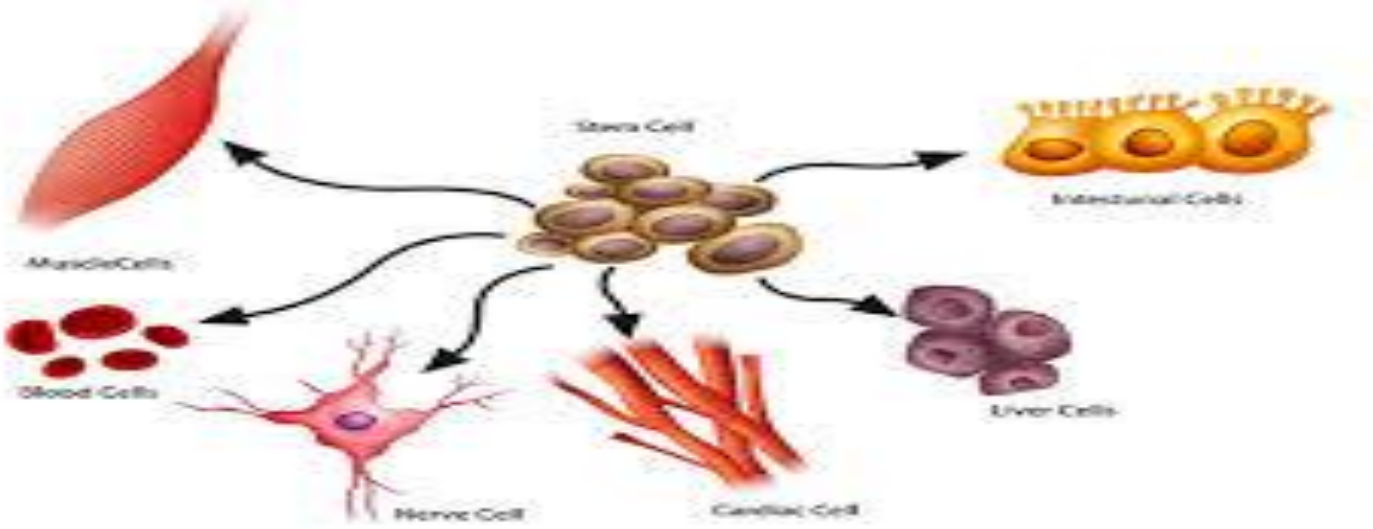


The role of menstrual blood-derived stem cells in the treatment of POR

Dr Simin Zafardoust.M.D

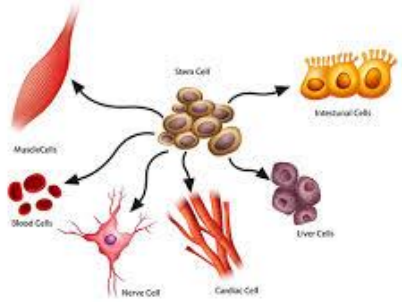
Assistant Professor of Obstetrics &Gynecology

Fellowship in Infertility and Reproductive Medicine

- The incidence: 9-24%
- The rate is reported as **50 %** in women over 40 years old

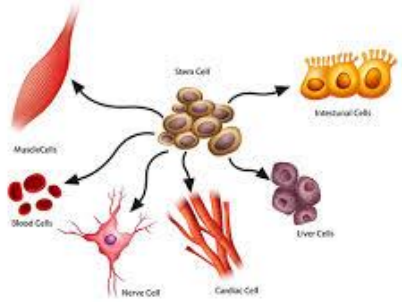
- FSH begins increasing 13 years before menopause. With increasing FSH, the number of follicules, oocytes, embryos and implantation rates decrease, and cycle cancellation rates increase.
- Despite improvements in ART, there is **no consensus** on the management of patients with poor responses.

Poor Ovarian Responders



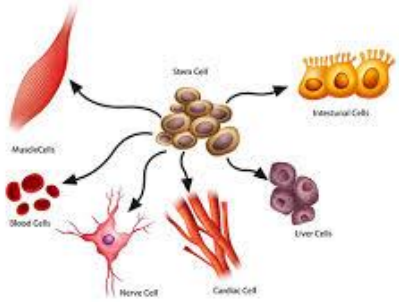
- In patients with POR, the incidence of poor response at the **second cycle** was reported as **62%**.
- A **decreased ovarian reserve** is related to decreased **oocyte quality**.

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

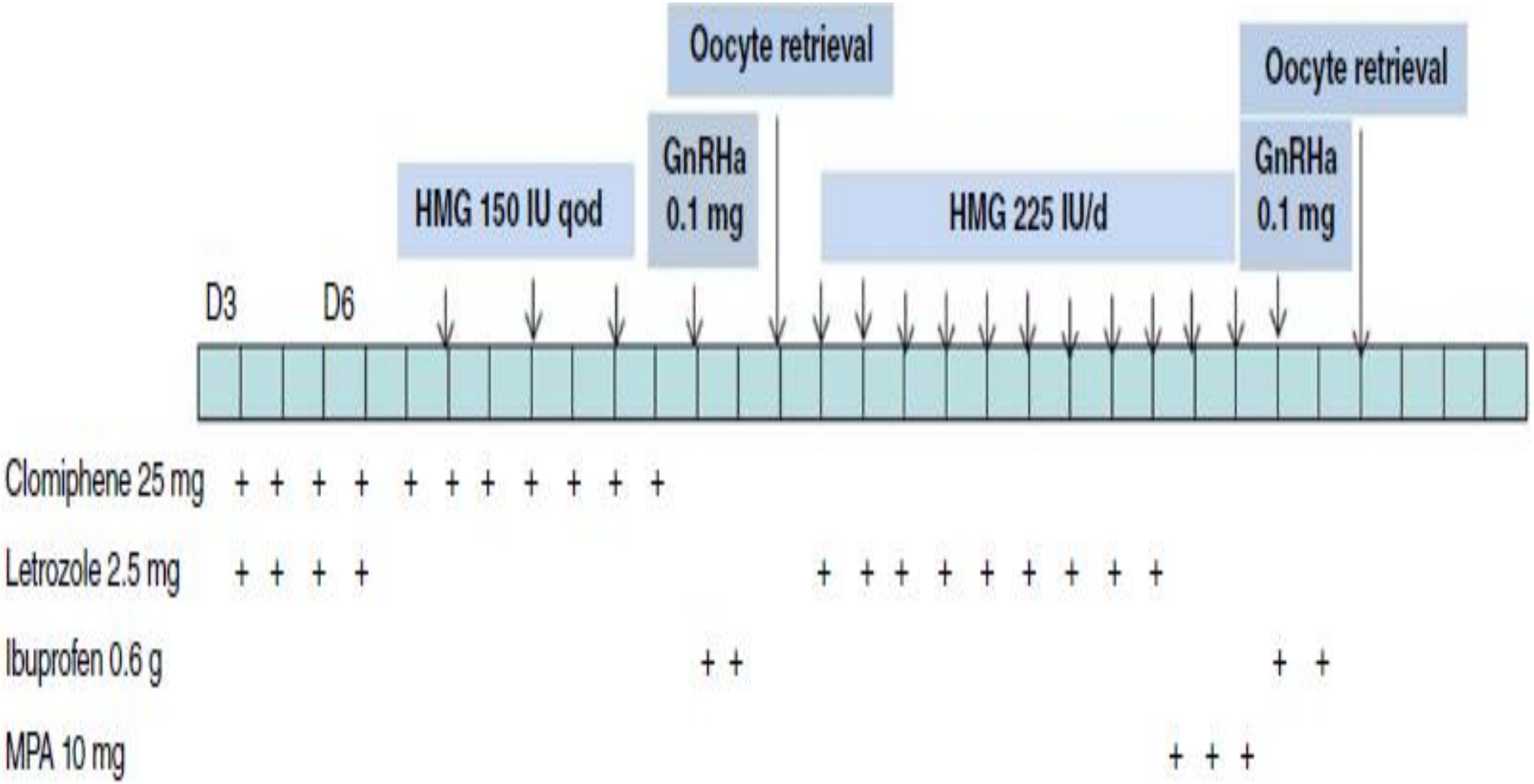


- 2015
- The **live birth rate** ranged between **5.5** and **7.4 %** and was not statistically different in the five different categories of women defined as poor responders according to the Bologna criteria.
- In our group ,CPR and LBR are **13.4%** and **6/7%**

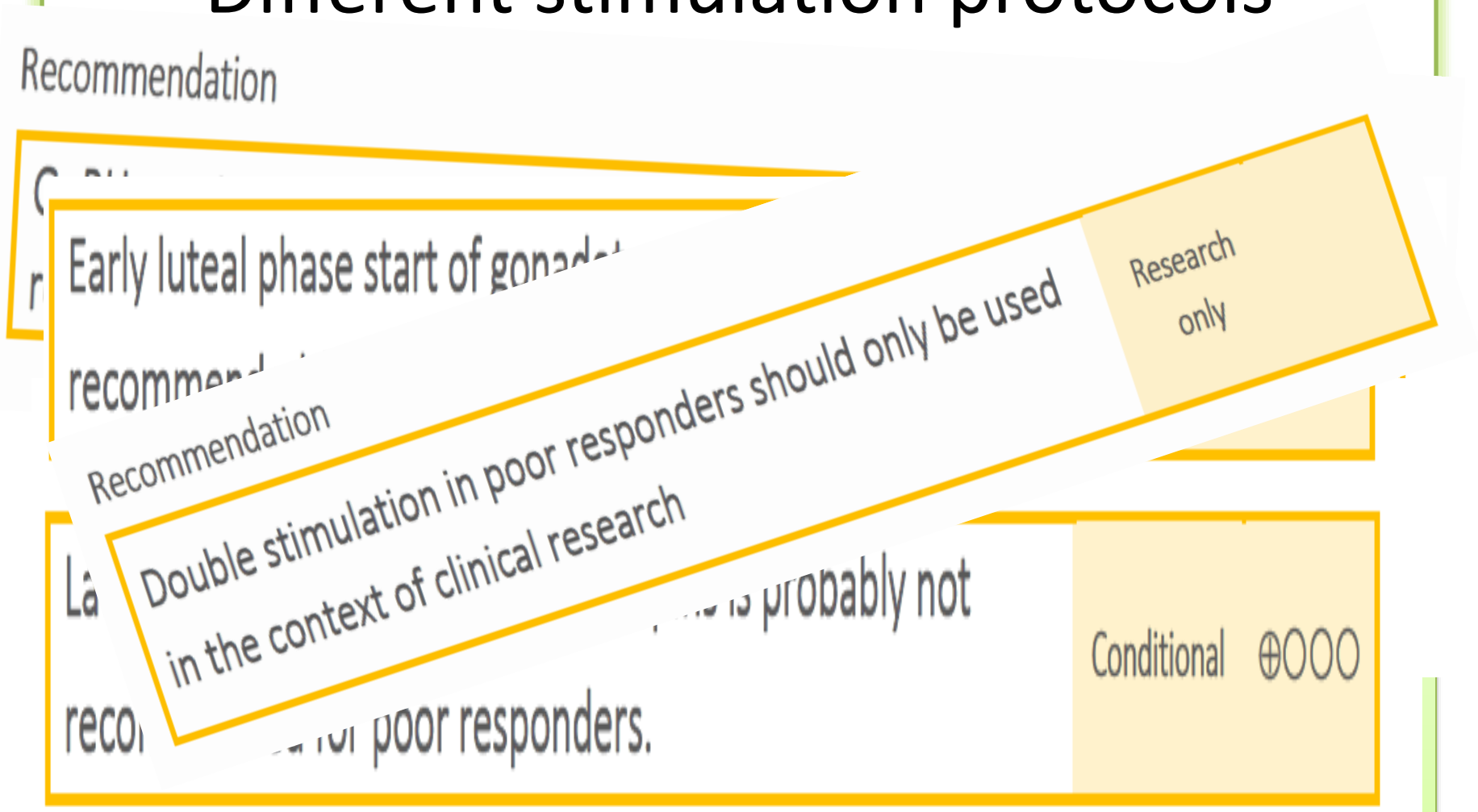
Treatment

Different stimulation protocols

- An RCT- Schimberni, et al., **2016**- 146 POR- compared the **short GnRH agonist** with the **GnRH antagonist** protocol .
- **Double stimulation** or “dual stimulation” or “duostim” (Vaiarelli, et al., 2018) or “Shanghai protocol”(Kuang, et al., 2014) is experimented in poor responder patients or in urgent oncologic fertility preservation.


Oocyte retrieval
Oocyte retrieval
GnRHa 0.1 mg
GnRHa 0.1 mg
HMG 150 IU qod
HMG 225 IU/d
D3
D6
Clomiphene 25 mg
Letrozole 2.5 mg
Ibuprofen 0.6 g
MPA 10 mg

Different stimulation protocols

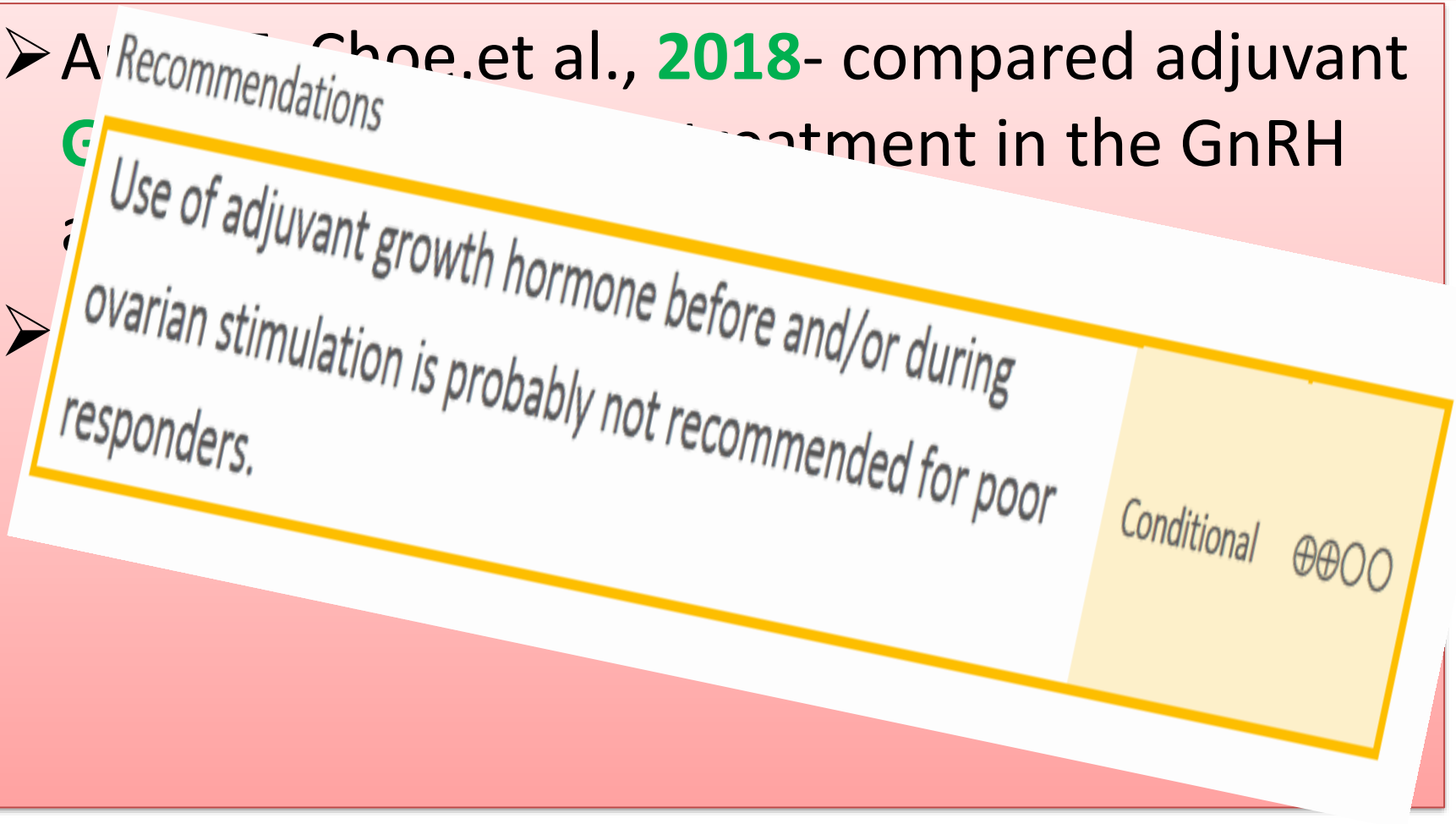


Recommendations

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

difference in live birth rate between the DHEA and control group.

- A[illegible] Choe.et al., **2018**- compared adjuvant **G**[illegible]atment in the GnRH [illegible]
-



At the moment systematic reviews and meta-analyses suggest that insufficient evidence exists to recommend most of the treatments proposed to improve pregnancy rates, with the poor ovarian response remaining one of the most challenging tasks in reproductive medicine.

Novel Treatments

- 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).

- **Rich** and **easily accessible** source of adult stem cells.
- **High rate of proliferation** and possess multi lineage **differentiation potency**.
- MenSCs come from body discharge and obtaining them **is non-invasive** to the body.
- 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

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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.

ELSEVIER

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**.

ELSEVIER

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

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

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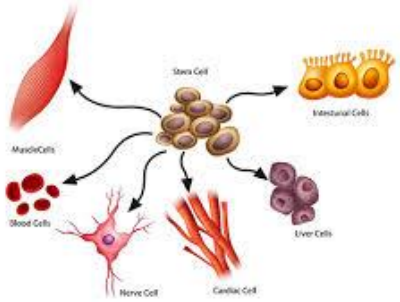
- **Result(s):**
- The ASCOT resulted in a significant improvement in AFC 2 weeks after treatment. With an increase in **AFC** of three or more follicles and/or two consecutive increases in **antimüllerian hormone levels** as success criteria,ovarian function improved in 81.3% of women. These positive 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%)

› 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

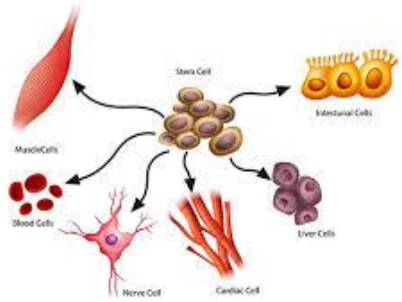
Simin Zafardoust [1], Somaieh Kazemnejad [2], Maryam Darzi [1], Mina Fathi-Kazerooni [1], Hilda Rastegari [1], Afsaneh Mohammadzadeh [1]

Isolation and culture of MenSCs

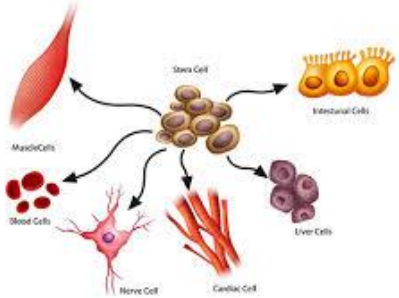


- 1) Menstrual blood was collected from women using a sterile Diva cup
- 2) The specimen was delivered into the collection tubes containing medium
- 3) Quickly conveyed to class B cell culture clean room

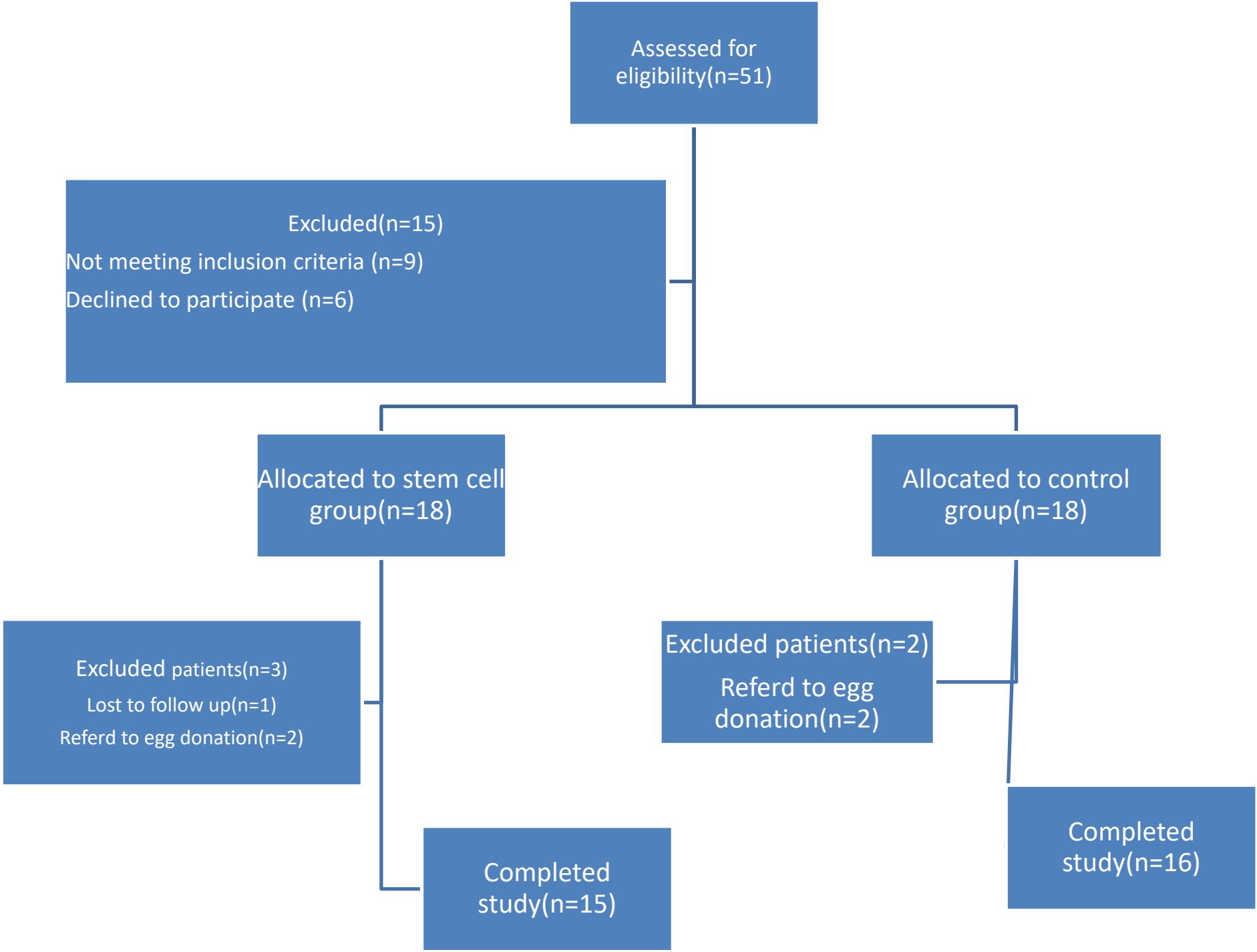
Final product preparation and administration into ovary



- 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
OVERALL	45	45
Until now (stem cell injection)	45	45
pregnancy	13(28.8%)	3
COH	24	25
Spontaneous pregnancy	11(24.4%)	3

Clinical Trial (phase III)

	Before	After	P-value
OOCYTE NUMBER	1.37±1.45	3.9±1.9	0.001
EMBRYO NUMBER	0.81±1.04	2.2±1.3	0.002

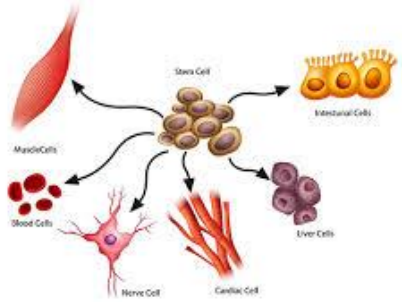
Process

Selection of patient → Sample collection →

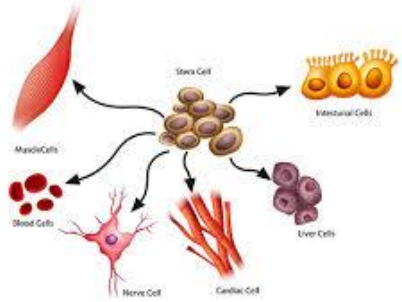
Injection → Follow up

Thanks for your attention

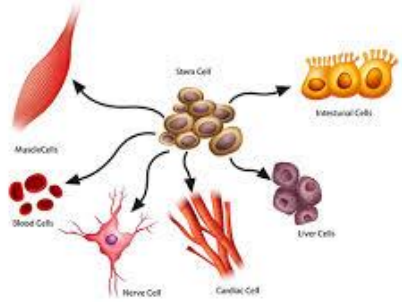
Treatment



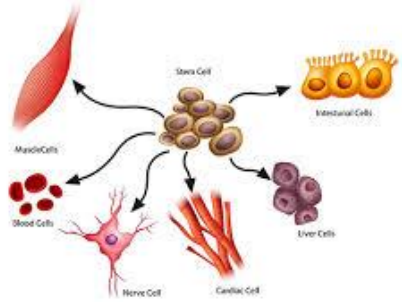
- Different stimulation protocols
- Increasing gonadotropin dosage
- DHEA(in maximum prosperity of **18-20%** in clinical pregnancy rate and **12-16.4%** in live birth rate)
- Testosterone(**30%** in clinical pregnancy rate and **27.3%** in live birth rate)
- Growth hormone(**22.2%** in clinical pregnancy rate and **14.7%** in live birth rate)



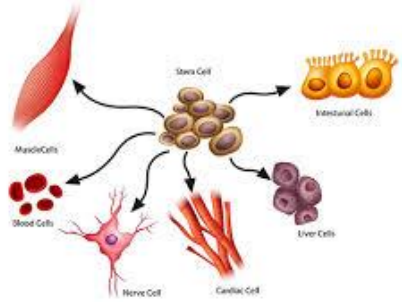
- Although BM-MSCs **have obtained great priorities** and predominant studies, the difficulty of separating BM-MSCs is a limiting factor owing to the requirements of invasive operation and ethical issue of donors.
- Recent research has indicated that the uterine lining, or endometrium, is a rich source 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) [12, 13].



- 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.



- Mesenchymal stem cells (MSCs), a heterogeneous subgroup of progenitor cells, have self-renewing capacity and differentiating potential into various specialized cell types, including osteoblasts, chondrocytes, and adipocytes [1].

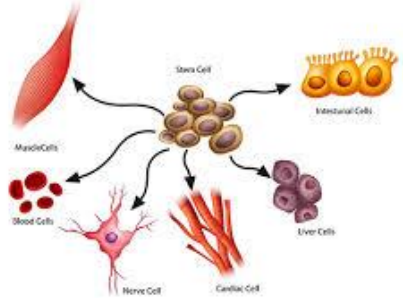


- Compared to SCs from bone marrow and adipose tissues, MenSCs come from body discharge and obtaining them **is non-invasive** to the body, they are easy to collect, and there are no ethical concerns.
- There is, hence, a growing interest in the functions of MenSCs and their potential applications in regenerative medicine.
- MenSCs are attracting more and more attention since their discovery in 2007.

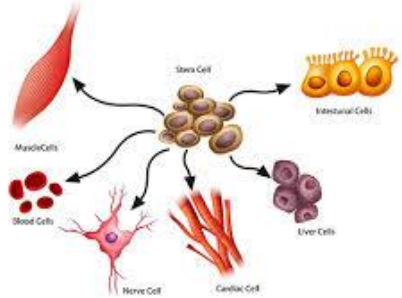
The existing nomenclatures for MenSCs in different Literatures

- Endometrial regenerative cells
- Menstrual blood-derived stem cells
- Menstrual-derived stem cells
- Menstrual blood stem cells
- Menstrual blood stromal stem cells
- Menstrual stem cells
- Menstrual blood-derived stromal stem cells
- Endometrial stem cells
- Menstrual blood-derived endometrial stem cells
- Menstrual blood-derived mesenchymal stem cells
- Menstrual blood progenitor cells
- Endometrial mesenchymal stem cells

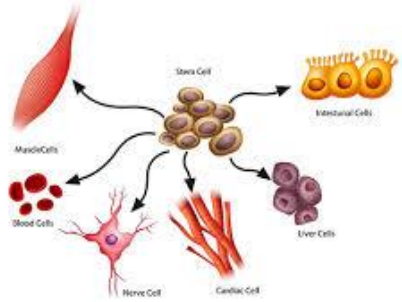
Methods



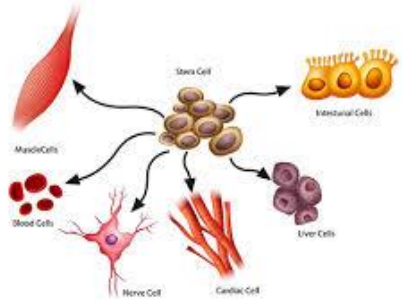
1) About **5-10** ml of menstrual blood (MB) was collected using sterile Diva cups inserted into vagina during menstruation from volunteered healthy fertile women aged between 22-30 years.
2) MB was transferred into **Falcon tubes containing phosphate buffered saline (PBS) without $Ca2^{+}$ or $Mg2^{+}$** supplemented with 2.5 µg/ml fungizone, 100 µg/mL streptomycin, 100 U/mL penicillin and 0.5 mM EDTA.
3) Mononuclear cells were separated using Ficoll-Hypaque density gradient centrifugation and washed out in PBS.
4) The cell pellet was suspended in DMEM-F12 medium supplemented with 10% FBS and cultured in tissue culture plates.
5) The isolated cells were co-cultured with keratinocytes derived from the foreskin of healthy newborn male aged 2-10 months who was a candidate for circumcision for differentiation into epidermal lineage.



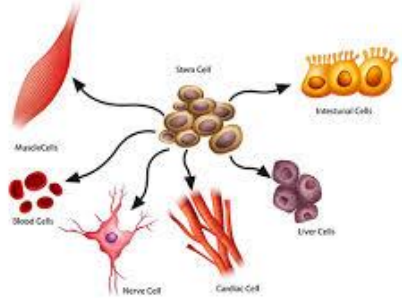
- **MenSCs were positive for several surface molecules, such as**
- CD9, CD29, CD44, CD73, CD90, CD105, octamer binding transcription factor 4 (OCT-4), CD166, major histocompatibility complex I (MHC I), and C-X-C chemokine receptor type 4 (CXCR4). **Among these molecules above, CD29, CD73, CD90, and CD105 were commonly identified for MSC markers.**
- MenSCs also remained to have negative expressions for hematopoietic stem cell markers, such as CD34, CD45, and CD133. And CD14, CD38, and human leukocyte antigen-DR isotype (HLA-DR) were also negative.
- Interestingly, some papers were reported for the positive expression of embryonic markers and intracellular multipotent markers, such as OCT-4, c-kit proto-oncogene (c-kit)/CD117, and stage-specific embryonic antigen-4 (SSEA-4), which have not existed in MSCs from other sources. However, these findings were controversial, and some researchers showed that the expressions of c-kit and SSEA-4 were negative .



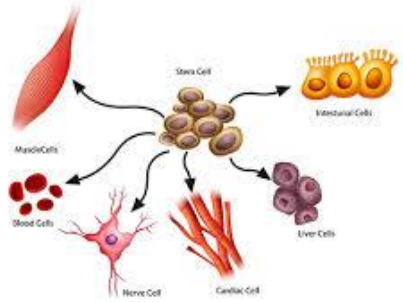
- Studies by Meng et al. and another group have reported that MenSCs ***from young and healthy women*** could increase to one doubling every 20 h supplied with sufficient culture conditions, which was **twice as fast as BM-MSCs** (estimated 40–45 h) [12, 35].
- MenSCs have similar phenotypes and properties compared with BM-MSCs, including spindles, classical three-line differentiation, and surface marker expression.



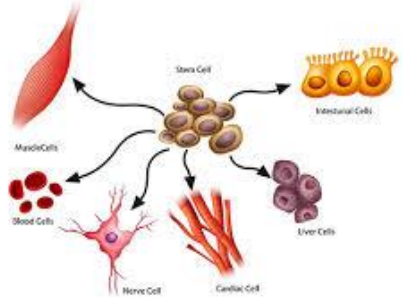
- MenSCs have been extensively expanded in vitro and hardly showed obvious **chromosomal abnormalities** by our group and others [12, 23, 35].
- Such a highly proliferating rate and stably genetic characteristic, as well as the apparent pluripotency, suggest that the novel stem cells may exhibit unexpected therapeutic properties.



- MenSCs are also remarkable for their broad differentiation capacity. Currently, MenSCs can be induced as **endothelial**, **cardiomyocytic**, **neurocytic**, **cartilaginous**, **myocytic**, **respiratory epithelial**, **pancreatic**, **hepatic**, **adipocytic**, and **osteogenic** parts using appropriate differentiation techniques [12, 14, 26].
- Hida et al. found that MenSCs exhibited cardiogenic differentiation in a scaffold culture system [45].
- Lai's team has confirmed that the differentiation of MenSCs into germ cells was induced in the appropriate medium [46].
- Similarly, Liu et.al also proved that MenSCs had the capacity to differentiate into ovarian tissue-like cells [22].
- Khanjani et al. have shown that MenSCs could differentiate into functional hepatocyte-like cells by checking mature hepatocyte functions [17, 33, 39]. In addition, MenSCs had a potential for differentiation into glial lineages (neurosphere-like cells) by examining the expression of glial fibrillary acidic protein, oligosaccharide-2, and myelin basic protein [47].



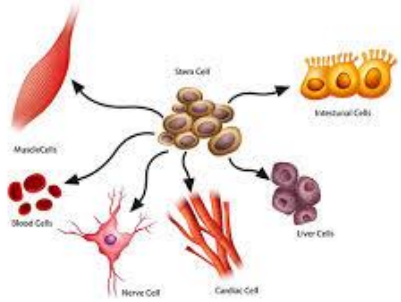
- It is worth noting that the endometrium is a part of the mucosal immune system, and MenSCs are extracted from menstrual blood, and their original sources are deciduous endometrial stem cells [44].
- Therefore, immunoregulatory properties of MenSCs are currently unrecognized despite the unified management mechanism of MenSC based therapy is explored in animal models and clinical researches.



- At present, more and more registrations for a variety of diseases support the therapeutic benefits of MSC transplantation in clinical trials (www.clinicaltrials.gov).
- In contrast, the registrations of MenSCs are still few, and no more than 10 clinical trials are presented by searching “menstrual blood stem cells”.
- Actually, the therapeutic potential of MenSCs has already been recognized in several kinds of diseases in pre-clinical research, which is fundamental for future clinical applications in tissue repair and regenerative medicine.
- Similar to BM-MSCs, MenSCs also have several merits, including the **ability to migrate into injury sites**, **differentiation into different cell** lineages, **secretion of soluble factors**, and regulation of immune responses. Therefore, more researches need to be explored before MenSC becomes a common use in clinical application and treatment.

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]



- 2016
- In this study, 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.

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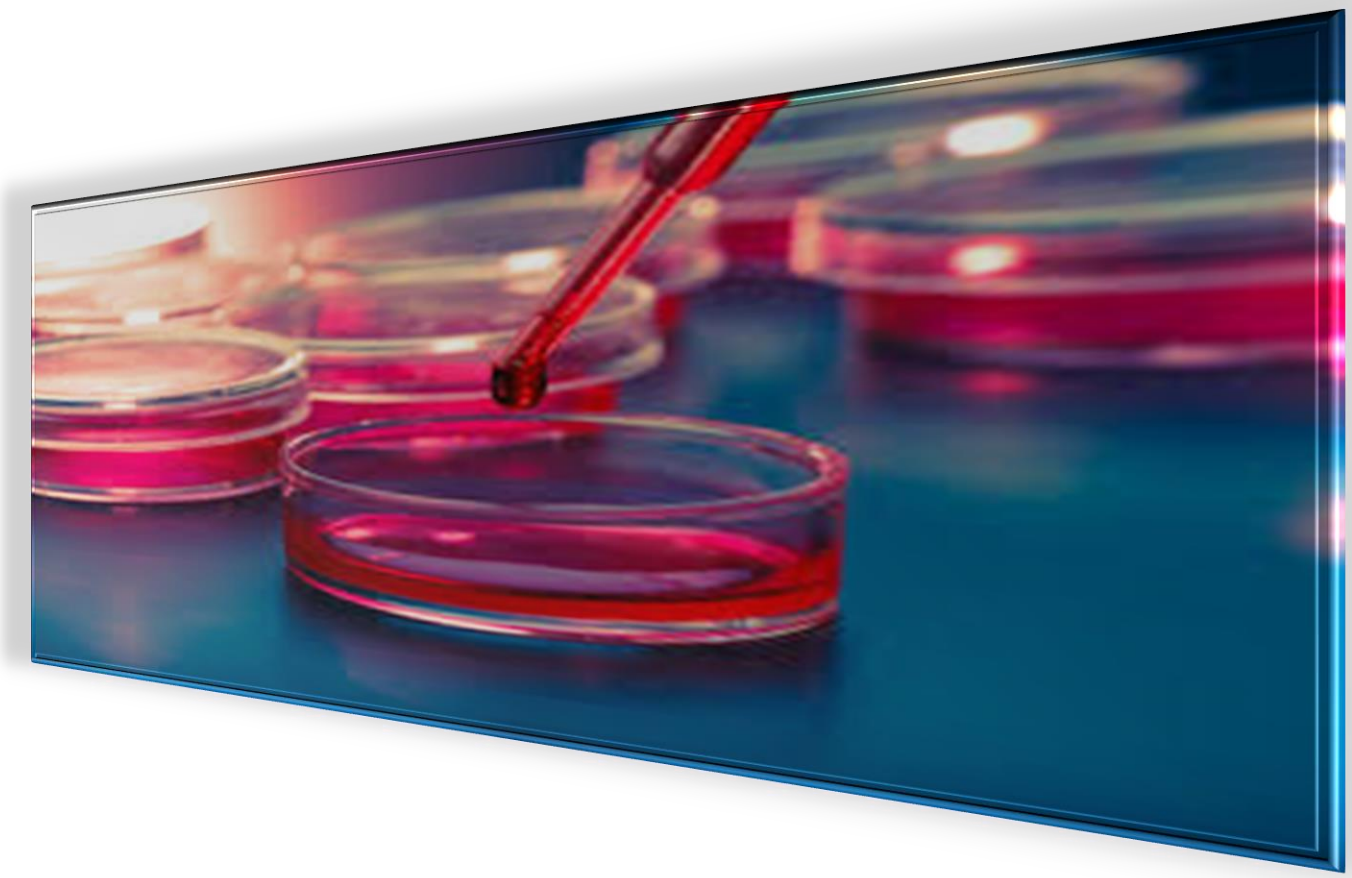


Transplantation of human menstrual blood stem cells to treat premature ovarian failure in mouse model

Te Liu [1], Yongyi Huang, Jian Zhang, Wenxing Qin, Huiying Chi, Jiulin Chen, Zhihua Yu, Chuan Chen

- 2016
- The results revealed that the HuMenSCs could survive within POF mouse ovaries for at least 14 days in vivo; further, ovaries of the HuMenSCs-transplanted group expressed higher levels of ovarian markers [AMH, inhibin α/β, and follicle-stimulating hormone receptor (FSHR)], and the proliferative marker Ki67. In addition, the ovarian weight, plasma E2 level, and the number of normal follicles increased over time in the HuMenSC group compared with the control group. Further, microarray analysis of cDNA expression patterns revealed that, after HuMenSC transplantation, the gene mRNA expression patterns in the ovarian cells following stimulation of the host ovarian niche became increasingly similar to those observed in human ovarian tissue compared with the pretransplantation mRNA expression pattern in HuMenSCs. 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.

Definition of Poor Ovarian Response: The "Bologna Criteria"

- Although the concept of poor ovarian response was introduced over 30 years ago, we **had not** had **a common** definition of poor responder patients until 2011.
- **Having at least two** of the following criteria:
  - **(1)** a previous episode of poor ovarian response (≤3 oocytes) with a standard dose of medication
  - **(2)** an abnormal ovarian reserve with AFC <5–7 follicles or AMH <0.5–1.1 ng/mL
  - **(3)** women above 40 years of age or presenting other risk factors for poor response such as previous ovarian surgery, genetic defects, chemotherapy, radiotherapy, and autoimmune disorders

- **Gonadotropins**

- Berkkanoglu and Ozgur confirmed that the increase of FSH starting dose does not result in higher pregnancy rates and also found no differences between the starting dose of 300UI, 450UI, and 600 UI of gonadotropins in terms of retrieved oocytes, number of embryos obtained, and pregnancy rates.
- It is today clear that these patients **have a reduced ovarian reserve**; the recruitable follicles **are fewer** and the gonadotropins, independently of the dosage administered, can only support the cohort of follicles receptive to stimulation without manufacturing follicles de novo.

- **Protocols**

- **A meta analysis** of randomized controlled trials demonstrated that stimulation protocols where GnRH antagonist is used result in **similar pregnancy** rates compared with long agonist or short flare up regimens.
- In a more recent meta-analysis of 14 randomized controlled studies, there was no significant difference in the number of oocytes retrieved, in the cycle cancellation rate, and in the clinical pregnancy rate.

- It is possible to assess the ovarian reserve by ultrasound on days 2-3 of the cycle in which controlled ovarian stimulation is planned and decide whether to initiate gonadotropins in the cycle where the probability of a favourable response is optimal. In fact, patients with a mean follicle count of <5 follicles **have significant cycle-to-cycle variability in antral follicle** count from −2 to +5 … to −3 to +7.

- **Addition of Estradiol in the Luteal Phase**
- The biological rationale might be that luteal estradiol priming could improve synchronization of the pool of follicles available to controlled ovarian stimulation .
- **Addition of Recombinant LH**

Addition of Growth Hormone

- It has been suggested that the use of growth hormone (GH) might **modulate the action of FSH on granulosa cells** by upregulating the local synthesis of insulin-like growth factor-I (IGF-I).
- The IGF-I amplifies the effect of FSH at the level of both granulosa and theca cells.
- Two recent meta-analyses of 6 randomized trials (128 patients in total) suggested that addition of GH significantly increased the probability of live birth in poor responders.

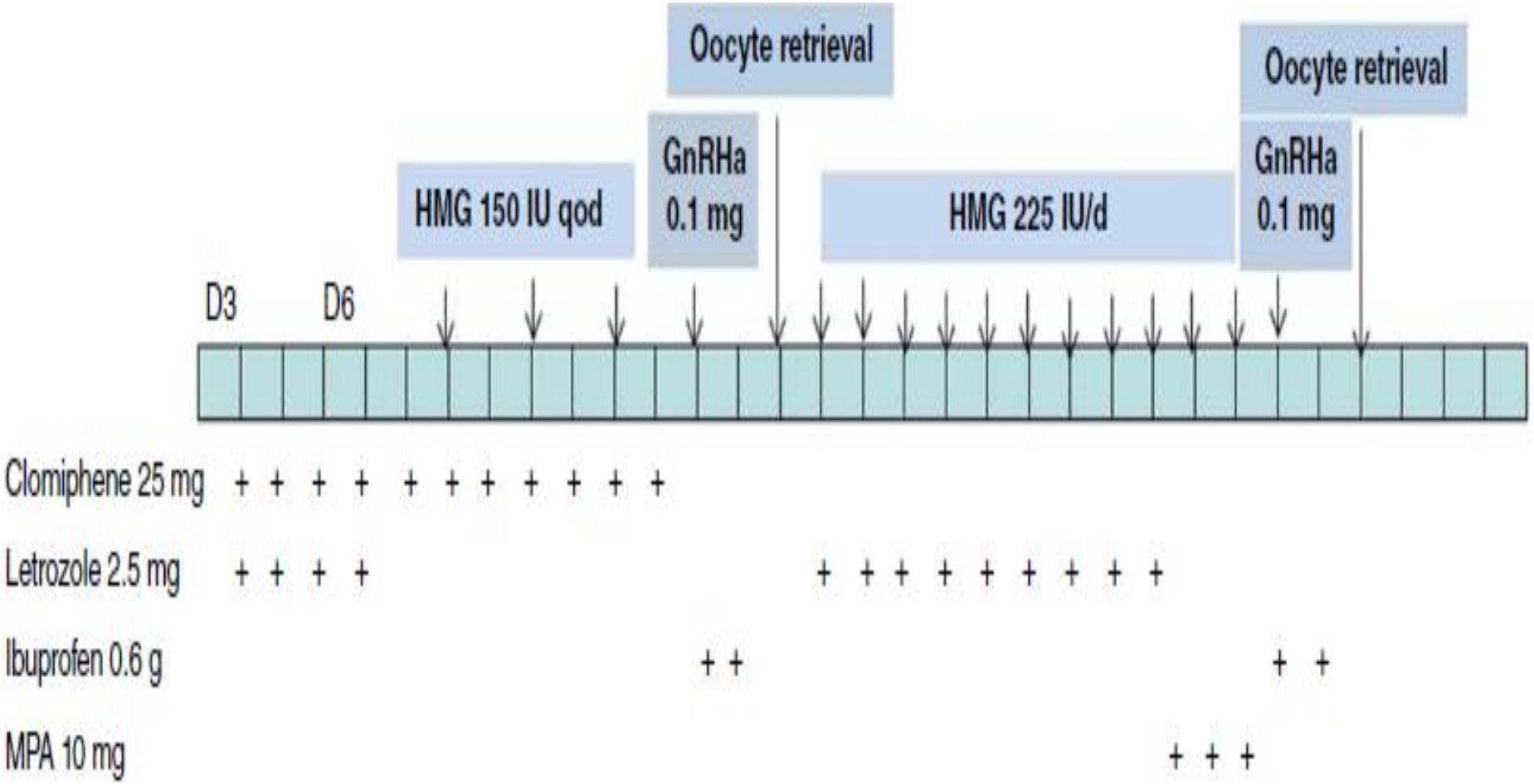
Addition of Androgens

- Androgens, produced primarily by theca cells, play a critical role for an adequate follicular steroidogenesis and for a correct early follicular and granulosa cell development .

- They are the substrate for the aromatase activity of the granulosa cells, which converts the androgens to estrogens.
- Androgens may increase FSH receptor expression in granulosa cells amplifying the effects of FSH and thus potentially enhance responsiveness of ovaries to FSH.
- Inadequate levels of endogenous androgens are associated with decreased ovarian sensitivity to FSH and low pregnancy rates after IVF.

- **Addition of Aspirin**
- Increased intraovarian vascularity has been linked to improved delivery of gonadotropic hormones or other growth factors required for folliculogenesis.

- **Natural Cycles IVF**
- A recent paper analyzed the effect of natural cycles IVF in women defined as poor responders according to the “Bologna criteria”: unexpectedly the data showed that the cumulative live birth per patient does not exceed 8%.


Oocyte retrieval
Oocyte retrieval
GnRHa 0.1 mg
GnRHa 0.1 mg
HMG 150 IU qod
HMG 225 IU/d
D3
D6
Clomiphene 25 mg
Letrozole 2.5 mg
Ibuprofen 0.6 g
MPA 10 mg

At the moment systematic reviews and meta-analyses suggest that insufficient evidence exists to recommend most of the treatments proposed to improve pregnancy rates, with the poor ovarian response remaining one of the most challenging tasks in reproductive medicine.

Thanks for your attention

Liver disease

- Liver fibrosis is the universal phase of various chronic liver diseases and causes a huge public health issue due to high rates for the morbidity and mortality worldwide
- At present, orthotopic liver transplantation (OLT) is the most effective strategy for patients at the end stage of liver disease. However, due to lack of organ donors, surgical complications, lifelong immunosuppression, and high cost, its application has been limited to a large number of patients in the current condition

Liver disease

we have studied the therapeutic effect of MenSC transplantation in a mouse model of liver fibrosis induced by CCl4 (carbon tetrachloride) [20]. The results showed that MenSC had the effect on treating liver fibrosis.

. The results suggest that MenSC may be an attractive treatment for chronic liver disease by targeting HSCs via paracrine mediators.

Liver disease

Fulminant hepatic failure (FHF) is a life-threatening and sharply pathological reaction, which results in relatively high mortality by rapid necrosis of liver cells with the stimulation of a variety of acute injuries, such as hepatotoxic drugs, immune-mediated attacks, or viral infections [52].

The exosomes contain microRNA/lncRNA and adhesion molecules as well as small vesicles of secreted proteins, which mediate cellular signaling pathways both in vivo and in vitro [53]. Our group proved that MenSC-derived exosomes (MenSC-Ex)

possessed therapeutic potential by inhibiting hepatocyte apoptosis in D-galactosamine (D-Gal) and lipopolysaccharide (LPS) induced FHF in mice, and we further showed that the levels of TNF-α, IL-6, and IL-1β were reduced by co-culture with AML12 hepatocytes (a normal mouse hepatocyte cell line) in vitro [19].

The study suggests that MenSC-Ex can improve liver function to increase the rate of survival in FHF model mice.

Liver disease

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Diabetes

- Type 1 diabetes mellitus (T1DM), known as a kind of autoimmune diabetes, is a multifactorial disease by the deficiency of secreting insulin in islet β cells to influence the normal organism metabolism, ultimately leading to elevated blood glucose levels and a severe decline in insulin secretion.
- Our team has studied the therapeutic effect of MenSCs and the underlying mechanism of β cell regeneration after MenSC transplantation in the T1DM mouse model [35]. From our study, MenSCs could facilitate β-cell regeneration and enhance the number of β cells

Ischemic stroke

- Ischemic stroke, one of the leading causes of long-termdisability, is a chain reaction of functional impairment that initially occurs during the identification phase of rapid physical and mental fluctuations.
- Currently ischemic stroke causes many patients producing permanent nerve damage, and stem cell therapy will help to
- improve and possibly restore the nerve function. Borlongan et al. demonstrated that MenSCs improved ischemic stroke in an oxygen glucose deprivation (OGD) rat model in vitro [40]. The behavioral and histological disorders were also significantly improved in the rat model of ischemic stroke by intracerebral/intravenous transplantation.

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Critical limb ischemia

- Critical limb ischemia (CLI) refers to the final clinical stage along with the limb damage due to severe blood loss causing a series of pathological and physiological abnormalities that lead to limb pain or insufficient nutrition to support the legs
- Currently,clinical trials have reported that autologous stem cells improve their symptoms by stimulating angiogenesis, the appropriate cell population of MSCs is still needed to explore. Murphy et al. demonstrated that administration of MenSCs improved CLI in a mouse model [58].

Duchenne muscular dystrophy

- Duchenne muscular dystrophy (DMD) is a deadly x-linked muscle degeneration disease that consists of a potential genetic defect characterized by an enhanced inflammatory response [56].
- DMD is an important part
- of the muscular dystrophy glycoprotein complex (DGC),
- which is involved in the relative stabilization of the
- sarcolemma and regulation of the interaction between
- the cytoskeleton and skeletal muscle and myocardial
- ECM. Umezawa's team showed that MenSCs could restore muscle degeneration and repair skeletal muscle
- abnormalities by increasing muscle-like protein expression in immunodeficient DMD model mice [13]

Ovarian-related disease

Ovarian-related disease

Ovarian-related disease