به‌نام خدای بخشاینده مهربان

In the name of Allah, the Beneficent, the Merciful.

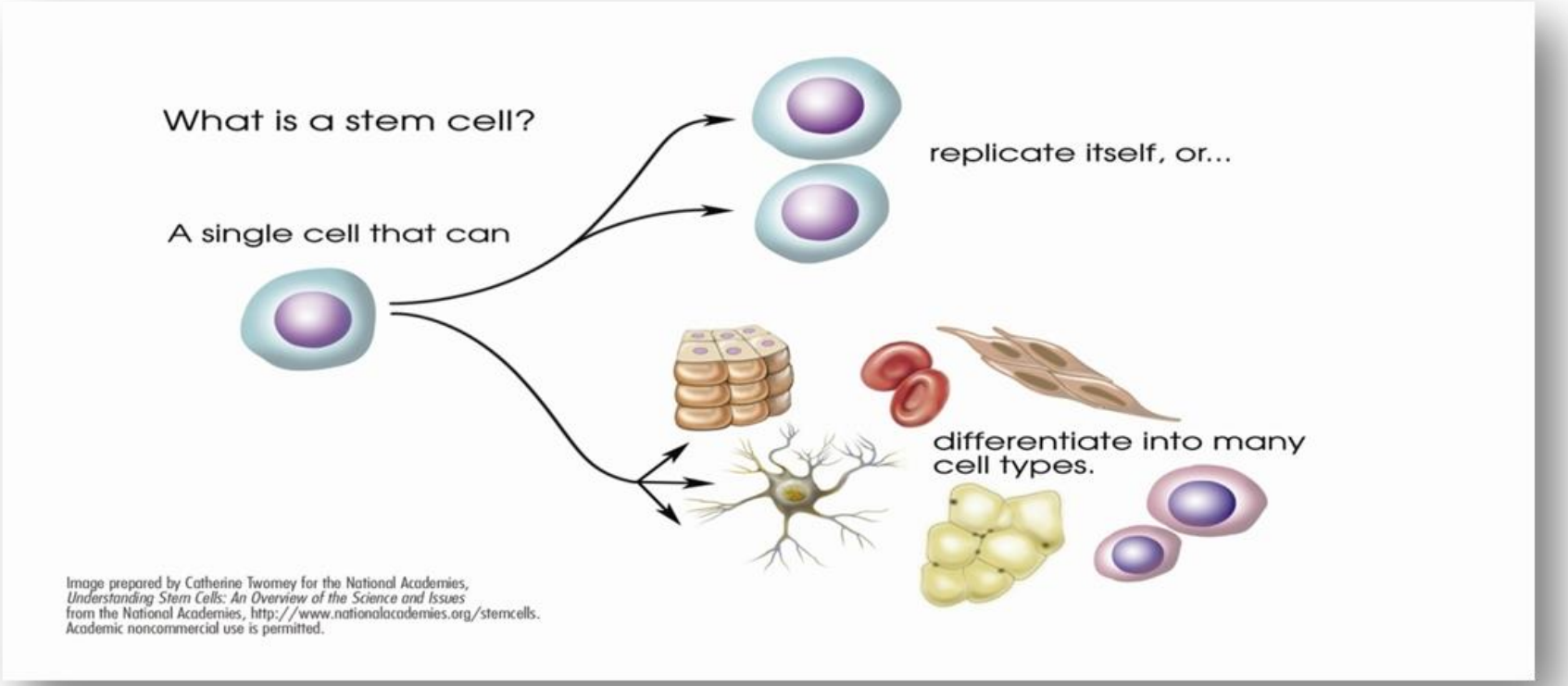
Potential and Challenges of Mesnchymal Stem Cell Therapy in Infertility Treatment

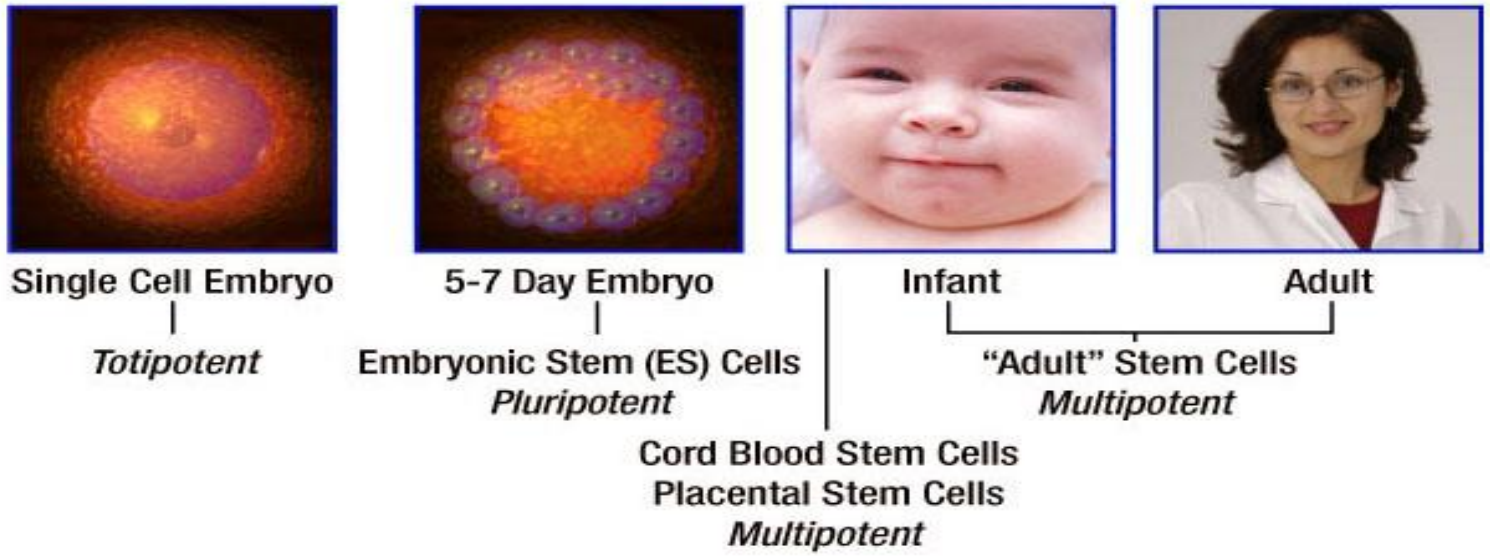
Somaieh Kazemnejad, Ph.D.

Clinical Embryologist
Director of Tissue Engineering and Regenerative Medicine Department,
Avicenna Research Institute, ACECR, Tehran

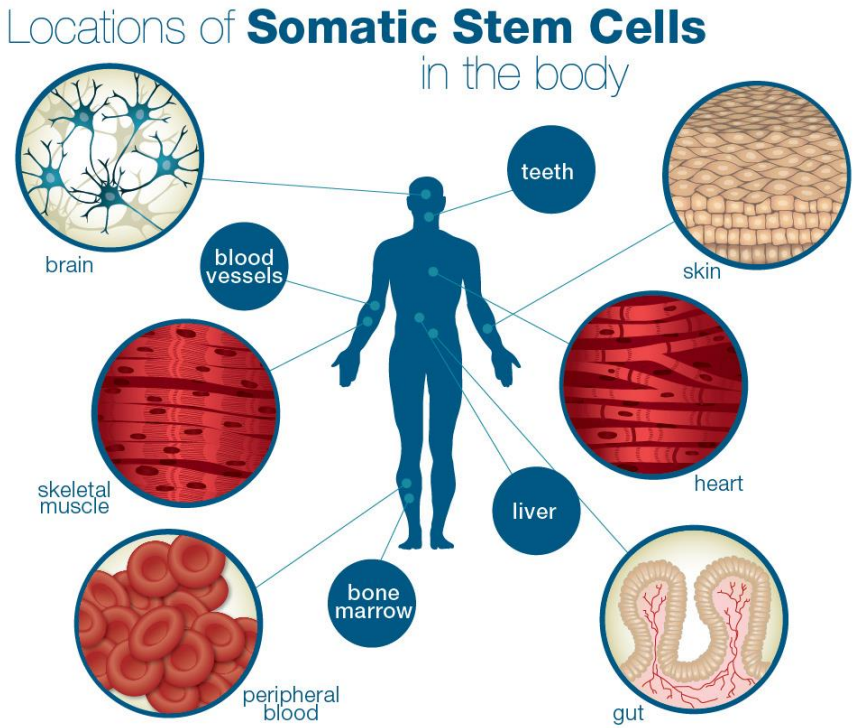
the only hope

What dose stem cell make as interesting?

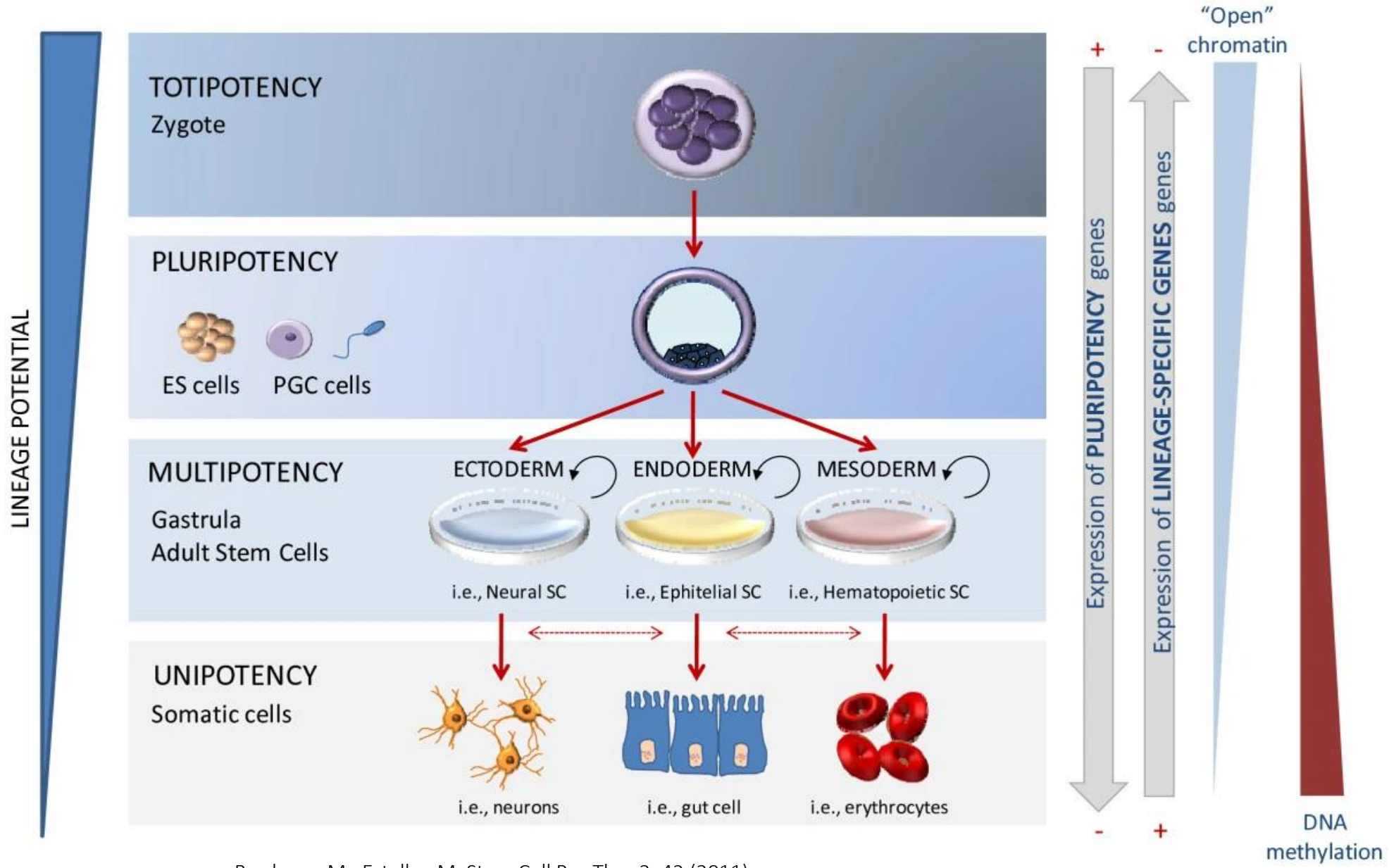




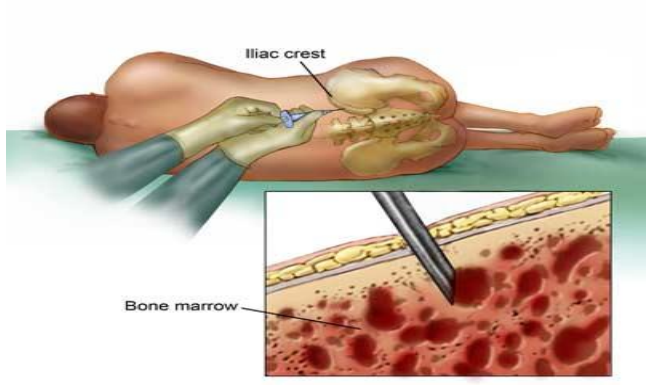
Stem cells in all careers of life



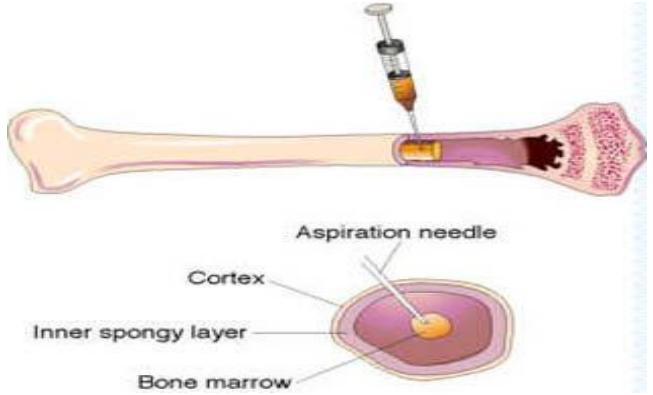
Stem cells are different based on plasticity



Berdasco, M., Esteller, M. Stem Cell Res Ther 2, 42 (2011).



Mesenchymal stem cell sources



<u>Bone marrow</u>

<u>Peripheral blood</u>

<u>Umbilical cord blood</u>

Menstrual blood

Adipose tissue

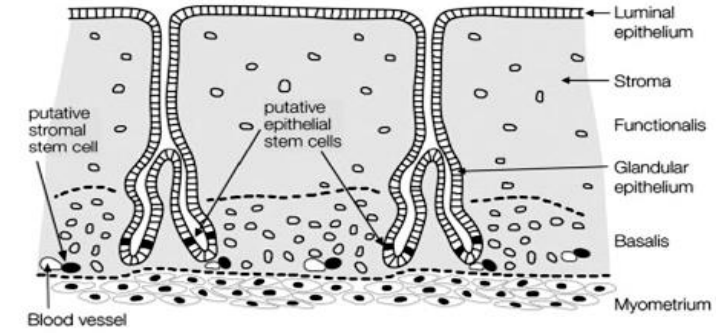
Amniotic fluid

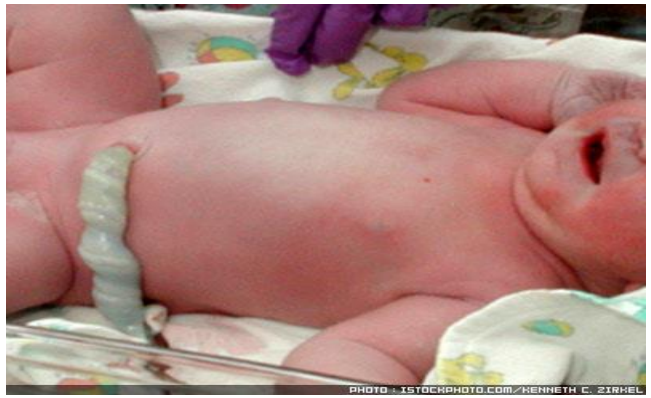
Endometrium

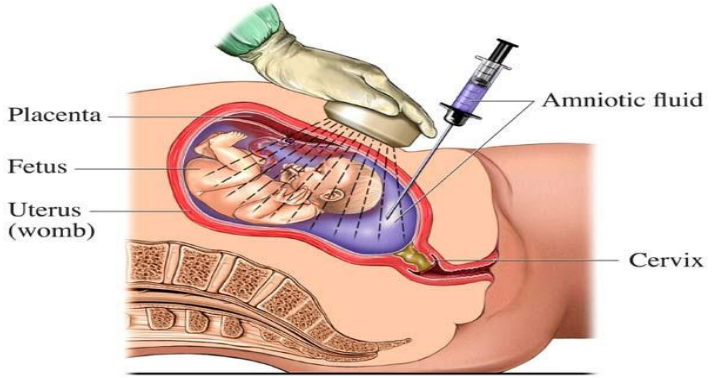
Placenta

Dental pulp

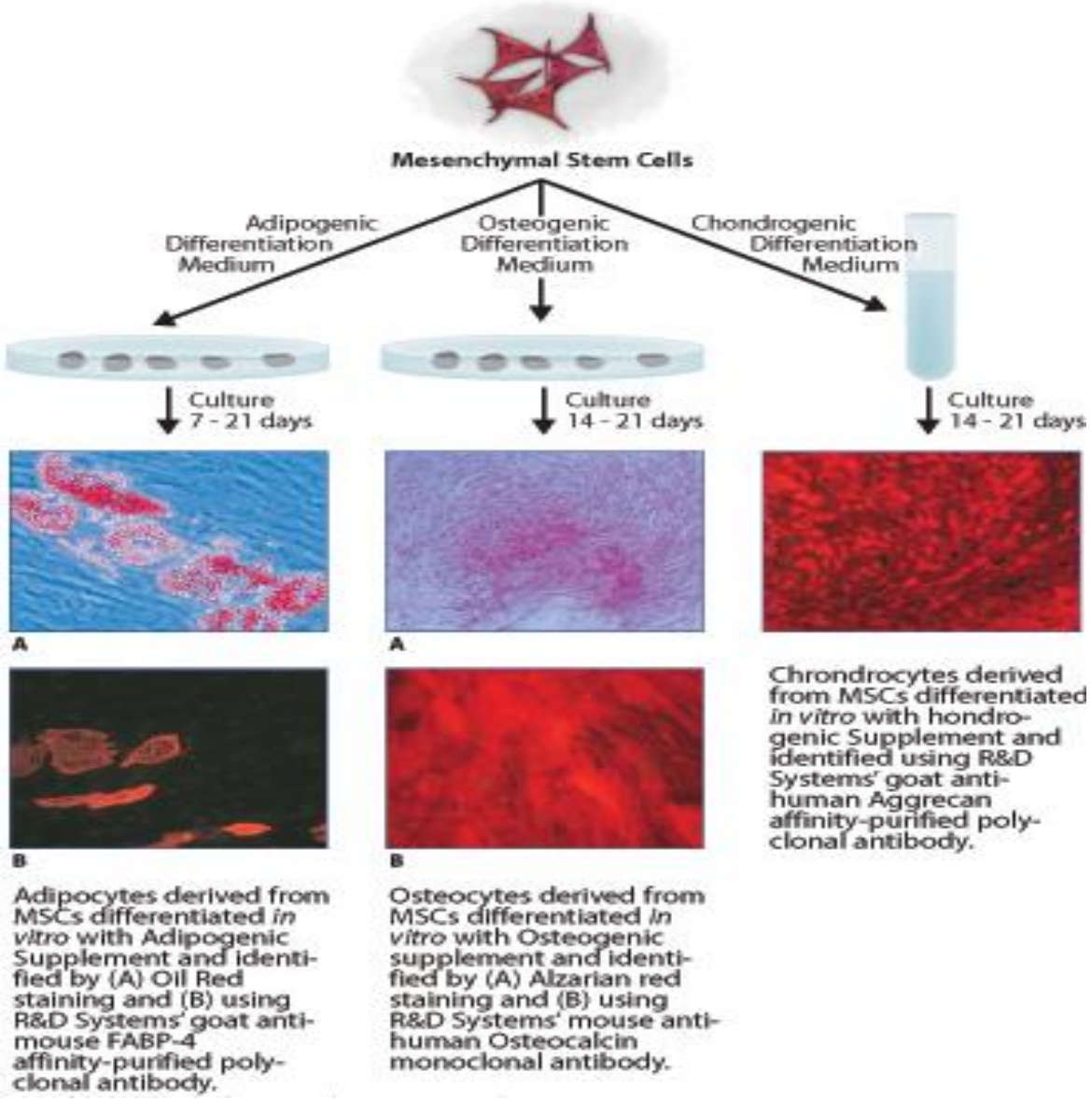
Cord (Wharton's Jelly)



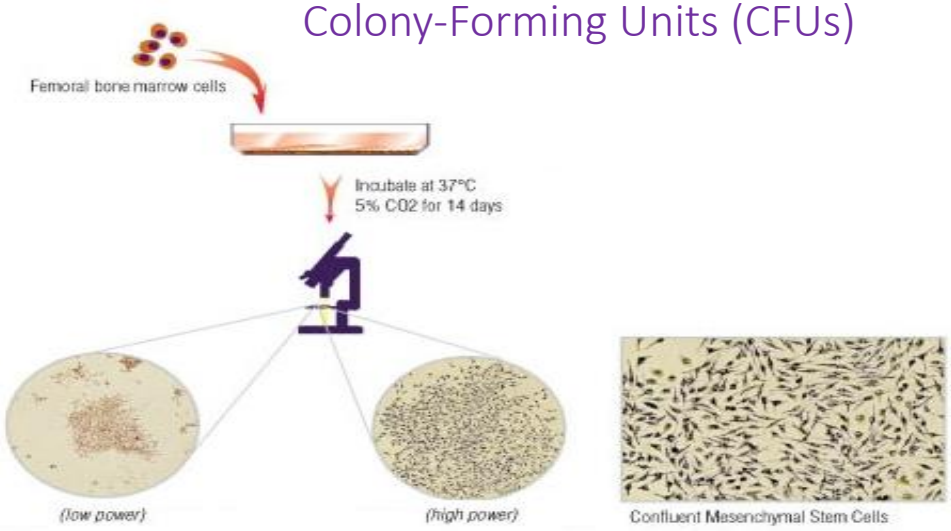




Characteristics of Mesenchymal stem cells



Colony-Forming Units (CFUs)

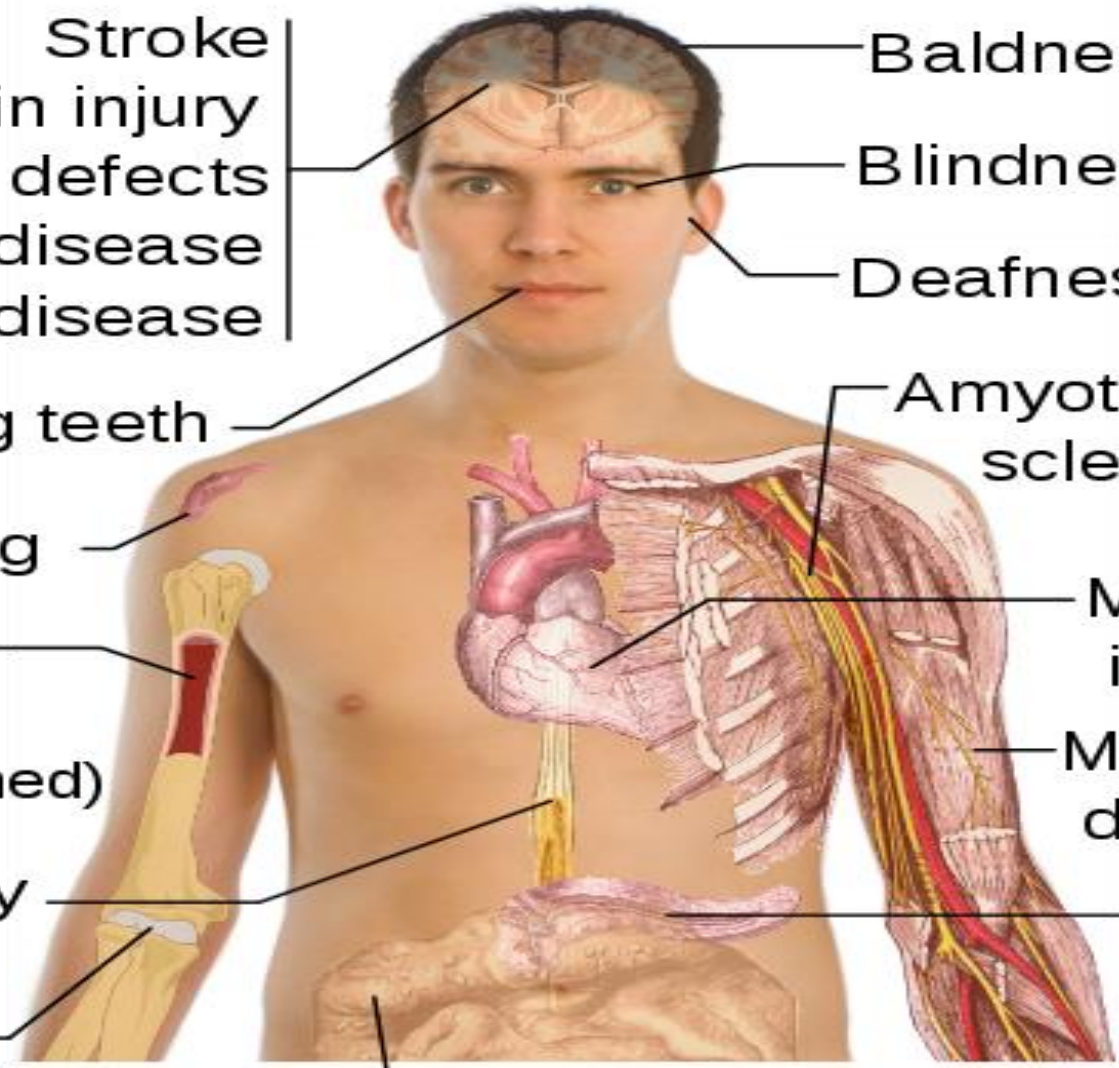


The mesenchymal markers

- **CD29(**Adhesion molecule on mesenchymal and hepatic stem cells)
- **CD44 (**Hyaluronic acid receptor)
- **CD73(**Ecto-5'-nucleotidase, involved in migration of MSC)
- **CD105 (**Marker of tissue and MSC)
- **CD106** (Vascular cell adhesion molecule-1)
- **CD146 (**Melanoma Cell associated Adhesion Molecule)
- **CD90 (**Fibroblast marker)
- **STRO1**(MSCs marker)

Potential uses of

Stem cells



Stroke
Traumatic brain injury
Learning defects
Alzheimer's disease
Parkinson's disease

Baldness

Blindness

Deafness

Missing teeth

Amyotrophic lateral-sclerosis

Wound healing

Myocardial infarction

Bone marrow transplantation (currently established)

Muscular dystrophy

Spinal cord injury

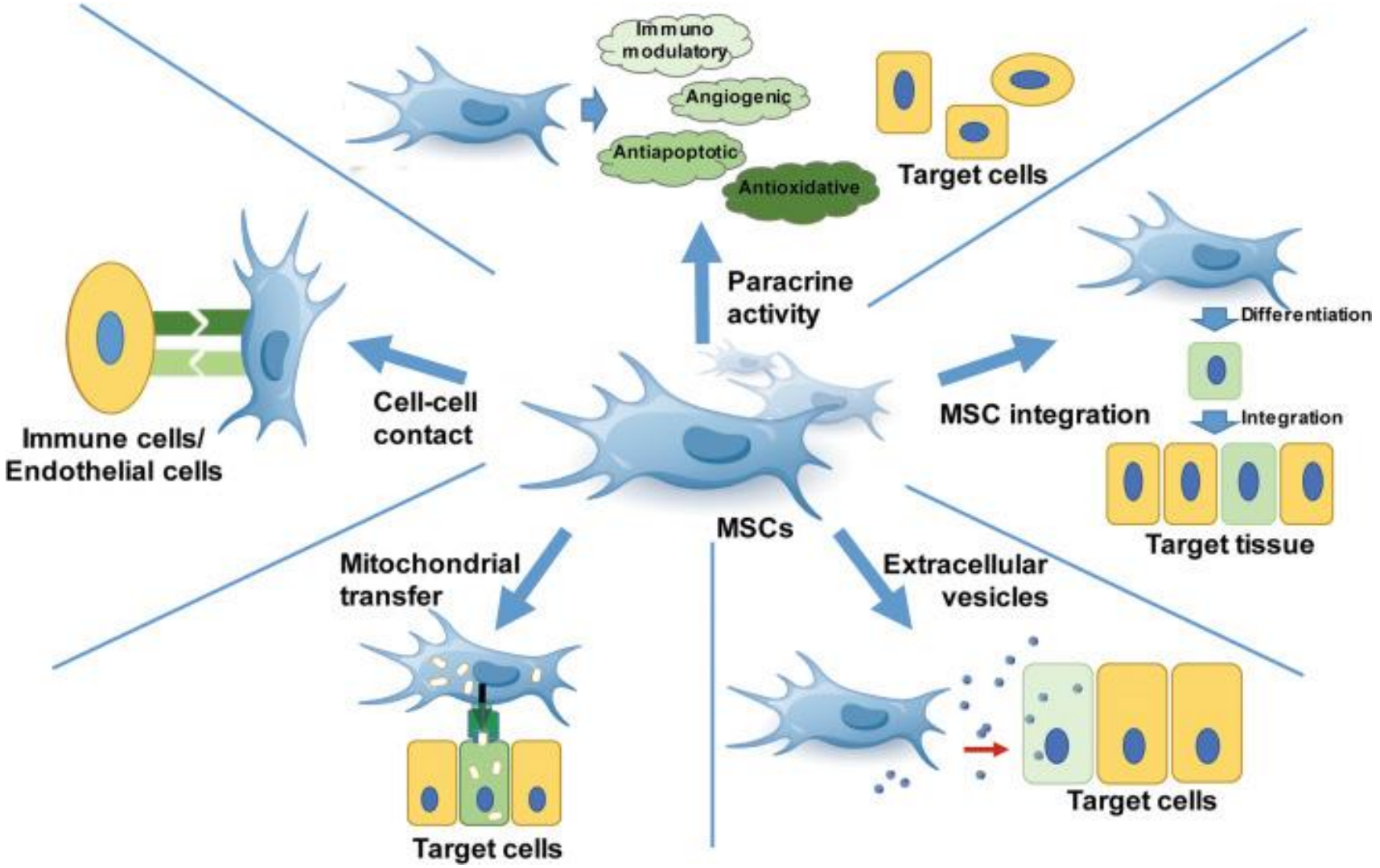
Diabetes

Osteoarthritis
Rheumatoid arthritis

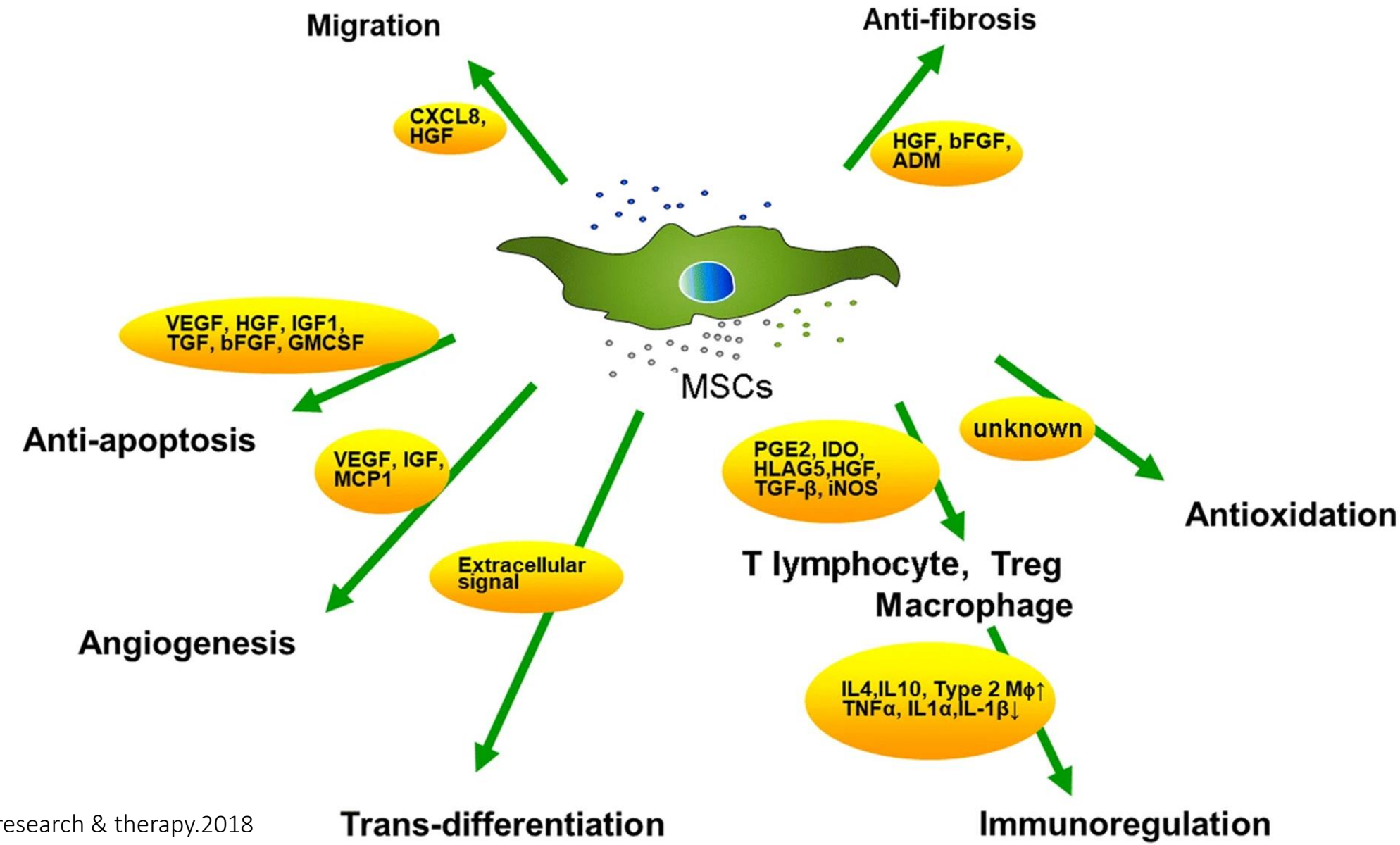
Crohn's disease

Multiple sites: Cancers

Proposed mechanisms underlying efficacy of stem cell therapy



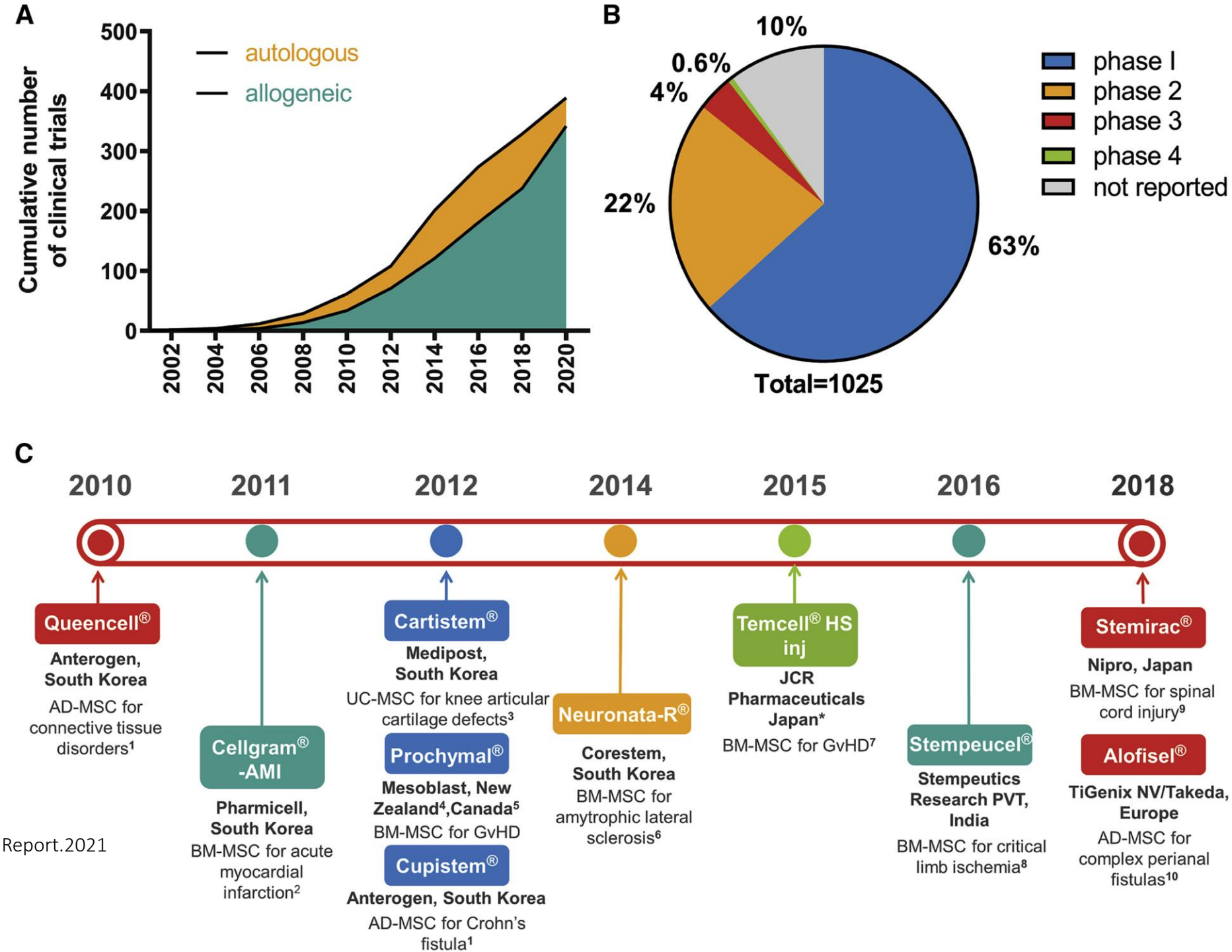
Xing-Liang Fan, et al. Cell Mol Life Sci. 2020



Yantao et al. Stem cell research & therapy.2018

Stem cell therapy based on donor type

Autologous transplantation	-they are potentially much more expensive to manufacture -greater inherited variability
Allogenic transplantation	the potential immunorejection is of concern.



Zhang et al. Stem Cell Report.2021

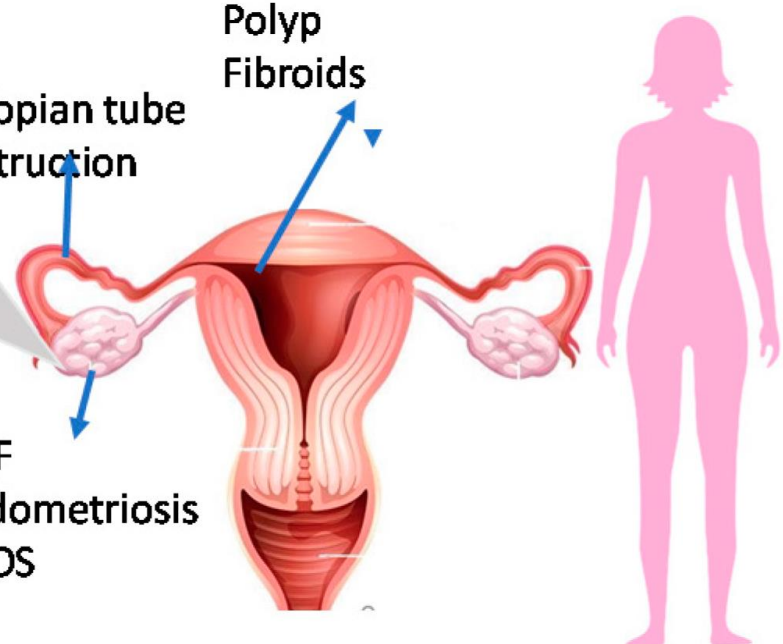
Hypothalamic amenorrhea

Pituitary adenoma

Stem cell therapy
-Bone marrow MSC
-Adipose tissue MSC
-Endometrial MSC
-Menstrual blood MSC
-Ovarian stem cell

Asherman syndrome
Polyp
Fibroids

Fallopian tube obstruction

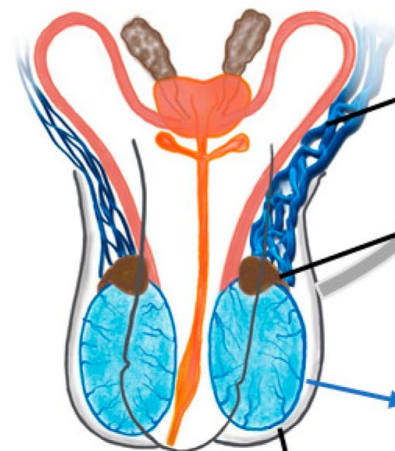


POF
Endometriosis
PCOS

Stem cell therapy
-Bone marrow MSC
-Adipose tissue MSC
-Spermatogonial stem cell

Kallmann syndrome

Hemochromotasis



Obstruction / absence of the vas deferens

absence of the epididymis

Azoospermia
Small testis
cryptorchidism
Y deletion,XXY

Varicocele

The potential of stem cell therapy in infertility

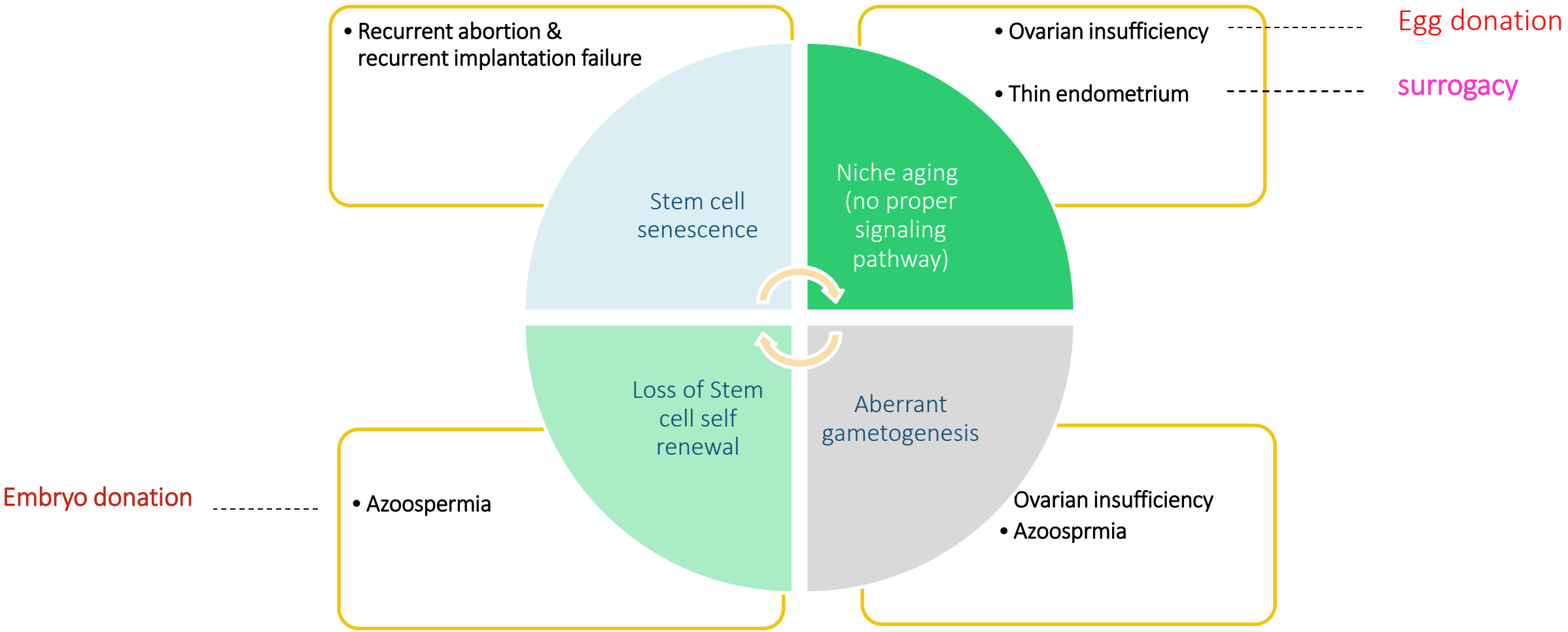


Table 1. Clinical trials related stem cell therapy performed or underway for improvement of infertility.

Trial Identifier	Est. # of Subjects	Status	Site	Conditions	Interventions	Outcome of Trial
NCT04706312	12	Not yet recruiting	Nanjing Medical University	Diminished Ovarian Response	Human Amniotic Mesenchymal Stem Cells (Hamscs) Transplantation	No results posted
NCT04676269	40	Recruiting	Indonesia University	Thin Endometrium Infertile Patients	Amnion Bilayer and Stem Cell Combination Therapy	No results posted
NCT03207412	20	Unknown	Chongqing Medical University, China	Premature Ovarian Failure	Human Amniotic Epithelial Cells	No results posted
NCT02696889	3	Active	University of Illinois at Chicago	Primary Ovarian Insufficiency, Low Ovarian Reserve	Autologous Stem Cell Therapy	Report of 2 cases revealed a significant improvement in clinical features related to POI. There was an increase in size as well as estrogen production in the MSC engrafted ovary [174]
NCT02713854	240	Recruiting	The University of Hong Kong	Subfertility	Human Embryonic Stem-Cell-Derived Trophoblastic Spheroid (Bap-Eb) as a Predictive Tool	No results posted
NCT03592849	50	Enrolling by invitation	Nanjing Drum Tower Hospital, China	Infertile Women with Thin Endometrium or Endometrial Scarring	Procedure: Collagen Scaffold Loaded with Umbilical-Cord-Derived Mesenchymal Stem Cells Therapy	No results posted
NCT03166189	46	Completed	D.O. Ott Research Institute of Obstetrics, Gynecology, Russian Federation	Infertility of Uterine Origin Asherman Syndrome	Biological: Bone Marrow-Derived Msc and Hrt Other: Hormonal Replacement Therapy	No Results Posted
						Phase 1 trial revealed that transplantation of clinical

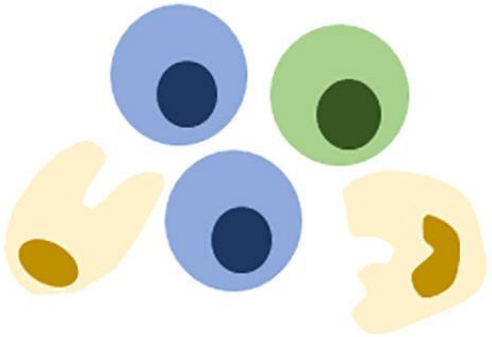
Trial Identifier	Est. # of Subjects	Status	Site	Conditions	Interventions	Outcome of Trial
			Gynecology, Russian Federation		Replacement Therapy	
NCT02313415	26	Completed	Nanjing Drum Tower Hospital, China	Infertility with Intrauterine Adhesions	Procedure: Umbilical Cord Mesenchymal Stem Cells	Phase 1 trial revealed that transplantation of clinical grade human UC MSC could improve the proliferative and differentiation efficiency of endometrium [175]
NCT02025270	100	Unknown	Al Azhar University, Egypt	Azoospermic Patients	Bone-Marrow-Derived Mesenchymal Stem Cells	No results posted
NCT02641769	50	Recruiting	Stem Cells of Arabia, Amman, Jordan	Non-obstructive Azoospermia	Intratesticular Transplantation of Autologous Stem Cells	No results posted
NCT02414295	1	Completed	Man Clinic for Andrology and male infertilit, Cairo, Egypt	Klinefelter Syndrome Azoospermia	Mesenchymal Stem Cell Injection	No Results Posted
NCT02062931	60	Unknown	Al-Azhar University hospitals, Egypt	Premature Ovarian Failure	Biological: Stem Cell Preparation and Injection	No results posted
NCT02603744	9	Unknown	Royan Institute	Premature Ovarian Failure	Intraovarian Injection of Adipose-Derived Stromal Cells (Adscs)	Intraovarian engrafting of ADSCs were found to be safe and feasible and linked to reduction in FSH level [176]
NCT02204358	30	Unknown	Nanjing University Medical School	Intrauterine Adhesions, Endometrial Dysplasia	Collagen Scaffold Loaded with Autologous Bone Marrow Stem Cells Testicular Injection of Autologous Human Bone Marrow	No results posted

Trial Identifier	Est. # of Subjects	Status	Site	Conditions	Interventions	Outcome of Trial
NCT02041910	60	Unknown	Hesham Saeed Elshaer, El-Rayadh Fertility Centre	Azoospermia	Derived Stem Cells	No results posted
NCT02151890	10	Completed	Al Azhar University, Cairo, Egypt	Premature Ovarian Failure	Biological: Stem Cell	No results posted
NCT02372474	112	Completed	Al Azhar University, Cairo, Egypt	Premature Ovarian Failure	Biological: Stem Cell	No results posted
NCT01742533	40	Unknown	Shenzhen People's Hospital, Shenzhen, Guangdong, China	Premature Ovarian Failure	Biological: Human Umbilical Cord Mesenchymal Stem Cells and Human Cord Blood Mononuclear Cells Drug: Hormone Replacement Therapy	No results posted
NCT03069209	50	Active, not recruiting	Stem Cells Arabia, Amman, Jordan	Premature Ovarian Failure	Biological: Stem Cells	No results posted
NCT00429494	60	Completed	UT MD Anderson Cancer Center, United States	Amenorrhea Premature Ovarian Failure Ovarian Function Insufficiency	Procedure: Hematopoietic Stem Cell Transplantation (Hsct) Drug: Leuprolide Acetate Behavioral: Questionnaire	Phase II trial revealed that Leuprolide could not preserve ovarian function in HSCT patients [177]

Saha et al. Cells 2021, 10(7), 1613

Trial Identifier	Est. # of Subjects	Status	Site	Conditions	Interventions	Outcome of Trial
NCT04009473	100	Enrolling by invitation	Multicenter	Ovarian Failure Premature Ovarian Failure	Combination Product: SEGOVA Procedure Includes Stem Cell Therapy, Growth Factor, and Platelet Plasma Rich Therapy	No results posted
NCT02240823	30	Unknown	Odense University Hospital	Erectile Dysfunction After Prostatectomy	Adipose-Derived Stem Cells (ADMSC)	Intracavernous injection of ADMSC is a safe procedure and resulted in improvement of erectile function [178]
NCT02414308	20	Unknown	Man Clinic for Andrology, Male Infertility, and Sexual Dysfunction	Erectile Dysfunction Peyronie' Disease	Adipose Tissue Stem Cell Injection	No results posted
NCT02008799	20	Recruiting	Man Clinic for Andrology, Male Infertility, and Sexual Dysfunction	Azoospermia	Intratesticular Artery Injection of Bone Marrow Stem Cell	No result posted

Immunomodulatory effect

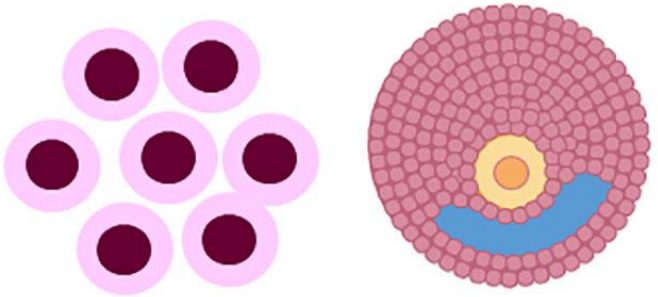


TNF-α
Anti-inflamatory cytokines
(Zhang et al., 2015; Li et al., 2017)

-Balance between pro-inflamatory and anti-inflamatory cytokines (Yin et al., 2018)
-Balance between macrophagues and regulatory T-cells populations (Abumaree et al., 2013; Yin et al., 2018)
-Regulation of oxidative estress (Seok et al.,2020)

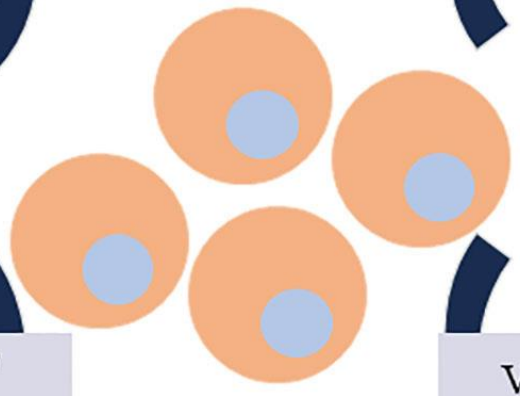
Folliculogenesis and regulation of GC survival

VEGF
HGF
IGF-1
FGF2
(Uzumcu et al., 2006; Fu et al., 2008)

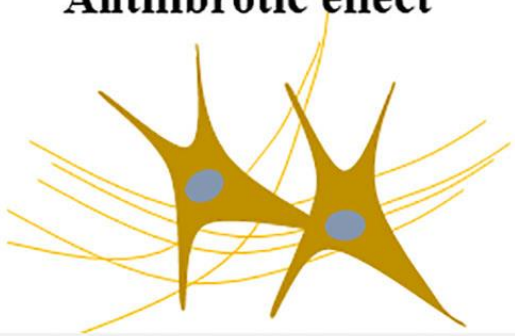


-Redution of apoptosis and atresia (Herraiz et al., 2018)
-Regulation of proapoptic proteins (Liu et al., 2020; Guo et al., 2013)
- Promotion of cell proliferation (Zhou et al., 2013)

STEM CELLS



Antifibrotic effect

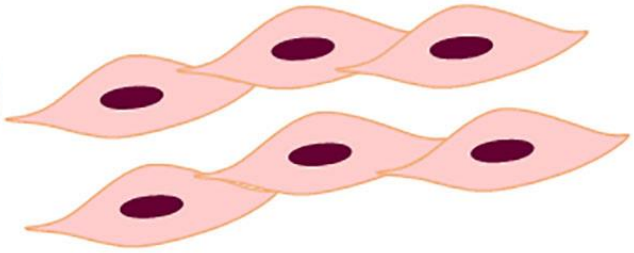


bFGF
Adrenome-dullim
HGF
(Figueroa et al., 2012)

-Collagen decrease (Affifi et al., 2013)
-Inhibition of fibroblasts proliferation and extracelular matrix deposition (Wang et al., 2017; He et al., 2018)

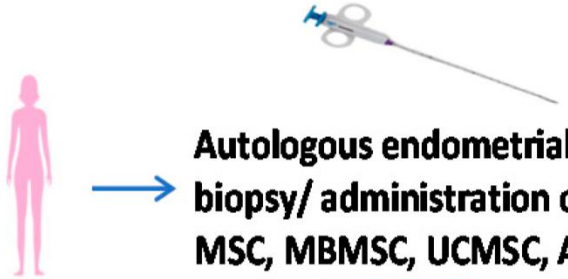
Regulation of angiogenesis

VEGF
FGF2
IL-6
PDGF
angionegin
(Kiinaird et al., 2004; Liang et al., 2017)



-Neoangiogenesis and vasculogenesis (Carrion et al., 2013; Abd-Allah et al., 2013))

Polonio et al. Front. Endocrinol., 2021



Autologous endometrial biopsy/ administration of BMD MSC, MBMSC, UCMSC, ADMSC

Trans-differentiation into endometrial stem cells

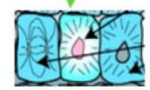
Activation of endogenous endometrial stem cells

Stromal stem cells

Reside close to endothelial cells in micro-vessel in both basalis and functionalis
CD44+, CD73+, CD 90+, CD105+
CD34-, CD45-

Endothelial stem cells

Reside in vascular endothelium of basalis
CD34+, CD31+

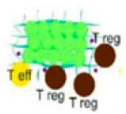


Epithelial progenitor cells

Reside at base of the glands in basalis
SSEA-1+, N cadherin

Secretion of paracrine factors

CXCL8



Immunemodulation

Angiogenesis

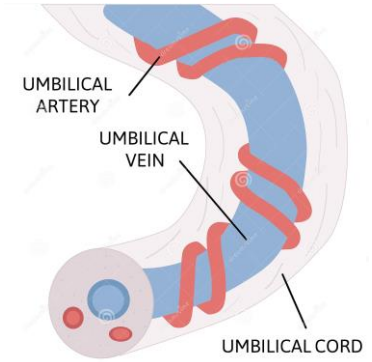
Anti fibrosis

Anti apoptosis

Endometrial regeneration → **Improve endometrial receptivity and implantation** → **Restoration of fertility**

STEM CELL THERAPY

Bone Marrow



Umbilical cord

Adipose Tissue

Menstrual Blood

COLLECTION

- Stem Cells are collected from Bone Marrow / Umbilical cord / Fat / Menstrual Blood

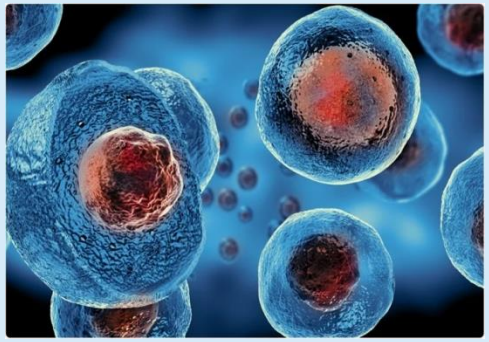
PROCESSING

- Blood or Bone Marrow is processed in the laboratory to purify and concentrate the stem cells

REINFUSION

- The Stem Cells are reinfused into the patient through intravenous injection

Main challenges



Stem cell type and source

Degree of disease

Stem cell preparation

Dosage and Route of administration

Critical points for selection of stem cell type

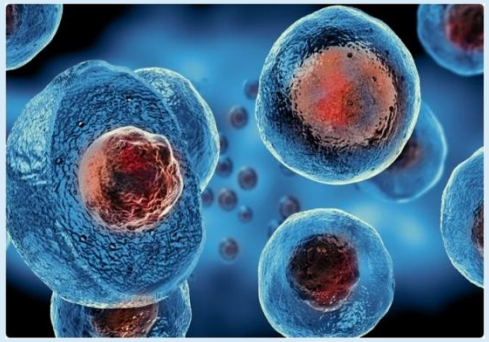
- **Accessibility**
- **Ethics**
- **Sterility**
- **Teratoma formation**
- **Immune rejection**
- **Genome stability**
- **Proliferation and differentiation ability**

Pros and cons of each stem cell type

Criteria	Stem Cell Types				
	Embryonic	**Bone marrow-Mesenchymal**	**Hematopoietic**	**Umbilical cord blood**	**Menstrual blood**
Plasticity	Pluripotent	Multipotent	Multipotent	Multipotent	Multipotent
Proliferation ability	+++++	+++	+++	++++	++++
Tumor formation (safety consideration)	High	no	no	no	no
Last of cell line	+++++	++	++	+++	+++
Immune rejection (safety consideration)	high	no	no	no	no
Accessibility	Low	Low	Low	Medium	High
Repeatability	Very low	low	low	low	High
Genome Stability(safety consideration	Instable	Stable	Stable	Stable	Stable
Cost	+++++	+++	+++	+++	+++
Ethical issue	significant	low	low	Very low	Very Low

Main challenges

2



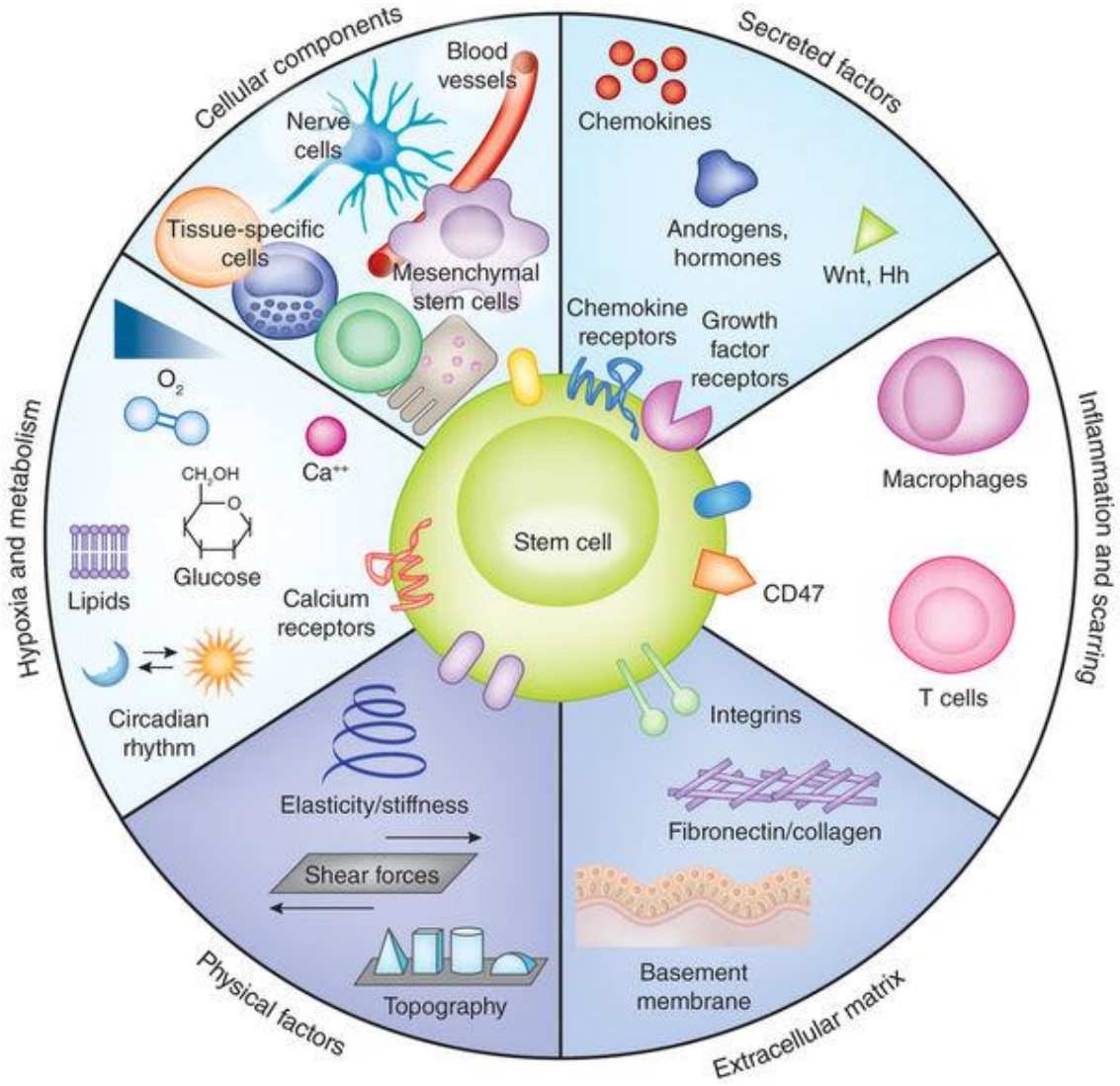
Stem cell type and source

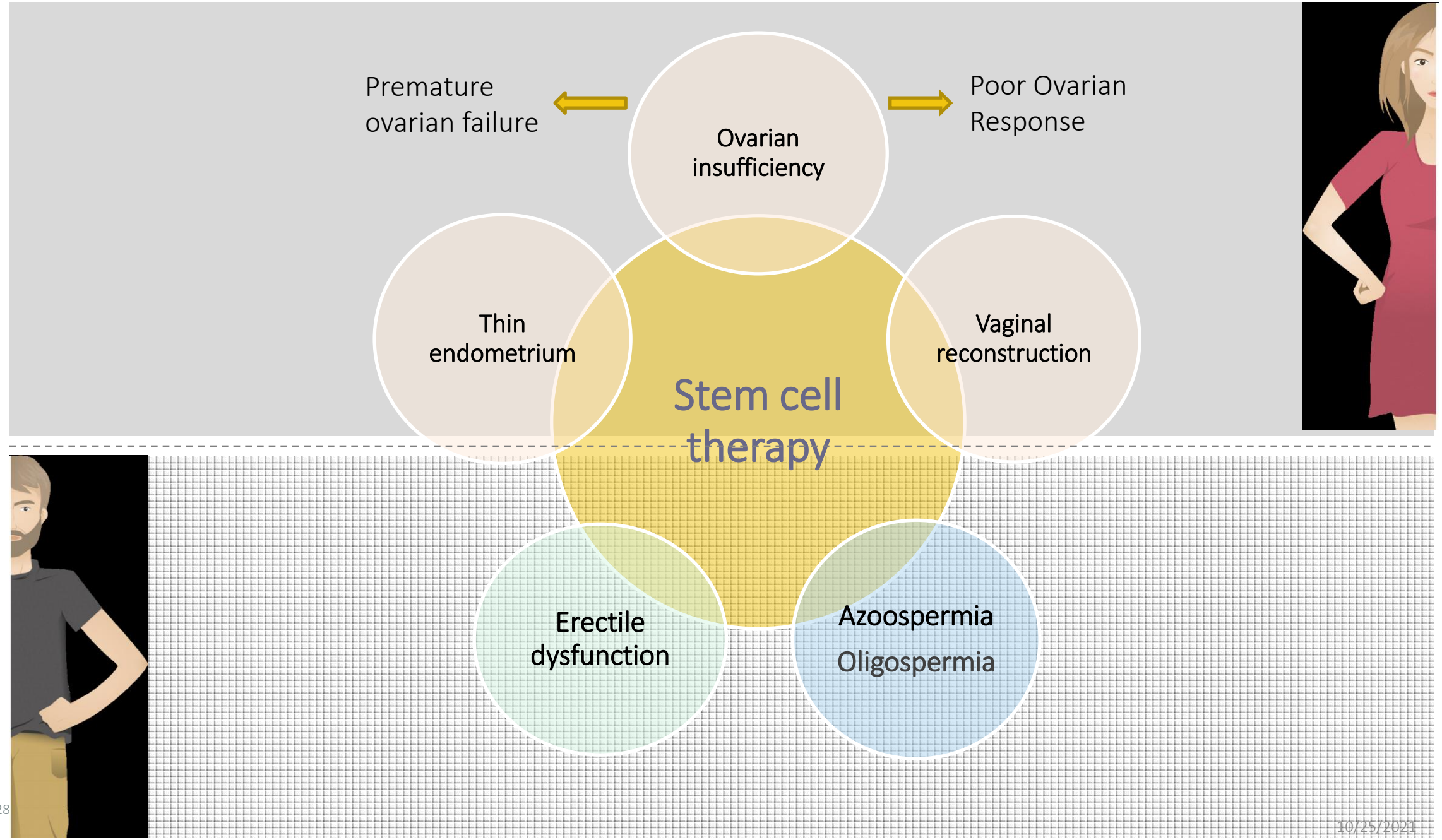
Degree of disease

Stem cell preparation

Dosage and Route of administration

Dialogue taking place between the stem cells and the niche




Premature ovarian failure
Ovarian insufficiency
Poor Ovarian Response
Thin endometrium
Vaginal reconstruction
Stem cell therapy
Erectile dysfunction
Azoospermia
Oligospermia

Autologous stem cell ovarian transplantation to increase reproductive potential in patients who are poor responders

Sonia Herraiz, Ph.D.,[a,b,c] Mónica Romeu, M.D.,[c,d] Anna Buigues, B.Sc.,[a,c,e] Susana Martínez, M.D.,[d] César Díaz-García, M.D.,[f] Inés Gómez-Seguí, M.D.,[g] José Martínez, M.D.,[h] Nuria Pellicer, M.D.,[d] and Antonio Pellicer, M.D.[a,c,i]

[a] Fundación IVI, [b] IVI-RMA Valencia, [c] Reproductive Medicine Research Group, IIS La Fe, [d] Women's Health Area, [g] Hematology Department, and [h] Radiology Department, La Fe University Hospital; [e] Department of Pediatrics, Obstetrics and Gynecology, University of Valencia, Valencia, Spain; [f] IVI-RMA London, London, United Kingdom; and [i] IVI-RMA Rome, Rome, Italy

Stem Cell Reviews and Reports
https://doi.org/10.1007/s12015-020-09969-6

Improvement of Pregnancy Rate and Live Birth Rate in Poor Ovarian Responders by Intraovarian Administration of Autologous Menstrual Blood Derived- Mesenchymal Stromal Cells: Phase I/II Clinical Trial

Simin Zafardoust[1] · Somaieh Kazemnejad[1] · Maryam Darzi[1] · Mina Fathi-Kazerooni[1] · Hilda Rastegari[1] · Afsaneh Mohammadzadeh[1]

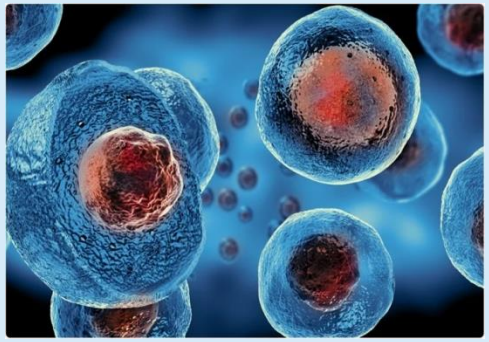
born babies using menstrual blood stem cells

Regenerative factor	Study population	Administration method	Main findings	Limitations	Reference
BM-MSC	1 perimenopausal woman (45-year old). AMH 0.4 ng/ml AFC = 1	BM-MSCs into both ovaries *via* laparoscopy.	-AFC and AMH increased 8 weeks after treatment. -1 live birth.	POR similar to that reported for POI patients without treatment	Gupta et al. (17)
BM-MSC	10 women with idiopathic POI (26–33 years old). AMH <0.1 ng/ml; FSH = 58 mIU/ml	BM-MSCs into both ovaries *via* laparoscopy.	-Resumption of menses in 20% patients after 3 months. -10% treatment POR. -One pregnancy and a live birth in one patient showing endometrial regeneration.	POR similar to that reported for POI patients without treatment	Edessy et al. (104)
BM-MSC	30 patients with POF (18–40 years old). Baseline characteristics not reported.	Direct laparoscopic infusion into the ovarian stroma and catheterism into the ovarian artery of one side.	-86.7% POF patients improved hormone profile 4 weeks after treatment. -60% showed ovulation. -3 patients underwent IVF. -1 spontaneous pregnancy.	-AFC not reported or compared between ovaries. -IVF outcomes were not reported.	Gabr et al. (105)
BM-MSC	33 patients with idiopathic/other POF/ POI and low ovarian reserves. Baseline characteristics not yet reported.	BM-MSCs into both ovaries *via* laparoscopy.	Not yet reported	Still ongoing	Al-Hendy et al. (NCT02696889)
BMDSC	20 POI patients (<39 years old). (10 patients included) Baseline characteristics not reported	One ovarian artery by intraarterial catheterism (ASCOT) (6 patients) and stem cells mobilization to peripheral blood by means of GSC-F (4 patients).	-Follicular development in both arms (90–140 days after treatments). -AFC increase in 50% of patients (GSC-F arm) and 66.6% of women (ASCOT arm). -Statistically significant FSH decrease is not observed, although FSH decreased was decreased. -In G-CSF arm: COS initiated in 2/4 women and 1 embryo vitrified. Embryo transfer was performed but pregnancy was not achieved -In the ASCOT arm: COS initiated in 4/6 women, 1 embryo vitrified and transferred, having an ongoing pregnancy. -Menses recovery in 40% of patients and climacteric symptoms decrease in 50% of women.	Still ongoing. Preliminary data from interim analysis reported (106)	Herraiz et al., (NCT03535480)

BM-MSC, bone marrow mesenchymal stem cells; BMDSC, bone marrow derived stem cells; POI, premature ovarian insufficiency; POF, premature ovarian failure; POR, poor ovarian responder; AFC, antral follicle count; AMH, anti-Mullerian hormone; FSH, follicle stimulating hormone; COS, controlled ovarian stimulation; IVF, in vitro fertilization; GSC-F, granulocyte colony-stimulating factor; ASCOT, autologous stem cell ovarian transplantation. Modified from Herraiz et al. (106).

Main challenges

3

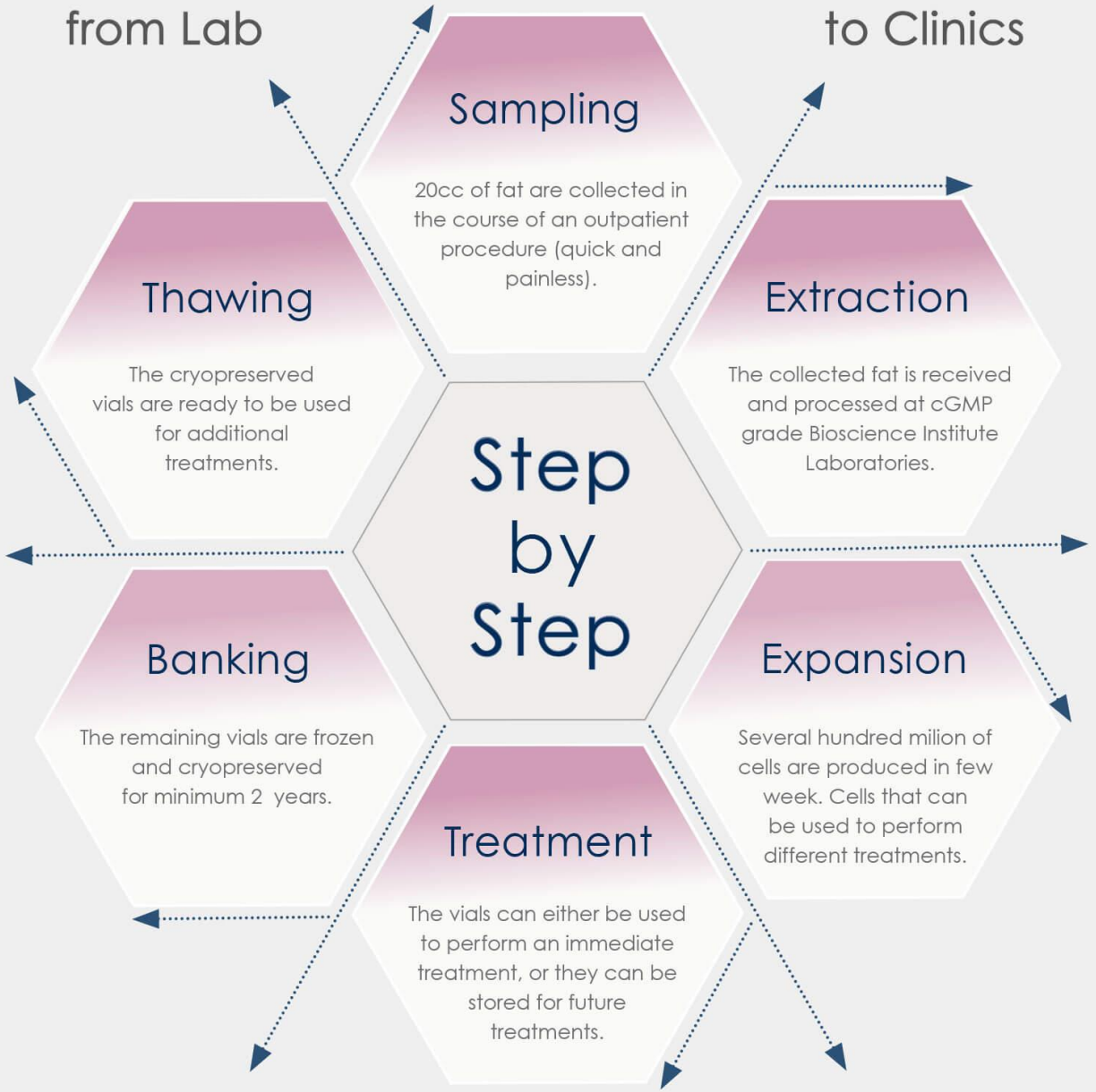


Stem cell type and source

Degree of disease

Stem cell preparation

Dosage and Route of administration


from Lab
to Clinics
Sampling
20cc of fat are collected in the course of an outpatient procedure (quick and painless).
Extraction
The collected fat is received and processed at cGMP grade Bioscience Institute Laboratories.
Expansion
Several hundred milion of cells are produced in few week. Cells that can be used to perform different treatments.
Treatment
The vials can either be used to perform an immediate treatment, or they can be stored for future treatments.
Banking
The remaining vials are frozen and cryopreserved for minimum 2 years.
Thawing
The cryopreserved vials are ready to be used for additional treatments.
Step by Step

Critical points to prepare stem cells for cell therapy

Facilities

- Class D
- Class C (preparation)
- Class B (processing)

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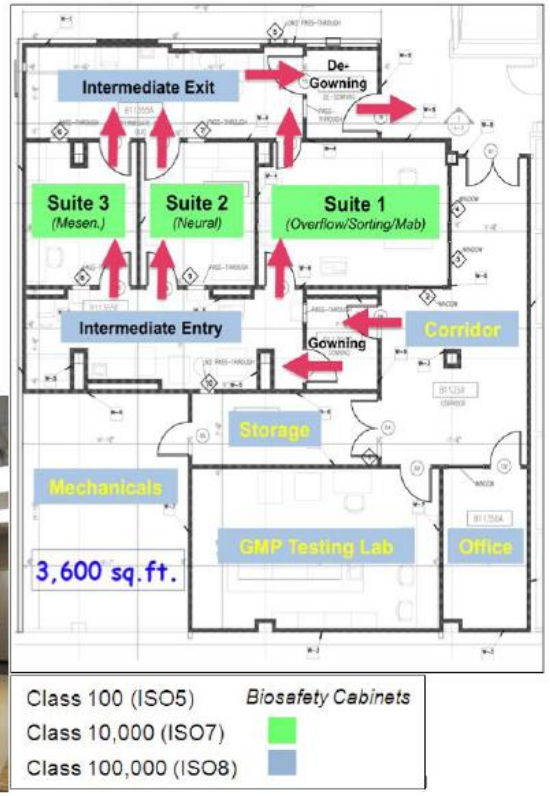


Quality Control Process

Materials & reagent
Active material
In process control
Final product

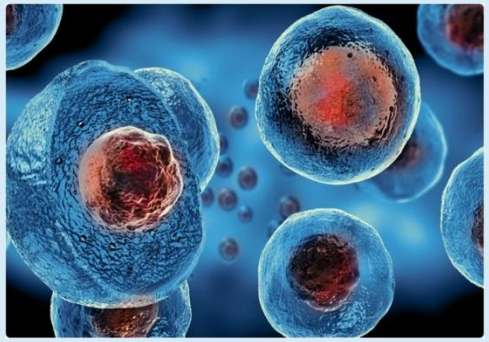
Stem cell purity: Establish sensitive analytical methods to detect cells with undesired characteristics
Microbiological safety: endotoxin, mycoplasma
Karyotyping
HLA
Suitable culture condition
Conservation of Functionality

Regulatory authorities are now demanding application of standardization and safety regulations protocols for cellular products, which include the use of Xeno-free culture media, recombinant growth factors in addition to “Good Manufacturing Practice” (GMP) culture supplies.

High cost

Main challenges

4



Stem cell type and source

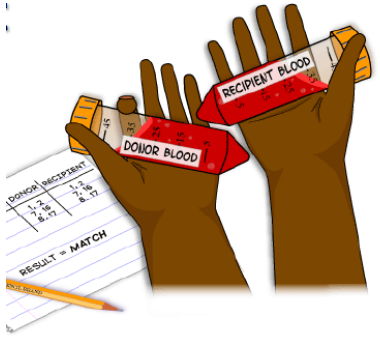
Degree of disease

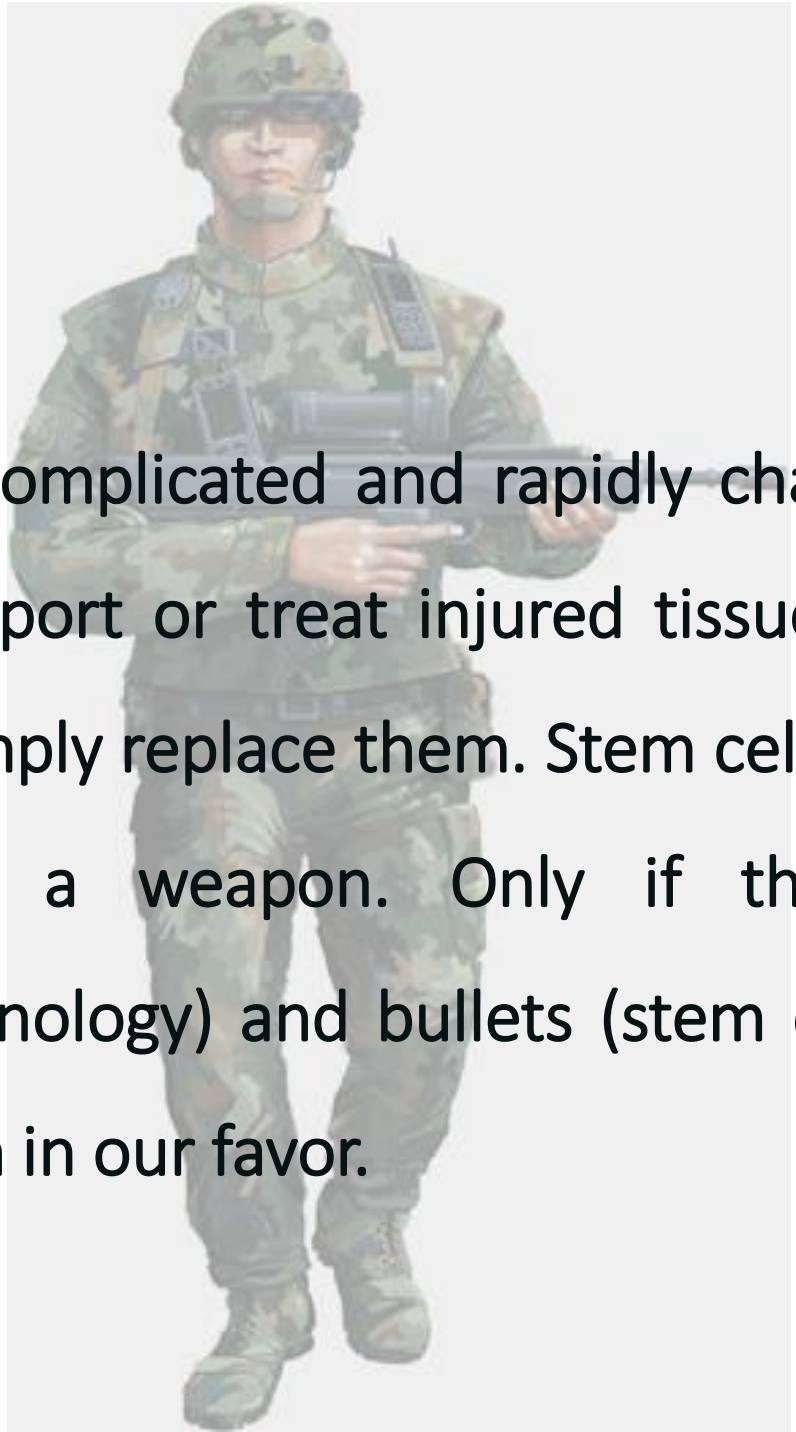
Stem cell preparation

Dosage and Route of administration

Challenges

- How many cells are needed?
- What is the optimal method/route to deliver the product?
- What is the optimal timing for product administration relative to the onset of disease/ injury?
- Will repeat administration be needed?
- What happens to the cells *in vivo* following delivery?
- What is the risk/benefit ratio for the intended patient population?





Stem cell research is complicated and rapidly changing. Today's medicine generally tries to support or treat injured tissues and organs, but stem cells may someday simply replace them. Stem cell therapy is considered as like a soldier with a weapon. Only if the soldier (experienced doctor),weapon (technology) and bullets (stem cells) all are in our hand than the fight will turn in our favor.

Thank you for your attention

Radin, first born baby using menstrual blood stem cells