Stem Cells and Their Applications in Infertility

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Stem Cells - Fact Sheet

What are stem cells ?

They are unspecialised cells that can replicate themselves through cell division over long periods of time**.**

Properties of stem cells

- They have characteristic properties of self renewal and differentiation.
- They enable the body to grow, repair and renew.

Reason for being major attraction for researchers

They can be manipulated, under certain conditions, to become mature cells with special functions, such as beating cells of the heart muscle or insulin-producing cells of the pancreas.

Types of stem cells based on potency

- Totipotent stem cells
- Pluripotent stem cells
- Multipotent stem cells

Types of stem cells based on source

- Embryonic stem cells
- Adult stem cells
- Induced pluripotent stem cells

Sources of stem cells in body

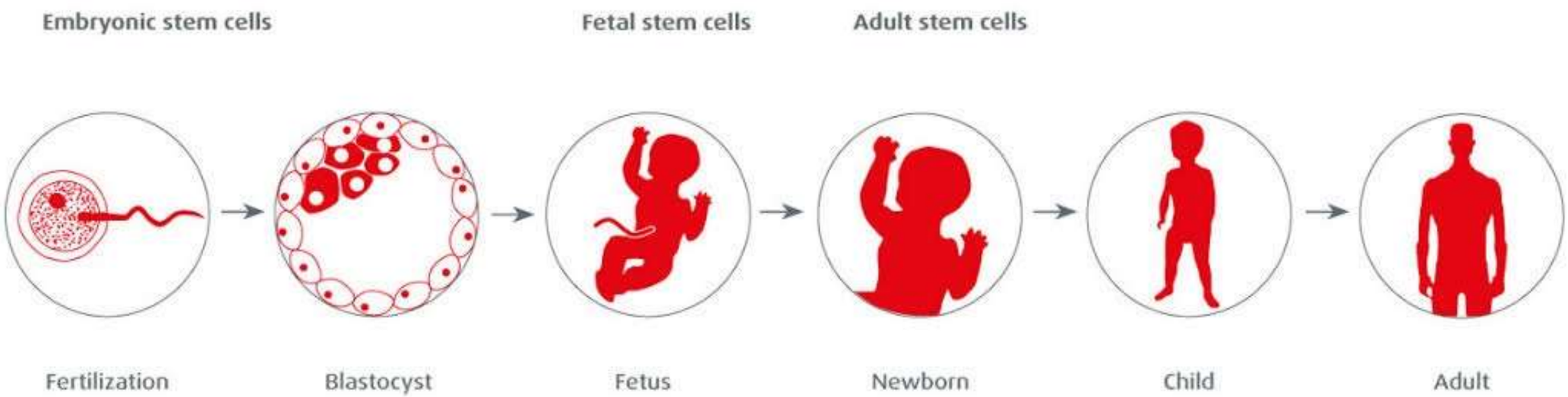
- **Hematopoietic sources :** Bone marrow, peripheral blood, umbilical cord blood.
- **Mesenchymal sources :** Dental pulp, bone marrow, adipose tissue, umbilical cord tissue.

Types of stem cell transplants

- Allogenic stem cell transplant
- Autologous stem cell transplant
- Syngeneic stem cell transplant

Scientists believe that stem cells can be used to treat medical conditions including spinal cord injury, stroke, burns, heart diseases, osteoarthritis, and rheumatoid arthritis.

Scientists also believe stem cells can be used to generate cells and tissues that could be used for cell based therapies as the need for donated organs and tissues outweighs the supply.



What are Stem Cells ?

- Foundation for every organ and tissue in our body.
- Undifferentiated biological cells; found in multicellular organisms.
- Can either divide into specialised cells or divide (through mitosis) to produce more stem cells.
- Unspecialised cells capable of renewing themselves through cell division without limit as long as the person is still alive.

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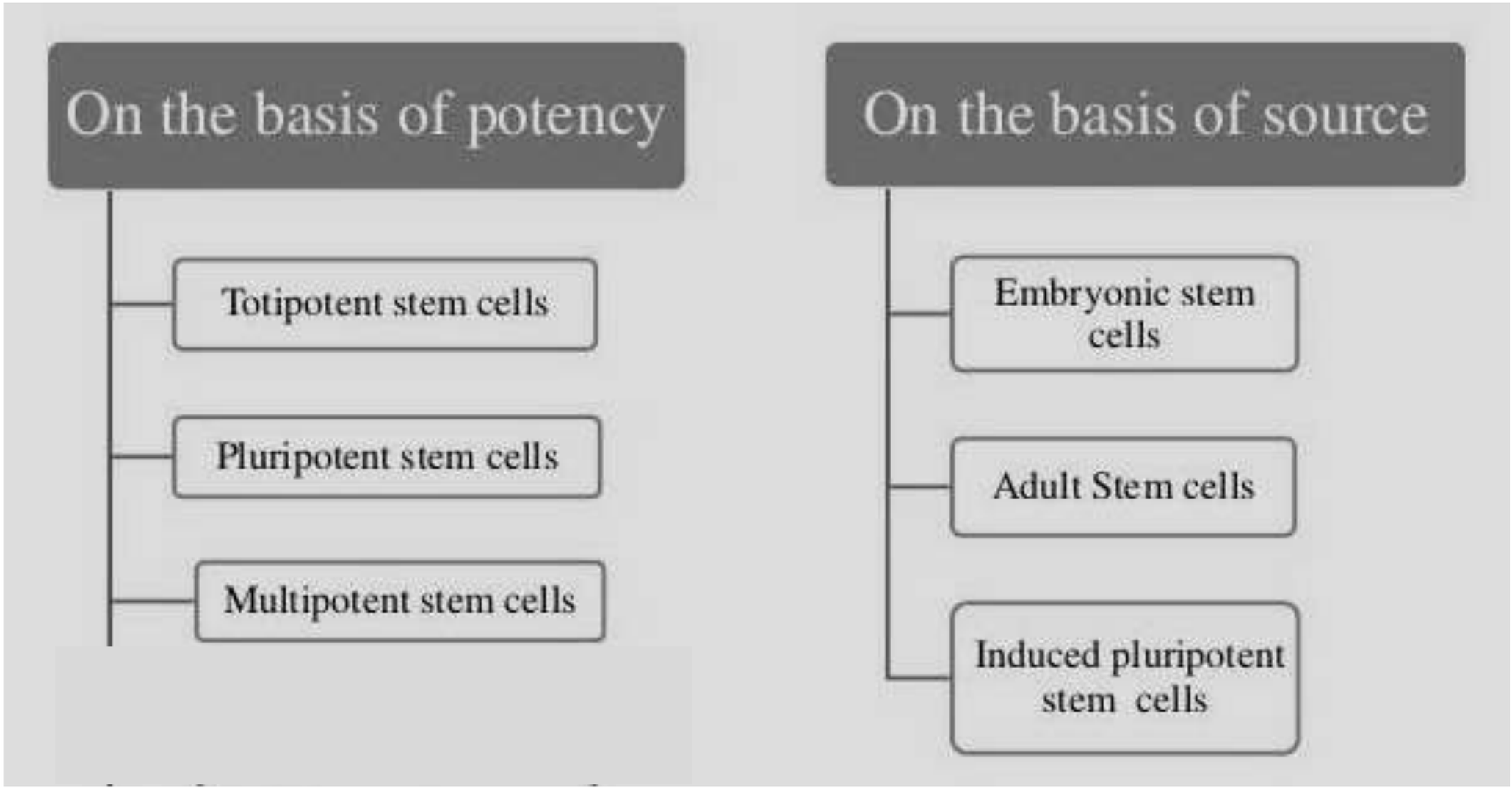
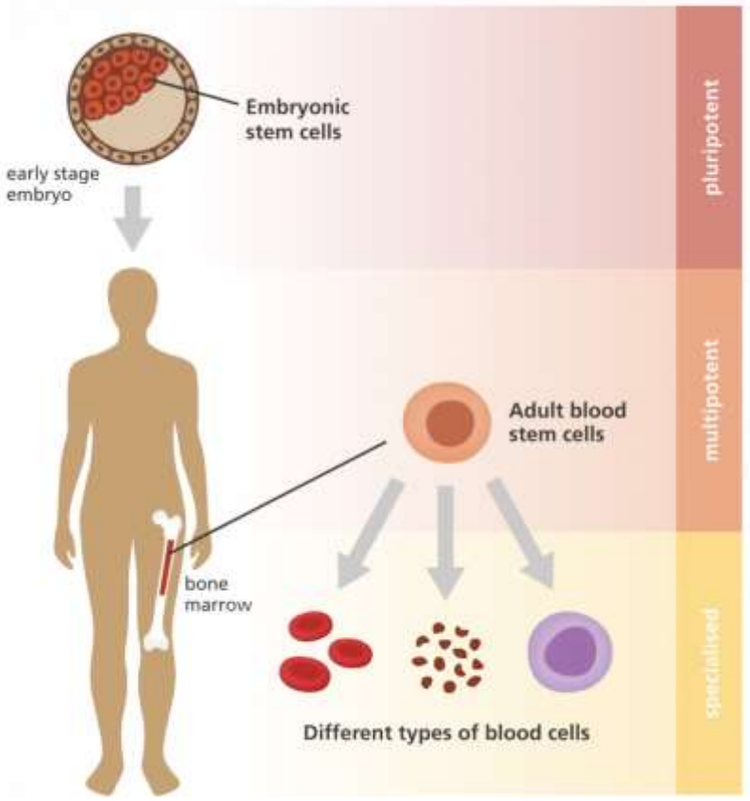
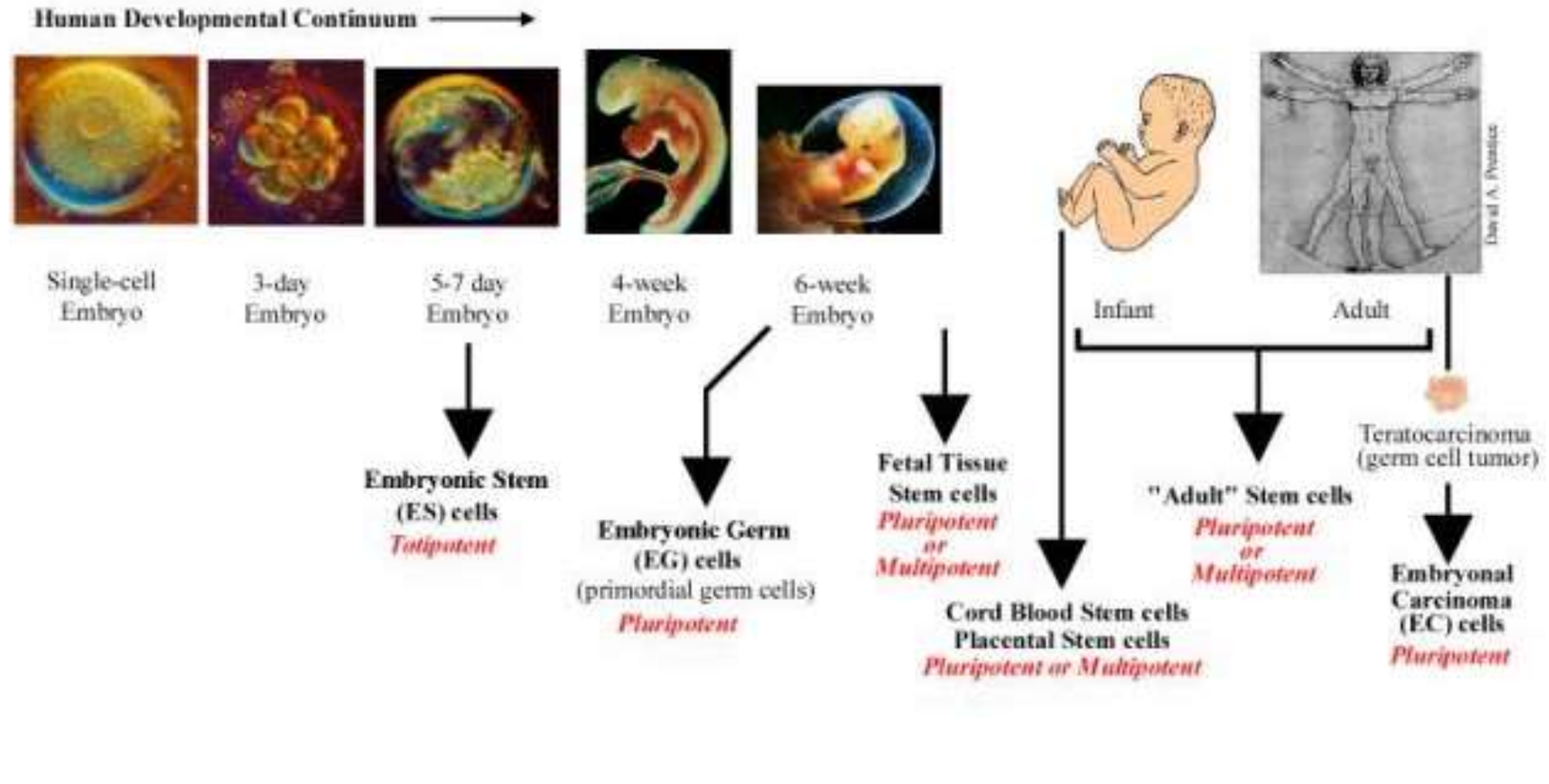
Stem Cell Classification



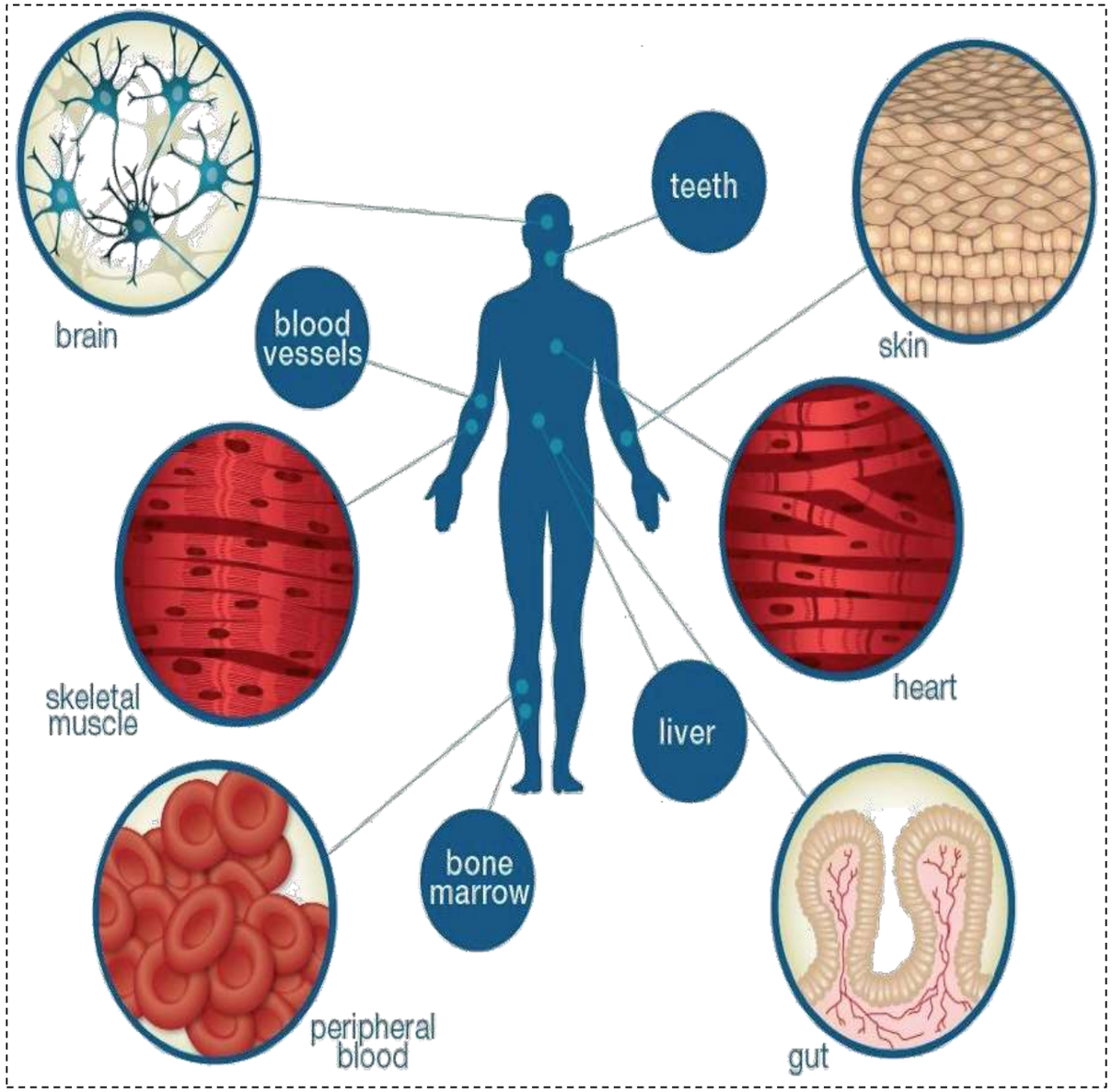
Types of Stem Cells based on Potency



Stem cell type	Description	Examples
Totipotent	Each cell can develop into a new individual	Cells from early (5-7 days) embryos
Pluripotent	Cells can form any (over 200) cell types	Some cells of blastocyst (5 to (6 weeks)
Multipotent	Cells differentiated, but can form a number of other tissues	Fetal tissue, cord blood, and adult stem cells



Adult Tissue Stem Cells



Undifferentiated cells, capable of proliferation, self-renewal, production of a large number of differentiated functional progeny, regenerating tissue after injury.

Have been identified in many organs and tissues, including bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, teeth, gut, liver, ovarian epithelium and testis.

Thought to reside in a specific area of each tissue called the ‘stem cell niche’.

Occur in many tissues and enter normal differentiation pathways to form the specialised cell types of the tissue in which they reside.

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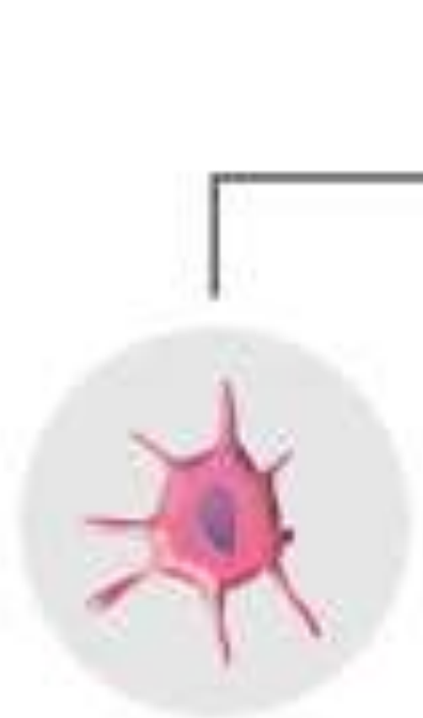
Sources of Stem Cells



Hematopoietic

- Bone Marrow
- Peripheral Blood
- Umbilical Cord Blood

Mesenchymal

- Dental Pulp
- Bone Marrow
- Adipose Tissue
- Umbilical Cord Tissue

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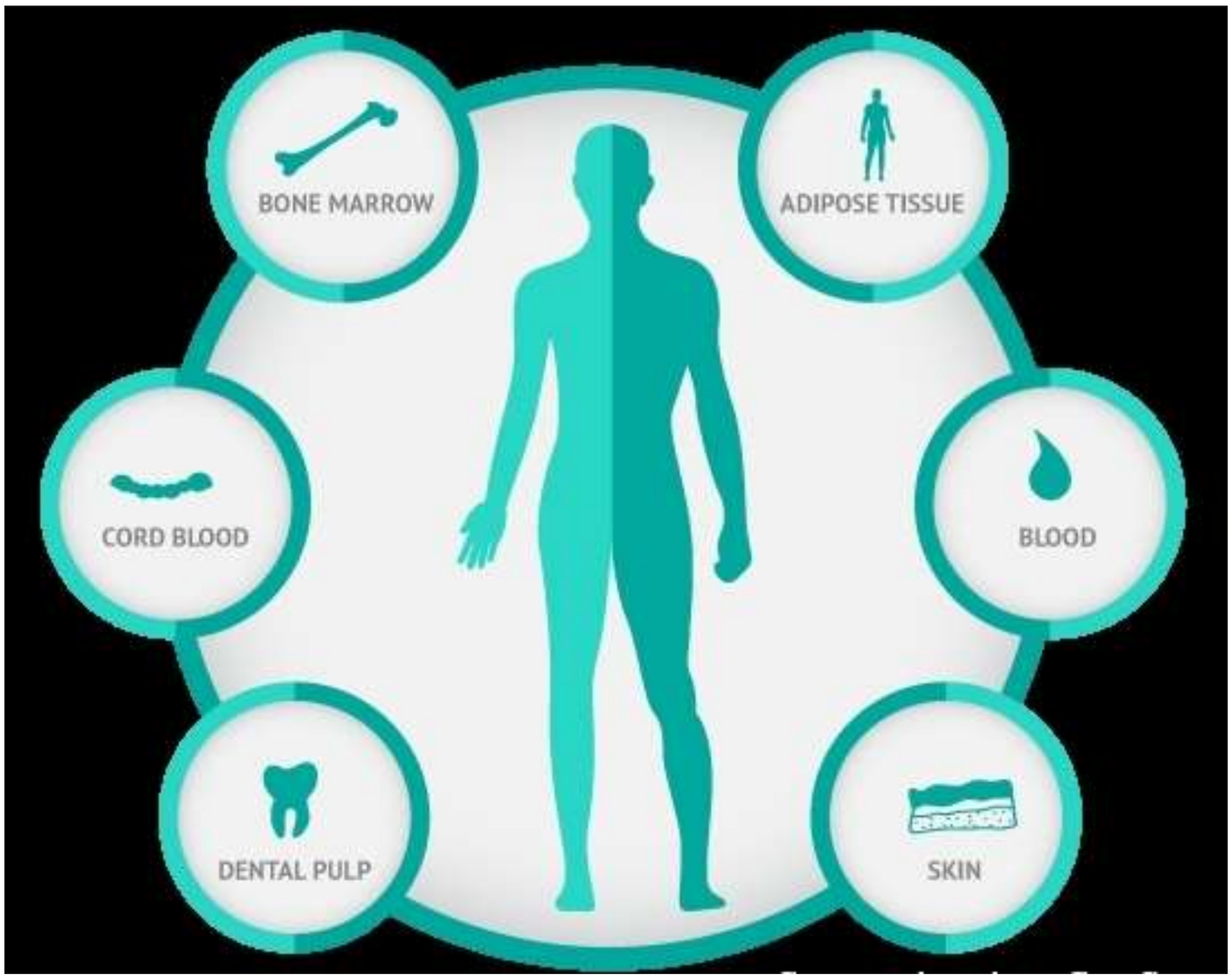
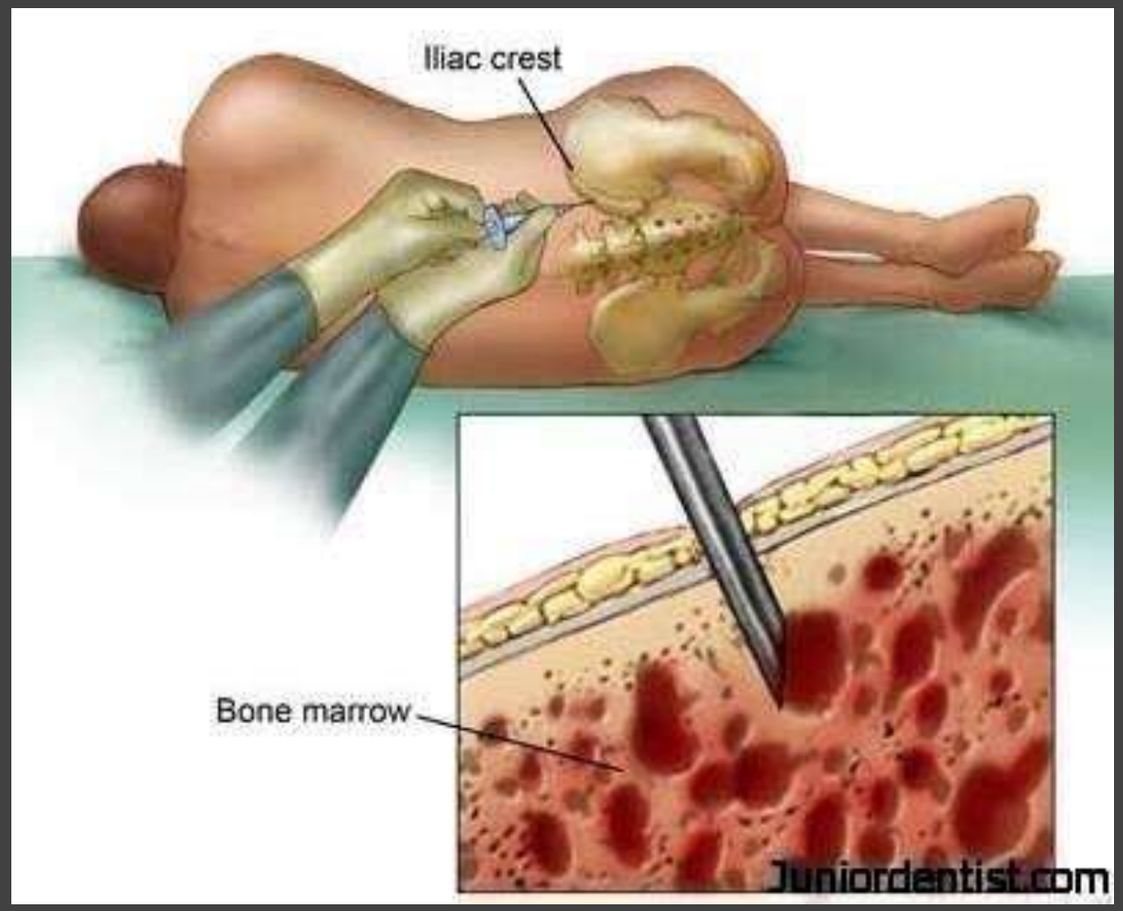
There are three accessible sources of autologous adult stem cells in humans:

- **Bone Marrow**, which requires extraction by harvesting, that is, drilling into the bone (typically the femur or iliac crest).
- **Adipose Tissue** (lipid cells), which requires extraction by liposuction.
- **Peripheral Blood**, which requires extraction through apheresis.



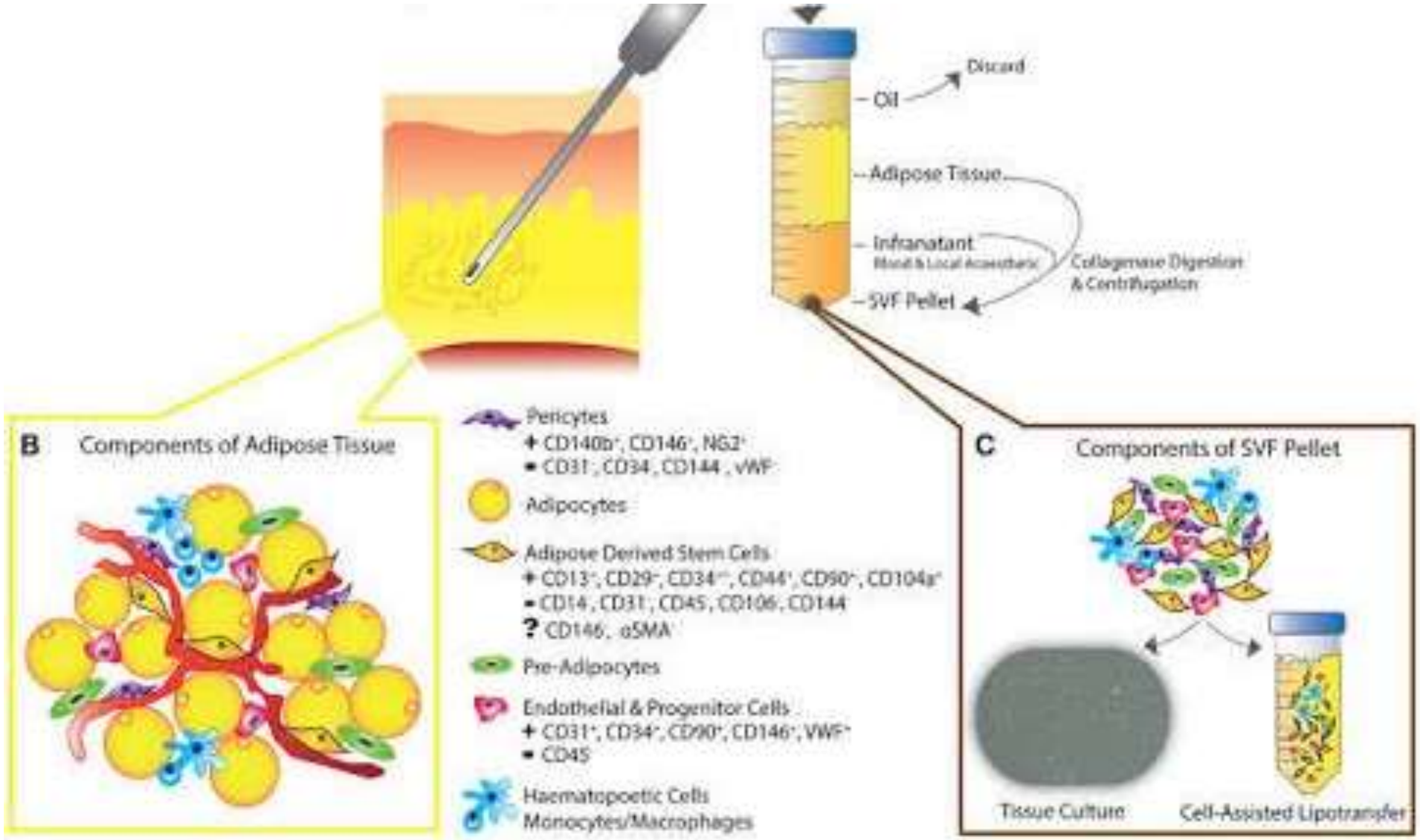
Stem cells can also be taken from umbilical cord blood just after the birth, skin and dental pulp of the patient.

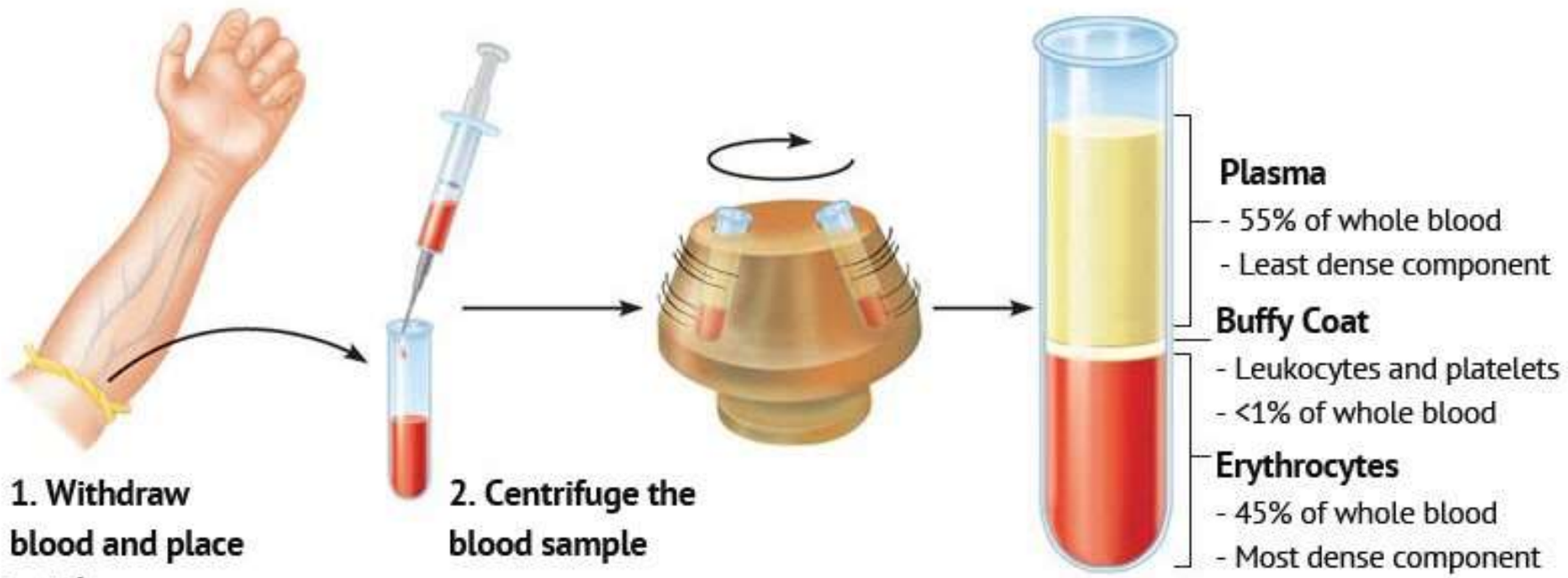


Preparation of Stem Cells

Bone Marrow Derived Stem Cells

Adipose Tissue Derived Stem Cells





Peripheral Blood Derived Stem Cells

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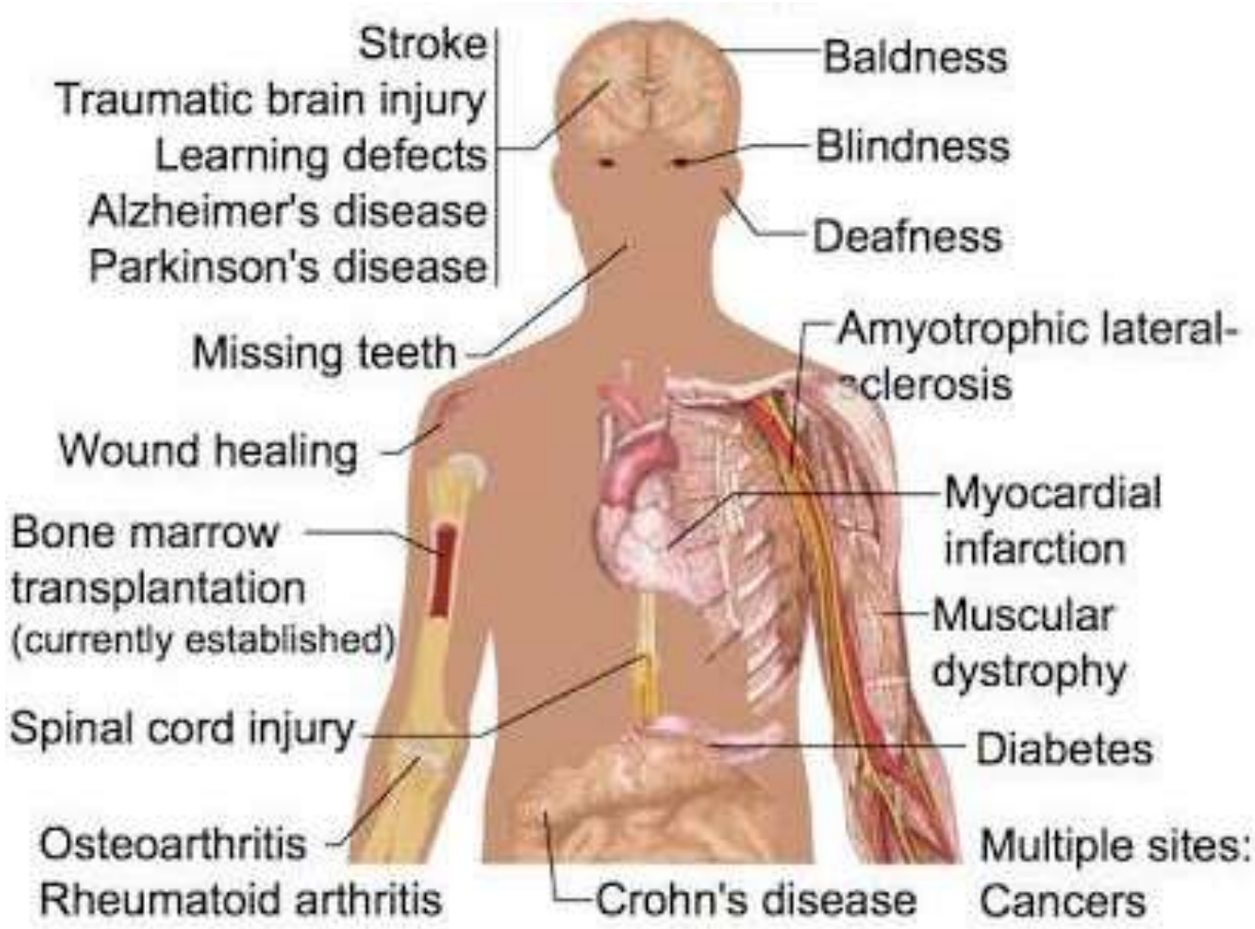
Possible Applications of Stem Cells

- Apart from these applications stem cells have emerged out to be applicable in managing different conditions of infertility both in males as well as females.



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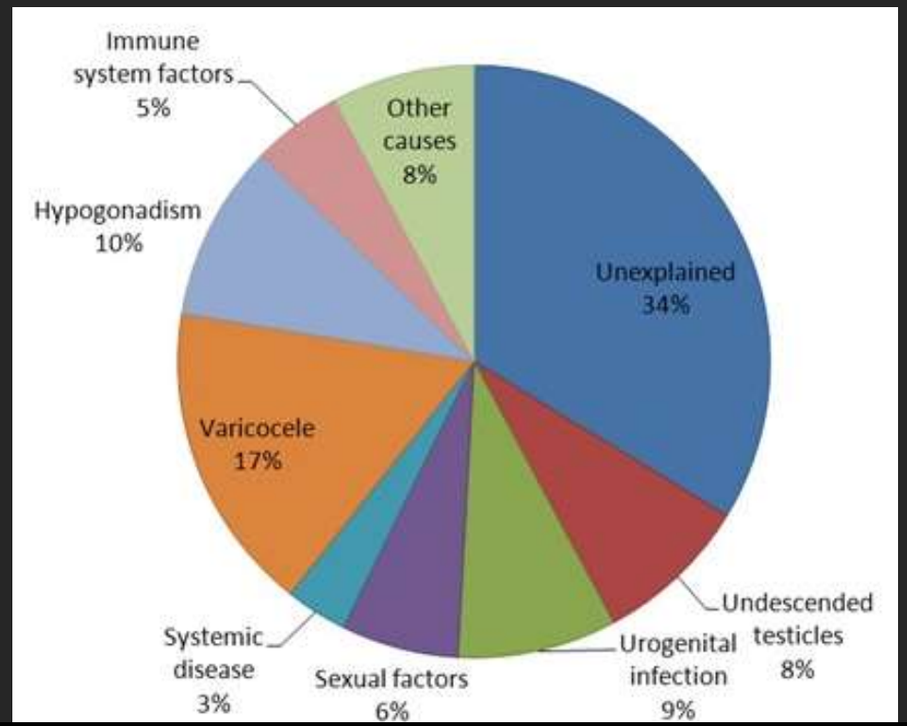
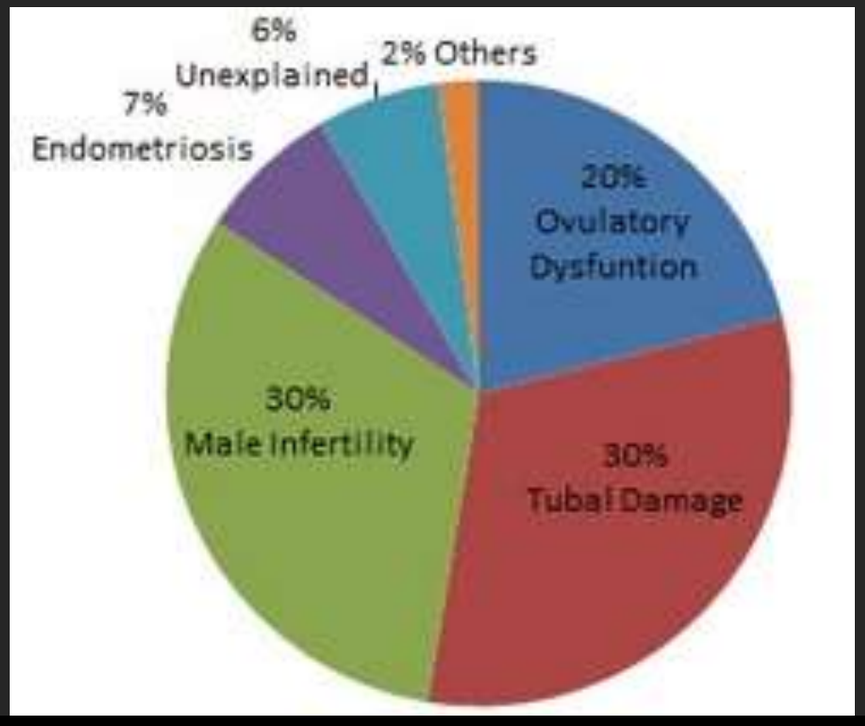
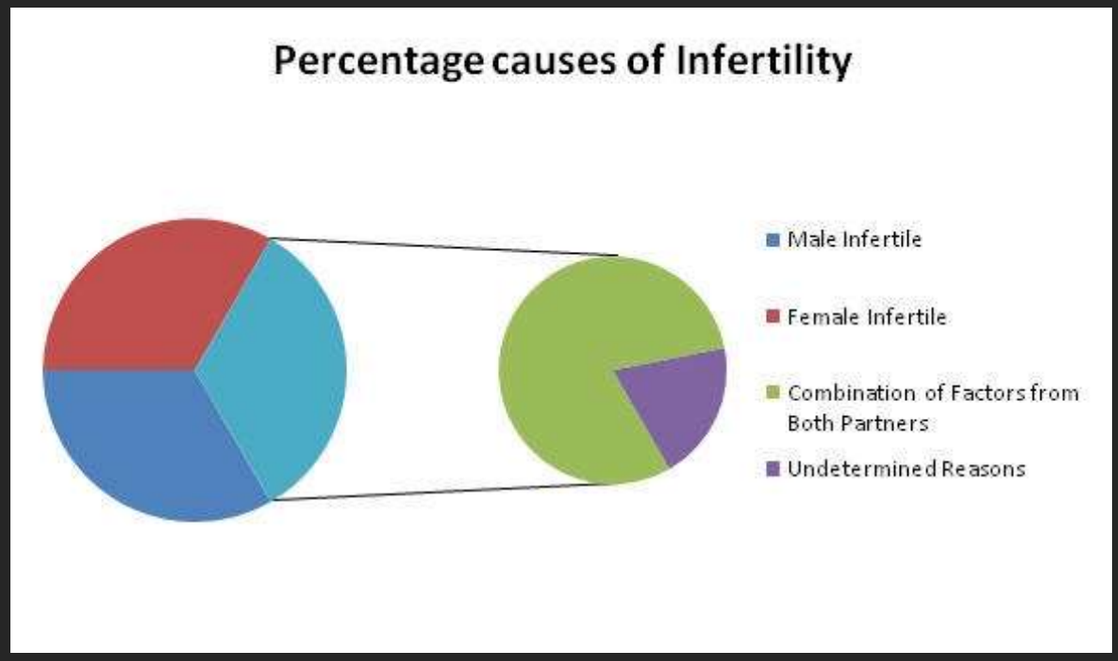


Causes of Male Infertility



Causes of Female Infertility



Stem Cells in Infertility

Conditions that can be managed using stem cells have have been listed below :

Female Infertility

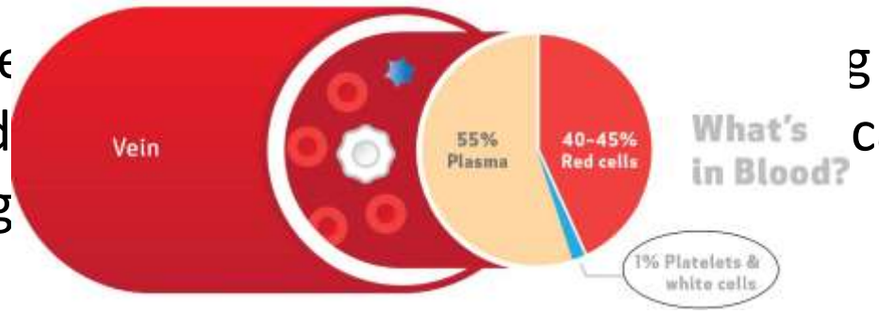
- Thin Endometrium
- Poor Ovarian Reserve or
- Premature Ovarian Failure
- Asherman's Syndrome

Male Infertility

- Oligospermia
- Azoospermia
- Erectile Dysfunction

What is PRP?

- Described as plasma with higher platelet count than that of peripheral blood
- Platelet-Rich Plasma (PRP) is also commonly termed as PRF (Platelet rich Fibrin matrix), GF-s (Platelet- rich growth factors), and platelet concentrate
- Over the years, the field has witnessed the use of PRP widely for tissue regeneration, skin rejuvenation, scars and wound healing, and for alopecia treatment owing to its property to kick start healing cascade
- With the remarkable succe g properties, the medical fields that use PRP are orthoped cardiac surgery, ophthalmology, plastic surgery, and urolog



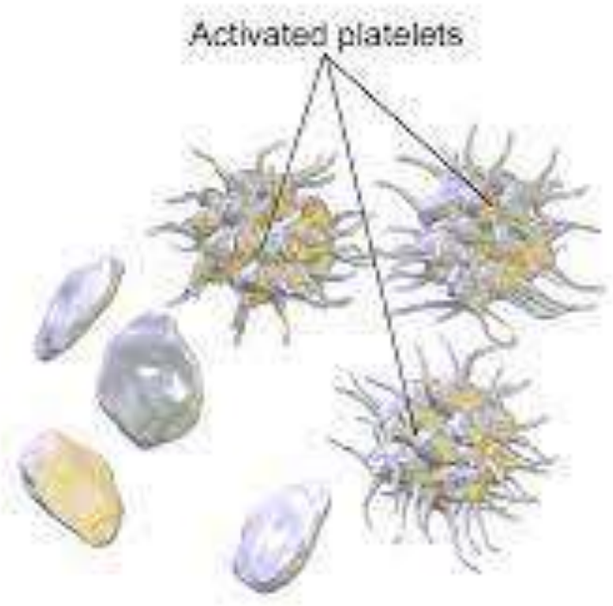
Understanding PRP

- Platelets are tiny blood cells called thrombocytes which are made in bone marrow

- Cytoplasmic fragments of megakaryocytes
  - Approx. 2 µm in diameter
  - Contain more than 30 bioactive proteins

- Platelets are known to be rich in cytokines, coagulation factors, adhesion molecules, immunologic molecules, and regulators of growth and angiogenesis which have peculiar roles in inflammatory processes, coagulation and immunity modulation.

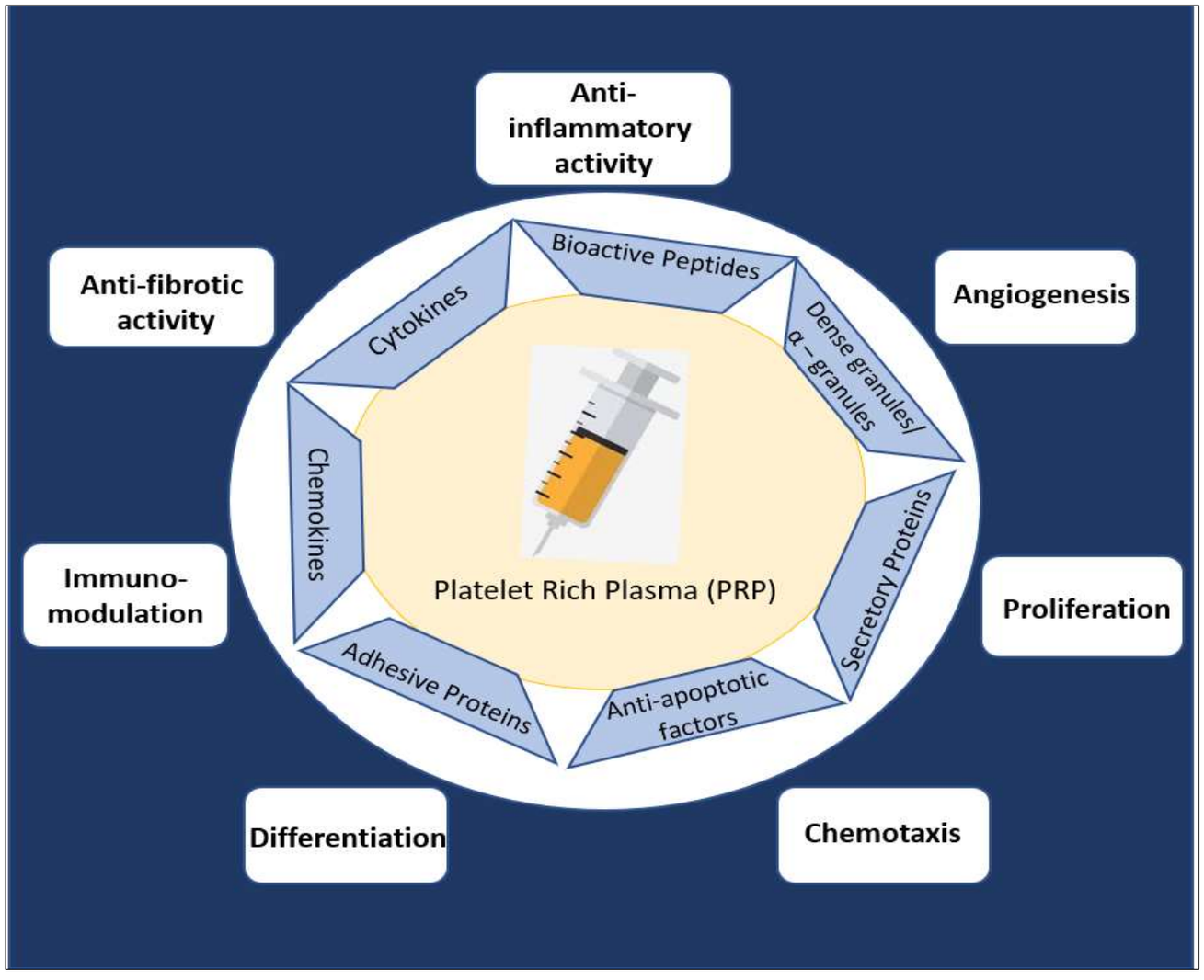
- They promote angiogenesis, tissue remodelling, and aid in wound healing process.



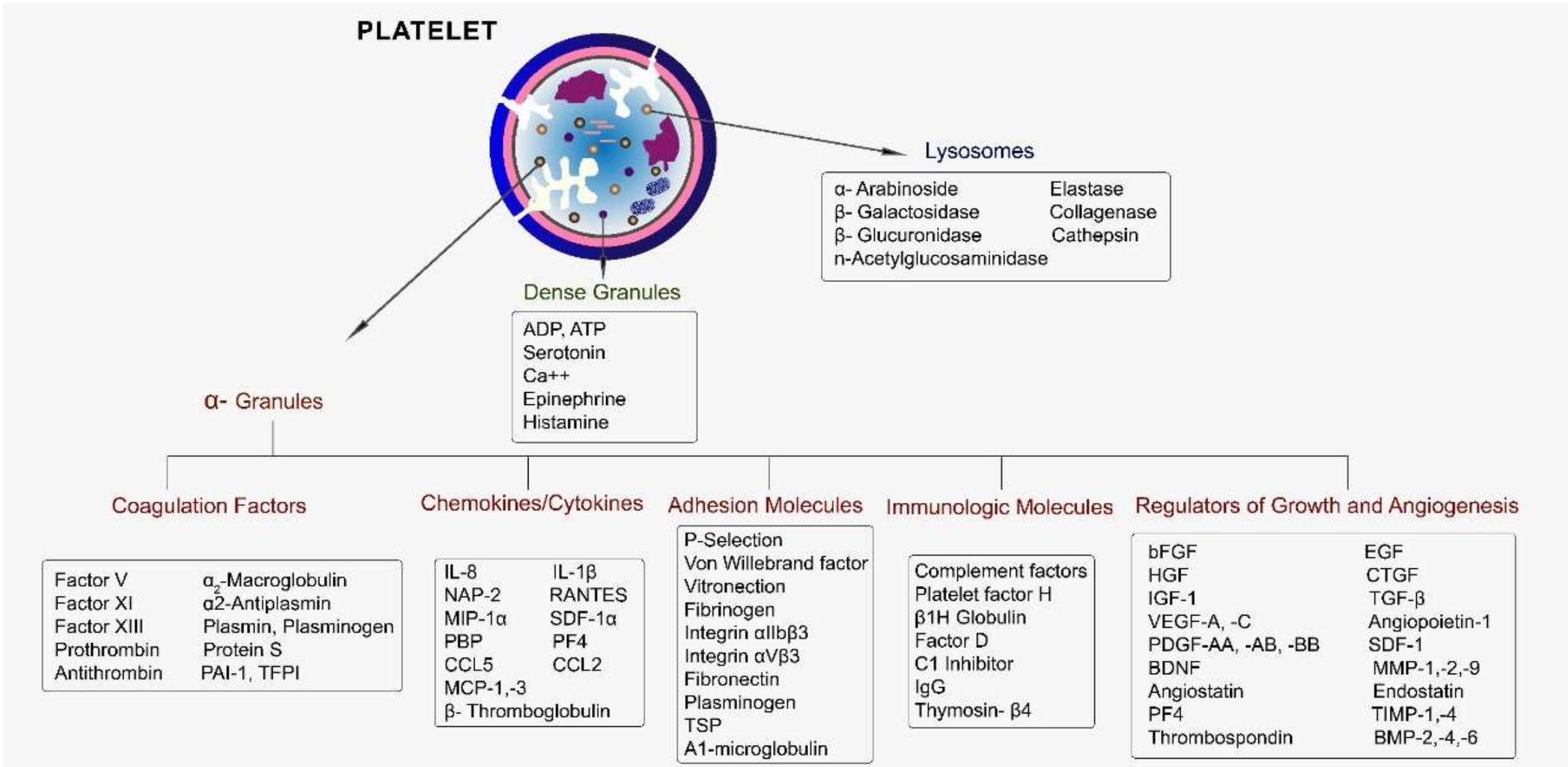
Platelets

Understanding PRP

What is PRP composed of and which pathways it stimulates?



Detailed composition of Platelets



Key Growth factors

EGF
(Epidermal Growth Factor)
Promotion of epithelial cell growth, angioegenesis, promotion of wound healing

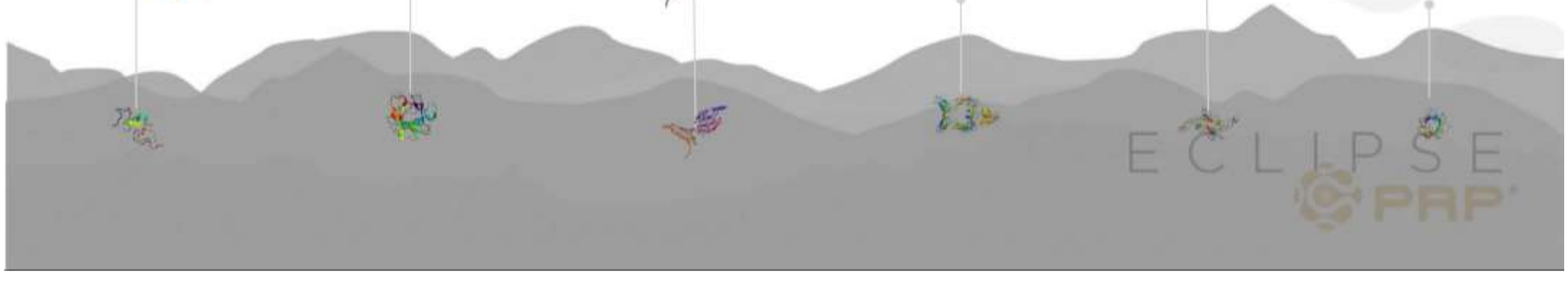
FGF
(Fibroblast Growth Factor)
Tissue repair, cell growth, collagen production

PDGF
(Platelet Derived Growth Factor)
Cell growth, new generation and repair of blood vessels, collagen production

VEGF
(Vascular Endothelial Growth Factor)
Growth and new generation of vascular endothelial cells

IGF
(Insulin-like Growth Factor)
Protective for cartilage cells and activates osteocytes

TGF-β1
(Transforming Growth Factor)
Induces cell apoptosis



Functions of Growth Factors

PDGF- Platelet-derived growth factor:

1. Chemotactic for fibroblasts and macrophages
2. Mitogenic for fibroblasts, smooth muscle cells and endothelial cells

TGF*- β1, β2 -(Transforming growth factor)

1. Chemotactic for fibroblasts, keratinocytes and macrophages
2. Mitogenic for fibroblasts and smooth muscle cells

Inhibits endothelial cells, keratinocytes and lymphocytes

3. Regulates matrix proteins, including collagen,

VEGF - Vascular endothelial growth factor

1. Chemotactic and mitogenic for endothelial cells
2. Mediates angiogenesis

EGF - Epidermal growth factor

1. Mediates angiogenesis Mitogenic for fibroblasts, endothelial cells and keratinocytes

HGF - Hepatocyte growth factor

1. Mediates regeneration

FGF - Fibroblast growth factor

1. Mediates tissue organization and regeneration
2. FGF-9 Aids generation of new follicles

PRP activation

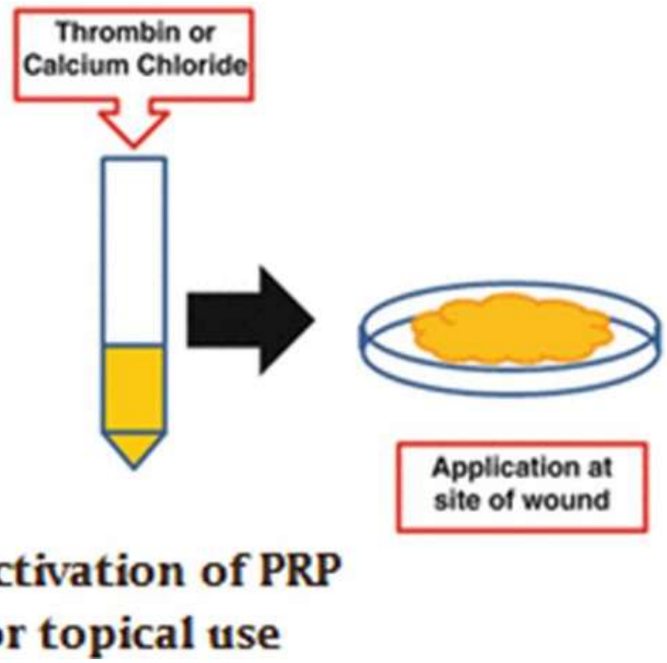
- Can be activated exogenously by thrombin, calcium chloride or mechanical trauma

- **Collagen is a natural activator of PRP**

– Thus, when PRP is used in soft tissue, it does not need to be exogenously activated

- After activation secretion of growth factors begins within 10 min

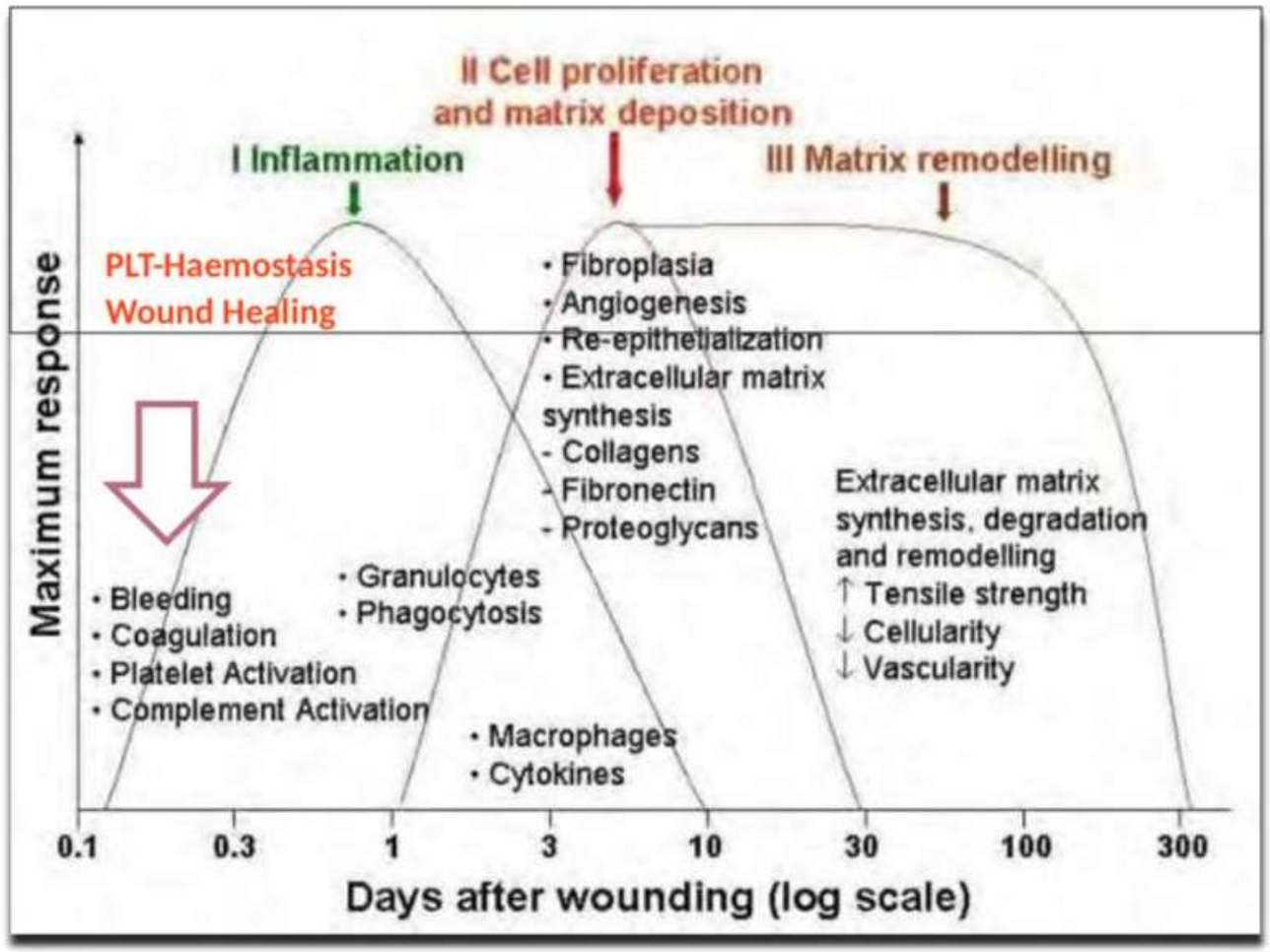
- More than 95% of pre-synthesized growth factors secreted within 1 h



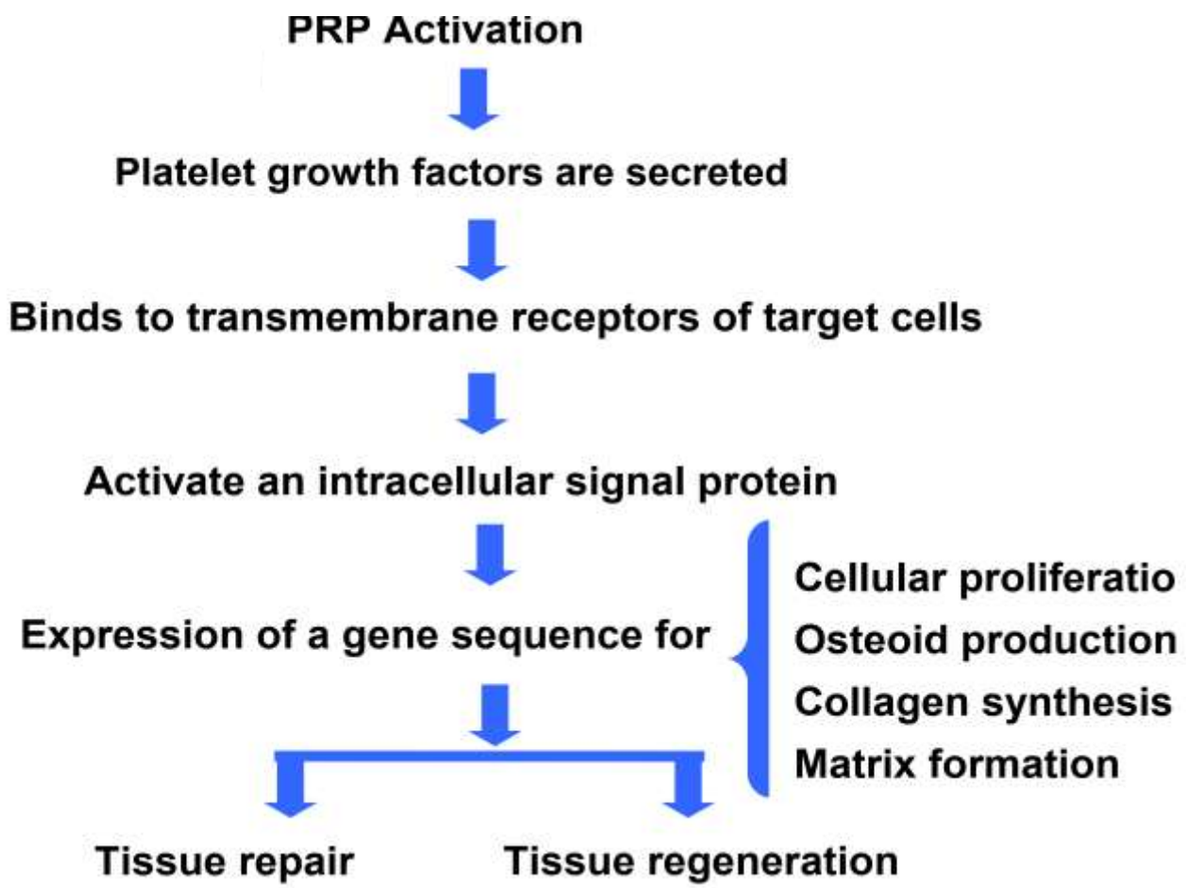
Activation of PRP
for topical use

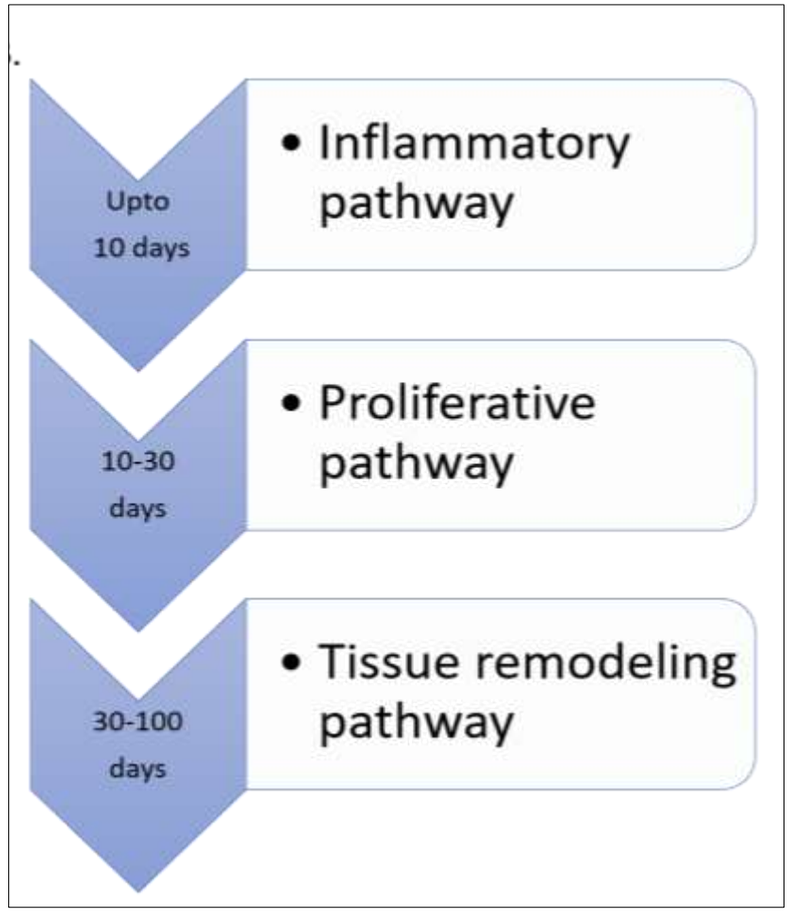
Mechanism of Action

HOW PRP WORKS?



Mechanism of Action





Classification of PRP

Depending on cell content and fibrin architecture

1. Pure Platelet-Rich Plasma (P-PRP) or leucocyte-poor PRP

- Without leucocytes and with a low-density fibrin network after activation

2. Leucocyte- and PRP (L-PRP) products

- With leucocytes and with a low-density fibrin network after activation
- Largest number of commercial or experimental systems exist in this family

3. Pure platelet-rich fibrin (P-PRF) or leucocyte-poor plateletrich fibrin preparations

- Without leucocytes and with a high-density fibrin network
- Only exist in a strongly activated gel form
- Cannot be injected or used like traditional fibrin glues

4. Leucocyte- and platelet-rich fibrin (L-PRF) or secondgeneration PRP products

Table 1. Platelet-containing preparations, as classified by Dohan Ehrenfest et al. [8]

Preparation	Acronym	Leukocytes	Fibrin density
Pure platelet-rich plasma	P-PRP	Poor	Low
Leukocyte- and platelet-rich plasma	L-PRP	Rich	Low
Pure platelet-rich fibrin	P-PRF	Poor	High
Leukocyte- and platelet-rich fibrin	L-PRF	Rich	High

Preparation of PRP

Draw whole blood (40-75ml) by venipuncture

↓

Collect in 15ml conical tubes containing anticoagulant (ACD-A sol, 10:1.5)

↓

Centrifuge at 160 g for 10 minutes at 20°C

↓

→Plasma

→Buffy coat (thin white layer containing WBCs and platelets)

→RBC layer

Aspirate plasma + buffy coat + superficial RBC layer

↓

Centrifuge at 400 g for 10 minutes at 20°C

↓

→PPP

→Platelet rich pellet

→Residual RBC's

↓

Reduce PPP to roughly ¼ volume

↓

Resuspend

↓

L-PRP (Leucocyte-and platelet-rich plasma)

Aspirate plasma + buffy coat

↓

Centrifuge at 400 g for 10 minutes at 20°C

↓

→ PPP

→ Platelet rich pellet

↓

Reduce PPP to roughly ¼ volume

↓

Resuspend

↓

P-PRP (Pure platelet-rich plasma)

↓

Mix PRP and "activator" (10% CaCl2) → "Activated PRP"

Factors influencing PRP yield

1. Draw of blood

- Clotting process is influenced from the time of the draw
- To avoid unintentional activation of platelets, most protocols use large bore needles (>22) to draw the blood

- **There was a downward trend in platelet counts with longer draw time**

(According to a study, which used two cell-salvage devices and two table top devices over the course of 260 clinical
cases)

Factors influencing PRP yield

2. Centrifugation

- To accelerate sedimentation, the effect of gravity is amplified using ‘centrifugal force’
  - Provided by a centrifuge
  - Can be many thousand times the force of gravity

Relative centrifugal field

- In centrifugation, RCF is the force required to separate two phases

Factors influencing PRP yield

3. Temperature

- Temperature during processing is crucial to prevent platelet activation

- 21°C-24°C for centrifugation of blood for obtaining PRP is recommended

- Cooling may retard platelet activation

- Temperature level of 12°C-16°C during centrifugation has been used by many investigators for best platelet recovery

Those who use an ordinary centrifuge to develop PRP, which are mainly developed for diagnostic purposes and not for PRP processing and hence may not produce a sufficient platelet yield

Factors influencing PRP yield

4. Anticoagulants

• Choosing an anticoagulant capable of preserving the platelets' best possible functionality, integrity, and morphology is important

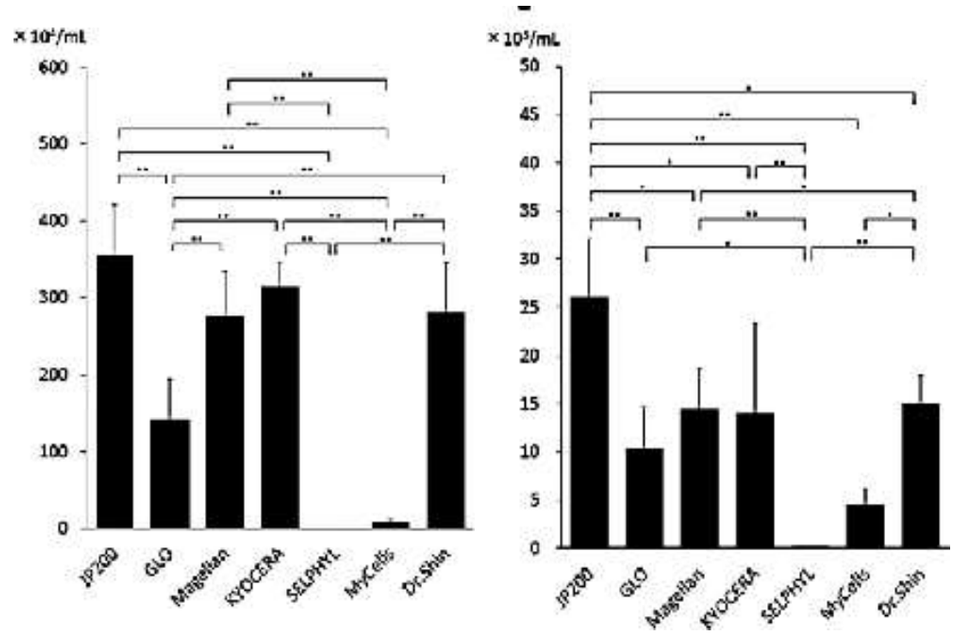
• Most authors agree on not using EDTA because it could damage the platelet membrane

• Anticoagulants with citrate and dextrose of sodium citrate are recommended

– ACD-A is the choice for collection of platelets by apheresis
– Trisodium citrate (3.2% or 3.8%) is the anticoagulant most commonly used for diagnostic evaluations of platelets

Performance Evaluation (1)

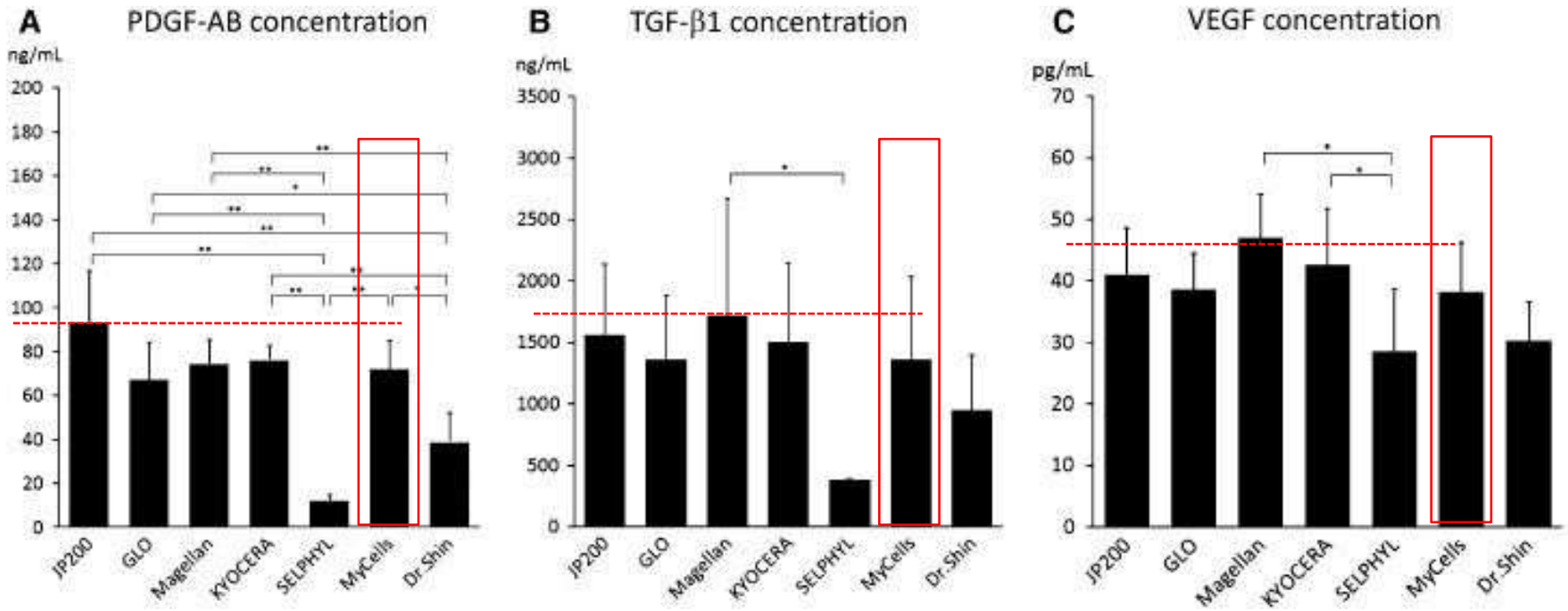
Tropocells™ PRP – 2ml	
Platelets concentration fold	X 4 - 5
RBC (10^6/ul)	0.0
WBC (10^3/ul	0.2
Granulocytes %	8.5
Mononuclear cells %	86.2
PDGF (pg/ml)	2048
VEGF (pg/ml)	220
EGF (pg/ml)	269



* “*Mycells*” , “*Tropocells*” and “Cellenis “ are commercial names of Estar’s PRP Systems.

Platelet and growth factor concentrations in activated platelet-rich plasma: a comparison of seven commercial separation systems Kushida S. et al J Artif Organs 2014

Performance Evaluation (1)



Platelet and growth factor concentrations in activated platelet-rich plasma: a comparison of seven commercial separation systems Kushida S. et al J Artif Organs 2014

Safety of PRP

- Being an autologous preparation, PRP is devoid of any serious adverse effects
- However, local injection site reactions like pain or secondary infection may occur
  - Can be avoided with proper precautions
- PRP has no issues regarding transmission of infections su
HIV

Overview of PRP applications

Therapeutic applications

Gynaecological conditions:
1. Cervical ectopy
2. Vulvar dystrophy
3. Reconstructive surgery of vulvar cancer

Reproductive Medicine:
1. Premature ovarian failure
2. Ovarian torsion
3. Refractory endometrium
4. Implantation failure

Urogenital disorders:
1. Genital fistula
2. Genital prolapse
3. Urinary incontinence

Orthopaedics/sports medicine:
Relieve pain in
1. Tendinitis,
2. Arthritis,
3. Ligament tears and

Aesthetic applications

Reconstructive surgery:
1. Breast reconstruction
2. Vulvar reconstruction

Gynaecological:
1. Female sexual dysfunction
2. Vulvovaginal rejuvenation
3. O shot therapy

Dermatological conditions:
1. Alopecia
2. Scars
3. Chronic non healing ulcers

PRP in Gynaecology

O-Cell ® procedure for PRP preparation

1. Venous blood (15–50 mL) is drawn from the patient's arm in anticoagulant-containing tubes;
2. The recommended temperature during processing is 21˚C–24˚C to prevent platelet activation during centrifugation of the blood;
3. The blood is centrifuged at 1,800 rpm for 12 minutes;
4. The blood separates into three layers: an upper layer that contains platelets and white blood cells, an intermediate thin layer (the buffy coat) that is rich in white blood cells, and a bottom layer that contains red blood cells; (Figure 2)
5. The upper and intermediate buffy layers are transferred to an empty sterile tube. The plasma is centrifuged again at 3,200 rpm for 6 minutes to help with the formation of soft pellets (erythrocytes and platelets) at the bottom of the tube;
6. The upper two-thirds of the plasma is discarded because it is platelet-poor plasma;
7. Pellets are homogenized in the lower third (5 mL) of the plasma to create the PRP;
8. The PRP is now ready for injection. Approximately 30 mL of venous blood yields 3–5 mL of PRP;

Mishra et al 2021

9. We then inject the prepared PRP either laproscopically (2.5cc in each ovary) at multiple site but preferably at cortex stromal site and sometimes in medulla of ovary in case of ovarian atrophy.

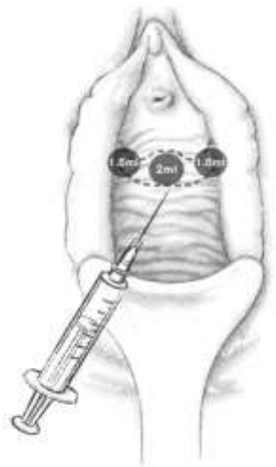
- topical anesthetic cream (23% lidocaine base, 3.5% tetracaine base, 3.5% tetracaine or 10-20% lidocaine cream) is applied to the anterior vaginal wall, around urethra and over clitoris before PrP injections
- 4-5 ml A-PRP, suburethral injections (1cc), skene glands (1cc each) and clitoris bilateral (1cc each side),
- use 1 ml syringe, 27-31G needle
- 2 treatment sessions, 1 month apart.
- touch up every 12-18 months (or as requested)

[Runels C, Melnick H, Debourbon E, Roy L. A pilot study of the effect of localized injections of autologous platelet rich plasma (PRP) for the treatment of female sexual dysfunction. J Womens Health Care. 2014;3:169]

Other techinque:
The patients are in the dorsal lithotomy position with empty bladder. The anesthetic cream is applied half an hour before the procedure (lidocaine 2.5% and prilocaine 2.5%). The cream is administered on top of the clitoris and on the lower one-third of the vagina. PRP is administered to form pili of 4 cc around the clitoris in the direction of clock positions of 12, 3, 6, and 9, each with 1 cc, 2 cc subcutaneously; right/left of paraurethral vaginal wall each with 1 cc and mid-urethral midline/right/left 1 cc. The PRP applications is administered using 31-G needles [Gökmen Sukgen, et al Platelet-rich plasma administration to the lower anterior vaginal wall to improve female sexuality satisfaction Turk J Obstet Gynecol. 2019 Dec; 16(4): 228–234]

SUI

- topical anesthetic cream (23% lidocaine base, 3.5% tetracaine base, 3.5% tetracaine or 10-20% lidocaine cream) is applied to the anterior vaginal wall and around urethra
- 4-5 ml A-PRP,
- use 1-3 ml syringe, 27-31G needle
- 2 treatment sessions, 1 month apart.
- touch up every 12-18 months (or as requested)



With a 27-31-gauge needle, PRP is injected into the anterior vaginal mucosa around mid-urethra, which is approximately 1 cm below the urethra meatus with a depth about 15 mm. 2 mL is injected underneath mid-urethra and 1.5 mL for each side of urethra (Fig.1). No anesthesia is needed in this procedure, but is possible. The treatment is reapeted 3 times in 1 month apart.

skin wound healing

<u>cesarean sections</u>

- anesthesia is not necessary
- 4-5 ml A-PRP,
- use 3-5 ml syringe, 27-31G needle
- 1 treatment sessions,

It was applied by Tehranian et al. [Tehranian A, et al. Application of autologous platelet-rich plasma (PRP) on wound healing after caesarean section in high-risk patients. Iran. Red Crescent Med. J. 2016;18:e34449.] in wound healing in high-risk women undergoing cesarean sections. After closure of the fascia and prior to skin closure, PRP is directly applied to the subcutaneous tissue of the wound site by using a sterile syringe. both inject into the tissue with a needle or pour over the tissue is possible. The same technique author is using during labioplasty and perineoplasty. 5 ml of plasma is injected into the tissue just before it is closed and sutured.
Another authors found that autologous platelet grafts applied in gynecological surgery were effective for pain reduction and were not associated with any adverse effects [Fanning J, Murrain L, Flora R, Hutchings T, Johnson JM, Fenton BW. Phase I/II prospective trial of autologous platelet tissue graft in gynecologic surgery. J Minim Invasive Gynecol. 2007;14:633–637].

vulvar dystrophy

- topical anesthetic cream (23% lidocaine base, 3.5% tetracaine base, 3.5% tetracaine or 10-20% lidocaine cream) is applied to the diseased area
- 4-5 ml A-PRP,
- use 1-3 ml syringe, 27-31G needle
- 2 treatment sessions, 1 month apart.
- touch up every 12-18 months (or as requested)

PRP is injected into the vulva in a fanning pattern. Punctual or retrograde administration of plasma is also possible. Patients are receiving three PRP treatments 4 to 6 weeks apart and again at 12 months. [Behnia-Willison F, Pour NR, Mohamadi B, Willison N, Rock M, Holten IW, et al. Use of platelet-rich plasma for vulvovaginal autoimmune conditions like lichen sclerosus. Plast Reconstr Surg Glob Open. 2016;4:e1124.]

Vaginal rejuvenation, dryness, GSM symptoms

-topical anesthetic cream (23% lidocaine base, 3.5% tetracaine base, 3.5% tetracaine or 10-20% lidocaine cream) is applied to the walls of vagina before PrP injections
-4-5 ml A-PRP into vaginal mucosa
- use 1 ml syringe, 27-31G needle
- 2 treatment sessions, 1 month apart.
- touch up every 12-18 months (or as requested)

Treatment is carried out using a speculum. After the speculum has been inserted into the vagina using the mesotherapy technique, approximately 5 ml of plasma are injected into the anterior and posterior walls of the vagina. Prp is administering papules of 0,1cc volume with 1,5centimeter intervals.

Dysuria, Recurrent urinary infections

-topical anesthetic cream (23% lidocaine base, 3.5% tetracaine base, 3.5% tetracaine or 10-20% lidocaine cream) is applied to the treated area before PrP injections

-4-5 ml A-PRP suburethral injections and posterior and anterior wall of vagina

- use 1 ml syringe, 27-31G needle
- 1 treatment sessions
- touch up every 6-12 months (or as requested)

We do not have publication but author is doing injection of 1 cc under the urethra and 4-5cc

genital fistulae

- topical anesthetic cream (23% lidocaine base, 3.5% tetracaine base, 3.5% tetracaine or 10-20% lidocaine cream)
- 4-5 ml A-PRP,
- use 1-3 ml syringe, 27-31G needle
- 1 treatment sessions or as requested

PRP is injected around the fistula into the tissue, and platelet-rich fibrin (PRF) glue was interpositioned in the tract.

genital prolapse

- patient is usually under general anesthesia
- 4-5 ml A-PRP,
- use 1-3 ml syringe, 27-31G needle
- 1 treatment sessions after operation or mesh implementation

Prp is injected into operated site or the site of mesh implementation [Gorlero F, Glorio M, Lorenzi P, Bruno-Franco M

Post-natal rehabilitation, scars after episiotomy

-topical anesthetic cream (23% lidocaine base, 3.5% tetracaine base, 3.5% tetracaine or 10-20% lidocaine cream) is applied to the treated area before PrP injections
-4-5 ml A-PRP deep injections point by point, underneath the scar
- use 1 ml syringe, 27-31G needle
- 1 treatment sessions or as requested

Usually is a nappage around the scars (transdermal injections + multiple superficial injections in the papillary dermis). But also we do deep injections point by point, underneath the scar. If the scar is drawn, it is worth cutting it with a needle beforehand [Osaid H Alser 1, Ioannis Goutos The evidence behind the use of platelet-rich plasma (PRP) in scar management: a literature review Scars Burn Heal 2018 Nov 18;4:2059513118808773]

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Thank you so much for your Attention

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PRP in Gynaecology

PRP for Ovarian Rejuvenation

- PRP therapy is an emerging promising treatment in the reproductive context as it enhances the development of primordial and primary preantral follicles.
- PRP has not only been proven to prevent possible ischemia following ovarian injury but it is explicitly clear that the use of PRP to target various issues regarding the reproductive system is highly positive and beneficial.
- According to a report by Sfakianoudis et al in 2019, autologous PRP application lead to pregnancy in menopause for a woman aged 40 and diagnosed with premature menopause.
- The patient chose PRP therapy with the aim to rejuvenate the ovarian tissue to enable the employment of her own gametes through IVF.
- The results showed a significant reduction in FSH levels after six weeks following the autologous PRP treatment.

PRP in Gynaecology

PRP for Ovarian Rejuvenation

- Melo et al in 2020 studied the impact of intracortical injections of autologous PRP on ovarian reserve markers in women observed with low ovarian reserve before undergoing ART.

- 83 women were included, of which 46 received PRP treatment and 37 underwent no intervention. Overall median age was 41 years (IQR 39–44).

- At the 3-month follow-up, women treated with PRP experienced a significant improvement in FSH, AMH and AFC, whereas there was no change in the control group.

- Furthermore, overall rates of biochemical (26.1% versus 5.4%, *P* = 0.02) and clinical pregnancy (23.9% versus 5.4%, *P* = 0.03) were higher in the PRP group, while there was no difference in the rates of first trimester miscarriage and live birth between groups.

- A significant improvement in the levels of FSH, AFC, and AMH was seen with no change in the control group. The group concluded that PRP injections improved these markers of low ovarian reserve and the therapy was safe and effective

PRP in Gynaecology

PRP for Ovarian Rejuvenation

- Sfakianoudis et al in 2020 aimed to provide pilot data regarding PRP application for ovarian rejuvenation.

- Four pilot studies were conducted on poor ovarian response (POR), premature ovarian insufficiency (POI), perimenopause, and menopause, respectively.

- Each pilot study reports on thirty patients, 120 participants were recruited in total.

- All participants provided written informed consent prior to treatment. Primary outcome measures for the POR pilot study were levels of anti-müllerian hormone (AMH), antral follicle count (AFC) and oocyte yield.

- For the POI, perimenopausal and menopausal pilot studies primary outcome measures were restoration of menstrual cycle, and Follicle Stimulating Hormone (FSH) levels.

PRP in Gynaecology

PRP for Ovarian Rejuvenation

- A significant improvement on the hormonal profile and the ovarian reserve status was noted, along with improved intracytoplasmic sperm injection (ICSI) cycle performance concerning POR participants.
- Menstruation recovery was observed in 18 out of 30 POI patients, along with a statistically significant improvement on levels of AMH, FSH, and AFC.
- Similarly, 13 out of 30 menopausal women positively responded to PRP treatment.
- Finally, menstruation regularity, improved hormonal levels and AFC were reported for 24 out of 30 perimenopausal women.
- To conclude, PRP infusion appears to convey promising results in addressing ovarian insufficiency.

PRP in Gynaecology

PRP for Ovarian Rejuvenation

Details regarding study design for the four pilot studies.

Pilot Study	Screening for Eligibility	Standard Examination Prior to PRP Treatment	PRP Intraovarian Infusion	Follow-Up Monitoring
POR (n = 30)	Bologna Criteria	- Assessment of AFC, serum FSH, LH, AMH and E_2 - HSG and semen analysis	Performed at least 2 months following the last ICSI-ET cycle	- AFC, serum FSH, LH, AMH and E_2 assessment for three consecutive months following PRP treatment - In the third month following PRP participants were subjected to an ICSI-ET cycle
POI (n = 30)	- Age <40 years - Amenorrhea for at least four months, and elevated FSH >25 IU/L	-Assessment of AFC, serum FSH, LH, AMH and E_2 -HSG and semen analysis	Performed immediately following standard investigation or at least six months following HR discontinuation	- AFC, serum FSH, LH, AMH and E_2 assessment for three consecutive months following PRP treatment. - Participants were invited to conceive naturally
Perimenopause (n = 30)	-Age ≥40 years -Menstrual cycle irregularities			
Menopause (n = 30)	- Age 45–55 years - Amenorrhea for at least 12 months without HR supplementation, and FSH >30 IU/L			

POR: Poor Ovarian Response; POI: Premature Ovarian Insufficiency; FSH: Follicle Stimulating Hormone; LH: Luteinizing Hormone; AMH: Anti-Müllerian Hormone; E_2: Estradiol; AFC: Antral Follicle Count; HSG: Hysterosalpigography; HR: Hormone Replacement; PRP: Platelet-Rich Plasma; ICSI: Intracytoplasmic Sperm Injection; ET: Embryo Transfer.

Inclusion Criteria

Sfakianoudis et al 202

PRP in Gynaecology

PRP for Ovarian Rejuvenation

- Women presenting with autoimmune disorders, sexually transmitted diseases, infectious diseases, tubal factor infertility/tubal obstruction, chronic inflammatory diseases, endometriosis, chronic endometritis, and endocrine disorders such as thyroid dysfunction, were excluded in general.

- Body Mass Index (BMI) above 30 or less than 18.5, hypothalamic-pituitary disorders, and medical history including surgeries of the reproductive tract were further considered to be general exclusion criteria.

- Patients presenting with anemia, thrombophilic disorders, current cancer diagnosis or a medical history of familiar cancer, were also excluded.

Exclusion Criteria

- Finally, all couples presenting with an abnormal semen analysis were excluded.

Sfakianoudis et al 202

PRP in Gynaecology

PRP for Ovarian Rejuvenation

Protocol Followed

- According to the protocol employed, PRP administration took place during the early follicular phase of the cycle.

- For women presenting with menstrual cycles, such as POR and perimenopausal women that was day 3 of the menstrual cycle. For POI and menopausal women being amenorrheic, PRP administration was performed on a random day.

- With respect to preparation timing, PRP was always prepared earlier on the day of administration for all groups. Preparation of PRP was performed immediately following blood sample collection.

- Blood samples were collected from the median antebrachial vein. PRP was prepared according to the manufacturer's instructions employing a RegenACR®-C Kit (Regen Laboratory, Le Mont-sur-Lausanne, Switzerland).

Sfakianoudis et al 202

- Approximately 60 mL of the patient's peripheral blood was required in order to yield the required volume of PRP.

PRP in Gynaecology

PRP for Ovarian Rejuvenation

Protocol Followed

- Following PRP preparation, the technique of injection can mainly be described as an empirical approach, resembling the transvaginal paracentesis performed during the oocyte pick-up procedure

- Briefly, both ovaries were visualized via transvaginal ultrasound monitoring, and they were intramedullary injected on multiple sites using a 17-gauge single lumen needle, with the patient under inhaled minimal sedation.

- The technique included penetration across the central part of each ovary respectively, and thereafter gradual infusion of 4 mL of activated PRP, via a syringe attached to the transvaginal probe transducer. Following the infusion procedure, the pelvis was thoroughly examined via ultrasonography, in order to check total vascular integrity.

Sfakianoudis et al 202

PRP in Gynaecology

O-Cell ® procedure for PRP preparation

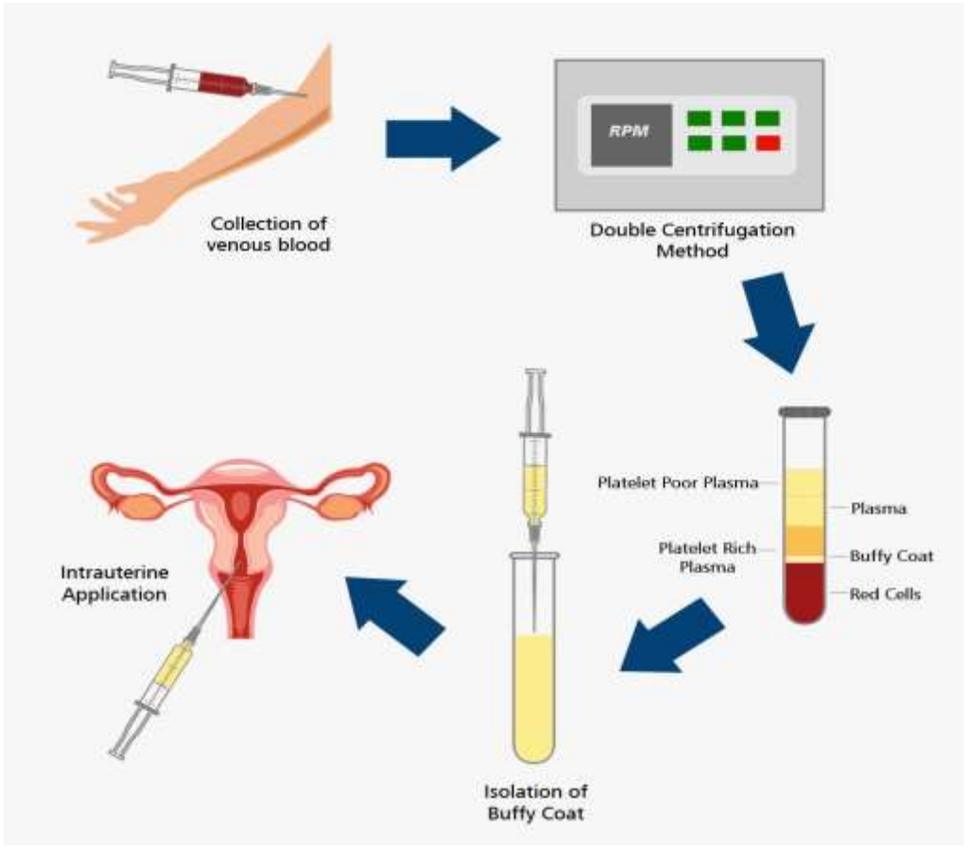
1. Venous blood (15–50 mL) is drawn from the patient's arm in anticoagulant-containing tubes;
2. The recommended temperature during processing is 21˚C–24˚C to prevent platelet activation during centrifugation of the blood;
3. The blood is centrifuged at 1,800 rpm for 12 minutes;
4. The blood separates into three layers: an upper layer that contains platelets and white blood cells, an intermediate thin layer (the buffy coat) that is rich in white blood cells, and a bottom layer that contains red blood cells; (Figure 2)
5. The upper and intermediate buffy layers are transferred to an empty sterile tube. The plasma is centrifuged again at 3,200 rpm for 6 minutes to help with the formation of soft pellets (erythrocytes and platelets) at the bottom of the tube;
6. The upper two-thirds of the plasma is discarded because it is platelet-poor plasma;
7. Pellets are homogenized in the lower third (5 mL) of the plasma to create the PRP;
8. The PRP is now ready for injection. Approximately 30 mL of venous blood yields 3–5 mL of PRP;

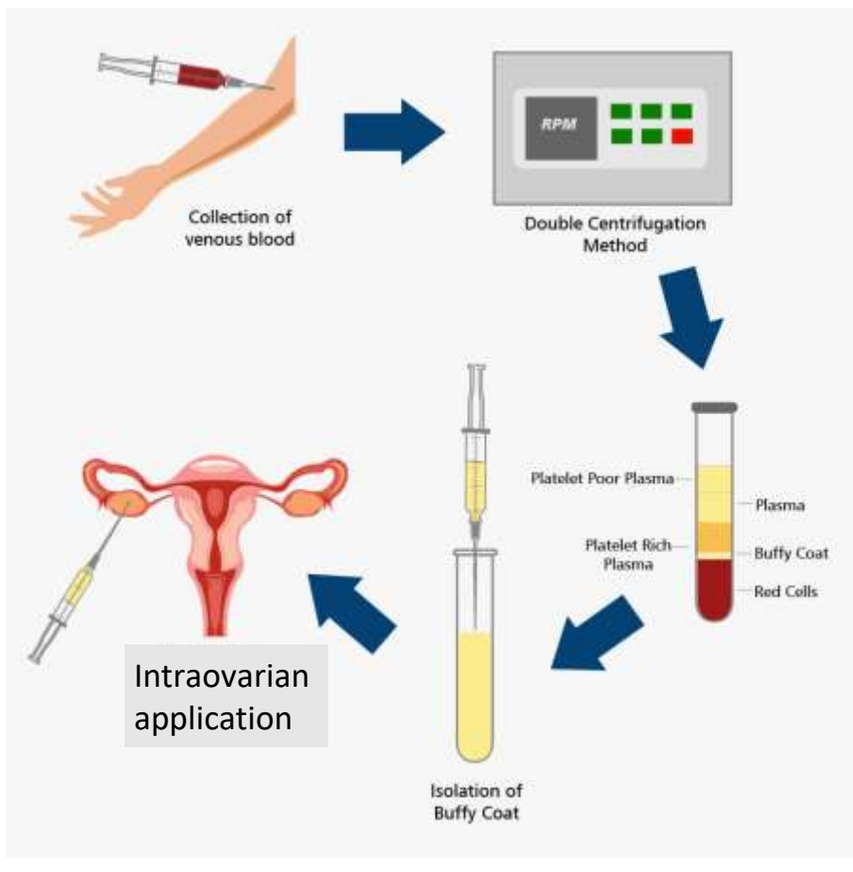
Mishra et al 2021

9. We then inject the prepared PRP either laproscopically (2.5cc in each ovary) at multiple site but preferably at cortex stromal site and sometimes in medulla of ovary in case of ovarian atrophy.

PRP in Gynaecology

O-Cell ® procedure for PRP preparation





Mishra et al 2021

PRP for Premature ovarian failure

- Premature ovarian failure (POF) is one of the leading causes of premature menopause and is diagnosed in approximately 1% women aged under 40 years.
- Women who experience premature menopause are at a higher risk of neurological diseases, psychosexual dysfunction, osteoporosis, mood disorders, ischemic heart disease, infertility and premature death
- The treatments that are readily available at hand to mitigate some of these adverse outcomes include estrogen treatment which can be initiated after the onset of menopause or hormone replacement therapy
- In scenarios where patients with irregular menstrual cycles or showing the symptoms of menstruation absence wish to address the concern of infertility, may consider the option of oocyte cryopreservation to preserve their fertility or in-vitro fertilization treatment (IVF) but the success of these treatments in the light of premature menopause onset is almost

PRP in Premature ovarian failure

- Pantos et al 2019 reports case series on two women with premature ovarian failure (POF) aged 40 and 27 years, respectively, and one menopausal woman aged 46 years.

- All patients presented with lack of menstrual cycle for over a year. The women reported previous failed in vitro fertilization (IVF) attempts, and, after rejecting the option of oocyte donation, they opted for the approach of autologous ovarian PRP treatment.

- Following PRP treatment, the three patients were invited to conceive naturally.

- The primary outcome was the restoration of menstruation following autologous ovarian PRP treatment, as well as an improvement in hormonal profile, a decrease in follicle-stimulating hormone (FSH) levels, and a concurrent increase in anti-Müllerian hormone (AMH) levels.

- Further to that, patients achieved pregnancy through natural conception within 2–6 months following PRP treatment, resulting in currently ongoing complication-free clinical pregnancies – a first report in the literature for menopausal and POF patients. Implementation of PRP should be further investigated through randomized controlled trials (RCTs), as it may hold the key to successful treatment for a certain cohort of patients exploring reproductive treatment options following menopause.

PRP in Premature ovarian failure

Table 1. Reproductive Background Prior to PRP Treatment.

	Patient 1	Patient 2	Patient 3
Age	46	40	27
Duration of amenorrhea	12 months	16 months	26 months
FSH prior to PRP (mIU/ml)	119	65	46.5
AMH prior to PRP (ng/ml)	0.16	0.06	0.17
E_2 prior to PRP (pg/ml)	19	17	15
LH prior to PRP (mIU/ml)	53.2	42	21
AFC/mm prior to PRP	0	0	0
Screening for endometrial pathologies through hysteroscopy	Negative	Negative	Negative
Bacteriologic screening	Negative	Negative	Negative
Intervention	HR for 6 months	None	None
HR discontinued	3 months prior to PRP	N/A*	N/A*

PRP, platelet-rich plasma; FSH, follicle-stimulating hormone; AMH, anti-Müllerian hormone; E_2, estradiol; LH, luteinizing hormone; AFC, antral follicle count; HR, hormone replacement.
*Patient did not receive HR

Table 2. Post PRP Follow-up in Patient 1 for 1 Month.

Follow-up	1st month
Menstruation recovery	Yes
FSH following PRP treatment (mIU/ml)	27
AMH following PRP treatment (ng/ml)	0.22
E_2 following PRP treatment (pg/ml)	18.1
LH following PRP treatment (mIU/ml)	4.8
AFC/mm post PRP during follicular phase (day 8)	Left ovary: 1 follicle (4 mm) 1 follicle (6 mm) Right ovary: 1 follicle (5 mm) 1 follicle (7 mm)

Table 3. Post PRP Follow-up in Patient 2 for 3 Months.

Follow-up	1st month	2nd month	3rd month
Menstruation recovery	No	Yes	Yes
FSH following PRP treatment (mIU/ml)	10	10	10
AMH following PRP treatment (ng/ml)	0.13	0.20	0.20
E_2 following PRP treatment (pg/ml)	86.5	78.5	101.5
LH following PRP treatment (mIU/ml)	13	16.5	18.0
AFC/mm post PRP during follicular phase (day 8)	Left ovary: 0 Right ovary: 1 follicle (6 mm) 1 follicle (8 mm)	Left ovary: 0 Right ovary: 1 follicle (8 mm)	Left ovary: 1 follicle (6 mm) Right ovary: 0

PRP in Premature ovarian failure

Table 4. Post PRP Follow-up in Patient 3 for 5 Months.

Follow-up	1st month	2nd month	3rd month	4th month	5th month
Menstruation recovery	Yes	Yes	Yes	Yes	Yes
FSH following PRP treatment (mIU/ml)	20.09	20.27	19.42	16.30	15.05
AMH following PRP treatment (ng/ml)	0.25	0.25	0.25	0.27	0.30
E_2 following PRP treatment (pg/ml)	107	98	78	111	120
LH following PRP treatment (mIU/ml)	19.59	19.22	17.37	20.14	20
AFC/mm post PRP during follicular phase (day 8)	0	Left ovary: 0 Right ovary: 1 (6.5 mm)	Left ovary: 0 Right ovary: 1 follicle (6.5 mm)	Left ovary: 1 follicle (7 mm)	Left ovary: 0 Right ovary: 1 follicle (10 mm)

Table 5. Reproductive Outcome Post PRP.

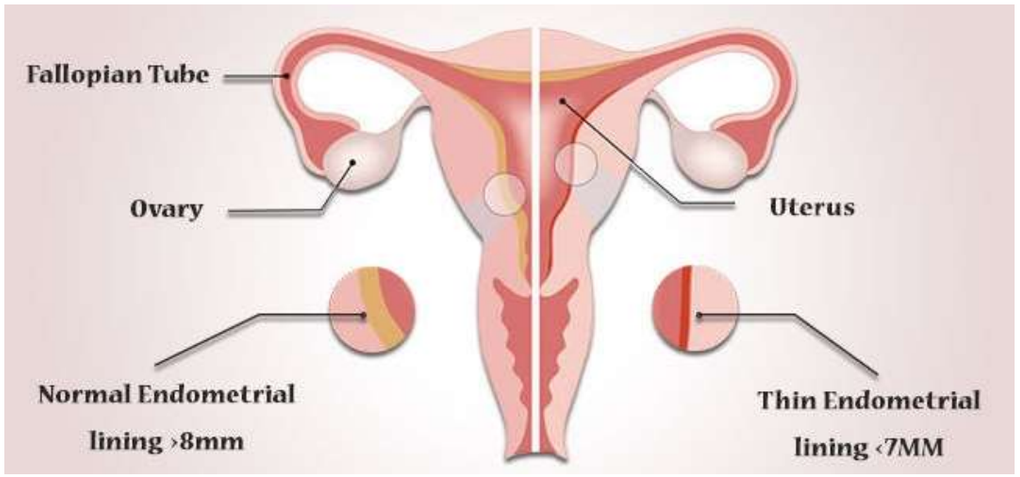
Patients	Patient 1	Patient 2	Patient 3
Time frame required to observe menstrual cycle restoration following PRP treatment	1 month	2 months	1 month
Time frame required for achieving pregnancy	2 months	4 months	6 months
Current pregnancy status	37 weeks Complication free	37 weeks Complication free	26 weeks Complication free

Pantos et al 2019

PRP for thin endometrium

- Endometrium thickness <7mm on ultrasound is considered as a thin endometrium which usually results in cycle cancellation and hence is non-responsive to standard treatments posing a challenge in Assisted Reproductive Technology.

- Numerous strategies have been utilized for the treatment of thin endometrium such as use of low-dose aspirin, use of exogenous estrogen, vitamin E, electroacupuncture, vaginal sildenafil citrate, and application of G-CSF (Granulocyte colony stimulation factor).



- Ironically despite performing these remedies, many women do not respond to the ART

PRP for thin endometrium

Kim et al (2019) conducted a pilot study to unravel the effect of PRP therapy on thin endometrium. They considered women who presented with a history of failed IVF cycles atleast twice, refractory thin endometrium (EMT < 7mm), undergone more than two cycles of previous therapy for increasing the EMT, such as, hysteroscopic adhesiolysis following hormone replacement therapy, high dose estradiol valerate, transvaginal sildenafil administration, or pentoxifyilline combination with vitamin E, and frozen embryo available for embryo transfer.

- Intrauterine autologous PRP administration was performed at the estrogen-primed FET cycle.
- The first autologous PRP infusion was performed on menstrual cycle day 10 which was repeated at 3 days intervals until the thickness of endometrium reached 7 mm.
- ET was conducted 3 days after the final autologous PRP administration.
- The serum β-hCG levels were measured from peripheral blood 2 weeks after ET.
- The authors found that the use of autologous PRP significantly improved implantation, pregnancy, and live birth rates of the patients with refractory thin endometrium .

PRP for thin endometrium

Inclusion and Exclusion Criteria

- The inclusion criteria were as follows: (a) age of 20–45 years at the time of enrollment, (b) endometrial thickness (EMT) of <7 mm on the human chorionic gonadotropin (hCG) administration day in fresh ET cycles or on the end of estrogen priming day in frozen ET cycles in all of the previous cycles, (c) two or more failed IVF cycles, (d) more than two cycles of previous therapy for increasing the EMT, such as, hysteroscopic adhesiolysis following hormone replacement therapy, high dose estradiol valerate, transvaginal sildenafil administration, or pentoxifyilline combination with vitamin E, (f) frozen embryo available for ET, and (g) informed consent form signed.

- The exclusion criteria were as follows: (a) hematologic disorders, hemoglobin level of <9.0 g/dL or platelet count of <100,000/μL, (b) auto-immune disease, (c) chromosomal abnormality in the patient or spouse, (d) peripheral NK cell proportion of ≥12%, (e) body mass index (BMI) of ≥30 kg/m^2, and (f) uncontrolled endocrine or other medical conditions, such as prolactinemia or thyroid diseases.

Kim et al (2019)

PRP for thin endometrium

Protocol Followed

- On each PRP administration day, 18 mL of venous blood was drawn from the patients using 30 mL syringes coated with 2 cc of acid citrate A, anticoagulant solution (ACD-A; Arya Mabna Tashkhis, Iran).

- The blood samples were then moved into an aseptic PRP centrifuge kit (PROSYS PRP; Prodizen, Korea) and centrifuged at 1017 G for 3 min.

- The buffy coat and plasma just above the buffy coat were collected, and 0.7–1.0 mL of PRP was produced and infused into uterine cavity.

- Based on the data provided by the manufacturer, the platelet concentration of PRP ranged from 717 × 10^3 to 1565 × 10^3/μL, and the WBC concentration varied from 24,000 to 37,000/μL.

Kim et al (2019)

Elements of informed consent

Appendix 1. Consent Form (sample)

Informed Consent Form For Patients Undergoing Platelet Rich Plasma (PRP) Treatment

(Name of Healthcare Professional) _____
(Name of Health Facility) _____
(Name of Patient) _____
This Informed Consent Form has two parts:
- **Information Sheet (to share information about the treatment with you)**
- **Certificate of Consent (for signatures if you agree to go ahead with the treatment)**

You will be given a copy of the full Informed Consent Form

PART I: Information Sheet

Introduction:
I, Dr. _____ with license No:_____ shall be performing the PRP treatment on Miss/Mrs./Mr. _____ aged _____ years, on date _____.

Description of the Process
Describe to the patient or customer, what will happen on a step-by-step basis. The patient shall be informed that procedure is newly introduced and the amount of supporting research and study available.

Side Effects
Potential patients should be told if there are any known or anticipated side effects and what will happen in the event of a side effect or an unexpected event.

Risks
Explain and describe any possible or anticipated risks. Describe the level of care that will be available in the event that harm does occur, who will provide it, and who will pay for it.

Complications (Not limited to)
Inform and explain any possible complications that could be caused as a result of the PRP treatment.

Discomforts
Explain and describe the type and source of any anticipated discomforts that are in addition to the side effects and risks discussed above.

Benefits
Mention only those activities that will be actual benefits of the PRP treatment.

Confidentiality
Explain how the clinical team will maintain the confidentiality of data, especially with respect to the information about the patient .

Right to Refuse treatment/procedure
This is a reconfirmation that the patient has the right to refuse the treatment.

Alternatives to clinical procedure or treatment
It is important to explain and describe the established standard treatment or procedure for the patient's condition.

PART II: Certificate of Consent
This section can be written in the first person. It should include a few brief statements about the treatment and be followed by a statement similar to the one in bold below. The healthcare professional performing the PRP treatment and the person going over the informed consent must sign the consent.
Example:
Patient Consent statement
I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to try this new treatment and understand that I have the right to withdraw from the procedure or treatment at any time without in any way affecting my medical care.
Name of Patient: _____ _____
Signature of Patient: _____ _____
Date: _____

Witness statement
I have accurately read or witnessed the accurate reading of the consent form to the potential patient, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.
Name of witness: _____
Signature of witness: _____
Date: _____

Healthcare Professional Declaration:
I have adequately explained to the patient about the procedure along with risks, adverse effects and the standard alternatives that are available for the procedure. I have permitted time and opportunity for the patient to ask questions and all questions have been answered to my knowledge
Name of healthcare professional: _____
Signature of healthcare professional : _____
Date: _____

Published Articles

Therapeutic Efficacy of Mononuclear Cells in Thin Endometrium: A Case Report

Prabhu Mishra, Mayuri Mohapatra, Venus Khanna, Natalia Kaszuba

DOI: 10.25096/jrafm.00108.052017

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Inclusion & Exclusion Criteria

Inclusion Criteria

- Women (22-40 years old) undergoing IVF cycles
- Endometrial lining <7 mm with poor vascularity despite standard dose of Estradiol valerate (up to 12mg/day),
- Repeated cancelled cycles due to poor lining
- Recurrent implantation failure due to poor lining
- Normal liver, kidney and heart function
- Presence of menstrual bleeding with natural cycle of HRT and
- Beta hCG negative patients

Exclusion Criteria

- Any other known cause of implantation failure such as poor embryo quality,
- Women with known Asherman's syndrome, HIV and Hepatitis B positive women,
- Women with uncontrolled diabetes with ketoacidosis,
- Presence of active infection in the form of vaginitis, cervicitis or hydrosalpinx and presence of any pathology distorting the uterine cavity.

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Patient's Characteristics

Patient	Age(Years)	Endometrium Pre/Post	PRP/G-CSF	Pregnancy	Outcome
1.	25	4.5mm/1.1cm	PRP	YES	Ongoing
2.	38	6.1mm/6.9mm	PRP	YES	Missed abortion
3.	27	7.2mm/1.1cm	PRP	YES	Ongoing
4	39	6.5mm/7.5mm	PRP	NO	NO
5	35	5.7mm/9.7mm	PRP	YES	Ongoing
6	31	7.7mm/8mm	G-CSF	ET Not done	
7	31	5.5mm /9.7mm	G-CSF	NO	NO
8	25	8mm/9.7mm	PRP	NO	NO
9	25	5.8mm/1.1cm	PRP	YES	Ongoing
10	35	5mm/7mm	PRP	YES	Ongoing

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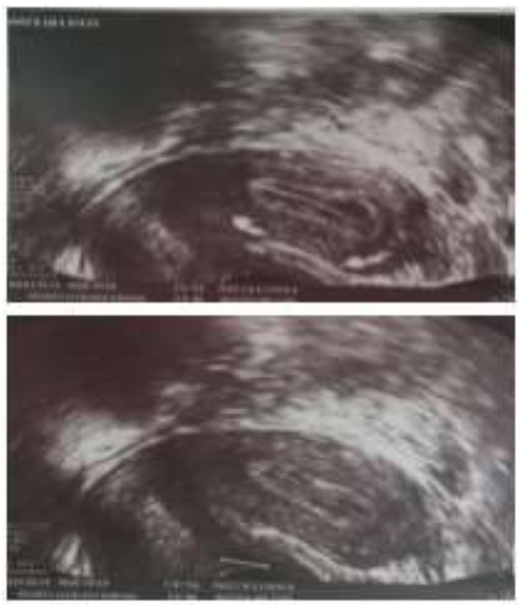
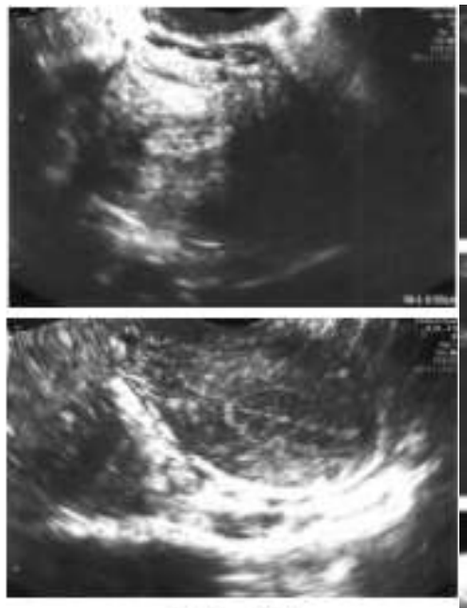
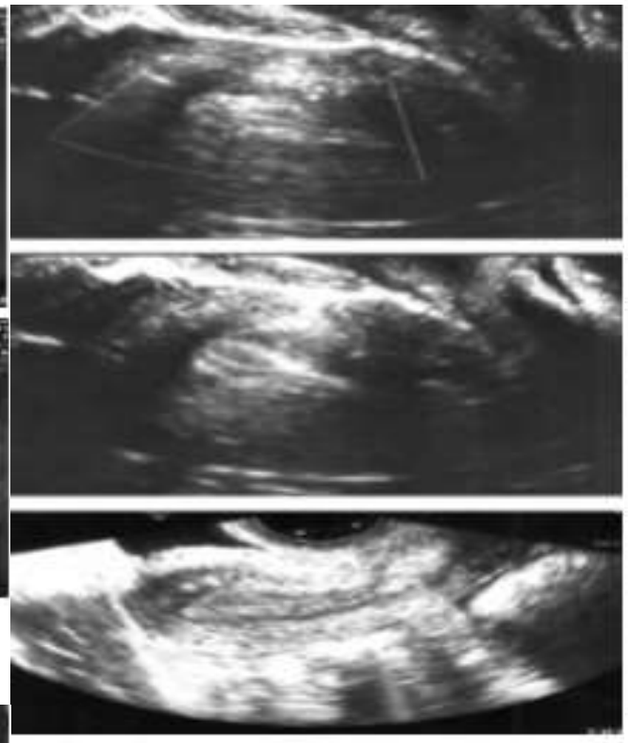
Ultrasonography outcomes



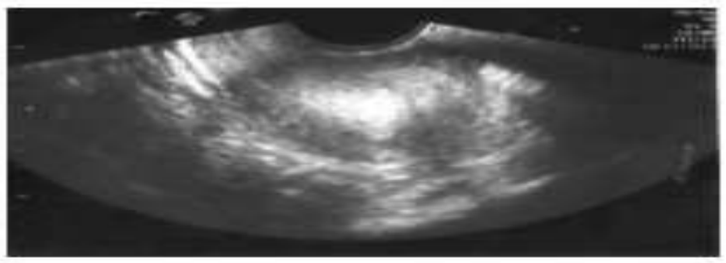
Patient 1



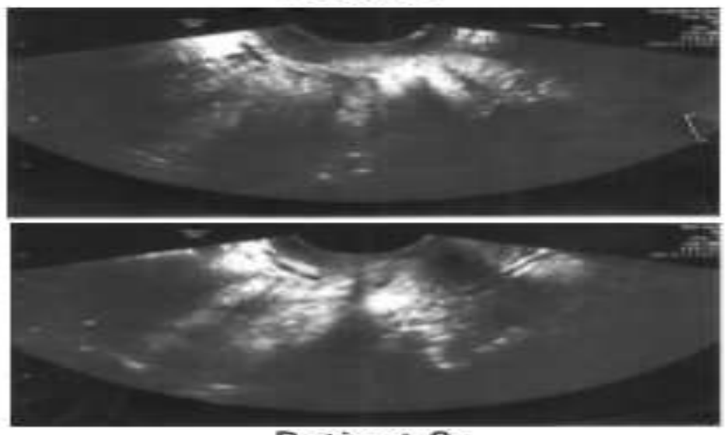
Patient 2



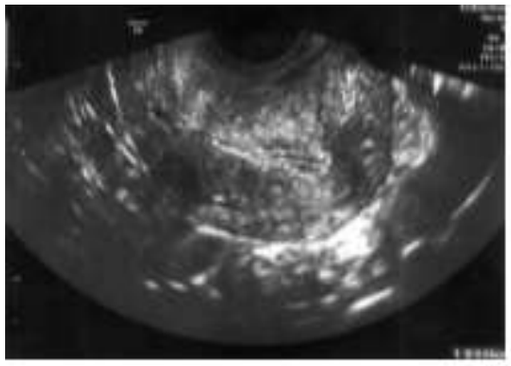
Patient 3

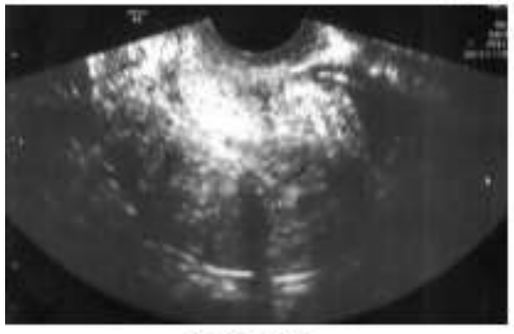


Patient 7



Patient 8





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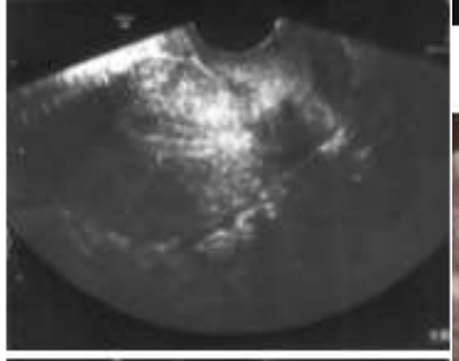

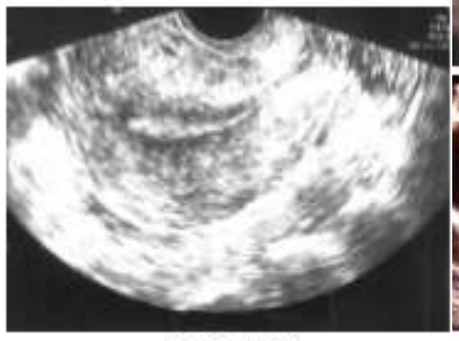

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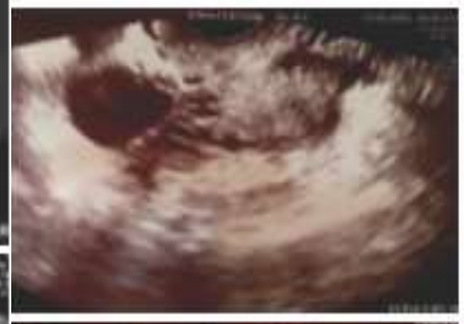

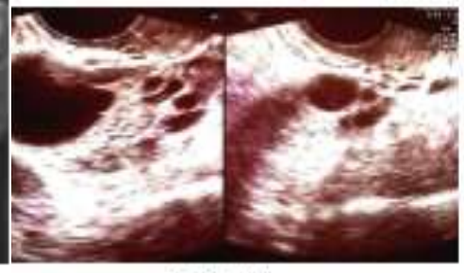

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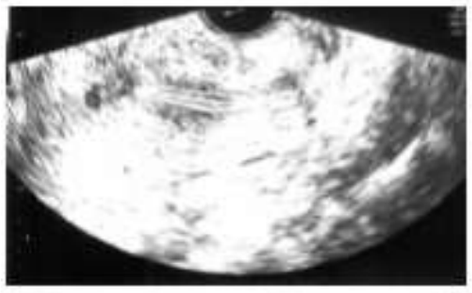
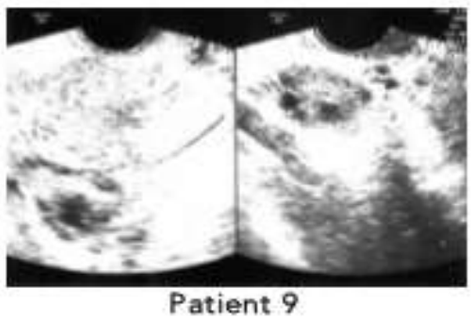
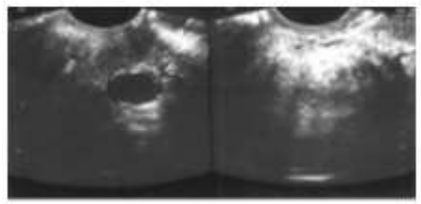
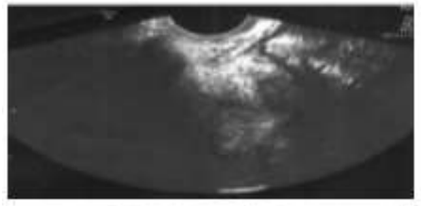
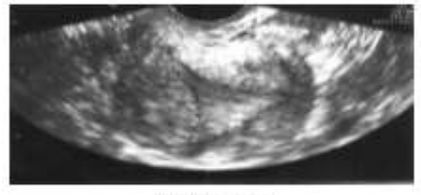




Patient 9







Patient 10

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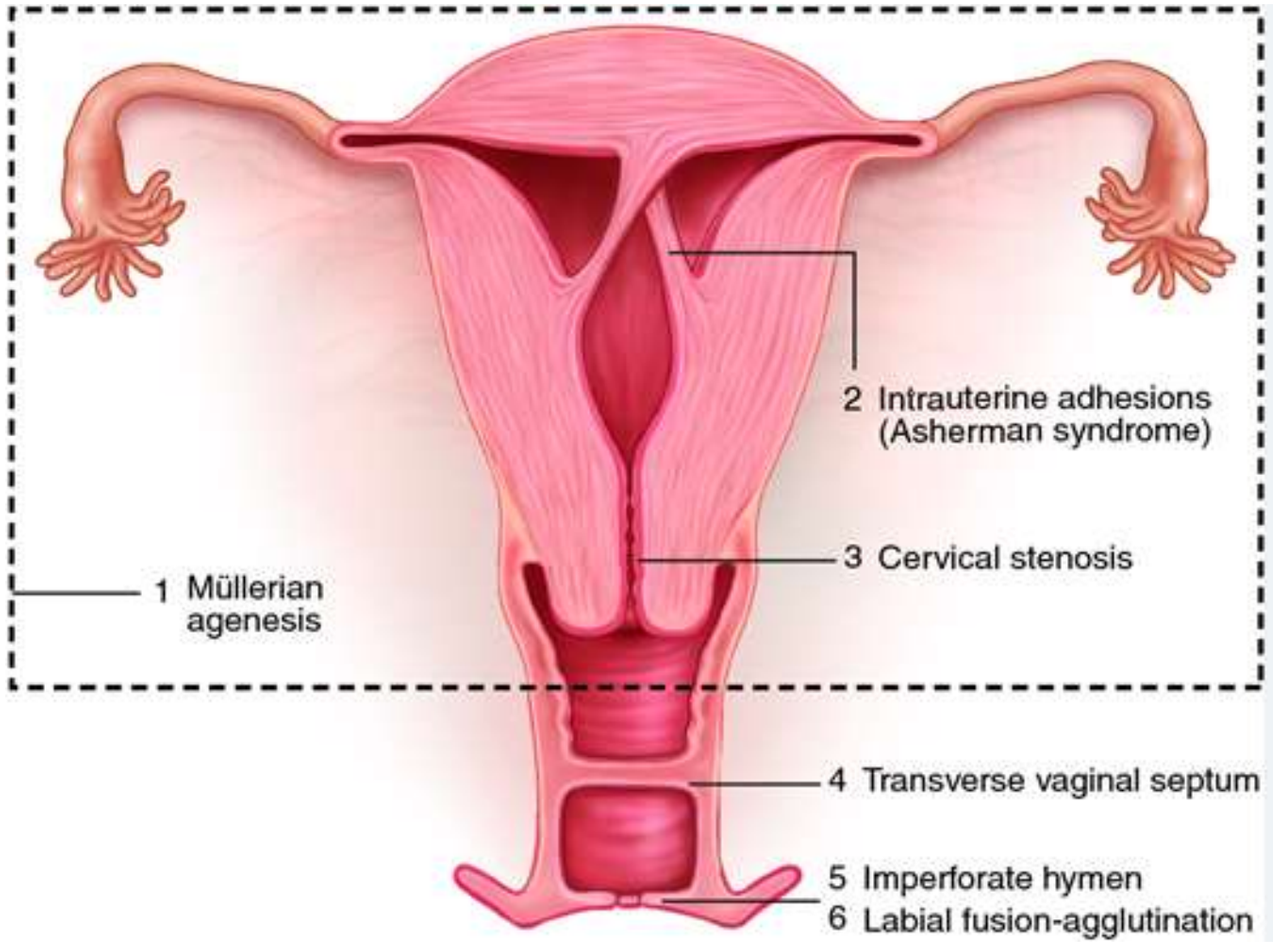
Asherman's Syndrome

- Also referred to as intrauterine adhesions (IUA).
- An acquired uterine condition that occurs when scar tissue (adhesions) form inside the uterus and/or the cervix.
- In most cases, occurs in women who have had several dilation and curettage (D&C) procedures.
- Can also be caused due to severe pelvic infection unrelated to surgery; after infection with tuberculosis or schistosomiasis.
- With stem cells, this condition is managed by injecting bone marrow derived stem cells and GCSF mobilised peripheral blood derived stem cells hysteroscopically in the sub-endometrial zone.
- Results vary between 50-55% till date and different for each patient.



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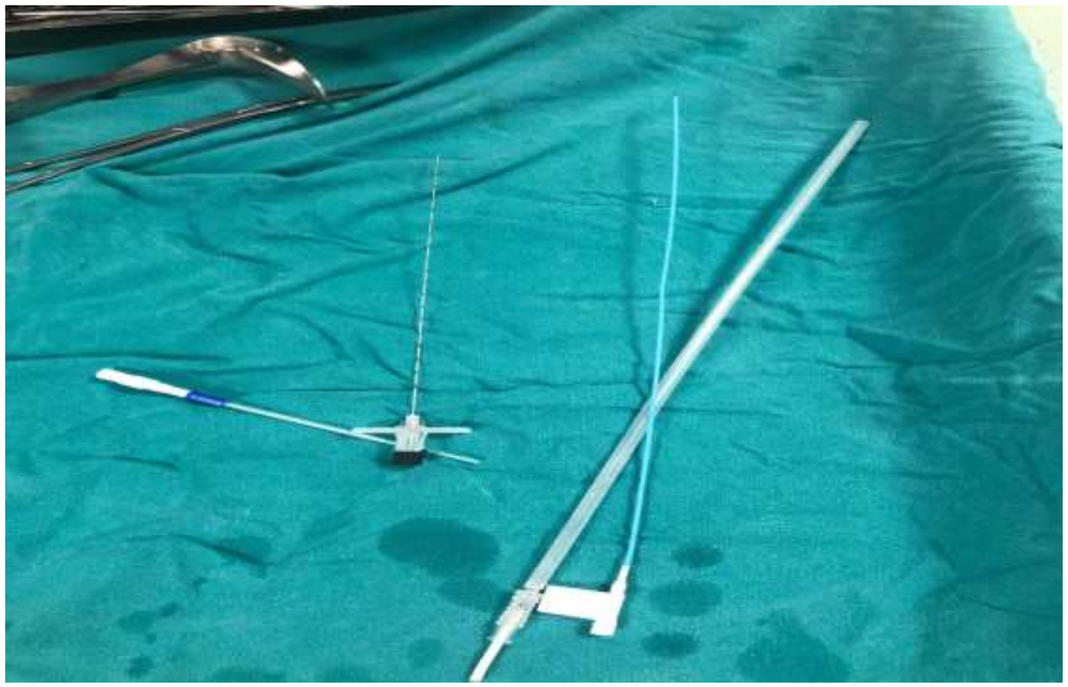
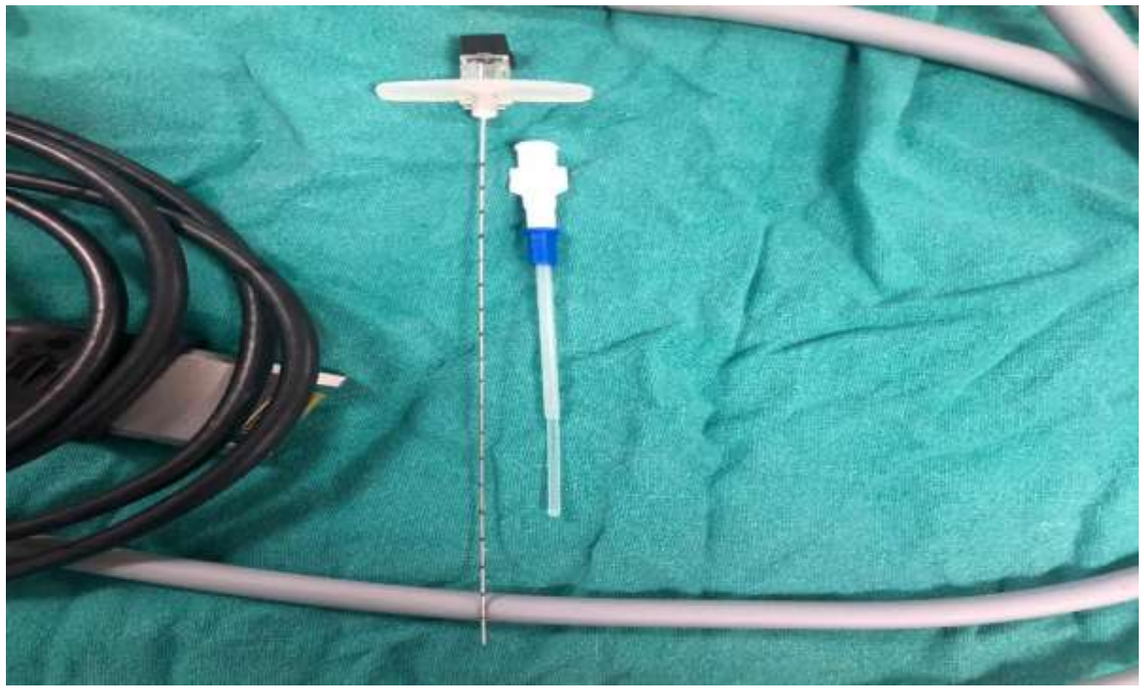
Hysteroscopic Infusion Of Bone Marrow Aspirated Concentrate In Asherman's Syndrome





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Journal of Human Reproductive Sciences
Wolters Kluwer -- Medknow Publications

Autologous stem cell transplantation in refractory Asherman's syndrome: A novel cell based therapy

Neeta Singh, Sujata Mohanty, [...], and Sona Dharmendra

Additional article information

Abstract

BACKGROUND:

There is substantial evidence that adult stem cell populations exist in human endometrium, and hence it is suggested that either endogenous endometrial stem/progenitor cells can be activated or bone marrow derived stem cells can be transplanted in the uterine cavity for endometrial regeneration in Asherman's syndrome (AS).

Review

Stem cell therapy in Asherman syndrome and thin endometrium: Stem cell- based therapy

Ramyar Azizi [a, b] ... Mehdi Yousefi [d, e]

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https://doi.org/10.1016/j.biopha.2018.03.091

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Abstract

The endometrium is one of the essential components of the uterus. The endometrium of human is a complex and dynamic tissue, which undergoes periods of growth and turn over during any menstrual cycle. Stem cells are initially undifferentiated cells that display a wide range of differentiation potential with no distinct morphological features. Stem cell therapy method recently has become a novel procedure for treatment of tissue injury and fibrosis in response to damage. Currently, there is massive interest in stem cells as a novel treatment

Endometrial regeneration using autologous adult stem cells followed by conception by in vitro fertilization in a patient of severe Asherman's syndrome.

Nagori CB, et al. J Hum Reprod Sci. 2011.
Show full citation

Abstract

In a woman with severe Asherman's syndrome, curettage followed by placement of intrauterine contraceptive device (IUCD) (IUCD with cyclical hormonal therapy) was tried for 6 months, for development of the endometrium. When this failed, autologous stem cells were tried as an alternative therapy. From adult autologous stem cells isolated from patient's own bone marrow, endometrial angiogenic stem cells were separated using immunomagnetic isolation. These cells were placed in the endometrial cavity under ultrasound guidance after curettage. Patient was then given cyclical hormonal therapy. Endometrium was assessed intermittently on ultrasound. On development of endometrium with a thickness of 8 mm and good vascularity, in vitro fertilization and embryo transfer was done. This resulted in positive biochemical pregnancy followed by confirmation of gestational sac, yolk sac, and embryonic pole with cardiac activity on ultrasound. Endometrial angiogenic stem cells isolated from autologous adult stem cells could regenerate injured endometrium not responding to conventional treatment for Asherman's syndrome.

PMID: 21772740 [] PMCID: PMC3136069

Published Articles

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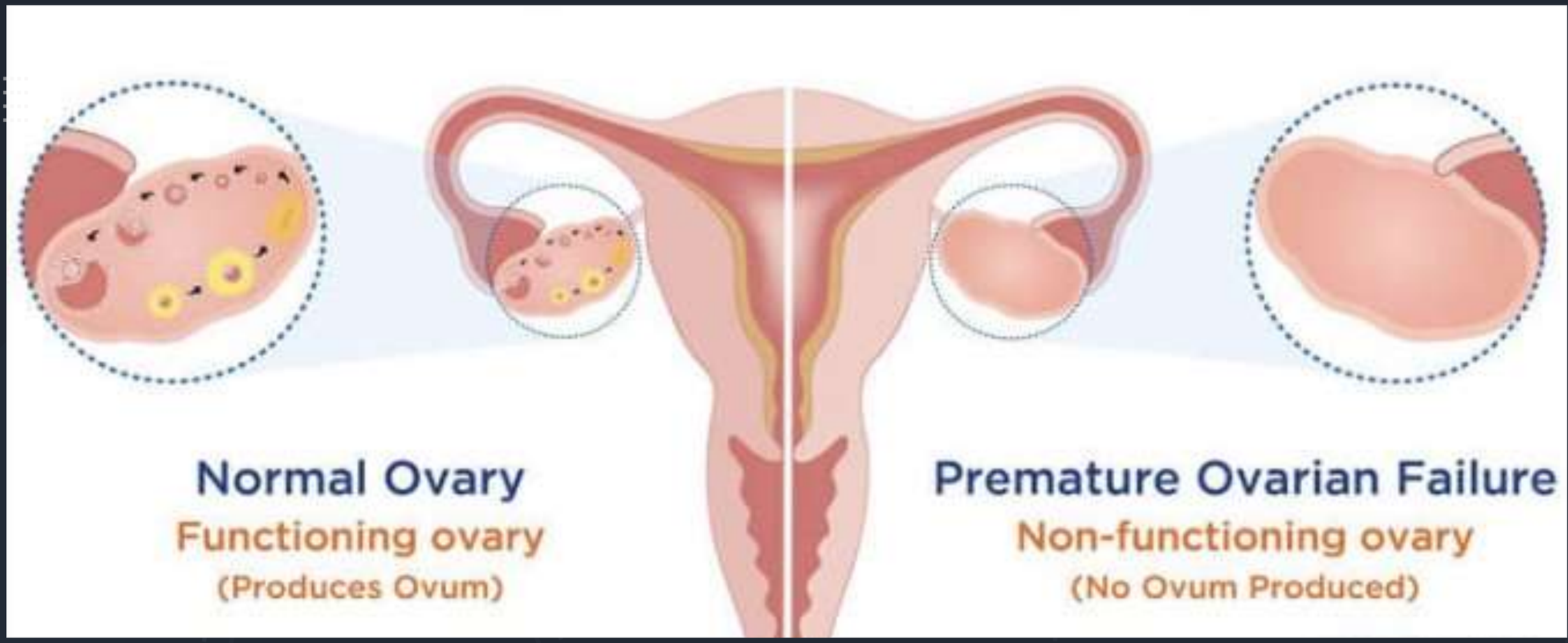


Poor Ovarian Reserve & Premature Ovarian Failure

- Important limiting factor for the success of any treatment modality for infertility.
- Indicates a reduction in quantity and quality of oocytes in women of reproductive age group.
- May be age related as seen in advanced years of reproductive life.
- May also occur in young women due to diverse etiological factors.
- With stem cells, this condition is managed by injecting bone marrow derived stem cells and GCSF mobilised peripheral blood derived stem cells either under the guidance of ultrasound or laparoscopically in the ovarian cortex.
- Majority of women require IVF treatment to attain pregnancy.
- Upto 65% results till date, varying from patient to patient.

Poor Ovarian Reserve & Premature Ovarian Failure

- **Primary Outcome Measures:** Antral follicle count (AFC) [Time Frame: 6 months] Every antral follicle is measured
- **Secondary Outcome Measures :** Time to Menses recovery [Time Frame: 6 months]Spontaneous menstrual cycle restoration and its characteristics
- **Serum follicle stimulating hormone (FSH) and estradiol** [Time Frame: 6 months] serum extraction for biological measurements
- **Ovarian reserve dynamics** [Time Frame: 6 months]ultrasound observation of follicular development
- **Controlled Ovarian Hyper stimulation (COH)** response [Time Frame: 6 months] ovarian response to gonadotropins
- **Pregnancy rate** [Time Frame: 2 years] pregnancy rate spontaneous and after COH
- **Number of good quality embryos** [Time Frame: 6 months] Morphological criteria and developmental potential
- **Number of participants** with treatment-related adverse events [Time Frame: 6 months] Secondary effects of the received interventions following hematological and gynecological medical criteria.

Protocol and Synopsis

Synopsis

Poor Ovarian Reserve/Premature Ovarian Insufficiency/Premature Ovarian Failure

Title:

Therapeutic efficacy and safety of autologous bone marrow aspirated concentrate in infertile women poor ovarian reserve/premature ovarian insufficiency/premature ovarian failure - a pilot study

Research site:

StemMax Research & Therapeutics Pvt. Ltd., New Delhi

Principal Investigators:

Dr. Deepti Dua, PhD, StemMax Research & Therapeutics Pvt. Ltd., New Delhi

SUMMARY:

Title:

Therapeutic efficacy and safety of autologous bone marrow aspirated concentrate in infertile women poor ovarian reserve/premature ovarian insufficiency/premature ovarian failure - a pilot study

Background:

Primary ovarian insufficiency (POI), also known as premature ovarian failure, happens when a woman's ovaries stop working normally before she is 40.

Many women naturally experience reduced fertility when they are about 40 years old. They may start getting irregular menstrual periods as they transition to menopause. For women with POI, irregular periods and reduced fertility start before the age of 40. Sometimes it can start as early as the teenage years.

POI is different from premature menopause. With premature menopause, your periods stop before age 40. You can no longer get pregnant. The cause can be natural or it can be a disease, surgery, chemotherapy, or radiation. With POI, some women still have occasional periods. They may even get pregnant. In most cases of POI, the cause is unknown.

Patients with POF, POI or Low Ovarian Reserve choosing to enroll will be provided informed consent for Rejuvenation of Premature Ovarian Failure With Stem Cells (ROSE-1). They will undergo diagnosis and screening confirming diagnosis including History and Physical Exams, Labs and Diagnostic Procedures. Following final approval and under anesthesia, bone marrow aspiration with separation of the bone marrow derived stem cell fraction will be performed. Diagnostic laparoscopy will allow for assessment of pelvic anatomy and subsequent injection of the bone marrow derived stem cells into the ovaries. In such patients, there is a need for an alternative therapy such as the use of autologous bone marrow stem cells.

Aim of the study:

This study aims at determining the efficacy of bone marrow aspirated concentrate on ovarian function recovery in subjects with poor ovarian reserve/premature ovarian insufficiency/premature ovarian failure

Trial site:

StemMax Research & Therapeutics Pvt. Ltd., New Delhi

Study design:

This is an open- label non- randomised prospective study to evaluate the effect of administration of autologous (patient derived) bone marrow aspirated concentration on ovulation/follicle formation in patients with infertility due to poor ovarian reserve/premature ovarian insufficiency/premature ovarian failure.

Study duration:

The expected total duration of this study is 1 year.

Total number of patients:

12+2 patients

Protocol and Synopsis

Type of patients:

Infertile women undergoing IVF (Frozen embryo transfer) cycles at _____.

Inclusion criteria:

- Able to understand and communicate in Hindi or English language
- Signed and dated informed consent
- Female over the age of 18
- Diagnosis of premature ovarian insufficiency: At least two menopausal FSH levels (≥ 40 IU/L) and/or Primary or secondary amenorrhea at least for 3-6 months OR Diagnosis of low ovarian reserve defined as: AMH < _0.42 ng/ML & FSH >20 IU/L, and/or failure of prior attempts of assisted reproductive techniques due to limited ovarian response (poor responder).
- Normal karyotype 46, XX.
- Presence of at least one ovary
- Acceptable uterine anatomy (by any clinically and/or imaging acceptable methods)
- Normal thyroid function as evidence by normal serum Thyroid Stimulating Hormone (TSH) levels.
- Agree to report any pregnancy to the research staff immediately.
- Willing and able to comply with study requirements and follow up instructions.
- No other causes of female infertility in the subject

If subject is planning to pursue pregnancy: Presence of at least unilateral tubal patency (with any clinically acceptable methods).

Exclusion criteria:

- Unable to understand and communicate in Hindi or English language
- Currently pregnant or breast-feeding
- Has a history of, or evidence of current gynaecologic malignancy within the past three years
- Presence of adnexal masses indicating the need for further evaluation
- Major mental health disorder that precludes participation in the study
- Active substance abuse or dependence
- Unfit or unwilling to undergo laparoscopy; has contraindication to laparoscopic surgery and/or general anesthesia
- Current or recent (within the past 2 weeks) use of the following medications: Oral or systemic corticosteroids, Hormones (estrogen, progestins, oral contraceptives), Danazol, anticoagulants, herbal or botanical supplements with possible hormonal effects. Washout will be allowed.
- Medical conditions that are contraindicated in pregnancy
- Type I or Type II diabetes mellitus, or if receiving antidiabetic medications
- Known significant anemia (Hemoglobin <8 g/dL).
- Untreated deep venous thrombosis, and/or pulmonary embolus
- Untreated cerebrovascular disease
- Known heart disease (New York Heart Association Class II or higher).
- Known Liver disease (defined as Aspartate Aminotransferase (AST) or Alanine Aminotransferase (ALT)>2 times normal, or total bilirubin >2.5 mg/dL).
- Known Renal disease (defined as Blood urea nitrogen (BUN)>30 mg/dL or serum creatinine > 1.6 mg/dL).

Test product:

Stem cells derived from patients' bone marrow aspirated concentrate which will include primarily the mononuclear cell fraction CD34+

Protocol:

45-60 mL of bone marrow aspiration is done from the patient's iliac crest, under general anaesthesia maintaining strict asepsis using a Jamshedi needle prewashed with heparinized medium. Collection of the aspirate is done in CPDA medium using 1ml of the medium for 7ml of the bone marrow in 15ml centrifuge tubes. The sample is processed manually using a centrifuge by the standard double spin procedure. Sample is centrifuged immediately at 1800 rpm for 12

Protocol and Synopsis

min to separate the red blood cells. The plasma and buffy coat is transferred again to fresh tubes and centrifuged again at 2200 rpm for 5 min to obtain the bone marrow derived mono-nuclear cells. Then, 2-2.5 ml of cells injected into the each of the ovaries through laparoscopy.

Primary end points:

- Improvement in diagnostic hormonal levels (Reduction in FSH and increase in AMH and estradiol levels).

Secondary end points:

- Resumption of menses.
- Improvement in hormonal levels toward normal ranges. Hormones may include FSH/LH; Estradiol/progesterone; Inhibin; Anti-Mullerian Hormone.
- Achievement of pregnancy by natural or assisted conception methods as may be deemed appropriate by the patient and her primary provider.

Statistics

Data will be expressed as mean and± SD, until otherwise specified. Baseline and post treatment data will be compared using Friedman test and Wilcoxon signed ranks test for tests of significance and linear regression analysis will be used to find correlation between independent variables. A probability (P) value of <0.05 will be regarded statistically significant. Analysis of Safety Variables Adverse events (AEs), serious adverse events (SAEs) and discontinuations due to adverse events (AEs) will be identified by the investigator during the study through interviews at each visit and will be reported descriptively.

Case Report

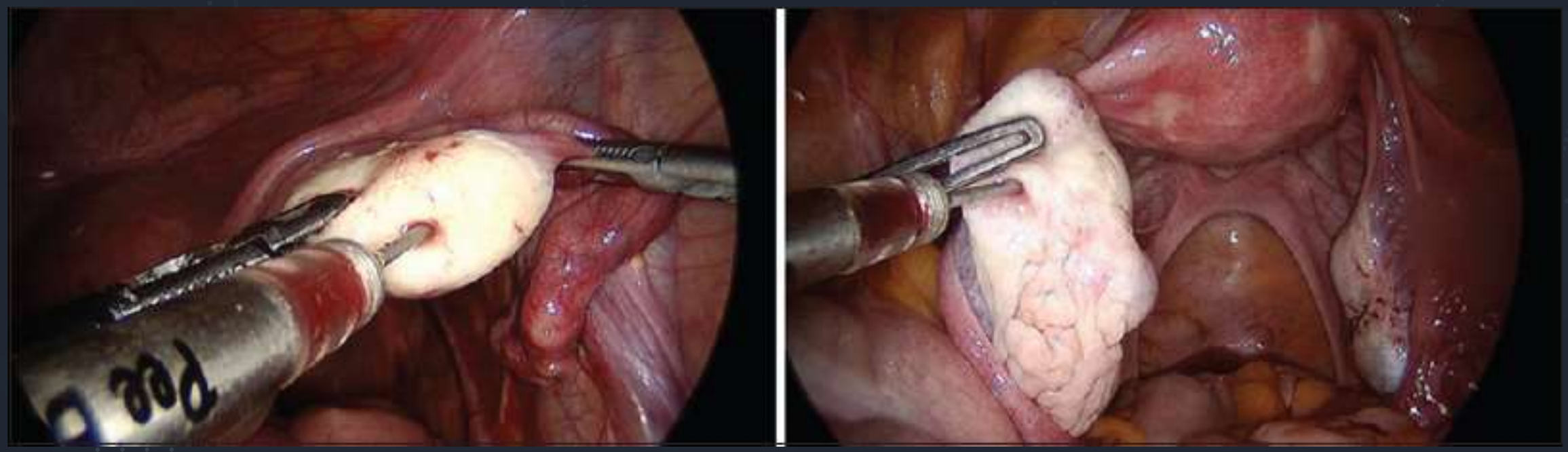
- A 45-year-old perimenopausal female, who was infrequently menstruating for the last 3 years, came to our fertility clinic. Her AMH level was low 0.4 ng/mL. On ultrasonography, ovaries were unremarkable with antral follicle count of one. Being a single mother, she did not wanted to take the option of assisted reproduction with the donor oocyte program.

- Several researchers have confirmed the presence of ovarian SCs, as well as bone marrow-derived SCs that have been able to colonize the ovaries and initiate folliculogenesis. Also few independent groups have shown the ability of autologous SCs to differentiate into primordial germ cells, which may form functional haploid gametes. Considering the above study, it was thought to use ABMDSC for the rejuvenation of functioning ovarian tissue and optimizing the success rate of achieving pregnancy through assisted reproduction.

- With the patient's written, audiovisual informed consent about the nature of the procedure to be undertaken and after explaining the pros and cons of this procedure, the patient was taken for the procedure. Her bone marrow aspiration was done from posterior iliac crest under local anesthesia maintaining strict asepsis. Aspiration was done using Jamshidi needle (13G) and 20 ml syringe prewashed with heparin. Around 120 ml of bone marrow was aspirated. 16 ml BMDSC were separated using the sepax (fully automated closed capability system) which uses optical sensor technology and simultaneous application of centrifugation and sedimentation. Considering the small size of ovaries which were not well approachable with the vaginal route and were difficult to fix for instillation, we preferred laparoscopic instillation of ABMDSC in ovaries. The patient was given general anesthesia; preparation for laparoscopy was done. Four-puncture laparoscopy with 5-mm telescope was performed. Ovaries were held at the cranial and caudal position with forcep and intraovarian instillation of about 1–2 ml of ABMDSC at 3–4 sites performed bilaterally as shown in Fig.

- After 8 weeks ABMDSCT, the ultrasound revealed two follicles in each ovary. In addition, the AMH improved to 0.9 ng/mL. Considering the age of the patient, the first cycle of egg pooling was planned immediately.

- We retrieved three eggs and one Grade A compacting embryo was frozen on day 3. On patient's insistance, we went ahead with frozen embryo transfer in subsequent cycle instead of second sitting of ABMDSCT. Her beta- human chorionic gonadotropin was 1280 on day 14 of embryo transfer, and a single intrauterine viable gestation was seen at 6 weeks on ultrasound. Noninvasive prenatal testing was done at 11 weeks which showed normal karyotype. The pregnancy was uneventful. A 2.7 kg female baby was delivery by cesarean birth at 38 weeks. The baby cried well after birth, had a good Apgar score, and has had an uneventful neonatal course so far.



What is Lichen Sclerosus?

•Lichen Sclerosus is a chronic inflammatory skin condition which can affect any part of the skin but most commonly effects the genital skin (vulva) and the skin around the anus. The condition causes the skin to have a white, shiny appearance which is usually thinned but can often become raised and appear thickened.

•What are the symptoms of Lichen Sclerosus?

•The most common symptom of vulval Lichen Sclerosus is itching, which may be severe and very sore if the skin breaks down or cracks. If breaking or cracking does occur in the genital area the scar-like healing process can tighten the skin making it uncomfortable to pass urine, stools or have sexual intercourse.

•Symptoms of Lichen Sclerosus include:

- •Mild to severe itching and soreness
- •Skin that appears fragile, pale, and/or white
- •Bruised skin with broken blood vessels or “blood blisters”
- •Small tears or fissures in the skin
- •Scar tissue covering the labia or clitoris
- •Bleeding when having bowel movements
- •Painful urination (peeing) due to urine flowing over irritated skin

•How can the Regenerative Clinic help treat Lichen Sclerosus?

•Liposuction was carried out from a donor region and processed lipoaspirate was injected in the damaged area. 5 ml of platelet- rich plasma was injected into the same areas in the intradermal-intramucosal, subdermal, and submucosal compartments. Fifteen days after intervention, symptoms improved, itching and burning disappeared within 1 month. Vulvar skin and mucosa appeared more elastic and soft, with a normal color. Four months after surgery, all patients reported total disappearance of pain and symptoms, and the anatomical features of the vulva were “quite normal”. All patients regained sexual activity. Patients with severe fibrosis and atrophy underwent the procedure again 3 months later, with satisfactory and stable results. Follow- up ranged from 6 to 24 months.

- Clinical applications of adipose derived stem cells and platelet rich plasma are thought to stimulate angiogenesis, fibroblasts and collagen synthesis for tissue reconstruction. 127 patients between 22 to 74 years received 1 to 4 treatments depending on the degree of the lesions, with 3 months intervals. 318 treatments were performed without complications. Improvement was observed after 1 month, in urethral dislocation, repositioning and normalization of the urinary flow was observed. Follow-up showed stable results.

StemGenn THERAPEUTICS

StemMAX

Protocol for SUI

- **Intimacell®**

- Protocol for SUI:
- Autologous adipocytes & secretomes is produced by well-established techniques including cell harvesting from lipoaspirates, expansion of adipose tissue derived stem cells, and differentiation into pure and immature adipocytes.

- A mixture of Micro-fragmented Adipsoe tissues and secretomes injected transurethrally via cystoscope under local anesthesia. The injections were placed directly under mucosa: 1.7 cm distal from the urethral neck at 3 and 9 o'clock, injected volume being 4-5 ml per patient. Two additional concomitant injections of MAT mixed with hd-PRP(volume 2.5 ml) were performed 1.5 mm more distally to bring the MAT in contact with the urethral musculature.

- We followed up with patients at 3, 6, and 12 months after the injections by a gynecological examination, a vaginal ultrasonography, a cough test, a 24-hour pad test, standardized questionnaires, and urodynamic evaluations (at 6 months).

- The primary outcome measure was the cough test. Other outcome measures were the 24-hour pad test, urodynamic evaluations , maximal urethral closure pressure [MUCP], and urethral stress profile, and patients' evaluations of their quality of life.

- The mechanisms underpinning the regenerative capabilities of mesenchymal stem cells (MSC) were originally thought to reside in their ability to recognise damaged tissue and to differentiate into specific cell types that would replace defective cells. However, recent work has shown that molecules produced by MSCs (secretome), particularly those packaged in extracellular vesicles (EVs), rather than the cells themselves are responsible for tissue repair.

IASRM

LIPOKRAFT ™

- LIPOKRAFT ™ is a cutting edge micro-fragmented Adipose tissue (MAT) Procedure that gently processes and uses your body's own fat tissue to cushion and support areas of injury or damage as your body heals itself . It’s autologous & same-sitting injection of adipose tissue. It enhances the healing capacities for cells as well as provide volumising effects .
- fat grafting technique using autologous micro-fragmented adipose tissue (Lipokraft) in female patients suffering from stress urinary incontinence

- Methods
- Patients with primary SUI or MUI with a predominant SUI component were recruited from a private academic urology practice. Women with a present diagnosis of cancer, untreated vaginal prolapse, incontinence of unknown etiology, overflow incontinence, neurogenic bladder, concomitant pelvic floor disorders, vulvar dermatosis, herpes simplex or active or recurrent urinary tract infections, chronic steroid use, or under the age of 18 were excluded. Prior to undergoing Lipokraft anti-incontinence medications were withheld for an appropriate washout period, and then video urodynamics were performed to obtain baseline filling and voiding parameters.

- Subjective (patient visual analog score) and objective (patient reported pad counts, and physician documented cough stress test) measurements were performed at baseline (pre-treatment), three, six, and twelve months post-operatively. Urodynamics were repeated at six months to measure the leak point pressure (LLP) if leakage was present.
- The patient was restricted from taking nonsteroidal anti- inflammatory medications for three days prior to and for two weeks after Lipokraft treatment to minimize risk of bleeding. Steroids were also withheld for three days prior to and for 12 months after so as not to affect the regenerative process.
- Harvesting of adipose tissue and micro-fragmentation of the lipoaspirate.

LIPOKRAFT ™

- The lipoaspiration procedure involves two steps: infiltration and aspiration. The harvesting site is chosen by the patient's body habitus: lower abdomen, lower back, hips, or outer thighs. The goal is to collect 60 ml, and then the aspirated adipose tissue is inserted into the Lipokraft procedure.The adipose is then micro-fragmented, filtered, and purified from oils and residual blood cells. Ultimately, ~20% of the original 60 mL is obtained as final lipoaspirate and used for mid- urethral injections through a needle of 21 G.

- Re-inoculation

- An injection needle, inserted via a cystoscope, is used to inject 10-20 mL of Lipokraft at the sphincteric level, typically at the 3, 6, 9 and 12 o'clock positions, as well as at the bladder neck and in the peri- urethral space. Injected volume is dictated by the individual patient and condition; more severe weakness requires greater volume of injection.

- After withdrawal of the cystoscope, the bladder is drained with an 8-French foley catheter and the patient is given the opportunity to void.

Managing Male Infertility

- **Oligospermia :** A male infertility issue characterised by low sperm count.

- **Azoospermia :** A complete absence of sperm from the fluid ejaculated during orgasm (semen).

- **Erectile dysfunction :** Occurs when a man can’t get or keep an erection firm enough for sexual intercourse.

- With stem cells, these conditions are managed by injecting bone marrow derived stem cells and GCSF mobilised peripheral blood derived stem cells in the testis (for oligospermia and azoospermia) and in corpora cavernosa (for erectile dysfunction).

- The success rate is upto 60% in all the three conditions.

Azoospermia

- 60 ml of Bone marrow will aspirated for stem cells isolation and preparation.
- 5 ml of stem cells prepared injected into testis.
- Cases Improvement Time Frame: 12 Weeks
- Serum Hormonal Profile: (Elevation of testosterone levels, decreasing of FSH, LH and Prolactine Levels); Testicular Size (increased size); and Sexual Potency (increased sexual potency).
- **Exclusion Criteria:**
- Patients with obstructive Azoospermia
- Men with previous surgery in testis
- Men with infectious genital diseases and anatomical abnormalities of the genital tract
- Those with major medical problems such as malignancy, hepatitis, etc. Chromosomal aberration (e.g. Y microdeleion, trisomy)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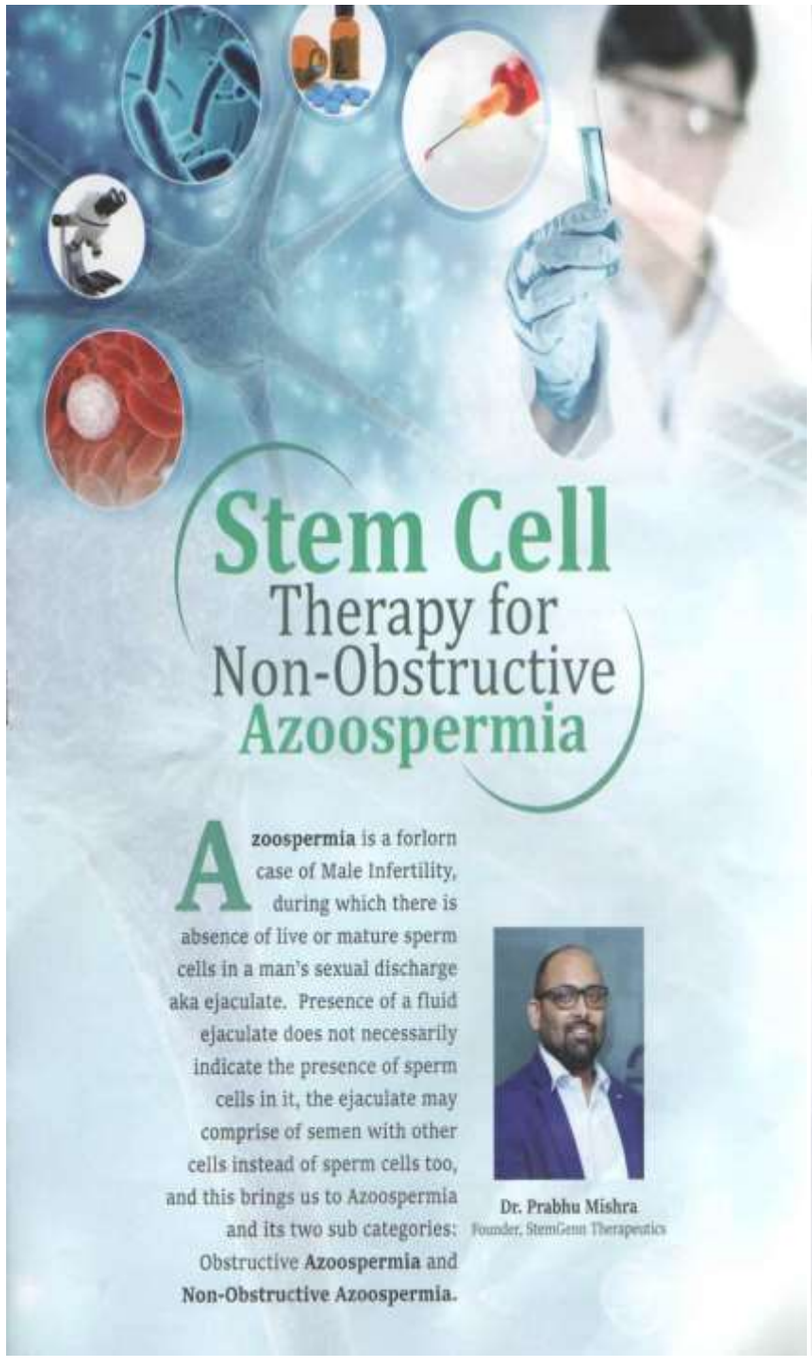
Published Articles



Stem Cell Therapy for Non-Obstructive Azoospermia

Azoospermia is a forlorn case of Male Infertility, during which there is absence of live or mature sperm cells in a man's sexual discharge aka ejaculate. Presence of a fluid ejaculate does not necessarily indicate the presence of sperm cells in it, the ejaculate may comprise of semen with other cells instead of sperm cells too, and this brings us to Azoospermia and its two sub categories: Obstructive **Azoospermia** and **Non-Obstructive Azoospermia.**

Dr. Prabhu Mishra
Founder, StemGenn Therapeutics

HEALTHWISE

Obstructive Azoospermia: As the name suggests, this is a condition where there is an obstruction; due to which the sperm cells are unable to release into the ejaculate in spite of an active sperm production taking place in the body.

The reasons behind Obstructive Azoospermia can be one to many, with the major one being vasectomy and this certainly is a reason that the patient himself would be aware of. Nonetheless, Vasectomy is not just the case, there are various other problems too which end up obstructing the sperm cells from entering into the ejaculate. Such cases of obstructive Azoospermia are taken care of- using surgical sperm extraction procedures during which an acceptable number of sperm cells are extracted from the male and used during an IVF cycle.

Non-obstructive Azoospermia: In case of non-obstructive Azoospermia, there is a problem with production and maturation of sperms which is why in such cases surgical methods involving sperm extraction are not liable of delivering desired outcomes because here the sperm cells are not being produced or being fully developed.

Thus, the alternative treatments for such cases are: Stem cell therapy and **IVF treatment** which is performed seeking sperms from a male donor. However, if the patient is not willing or is not ready to proceed with the IVF treatment using the donor sperm, an assessment can be carried out in order to examine his ability to undergo stem cell therapy. The assessment involves the conduction of a few necessary tests, which include:

During the First phase of tests, we aim at making the patient undergo all relevant test results, such as:

1) Semen Analysis + Biopsy - The results derived from thisparticular test relates to the diagnosis of non-obstructive Azoospermia.
2) Blood tests including Alpha-fetoprotein (AFP) and Beta human-chorionic gonadotropin (b-hCG) - The results of this test will allow us proceed to the next step, since we have confirmed the non-existence of a neoplastic change in the testicles.

Once the patient has been declared fit for carrying out stem cell therapy, the next step is to obtain results for a few standard tests; these tests are general and are normally performed prior to any operation/ surgery:

1) Complete blood count
2) Screening of an Infectious disease
3) Analysis of Patient's vitals

Onor these tests are performed and the conclusions have been made, Stem cell therapy can be conducted. Stem cell therapy for Azoospermia can be carried out in any which ways, out of the two given below:

a) Systemic infusion Method where systemic infusion of stem cells is carried out via IV infusion.
b) Direct Injection Method where the processed stem cells harvested from the patient's body are directly injected into the testicles.

However, the success ratio of the first method is comparatively low in comparison to the second method. The second method i.e. the direct injection method has served more desirable results based on the observations made so far.

Stem cell therapy revolves around using the patient's own adipose tissue for harvesting cells and this extraction procedure is carried out via liposuction. The entire procedure can be carried out within no downtime, since our aim is to obtain a very small amount of tissue. Once the required amount of tissue is obtained, it is processed and adipose derived stem cells are isolated in the lab, later combining these cells with the patient's own platelet-rich plasma (isolated from the patient's blood sample) making a cocktail in order to enhance the effect of the treatment. It is made sure that every single step that goes behind the preparation of the cocktail is carried out under most advance care possible. The cocktail is then injected into different areas of the testicles which might be one of the reasons behind the halt of the spermatogenesis cycle.

About the Author :
Dr. Prabhu Mishra is the President, CEO, Co-founder of StemGenn Therapeutics. Dr. Prabhu develops strategic alliances and implements national and international projects for the mission of advancing regenerative medicine and stem cell therapy.

Erectile Dysfunction

- The term "**Penoplasty**" refers to a combination of procedures the surgeon may use depending on the result the patient is looking for, Eg. penile lengthening, girth enhancement, or both. In order to obtain the desired result, the following procedure :
- Laser division of the suspensory ligament (penile lengthening)
- Suprapubic cutaneous laser-plastic surgery (V-Y flap surgery)
- Suprapubic vibroliposculpture (suprapubic lipectomy)
- Penoscrotal webbing plastic surgery
- Lipo-sculpture of the penis (girth enhancement)

Erectile Dysfunction

- It is now possible to increase the length and girth of the penis, individually or (better) in association, through quick and relatively simple surgical operations.

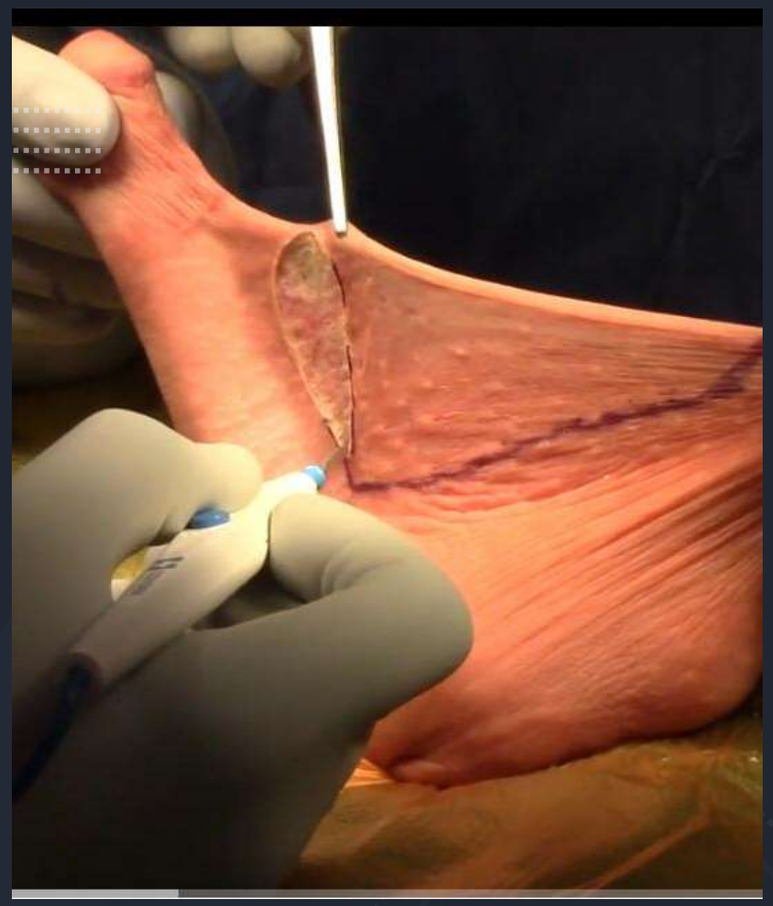
- At present, such surgical methods are standardised. All our procedures are outpatient procedures, with discharge within a few hours from the surgery.

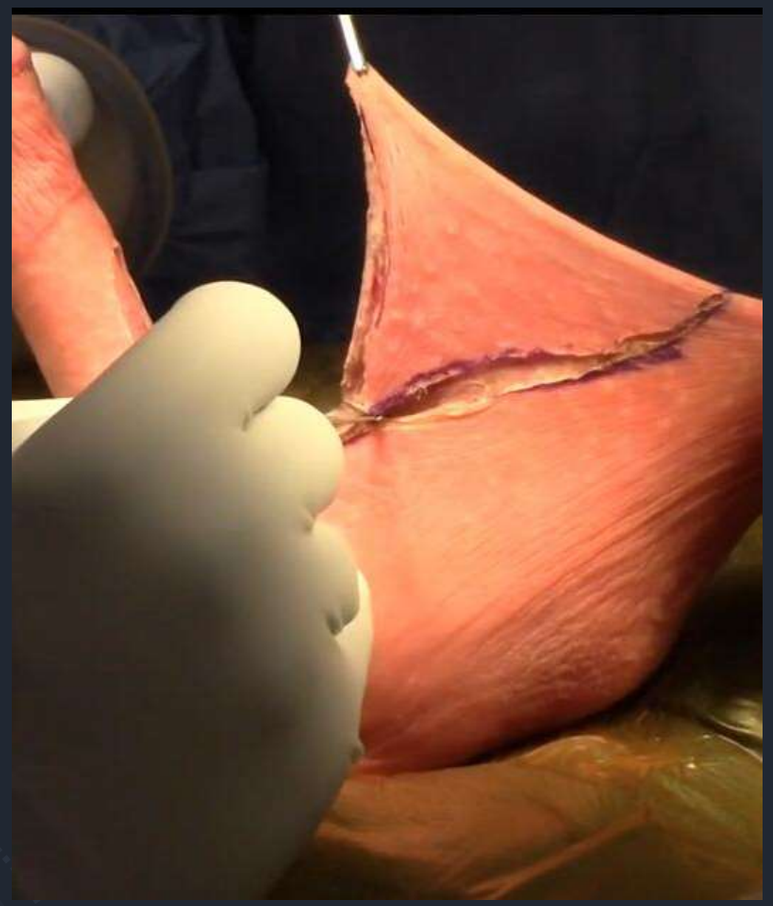
- Regenerative Medicine have added many advances . Length, Girth and Angiogenesis (Erection) in single procedure .

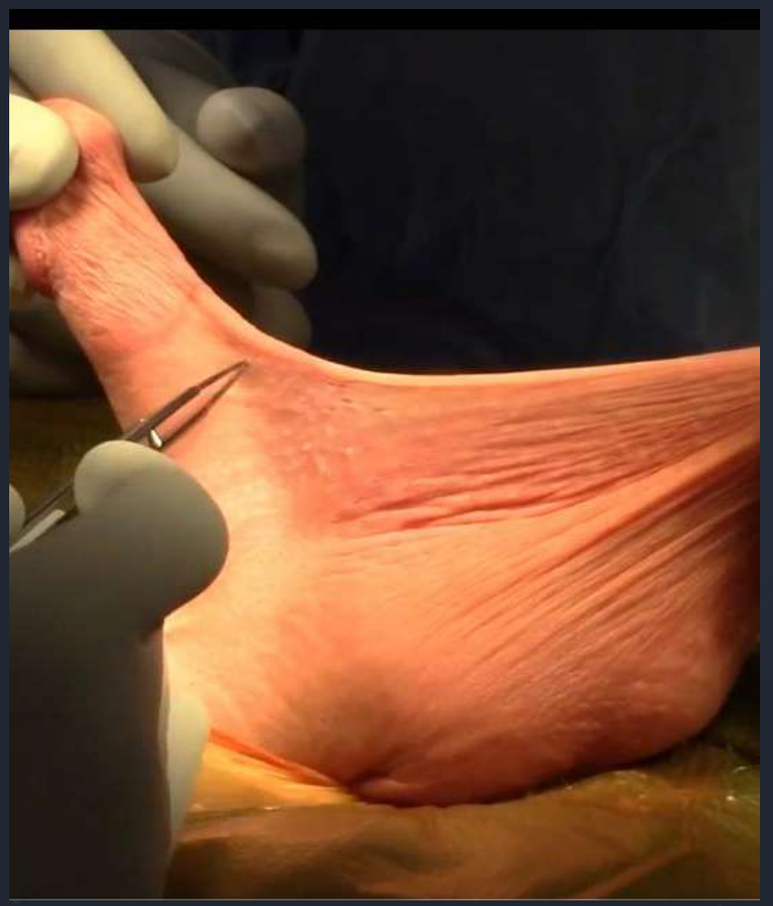
- Phallosplasty(For Length + Nanofat along SVF. (Girth)+ SVF mixed with PRP(Erection)

Erectile Dysfunction

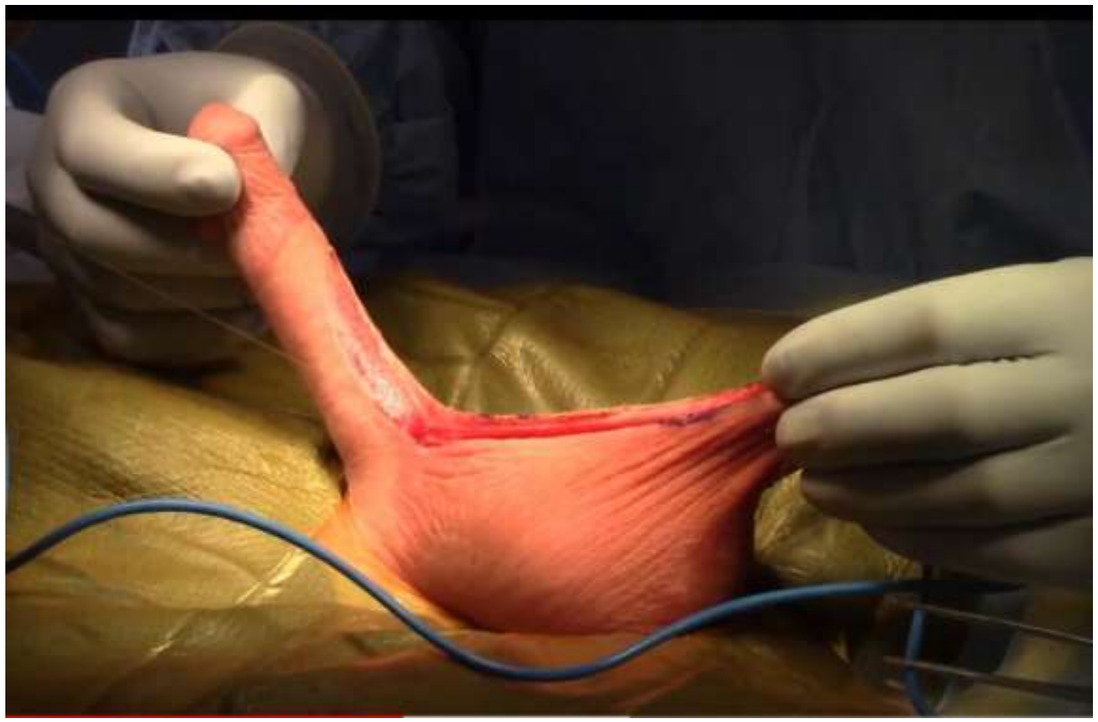
- Patients' satisfaction was assessed using 5-points Liken scale pre-operatively and 2 weeks, 3 months postoperatively. Also, the pen scrotal junction to glans tip length was measured pre and post-operatively to measure the percent of newly exposed ventral penile shaft skin.
- **Results:** Showed that patients satisfaction was significantly improved post-operatively (p-value 0.001). Concerning the post-operative complication; only one case had ecchymosis, one skin dehiscence, and one superficial skin infection.
- **Conclusion**: Ventral Phalloplasty is considered to be safe, none time consuming surgical technique, with minimal post-operative complications that improves the patients' satisfaction about their penile length after penile prosthesis implantation. Nanofat + SVF combined given enhancement of Girth in around 30%. PRP+SVF: given new angiogenesis which leads to erection.

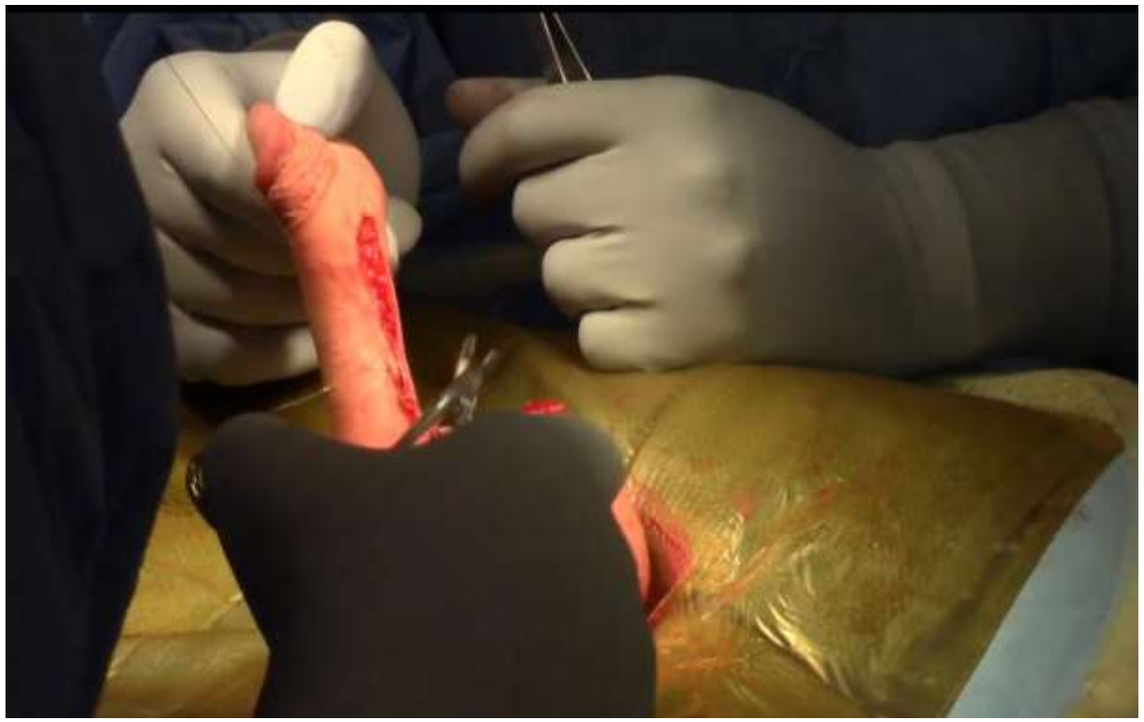


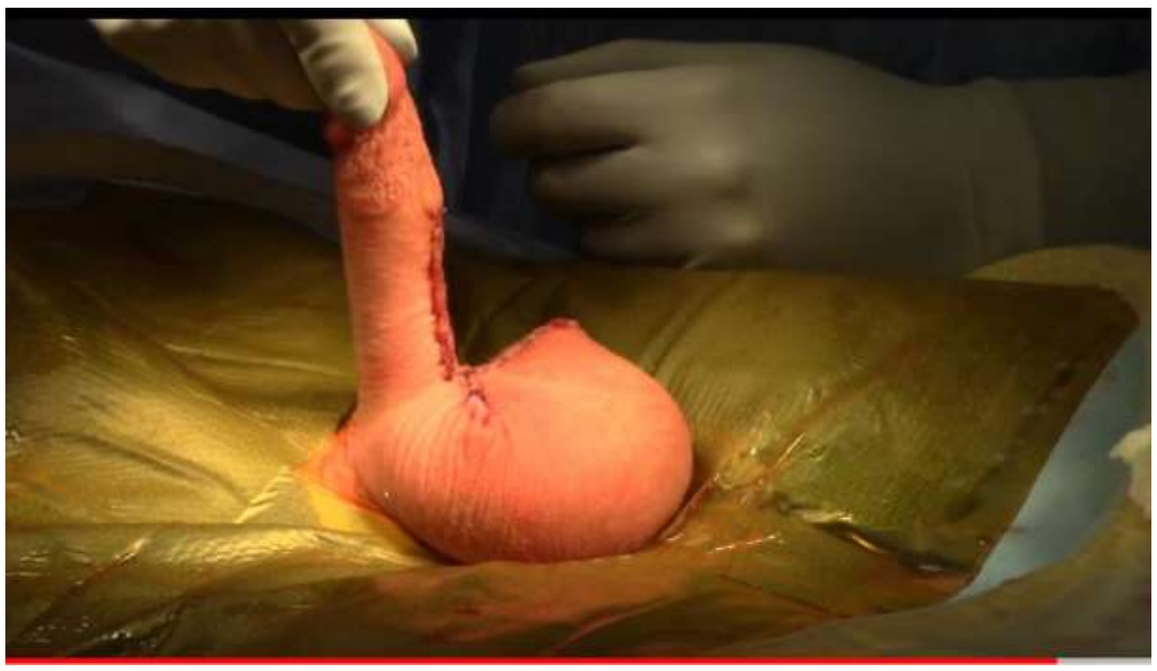




Intra Corporal cavernous Infusion







StemGenn THERAPEUTICS

StemMAX

Stem Cells and Cell based therapy in Cosmetic Gynecology

- PRP Fat Transfer PRF Gel
- NanoFat
- Adipose derived stem cells
- **Indications:**
- PRF gel and fat transfer for volume restoration, Rejuvenation of vulvar skin and vaginal wall, Vaginal recalibration, Labiaplasty of labia majora (Augmentation and Rejuvenation), vulvar dystrophy, post menopausal female genital atrophy lichen sclerosis, vaginal Tightening and rejuvenation

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Original Research Article

Role of autologous bone marrow derived stem cells and platelet rich plasma for endometrial regeneration and repair and ovarian rejuvenation

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ABSTRACT

Background: Stem cells are undifferentiated cells with a potential for self- renewal and differentiation into multiple mature cell types. PRP is blood plasma that has been enriched with platelets and multiple growth factors that can stimulate cellular processes and activate multi-potent stem cells to generate new, younger tissue and new blood vessels relevant to ovarian rejuvenation and endometrial regeneration.
Methods: This study was to evaluate the effectiveness of ABMDSC and PRP application in patients with thin endometrium refractory to treatment, premature ovarian failure, infertility and menopause. 37 symptomatic women between age group 18 years to 56 years were selected for ABMDSC and PRP instillation in endometrium and/ or ovaries. PRP was instilled in endometrium in 8 patients, in ovaries in 5 patients and in both endometrium and ovaries in 24 patients.
Results: There was significant improvement in endometrial thickness, along with improved blood flow to both endometrium and ovaries. FSH levels decreased and ovarian volume increased. There were three confirmed pregnancies with one delivery. Menopausal symptoms decreased in 2 patients and spontaneous resumption of menses was seen in 1 patient of POF.
Conclusions: Stem cell therapy serves as a game changer with their unique properties, offering solutions for scores of women suffering from POF, poor oocyte quality, endometrial degeneration/ damage or menopausal symptoms.

Keywords: Bone marrow-derived stem cell, Platelet rich plasma, Premature ovarian failure, Thin endometrium

INTRODUCTION

Current research has supported the knowledge that approximately 1000 quiescent residual primordial follicles remain in the ovaries at menopause. This is in contrast to the earlier concept that human ovaries are born with a finite number of ova, that determined her reproductive lifespan, the number depleting entirely during menopause.

Stem cells are undifferentiated cells with the ability for self-renewal producing exact copies of themselves by continuous division and to advance into specialized cells by differentiation. PRP is autologous blood plasma enriched with platelets containing several growth factors and cytokines. The growth factors released by the PRP also activates multiple stem cells instrumental in angiogenesis and formation of denovo younger tissue.

Inclusion criteria

For endometrium

Persistent thin lining <6 mm in previous IVF or FET cycle, moderate-to-severe Asherman's syndrome, severe oligomenorrhea/amenorrhea.

For ovaries

Primary or secondary amenorrhea at least for 3 months, infertile women having DOR and low AMH levels, diagnosis of DOR defined as: AMH ≤0.42 ng/ML & FSH ≥12 IU/L,diagnosis of POF with FSH levels ≥ 30 IU/L, normal karyotype 46, XX, presence of at least one ovary

Exclusion criteria

Age≤18 years old, pregnancy or lactation, history of, or evidence of malignancy, Hb≤8 g/dl, platelets≤150,000/mm^3, anticoagulation therapy, any contraindication to laparoscopic surgery and / general anesthesia, medical conditions that are contraindicated in pregnancy, any significant comorbidity or psychiatric disorder.

Results

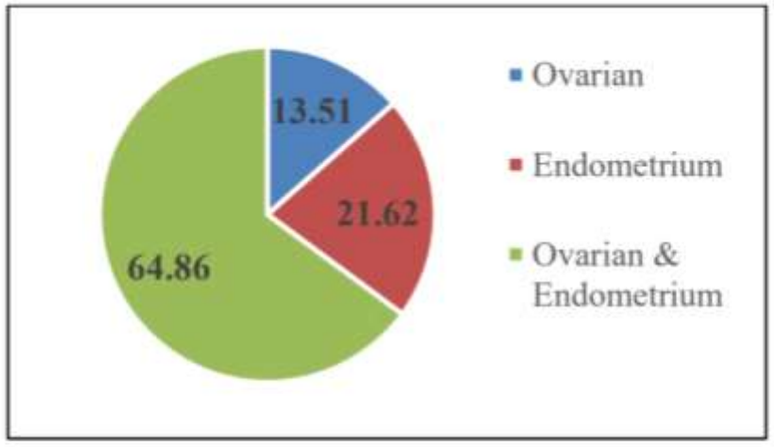

Figure 2: ABMDSC and PRP instillation.

Table 1: Improvement in endometrial thickness (Day 15).

Improvement in endometrial thickness	Frequency	Percent
Improved	31	100
Total	31	100

Comparison of initial ET and ET on day 15 showed P-Value of 0.000 which indicates significant improvement (Paired sample test 1).

Table 2: Improvement in endometrial blood flow (Day 15).

Improvement in endometrial blood flow	Frequency	Percent
Improvement	23	69.70
No improvement	10	30.3
Total	33	100

Comparison of initial endometrial blood Flow and blood flow on Day 15 showed P-Value of 0.000 which indicates significant improvement (Paired sample test).

16/37 patients had normal ovarian blood flow pre procedure. Post procedure ovarian intrastromal blood flow improved in 20/ 37 patients (Table 3).

Table 3: Improvement in ovarian blood flow (day 30).

Improvement in ovarian blood flow	Frequency	Percent
Improvement	4	19.05
No Improvement	17	80.95
Total	21	100

Results

FSH levels improved in 26/29 cases (89.66%) with no improvement in 3/29 cases (Table 4), FSH improvement (Day 30) with mean FSH of 32.86 before study to 10.73 at end of 30days (Table 5), Mean FSH values before and after procedure. FSH levels improved in 12/12 POF patients and 2/2 MP patients (Table 6, FSH improvements in POF and MP patients (Day 30).

Table 4: FSH improvement (Day 30).

Improvement in FSH Patients	Frequency	Percent
Improvement	26	89.66
No improvement	3	10.34
Total	29	100

Table 5: Mean FSH values before and after procedure.

	Mean	N	Std. deviation
FSH –Before	32.8686	29	28.62151
FSH - After	10.7359	29	9.41591

Table 6: FSH improvements in POF and MP patients (Day 30).

	Total patients	Improvement	Percent
POF	12	12	100
MP	2	2	100

Table 7: Paired samples statistics.

		Mean	N	Std. deviation	Std. error mean
Pair 1	ETB	5.5811	37	1.84810	0.30383
	ETA	7.7162	37	1.45173	0.23866
Pair 2	BFB	1.2162	37	.62960	0.10351
	BFA	2.5135	37	.86992	0.14301
Pair 3	FSHB	32.8686	29	28.62151	5.31488
	FSHA	10.7359	29	9.41591	1.74849

ETB – Endometrial Thickness Before; ETA – Endometrial thickness after (Day 15); BFB – Blood flow before; BFA – Blood flow after (Day15); FSHB – FSH before; FSHA – FSH after (Day 30)

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Original Research Article

The treatment analysis of the patients suffering from vaginismus and the correlation with the psychological issues

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ABSTRACT

Background: We aimed to present the demographic information, treatment protocol, and results of 482 female patients that presented to our clinic specialized in sexual dysfunction with the complaint of no or only partial sexual intercourse and were diagnosed with primary vaginismus.
Methods: The female patients were asked eight questions about demographics; 13 questions about marriage; seven questions about family structure and upbringing; three questions about history of psychiatric diseases and general phobias; and 17 questions about sexual history and previous treatments. The male spouses were asked seven questions concerning age, occupation, educational level, personality, sexual experience, and sexual dysfunction.
Results: The median age of the female patients was 28 and their spouses was 29. The mean duration of marriage was 18.2 months. Of the female patients, 65.4% reported that they felt they would have pain during sexual intercourse, 23.6% stated that they really had pain, 74.1% mentioned that they had heard horrifying stories about the first night of marriage in the pre-marital period. Cognitive behavioral therapy was performed alone in 85.7% of the patients, following hymenotomy in 5%, and following hymenectomy in 9.3%.
Conclusions: False and exaggerated information about sexuality being embedded in the subconscious of women is very effective in the development of vaginismus. On the other hand, traditional family structure, adolescent traumas, first night stories, and superstitions about sexuality are among the important causes of vaginismus.

Keywords: Female sexual pain, Genito-pelvic pain, Penetration disorder, Psychological issue in vaginismus treatment, Vaginismus, Vaginismus treatment

Research Article

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PLATELET RICH PLASMA (PRP) THERAPY: A PERSPECTIVE INTO TREATING GYNAECOLOGICAL DISORDERS

Prabhu Chandra Mishra[1]*, Jyoti Bandi[2], Nidhi Khurana[3], Diana Mihai[4], Nidhi Jha[5], Poonam Mishra[6], Sheryl Palad[7], Adrienne E. Lara[8], Saurabh Kumar Jha[9], Puja Sharma[10], Didem Kurban[11], Vishwa Chavda[12] and Manar Jabbar[13]

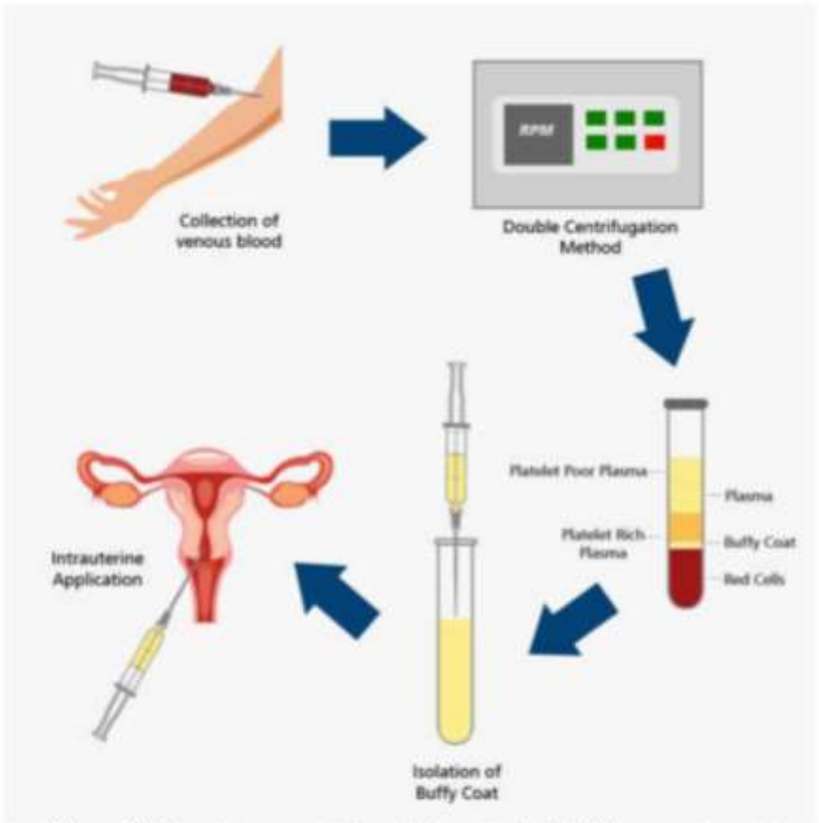

Figure 1 Schematic representation of the protocol for PRP preparation and collection for application. Venous blood is collected and subjected to double centrifugation method to separate the components of the blood. After centrifugation, the blood components (red blood cells, leukocytes, and platelets) are separated from the plasma due to their different densities. The platelets have the lowest density and hence float on top. The yellow part consisting of Platelet Rich Plasma is then collected via syringes and to heal pre-menopausal issues, is subjected to intrauterine application.

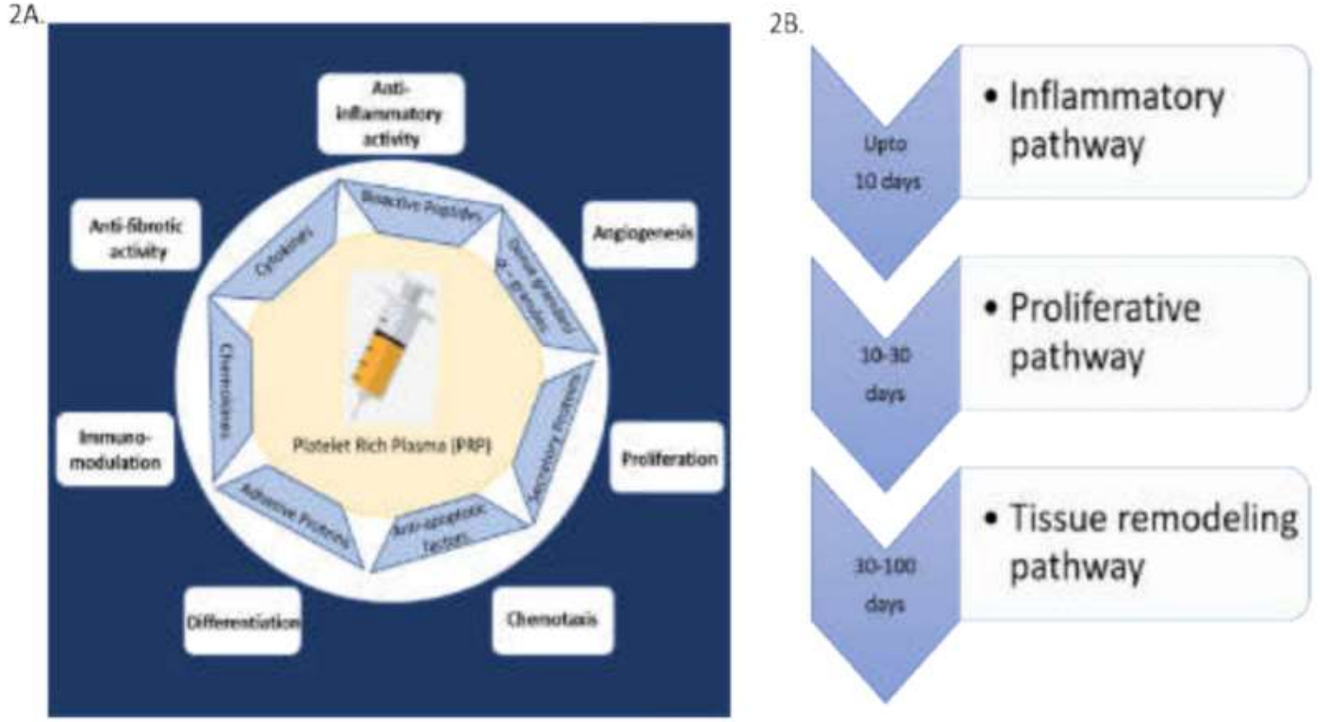

Figure 2 Mechanism of action of PRP. (2A) The figure depicts the composition of PRP and the various pathways that it is predicted to target to initiate the healing cascade. (2B). The timeline of the PRP induced process. The flowchart signifies when to repeat the next cycle of PRP.

Figure 3 Applications of PRP. The figure shows various therapeutic and aesthetic application of PRP and the conditions it is used to treat under each of the areas.[10]

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"The precious pages of this book contain the particularly modern, enhanced and condensed knowledge of Stem Cell and Regenerative Medicine strategies entirely aimed at reconciling fertility in men and women. The authors of this book have put together a remarkable bulk of research and insights that offer a safety net of regenerative treatment possibilities to patients in both high and low-income countries." - **Prof. Elvira Bratila MD Ph.D.**, Urogynaecologist,Bucharest,Romania

"This text provides the basics and delves into the detailed protocols of stem cell use as the new frontier in the armamentarium of infertility treatments. The authors have not only explained how the science of regenerative therapies have evolved but have delivered the specifics of the protocols so that any capable provider can begin to offer the treatments themselves."- **Dr. Carolyn A DeLucia, MD** FACOG, New Jersey, USA

This book brings you a comprehensive package on latest advancements in the field of stem cells.A unique feature that makes this book stand out is the access to the video protocols of techniques like PRP, BMAC, Adipose Stem cells in various gynaecological indications.
Dr. Nidhi Khurana, Chief Scientific Officer, StemMax Research and Therapeutics

Prabhu travels the world and teaches his gospel of stem cells and regenerative medicine to all who are interested. In this new book the clinical applications of his research are combined with the clinical experience of Romanian physician Diana Mihai. This is definitely not mainstream reproductive endocrinology so do not expect the usual and mundane. This book will stretch your boundaries.
Dr. Red M. Alinsod, MD
Alinsod Institute for Aesthetic Vulvovaginal Surgery, South Coast Urogynecology, California

Stemcell & Regenerative Medicine in Infertility.

This book discusses various avenues that stem cell therapy has opened for fertility treatment. One starts by learning about the basics of stem cells and how different kinds of infertility indications can be treated by various stem cell-based strategies like from bone marrow derived stem cells, adipose derived stem cells, and platelet rich plasma. Detailed protocol and information has been provided for the clinical trials, research, and treatment of asherman's syndrome, endometrial regeneration, ovarian rejuvenation, azoospermia, erectile dysfunction among other conditions leading to infertility in men and women.

About the Authors

Prabhu Chandra Mishra is the CEO of StemMax Research and Therapeutics Pvt. Ltd., India and President of International Association of Stem Cell and Regenerative Medicine (IASRM). He has marked his own place with his continuous efforts in the field of stem cells and anti-ageing in India and is today not just recognized nationally, but also internationally for his endeavours. His experience in the regenerative medicine industry for the last 15 years and his academic background (Master of Science in Molecular Medicine & Stem Cell Technology, Doctoral degree awarded, mini-MBA in addition to certificate courses in the field of Stem Cell Technology, Nanomedicine, and Translational medicine) contribute to his great success in the field. He has various publications published in well reputed national and international journals and 11 trademarks registered to his name. He has been featured in various national and international magazine and media.

Diana Mihai M.D. is a Gynaecologist, Obstetrician, Fertility specialist and Gynaecology Aesthetician from Bucharest, Romania. She is the pioneer of Aesthetic Gynaecology in Romania who built the path to opportunities for regenerative medicine in the gynecological field and currently brings both theoretical knowledge and practical skills to her aspiring colleagues as the National PRP and laser Trainer in Gynecology during her monthly 3-day courses. Passionate for the questions of Infertility, she competes both her PhD and her master degrees in the field of Human Reproduction and is additionally a bright member of European and American Human Reproductive Societies. She has multiple research publications and various meaningful accomplishments to her name that rightfully highlight her contribution to exploring the potential of infertility and regenerative medicine and offering modern techniques in treating complex gynaecological disorders.

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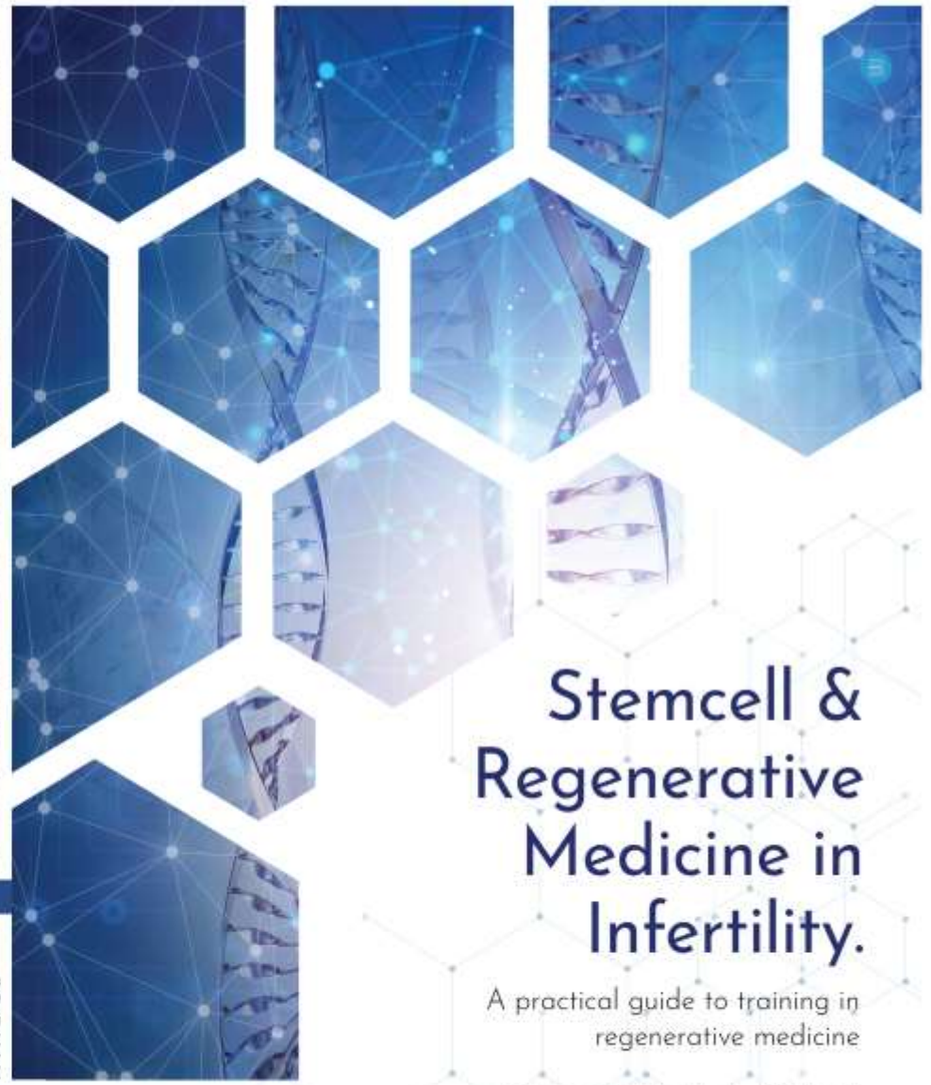
STEMCELL & REGENERATIVE MEDICINE IN INFERTILITY

DR. DIANA MIHAI
PRABHU CHANDRA MISHRA



Stemcell & Regenerative Medicine in Infertility.

A practical guide to training in regenerative medicine

DR. DIANA MIHAI
PRABHU CHANDRA MISHRA

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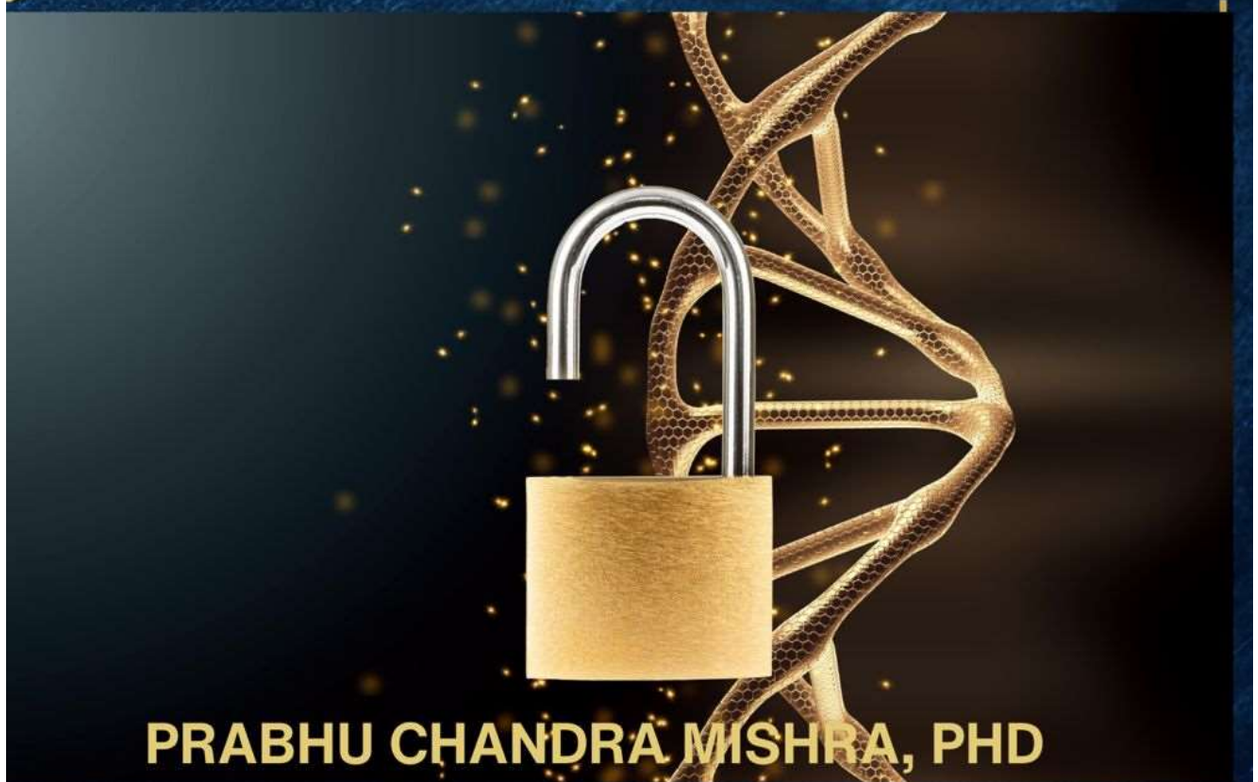

PRABHU CHANDRA MISHRA, PHD

Thank You
for
your patience
and
being a great audience.