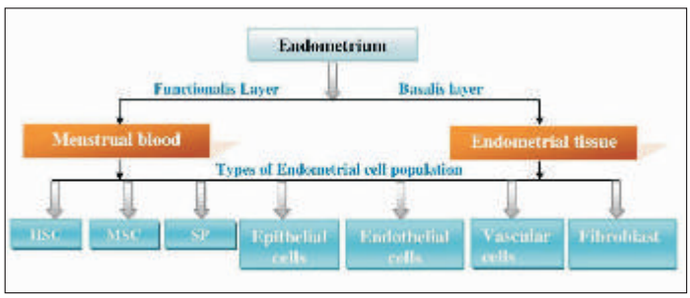


Fig 2: Location of endometrial stem cells in human endometrium.



Endometrial stem cells in-vitro

Understanding the importance of these stem cells, several researchers have studied their self renewal, differentiation and other properties in-vitro. A basic idea about its isolation and culture of these stem cells along with the culture and characterization are described below.

Isolation and culture protocol:

Endometrium biopsies were obtained from surgical procedures of dilatation and curettage done for various gynecological disorders like infertility or dysfunctional uterine bleeding. The samples were collected by aseptic technique and transported to the laboratory in normal saline **(**0.9% NaCl**)**. The tissue samples were washed in phosphate- buffered saline to remove blood and other debris. The washed samples were then minced mechanically and treated with collagenase for adequate cell separation. The digested tissues were centrifuged to obtain a single cell suspension. The cells were cultured in tissue culture flasks with DMEM supplemented with 10%h UCBS. The cultures were maintained CO2 incubator containing 5% CO2, 95% humidity at 37^{0}C.

Medium was changed every 48hr and replenished with DMEM supplemented with 10% h UCBS. At 80% confluency, cells were passage using Trypsin**-**ethylenediaminetetracetic acid (EDTA).

Flowcytometric characterization:

It was performed on a Becton Dickinson FACS Aria using a 488nm argon ion LASER and 632nm red LASER for excitation**.** Fluorescence emission was collected using the corresponding detectors. About 1x10^{5} cells were stained with saturating concentrations of fluro-chrome-conjugated antibodies. The cells incubated in Peridinin chlorophyll protein complex **(**PER CP**)** CD90; Allophycocyanin (APC) CD105; Phycoerythrin (PE) CD73, CD29.

The cells were incubated in the dark for 20 min at RT, washed thrice with wash flow buffer and resuspended in 500µl of phosphate buffer solution**.** Data analysis and acquisition was then performed using DIVA software **(**Becton Dickinson**)**. A minimum of 10,000 events were characterized and recorded.

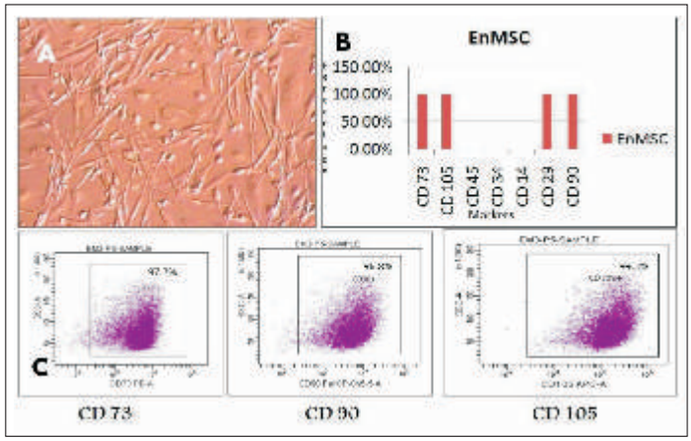


Fig: (A) Microscopic picture – Endometrial stem cells at confluence **(**P3**)**; (B); Percentage expression **(**C**)** Characterization of isolated cells using flow cytometry

Endometrial stem cells: A Double edged sword

It is evident that in highly regenerative human endometrium, remodeling occurs during each menstrual cycle, after resection, parturition, and in postmenopausal women using hormone replacement therapy. It has been suggested that endometrial stem cells must play a role in endometrial remodeling and regeneration during menstrual cycle and pregnancy. Besides, bone marrow-derived stem/progenitor cells are also able to incorporate themselves into the endometrium, contributing to the vascular remodeling or transdifferentiating into endometrial cells. Thus, the uniqueness of endometrial stem cells are known widely. In parallel,

emerging research results suggest that irregular function of these stem cells may contribute to the abnormalities substantially contributing to endometrial disorders, including endometriosis, causing dysmenorrhoea, adenomyosis, subfertility, endometrial hyperplasia and endometrial carcinoma. Such stem cells may also contribute to the pathogenetic process, because their high proliferation promotes rapid clonal expansion.

Thus, we call endometrial stem cells as a "double edged sword", wherein, on one side, it plays a dynamic role in normal physiology and development throughout women's life as well on the other side, it contributes to the pathogenesis of various endometrial disorders through its abnormal proliferation and differentiation.

Threats and challenges of endometrial stem cells

Endometrial stem cells are already known for its higher proliferation, differentiation, undergoes rapid angiogenesis during menstruation and immune tolerance for embryo during pregnancy. Thus, they are considered as a valuable source of stem cells. Several researches have demonstrated the multidifferentiation potency of endometrial MSCs both in-vitro and in-vivo. They had demonstrated to differentiate into various cell types such as, insulin producing cells, osteoblasts, neurons, myoblast, chondrocytes. Besides, pre-clinical and clinical experimental trial had also become successful in treating various diseases such as myocardial infarction, stroke, parkinson's disease and diabetes. However, on the other side of the coin, it possesses a threat to us by means of causing several gynecological disorders due to its abnormal proliferation and differentiation. It is postulated that several gynecological conditions are associated with abnormal endometrial proliferation, and it is possible that putative endometrial stem/progenitor cells may play a role in the pathophysiology of diseases such as endometriosis, endometrial hyperplasia, endometrial cancer, and adenomyosis. Alterations in the number, function, regulation, and location of epithelial/stromal endometrial stem/progenitor cells may be responsible for any one of these endometrial diseases. Furthermore, study of the clinical correlations of endometrial stem cells with gynecological diseases may unravel several unresolved barriers and lead to the use of endometrial stem cells as an ideal alternative source of curative therapeutics. Thus, addressing this barrier would be a great challenge and beneficial for the womankind. Therefore, endometrial stem cells could be considered as "dual role payer" or a "double edged sword", as mentioned above.

Currently, two major problems hinder the medical assistance to the women suffering from endometrial disorders: the lack of appropriate biomarkers useful in early diagnosis and the inexistence of conservative (medical) treatments with long - lasting effect. Overcoming these obstacles, threats and challenges may open up possibilities for rational development of novel and improved diagnostic and therapeutic strategies.

Endometrial Disorders	
Endometrial Cancer	Mutated stem/progenitor - tumor responsible for progression, metastasis, recurrence
Endometriosis	Normal stem/progenitor cell shed into peritoneal cavity - ectopic implant
Adenomyosis	Normal stem/progenitor cells, abnormal niche, inappropriate differentiation - ectopic growth, SMC hyperplasia
Asherman's Syndrome	Damage/loss of normal stem/ progenitor cells
Inadequate endometrium for IVF	Diminished activity of normal stem/progenitor cells

Gargett, 2007 13: 87-101

From the down table, it is apparent that underlying cause of all these diseases is stem cells. Thus, overcoming challenges facing endometrial diseases by targeting endometrial stem cells plays a significant role in diagnosis and treatment modalities of endometrial disorders.

Future perspectives

Once a mechanical or functional characteristic platform of these endometrial stem cells has been constructed under normal physiology and pathologic conditions, the following becomes possible to identify:

1. The underlying role of these endometrial stem cells in the normal physiological development of the uterus/endometrium.
2. The underlying role of these endometrial stem cells in causing pathophysiology of various gynecological disorders, as specified above.
3. To utilize endometrium, the trash source obtained from the uterus, for the treatment of wide horizon of diseases in two different approaches:
   a. As a treasure in regenerative medicine, whereby enhancing the applicability of endometrial stem cells, the highly regenerative cells for treating wide horizon of diseases.
   b. To design possible mechanism to target endometrial stem cells, thereby treating various gynecological disorders such as endometrial hyperplasia, endometrial carcinoma and so on.

Further readings:

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