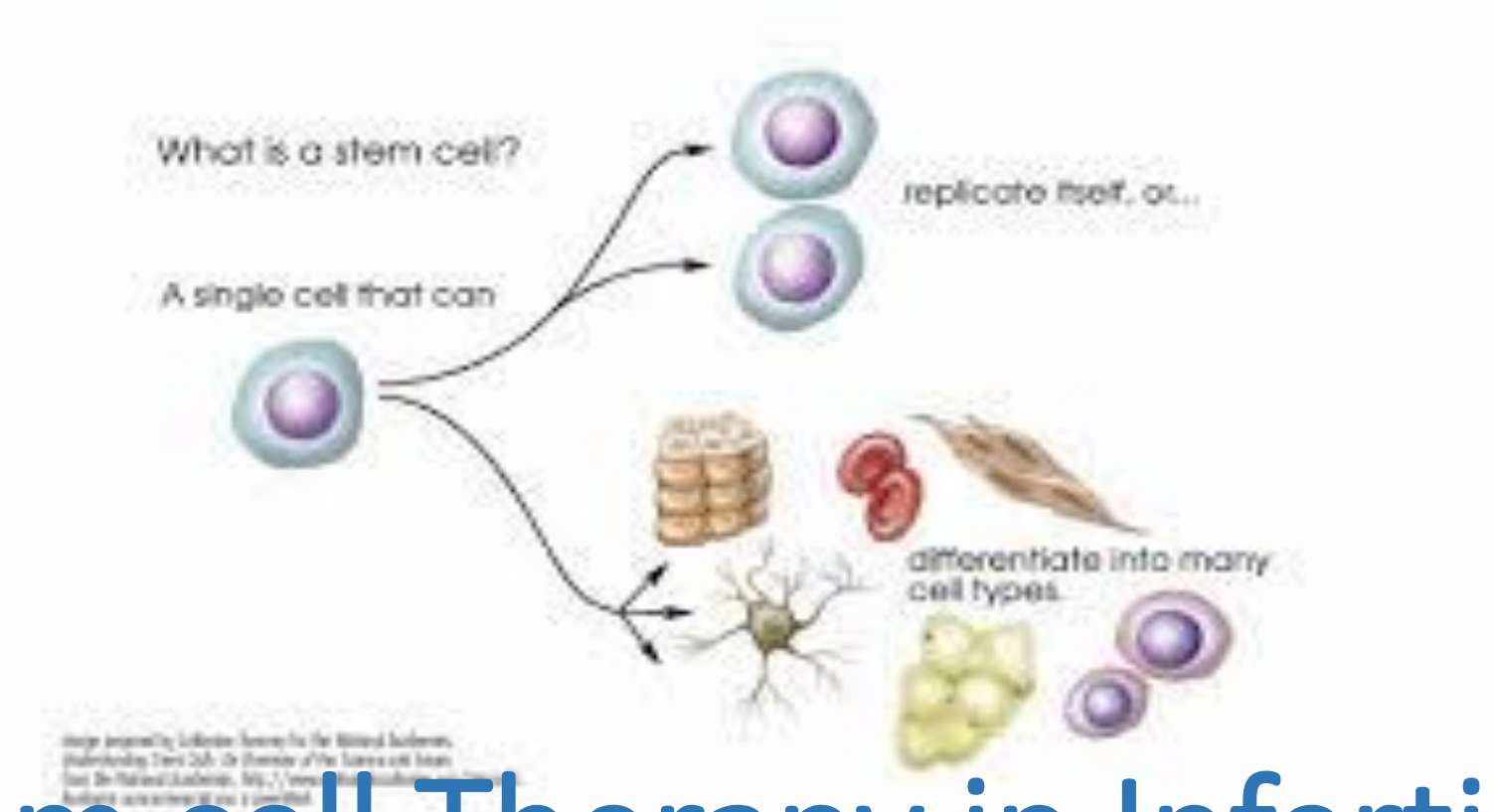
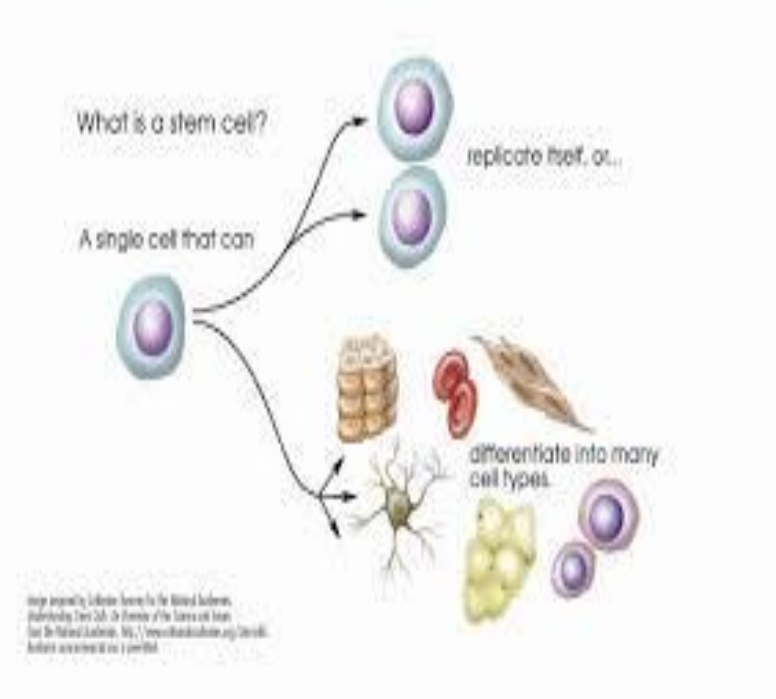


Stem cell Therapy in Infertility

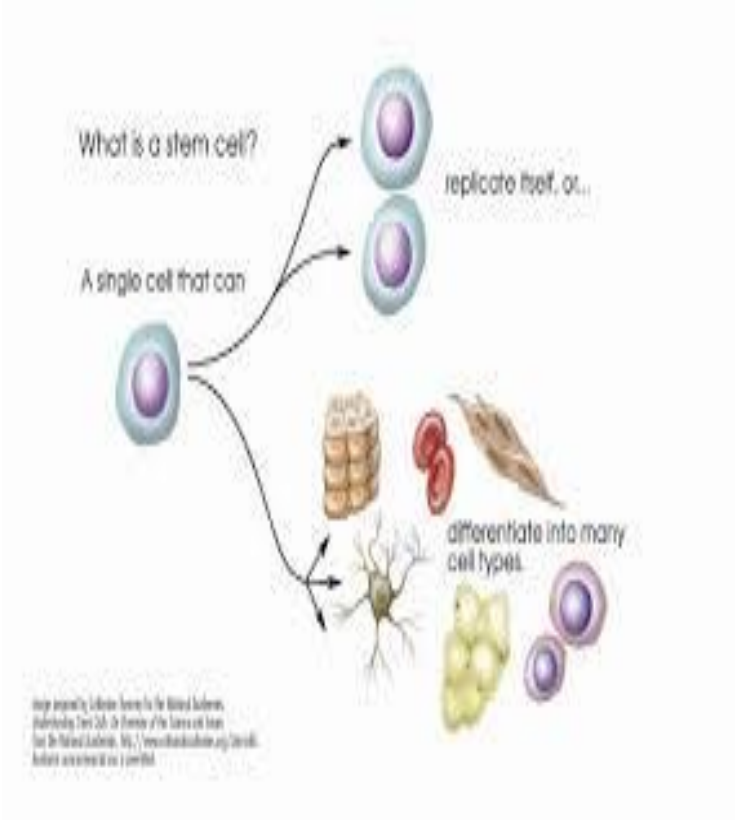
Zafardoust Simin.M.D.

Fellowship in infertility and reproductive medicine

- Infertility is **a major health problem**, and despite improved treatments over the years, there are still some conditions that **cannot be treated** successfully using a conventional approach. Therefore, new options are being considered and one of them is **cell therapy** using stem cells.



- Women are born with a **finite pool** of ovarian follicles that decreases dramatically during intrauterine life from a peak of about **7 million to 1 million at birth**. During childhood the descent continues, so that at the **age of menarche about 400,000** persist follicles. Finally at menopause there are only **less than 1,000** follicles in the ovaries.

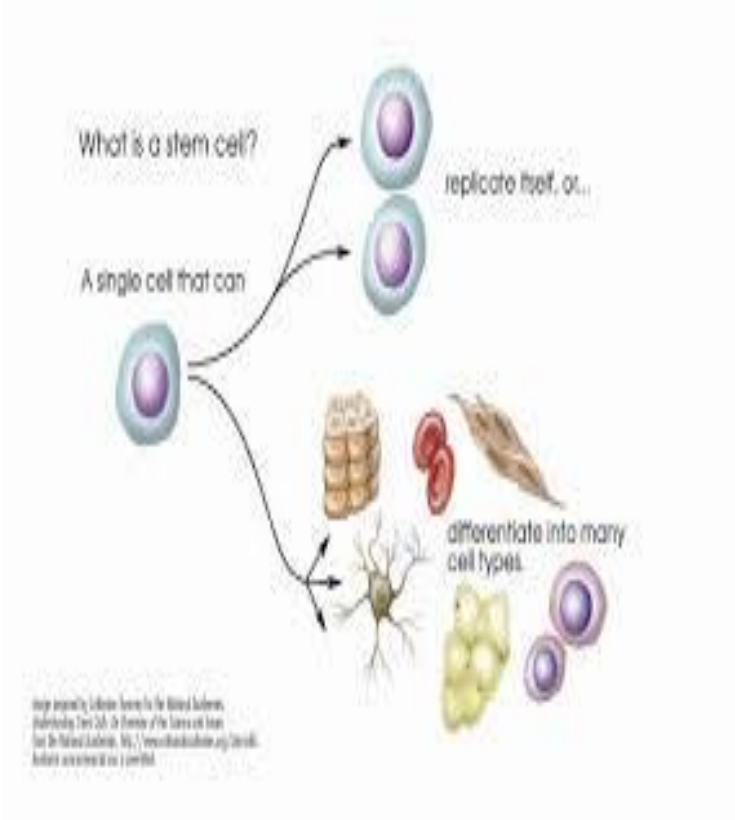


➢**According to these concepts, three different scenarios may occur:**

1) A normal decrease of ovarian reserve with age

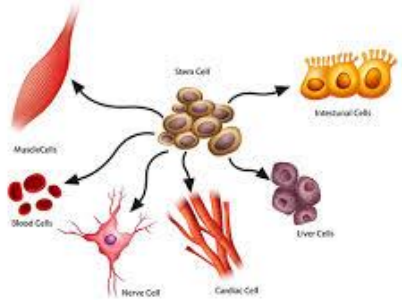
2) a lower ovarian reserve set prenatally with an usual postnatal decay

3) A decrease of ovarian reserve during adverse postnatal environmental or nutritional challenges



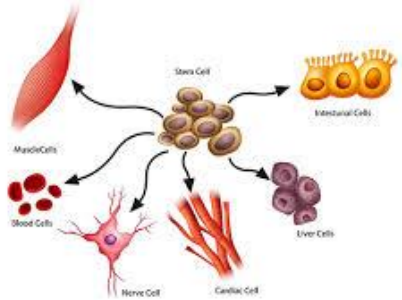
Anyhow, the diminished ovarian reserve (DOR) constitutes one of the most important therapeutic challenges in assisted reproduction.

Poor Ovarian Responders



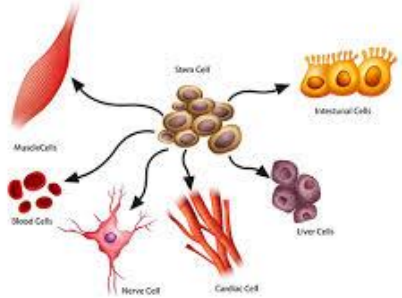
- Poor ovarian response is encountered in **9–25%** of patients undergoing IVF.
- The rate is reported as **50 %** in women over 40 years old
- FSH begins increasing 13 years before menopause. With increasing FSH, the number of follicules, oocytes, embryos and implantation rates decrease, and cycle cancellation rates increase.
- Despite improvements in ART, there is **no consensus** on the management of patients with poor responses.

Poor Ovarian Responders



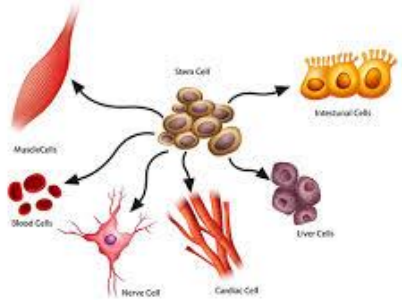
- In patients with POR, the incidence of poor response at the **second cycle** was reported as **62%**.
- A **decreased ovarian reserve** is related to decreased **oocyte quality**.

Bologna criteria



- 2011
- At least **two of** the following characteristics are needed to consider a poor ovarian responder:
- **1)** advanced maternal age (>40 years);
- **2)** a previous meager response, unerringly three or less oocytes after conventional ovarian stimulation protocol;
- **3)** an abnormal ovarian reserve test such as antral follicle count (AFC) less than five to seven follicles or anti-Mullerian hormone (AMH) below 0.5–1.1 ng/ml

Live birth rates in the different combinations of the Bologna criteria poor ovarian responders: a validation study



- 2015
- The **live birth rate** ranged between **5.5** and **7.4 %** and was not statistically different in the five different categories of women defined as poor responders according to the Bologna criteria.
- In our group ,CPR and LBR are **13.4%** and **6/7%**

POSEIDON GROUP 1

Young patients <35 years with adequate ovarian reserve parameters (AFC≥5; AMH≥1.2 ng/ml) and with an unexpected poor or suboptimal ovarian response.

- Subgroup 1a: <4 oocytes*
- Subgroup 1b: 4-9 oocytes retrieved*

*after standard ovarian stimulation

POSEIDON GROUP 2

Older patients ≥35 years with adequate ovarian reserve parameters (AFC≥5; AMH≥1.2 ng/ml) and with an unexpected poor or suboptimal ovarian response.

- Subgroup 2a: <4 oocytes*
- Subgroup 2b: 4-9 oocytes retrieved*

*after standard ovarian stimulation

POSEIDON GROUP 3

Young patients (<35 years) with poor ovarian reserve pre-stimulation parameters (AFC<5; AMH<1.2 ng/ml)

POSEIDON GROUP 4

Older patients (≥35 years) with poor ovarian reserve pre-stimulation parameters (AFC<5; AMH<1.2 ng/ml)

Recommendation

GnRH antagonists and GnRH agonists are equally recommended for predicted poor responders.	Conditional	⊕⊕◯◯

Recommendation

Clomiphene citrate alone or in combination with gonadotrophins, and gonadotropin stimulation alone are equally recommended for predicted poor responders.	Strong	⊕⊕◯◯

Recommendation

The addition of letrozole to gonadotropins in stimulation protocols is probably not recommended for predicted poor responders.	Conditional	⊕⊕◯◯

Recommendation

It is unclear whether a higher gonadotropin dose is recommended over 150 IU for predicted poor responders.	Conditional	⊕◯◯◯

Recommendations

Use of testosterone before ovarian stimulation is probably not recommended for poor responders.	conditional	⊕⊕⊕◯

Recommendations

Use of DHEA before and/or during ovarian stimulation is probably not recommended for poor responders	Conditional	⊕⊕⊕◯

Recommendation

Use of aspirin before and/or during ovarian stimulation is not recommended in the general IVF/ICSI population and for poor responders.	Strong	⊕⊕⊕◯

Recommendations

Use of sildenafil before and/or during ovarian stimulation is not recommended for poor responders	Strong	⊕◯◯◯

Recommendations

Late luteal phase start of gonadotropins is probably not recommended for poor responders.	Conditional	⊕◯◯◯
Early luteal phase start of gonadotropins is probably not recommended for normal and poor responders.	Conditional	⊕◯◯◯

- Recent research has indicated that the uterine lining, or endometrium, is a rich source of adult stem cells.
- Approximately **a decade ago**, Meng et al. and Cui et al. discovered a novel source of MSCs from human menstrual fluid, named menstrual blood-derived stem cells(MenSCs).
- MenSCs are attracting more and more attention since their discovery in 2007.

- menstrual blood appears to be a rich and **easily accessible** source of **adult** stem cells
- These **mesenchymal-like** stem cells have high rate of **proliferation** and possess multi lineage **differentiation potency**.
- Compared to SCs from bone marrow and adipose tissues, MenSCs come from body discharge and obtaining them **is non-invasive** to the body, they are easy to collect, and there are **no ethical concerns**.

Journal of Translational Medicine
BMC

J Transl Med. 2015; 13: 155.
Published online 2015 May 12. doi: 10.1186/s12967-015-0516-y
PMCID: PMC4490699
PMID: 25964118

Human endometrial mesenchymal stem cells restore ovarian function through improving the renewal of germline stem cells in a mouse model of premature ovarian failure

Dongmei Lai,✉ Fangyuan Wang, Xiaofen Yao, Qiuwan Zhang, Xiaoxing Wu, and Charlie Xiang✉

- Transplanted EnSCs were injected into the tail vein of sterilized mice (n=80).
- Non-sterilized mice were untreated controls (n = 80).
- EnSCs derived from menstrual blood, as autologous stem cells, **may restore damaged ovarian function** and offer a suitable clinical strategy for regenerative medicine.

STEM CELLS AND DEVELOPMENT
Mary Ann Liebert, Inc. publishers
Journals | Search | Alerts

Stem Cells Dev. 2014 Jul 1; 23(13): 1548–1557.
Published online 2014 Mar 4. doi: 10.1089/scd.2013.0371
PMCID: PMC4066227
PMID: 24593672

Transplantation of Human Menstrual Blood Stem Cells to Treat Premature Ovarian Failure in Mouse Model

Te Liu,[✉1] Yongyi Huang,[2] Jian Zhang,[3] Wenxing Qin,[4] Huiying Chi,[1] Jiulin Chen,[1] Zhihua Yu,[1] and Chuan Chen[✉1]

- HuMenSCs were injected into a cyclophosphamide-induced **mouse model of POF**.
- The results revealed that the HuMenSCs could survive within POF mouse ovaries for at least 14 days in vivo.
- The **ovarian weight**, plasma **E_2 level**, and the number of normal **follicles** increased over time in the HuMenSC group compared with the control group.
- Hence, we can safely conclude that the mesenchymal stem cell properties and in vivo survival of HuMenSCs make them ideal seed cells for stem cell transplantation in the treatment of POF.

Acta Biochim Biophys Sin (Shanghai). 2016 Nov;48(11):998-1005. doi: 10.1093/abbs/gmw090. Epub 2016 Sep 2.

Differentiation of human menstrual blood-derived endometrial mesenchymal stem cells into oocyte-like cells

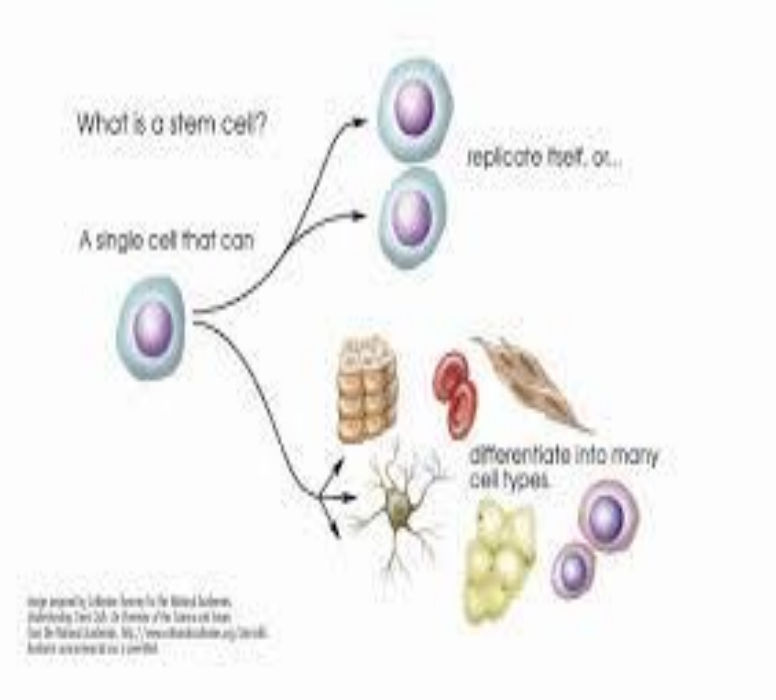
Dongmei Lai [1], Ying Guo [2], Qiuwan Zhang [2], Yifei Chen [2], Charlie Xiang [3]

- EnSCs were induced to differentiate into germ cells in a differentiation medium supplemented with 20% human follicular fluid.
- Our results demonstrated that EnSCs derived from human menstrual blood **form oocyte-like cells** and express **germ cell markers**.
- The induced cell aggregates contained not only oocyte-like structures but also cells expressing follicle stimulating hormone receptor and luteotropic hormone receptor, and produced estrogen and progesterone regulated by gonodatropin, suggesting that granulosa-like and theca-like cells were also induced.
- We further found that granulosa cells promote the development of oocyte-like cells and activate the induction of blastocyst-like structures derived from EnSCs. In conclusion, EnSCs may potentially represent an in vitro system for the investigation of human folliculogenesis.

Stem cell treatments for infertility

Direct transplantation of stem cells or their paracrine factors into reproductive organs

In vitro differentiation into germ cells or gametes




CLINICAL
TRIAL

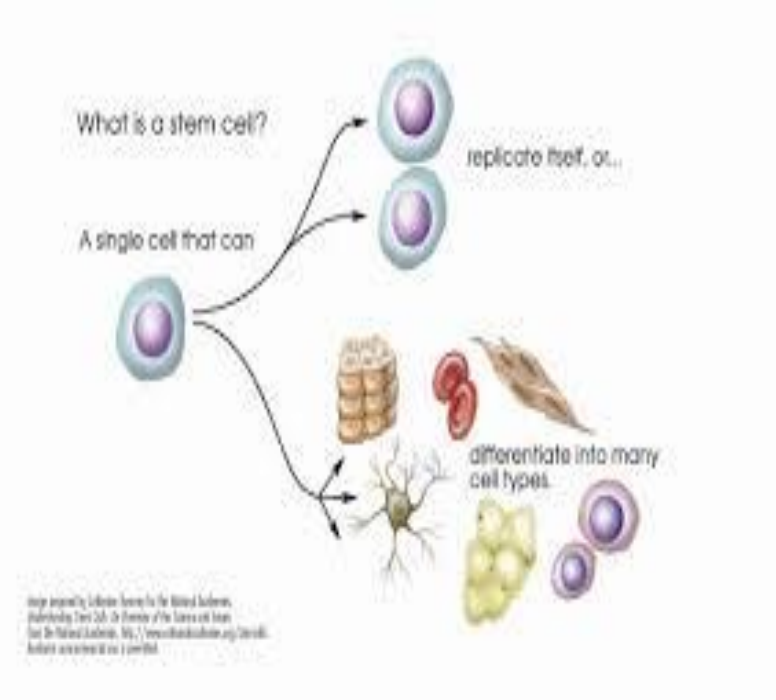
BONE MARROW DERIVED MESENCHYMAL STEM CELL RESTORE OVARIAN ACTIVITY IN CASES OF PREMATURE OVARIAN FAILURE.

H Gabr, WA Elkheir, H El Shaer Obstetrics & Gynecology, Cairo University, Cairo, Egypt

.2016

a pilot human clinical trial

- **20 cases** of premature ovarian failure were enrolled. Autologous bone marrow derived MSC were injected in to the ovaries laparoscopically. Follow up was done by measuring serum FSH& follicle counting by ultrasound.
- Human subjects showed normalization of FSH and E2 with sonographic evidence of ovulation in 5 out of 20 patients. These patients were referred to IVF centers.

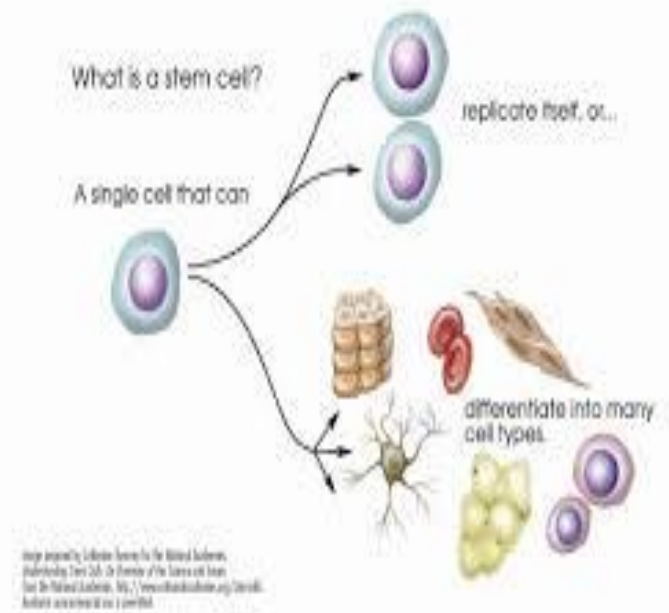


Autologous stem cells therapy, The first baby of idiopathic premature ovarian failure

M Edessy[1], Hala N Hosni[2], Y Shady[1], Y Waf[1], S Bakr[3], M Kamel[1]

2016

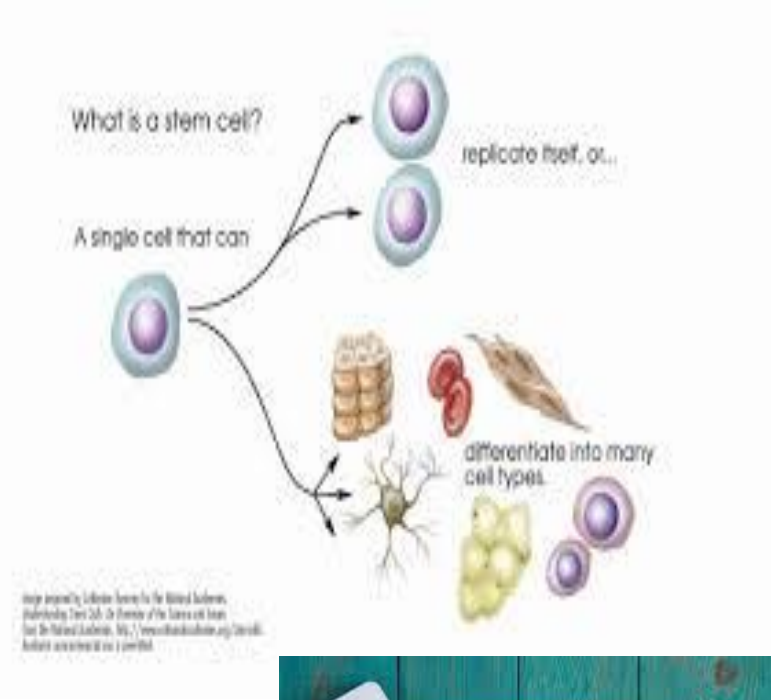
- **Aim:** To evaluate the therapeutic potential of autologous Mesenchymal sc (MSC) transplantation in women suffering from POF.
- 10 cases
- MSC preparation from the bone marrow of the iliac crest was laparoscopically injected into the ovaries.
- **Results:** Showed that after transplantation two cases (20%) (ESS = 5 and 6) resumed menstruation after 3 months, one of them (10%) (Case no 5) (ESS = 6) got pregnancy after 11 months and delivered a healthy full term baby (Zeinab).Ten months after transplantation EORS of patient who developed pregnancy (case no 6) was found to be 7 after being 0 before therapy. EORS of the other menstruating case (case no 10) was 5 after being 0. The 2 menstruating cases showed focal secretory changes after being atrophic endometrium in case 5 and distorted proliferative endometrium in case 10.



Role of Autologous Bone Marrow-Derived Stem Cell Therapy for Follicular Recruitment in Premature Ovarian Insufficiency: Review of Literature and a Case Report of World's First Baby with Ovarian Autologous Stem Cell Therapy in a Perimenopausal Woman of Age 45 Year

Shreya Gupta, Pooja Lodha, M. Selva Karthick, and Sunita Rajesh Tandulwadkar

- 2018
- 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.
- 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.
- laparoscopic instillation
- about 1–2 ml of ABMDSC at 3–4 sites performed bilaterally
- After 8 weeks (the AMH improved to 0.9 ng/mL)
- Three eggs were retrived and one Grade A compacting embryo was frozen on day 3,
- **A 2.7 kg female baby**

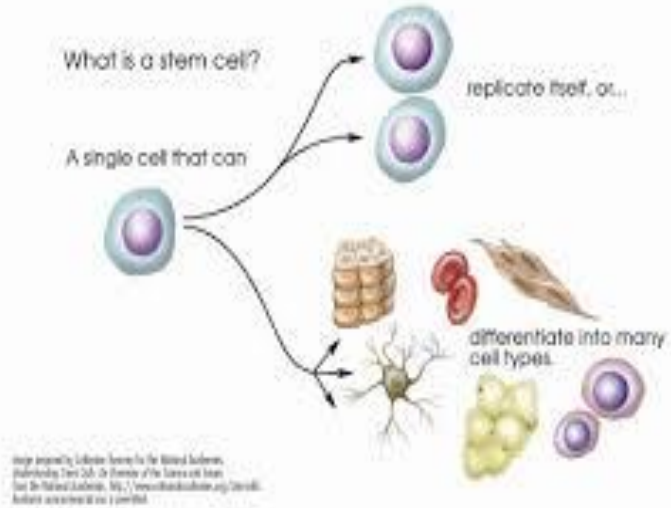


Fertility and Sterility
Volume 110, Issue 3, August 2018, Pages 496-505.e1

Original article

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

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



- Patient(s): Seventeen women who are poor responders.
- Intervention(s): Ovarian infusion of bone marrow-derived stem cells.
- **Five pregnancies** were achieved: **two after ET**, **three by natural conception**.

Fertility and Sterility
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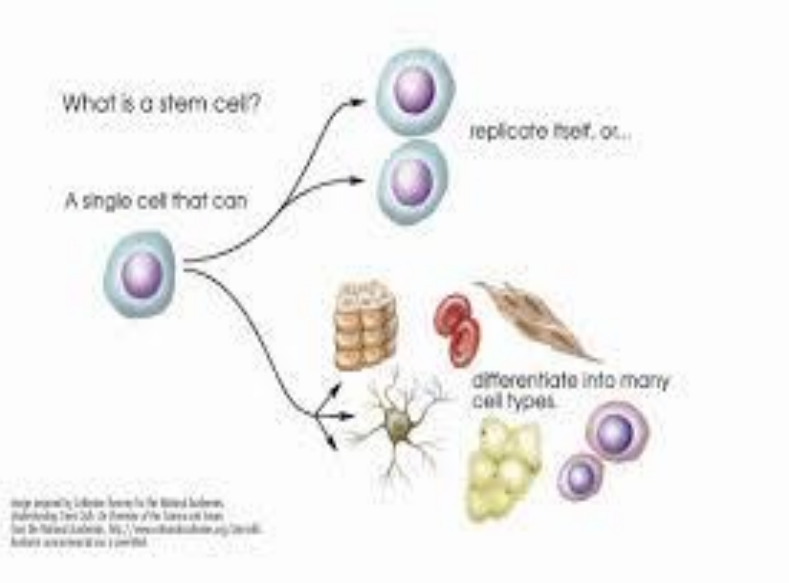
Original article

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

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



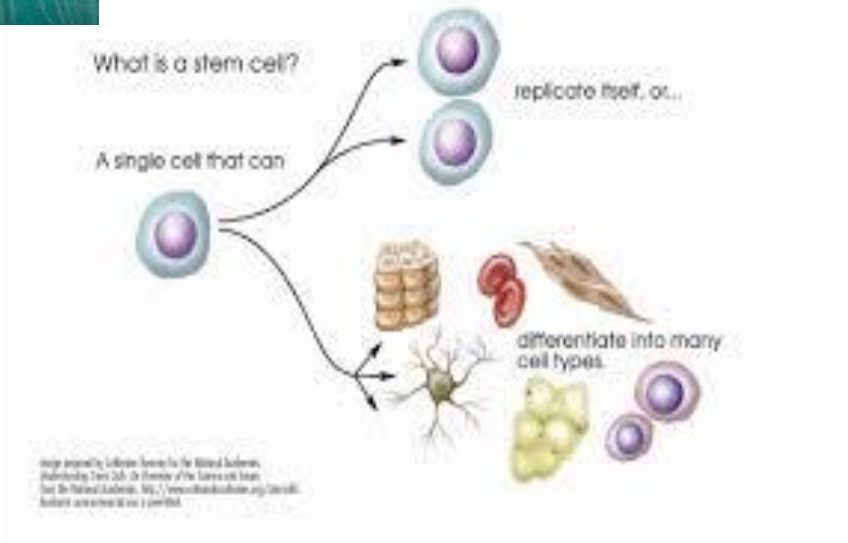
- **Result(s):**
  - The ASCOT resulted in a significant improvement in AFC 2 weeks after treatment. With an increase in **AFC** of three or more follicles and/or two consecutive increases in **antimüllerian hormone levels** as success criteria,ovarian function improved in 81.3% of women. These positive effects were associated with the presence of fibroblast growth factor-2 and thrombospondin. During controlled ovarian stimulation, ASCOT increased the number of stimulable antral follicles and **oocytes**, but the embryo euploidy rate was low (16.1%)

Igboeli et al. Journal of Medical Case Reports (2020) 14:108
https://doi.org/10.1186/s13256-020-02426-5

CASE REPORT Open Access

Intraovarian injection of autologous human mesenchymal stem cells increases estrogen production and reduces menopausal symptoms in women with premature ovarian failure: two case reports and a review of the literature

Prosper Igboeli[1], Abdeljabar El Andaloussi[2], Ujalla Sheikh[1], Hajra Takala[1], Amro ElSharoud[1], Ashley McHugh[1], Larisa Gavrilova-Jordan[3], Steven Levy[4] and Ayman Al-Hendy[1*]



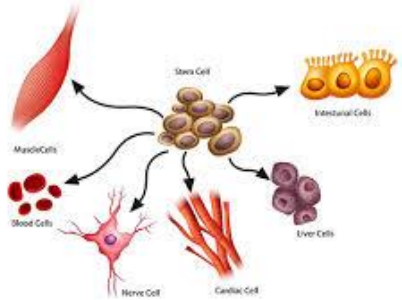
- 2020
- We present **two cases** of Caucasian women with premature ovarian failure who resumed ovarian estrogen production and menses 7 months following autologous bone marrow–derived mesenchymal stem cell injections into the ovary.
- The **bone marrow– derived mesenchymal stem cells** were harvested from the bone marrow of the iliac crest of the patients with premature ovarian failure and nucleated cells concentrated and enriched in bone marrow–derived mesenchymal stem cells intraoperatively, and then injected into the patient's **right ovary via laparoscopy.**
- Increases of approximately 50% in volume of the treated ovaries in comparison with the contralateral control ovaries.
- Serum levels of estrogen increased by approximately 150% compared with the preoperative levels.
- Each of the two patients had an episode of menses, and also both of them reported marked improvement of their menopausal symptoms that also persisted to the end of the study (1 year).

› Stem Cell Rev Rep. 2020 Aug;16(4):755-763. doi: 10.1007/s12015-020-09969-6.

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

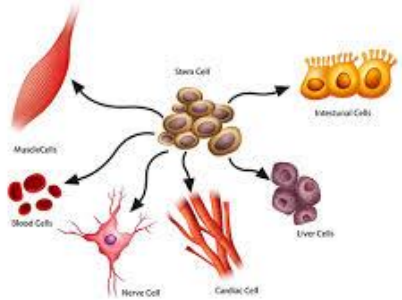
Simin Zafardoust [1], Somaieh Kazemnejad [2], Maryam Darzi [1], Mina Fathi-Kazerooni [1], Hilda Rastegari [1], Afsaneh Mohammadzadeh [1]

Isolation and culture of MenSCs

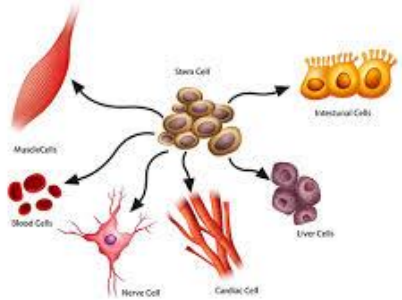


- 1) Menstrual blood was collected from women using a sterile Diva cup
- 2) The specimen was delivered into the collection tubes containing medium
- 3) Quickly conveyed to class B cell culture clean room

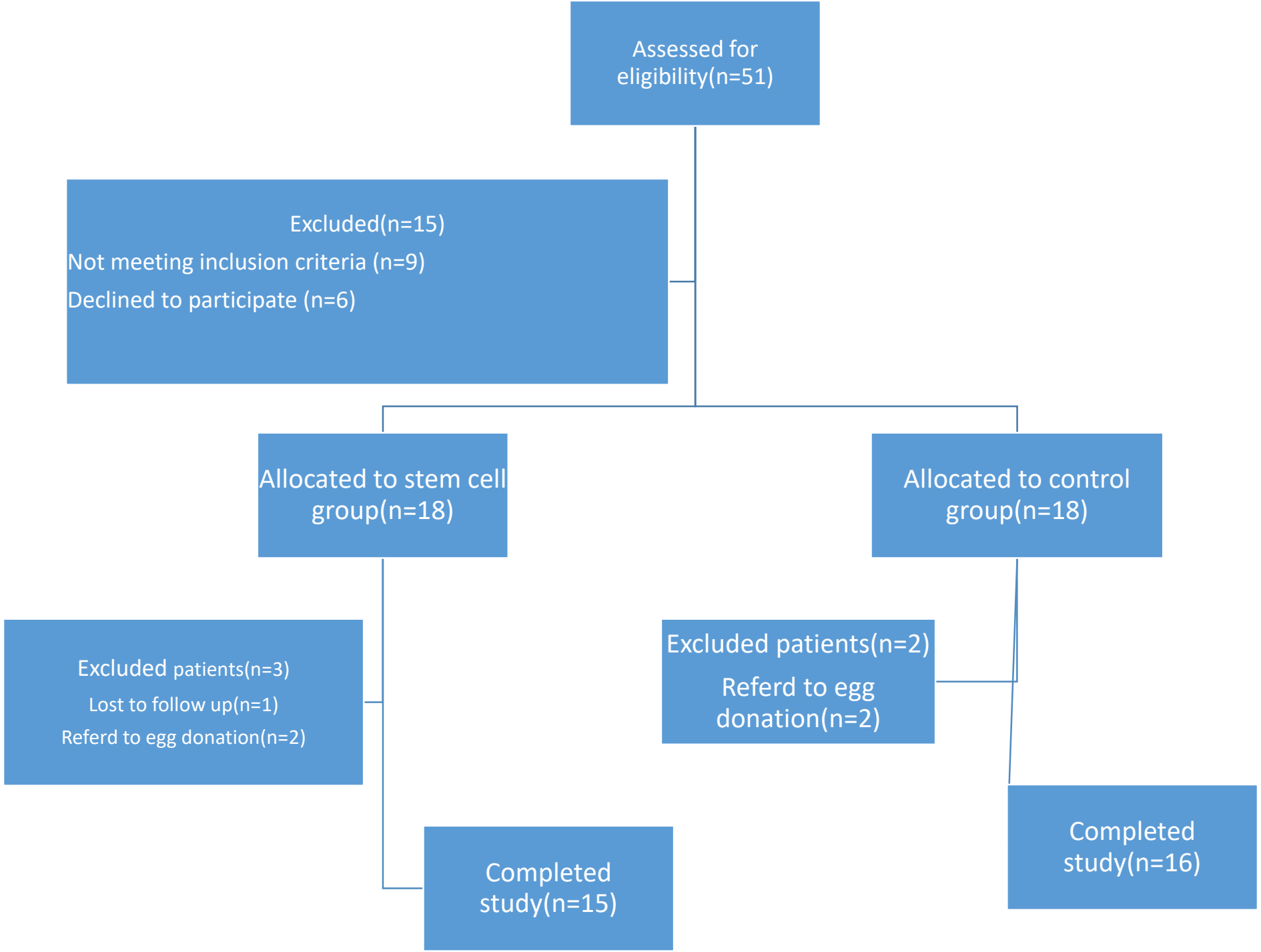
Final product preparation and administration into ovary



- At **day of cells injection**, the cultured and qualified cells were trypsinized, counted and suspended in normal saline included 10% human serum albumin to prepare the density of **20×10^6 cells/ml**.
- Thereafter, **150 µl** of prepared suspension was injected into ovary of patients after receiving general anesthesia.



- MenSCs isolated from all participants had **spindle-shaped** morphology in culture and were positive for **CD90**, **CD 73**,**CD44** and **negative** for hematopoietic marker **CD45.** Moreover, all cultured cells showed normal **karyotype** pattern.
- The sterility of the cells was confirmed for all cultures


Assessed for eligibility(n=51)
Excluded(n=15)
Not meeting inclusion criteria (n=9)
Declined to participate (n=6)
Allocated to stem cell group(n=18)
Allocated to control group(n=18)
Excluded patients(n=3)
Lost to follow up(n=1)
Referd to egg donation(n=2)
Excluded patients(n=2)
Referd to egg donation(n=2)
Completed study(n=15)
Completed study(n=16)

Group parameter	Stem cell group(n=11)	Control group(n=16)	P-value
AMH	0.5(0.9)	0.4(0.5)	**0.08**
AFC(right ovary)	3(2)	2(2)	**0.15**
AFC(left ovary)	2(3)	1(1)	**0.55**
Gonadotropin Ampoules number	50(18)	64(21)	**0.008**
Duration of HMG administration(days)	10(3)	10(4)	**0.04**
Number of follicles	4(6)	4(1)	**0.76**
Number of oocytes	3(5)	1(1)	**0.10**
Fertilization rate (%)	**90%,94%**	**62±0.30**	**0.04**
Number of embryos	**3(5)**	**1(1)**	**0.02**
Embryo number(GradeA)	**2(5)**	**0(1)**	**0.008**

Group / parameter	Stem cell group(n=15)	Control group(n=16)	P-value
Spontaneous clinical pregnancy	**4(26.7%)**	**0(0%)**	**0.032**
Spontaneous live birth	3(20%)	0(0%)	0.068
Total Clinical pregnancy rate	**7 (46.7%)**	**2(13.3%)**	**0.04**
Total live birth rate	**5 (33.3%)**	**1(6.7%)**	**0.06**
Sex of born babies	3 boys and 2 girls	1 girl	-
Babies weight	3200-3950g	3320 g	-

group	Stem cell(n=11)			Control(n=16)		
Therapy situation / parameter	before	after	P value	before	after	P value*
AMH	0.4(0.6)	0.5(0.9)	0.14	0.6(0.7)	0.4(0.5)	0.008
AFC (right ovary)	1(2)	3(2)	0.01	2(2)	2(2)	0.15
AFC (left ovary)	1(2)	2(3)	0.11	1(1)	1(1)	0.55
Gonadotropin Ampoules number	59(34)	50(18)	0.30	60(8)	64(21)	0.22
Duration of HMG administration(days)	8(3)	10(3)	0.92	10(2)	10(4)	0.06
Number of follicles	2(4)	4(6)	0.01	3(4)	4(1)	0.71
Number of oocytes	1(2)	3(5)	0.01	2(3)	1(1)	0.71
Fertilization rate (%)	75%,80%	90%,94%	0.01	80%,85%	60%,63%	0.27
Number of embryos	0(2)	3(5)	0.01	1(3)	1(1)	0.20
Number of embryos (Grade A)	0(0)	2(5)	0.01	1(2)	0(1)	0.03

Clinical Trial (phase III)

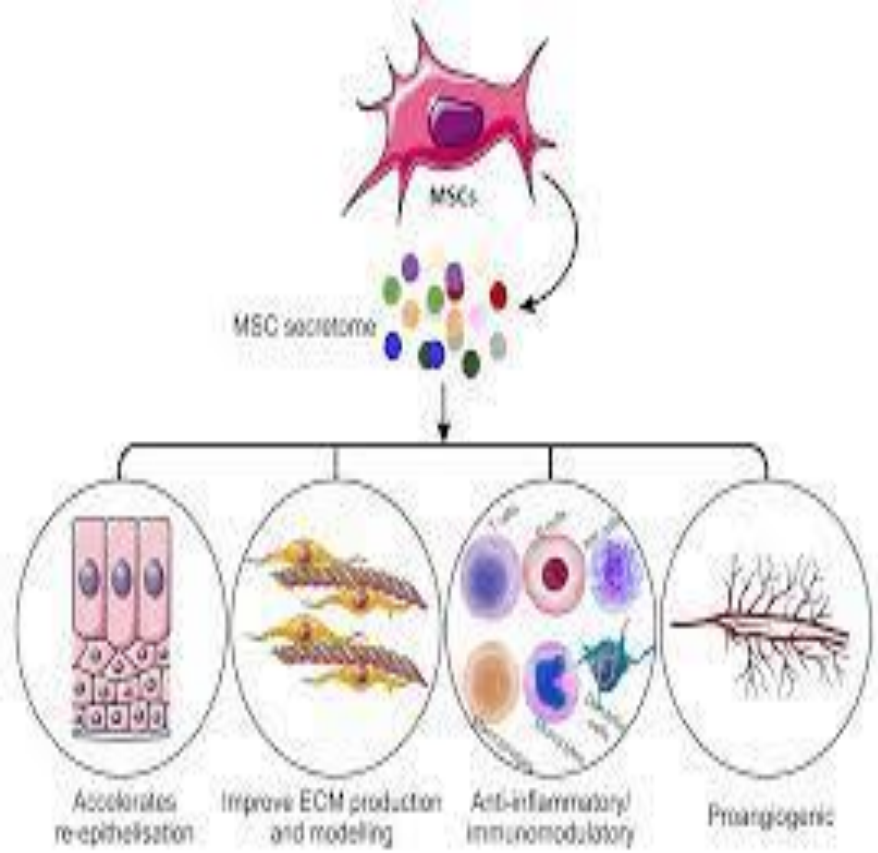
	Age<40		Age:40-45	
	Stem cell	control	Stem cell	control
OVERALL	45	45	45	45
Until now (stem cell injection)	45	45	-	-
Spontaneous pregnancy	10 (22.2%)	5 (11.1%)	5 (11.1%)	1 (2.2%)

	Stem cell (N=50)	Control (n=52)	P-value
oocyte	3.2±2.6	2±1.5	0.00
MII	2.5±2	1.7±1.3	0.02
embryo	2.1±1.7	1.2±1.2	0.07
Spontaneous pregnancy	15(16.6%)	5(5.5%)	0.02
CPR	16(32%)	8(19.2%)	0.05

These studies insinuate that patients whose only chance at having a baby previously was by using donated oocytes now have one more option to get their biological child before entering the donation program

Mechanisms

- By secreting growth factors, they could inhibit stromal cell apoptosis.
- VEGF secreted by MSCs induces vascular remodeling.
- Over 100 cytokines derived from MSCs, which are involved in apoptosis, angiogenesis, cell cycle, and immune response and seem to mediate ovarian function recovery.
- Directly involved in the formation of new primordial follicles



Thanks for your attention