THE EUROPEAN SOCIETY OF MEDICINE
Medical Research Archives, Volume 13 Issue 2

CLINICAL ARTICLE (Misc.)

Role of Embryo pooling in Low ovarian reserve

Kaberi Banerjee, MD, DNB, MRCOG, FRCOG[1], Bhavana Singla, MD, F.MAS, F.ART[1], Priyanka Verma, Resident Medical officer[1]

[1]Advance Fertility and Gynaecology Centre, New Delhi, India

OPEN ACCESS

PUBLISHED
28 February 2025

CITATION
Banerjee, K., et al., 2025. Role of Embryo pooling in Low ovarian reserve. Medical Research Archives, [online] 13(2). https://doi.org/10.18103/mra.v13i1.6325

DOI
https://doi.org/10.18103/mra.v13i1.6325

ISSN
2375-1924

ABSTRACT

Objective: To study the role of embryo pooling in low ovarian reserve.

Design: Retrospective analysis

Subjects: Forty-six (46) infertility patients with low ovarian reserve

Intervention: We had assessed 46 infertility patients with low ovarian reserve. All patients were started on dehydroepiandrosterone (DHEA) supplements. We had advised IVF with high dose hormones with pooling of embryo for 2 to 3 cycles with the criteria for getting at least 2 grade A embryos. First ovarian stimulation was done with antagonist protocol using Gonadotropins. Second stimulation was started in immediate next cycle with long agonist protocol. In 18 patients out of 46, third ovarian stimulation was done with antagonist protocol.

Main outcome measures: Pregnancy rate, clinical pregnancy rate and live birth rate were assessed.

Results: In 29 females, ovarian stimulation was done twice and in 18, three ovarian stimulations were done to pool embryos. In 3 patients, embryo transfer was not done as response was not good. Overall pregnancy rate was 44.4%, the clinical pregnancy rate was 35.5% and live birth rate was 26.6%.

Conclusions: Fertility treatment of patients with low ovarian reserve is challenging for fertility experts and assisted reproduction technologies are best option for these patients. Embryo pooling is the best option in these cases. But the couples should be properly counselled for the whole process, duration of treatment and the success rate of procedure. Further well-designed studies are required to predict the pregnancy rate and the clinical pregnancy rate more precisely.

Key words: Low ovarian reserve, embryo pooling, pregnancy rate and the clinical pregnancy rate

Introduction

Low ovarian reserve or Diminished ovarian reserve (DOR) is the condition in which fertility of the female reduces due to lesser quantity and low quality of oocytes in the ovaries. It can be due to many reasons like advanced maternal age, genetic factors, ovarian issues, drugs, environment. Various ovarian reserve tests include biochemical tests - follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E_2), inhibin B, and anti-Mullerian hormone (AMH), and transvaginal ultrasound imaging of the ovaries to check antral follicle count (AFC). AMH is one of the most commonly used markers to estimate ovarian reserve which shows the follicle pool without the effect of gonadotropins. Incidence of low Anti Mullerian hormone (AMH) is rapidly increasing in fertility clinics. In a recent study in India, it was found that more than 30% of women visiting such clinics were found to have low AMH.[1] Earlier poor ovarian response was described as Bologna criteria when at least 2 of the 3 criteria were present: a) advanced female age, b) a previous poor response and c) abnormal ovarian reserve tests and in the absence of the above criteria, two previous poor ovarian response following maximal stimulations.[2] However, with these criteria certain set of women were excluded and therefore the POSEIDON criteria (acronym for Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number) were established. In this, two new categories of impaired response were included: a) a sub optimal response which was defined as retrieval of 4 to 9 oocytes rather than their expected 10 to 15 oocytes and b) was a hypo response in which higher dose of gonadotropin and more prolonged stimulation was required to obtain an adequate number of oocytes (more than 3).[3] Low AMH is associated with lower number of oocytes retrieved and poor pregnancy rates.[4,5] AMH of < 0.5 ng/ml predicts an oocyte yield of < 4.[6]

Sunkara et al suggested that if number of oocytes retrieved up to 15 in number, then pregnancy rate can be maximized.[7] We know that in poor responders the total number of eggs in each stimulated cycle would be less, therefore if we try to replicate the high success model, it would mean that we continue with stimulation for more than one cycle and pool as many good quality oocytes and embryos as possible. In today's world with the excellent cryopreservation systems and protocols it is possible to pool such gametes/embryos and then do a single transfer of high-grade embryos to project the results of the good prognosis group.[8]

Materials and Methods

We conducted a retrospective study on 46 women at fertility clinic in New Delhi, India, who underwent embryo pooling. Table 1 shows the distribution of the patients according to the Poseidon criteria. Most of the women had AMH < 1 ng/ml and some of them had hypo response despite AMH of > 1.2 ng/ml (Fig 1). The approval from Independent Ethical Committee (F.1/IEC/IFS/2024/No.21) was obtained. The primary outcome was assessed with positive serum beta HCG rate after embryo transfer and the secondary outcome was assessed with clinical pregnancy rate.

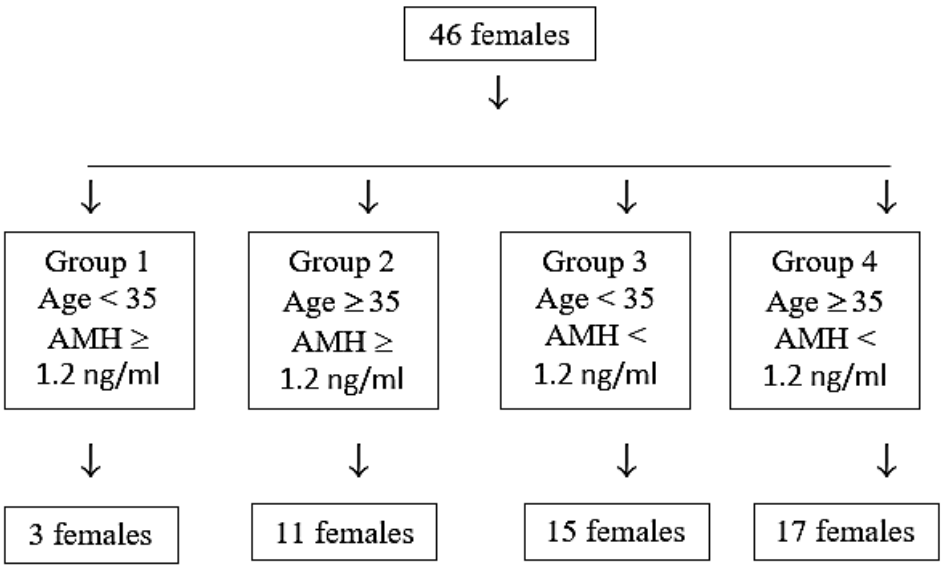

Figure 1. Flow diagram of study design (AMH – Anti Mullerian Hormone)

All couples were counselled for embryo pooling after reviewing all the fertility reports and were explained about need of second or third ovarian stimulation when less than two grade A cleavage stage embryos will be formed after the first stimulation cycle.

The first ovarian stimulation was an antagonist protocol. In this a total gonadotrophin dose of 350 IU to 450 IU was used; either a combination of follicle-stimulating hormone (FSH) and human menopausal gonadotropin (HMG) or pure HMG. The stimulation would go on for 10-12 days. Inj Cetrorelix acetate as antagonist 0.25 mg was added when the lead follicle was 14 mm. Trigger injection as Human chorionic gonadotropin (HCG) 10,000 was added when the lead follicle reached 18 mm and egg pick up was done 36 hours later. The second stimulation cycle was the long protocol in which leuprolide acetate 0.6 milligram was injected subcutaneously from the 21st day of the prior cycle and then from the first or second day of the cycle it was halved and high dose stimulation described above was started. HCG 10,000 IU was added when the lead follicle reached 18 mm and egg pick up was done 36 hours later. Before the 3rd stimulation protocol, one month of testosterone application gel (1% of 10mg/day transdermal) was given for local application on undersurface of arm and then the antagonist protocol was used. Stimulation 1 and stimulation 2 were done either back-to-back or with a gap of one month. After the egg collection procedure, the oocytes were fertilized using either In vitro fertilization (IVF) or Intracytoplasmic sperm injection ICSI, depending on the sperm parameters. If more than one grade A embryo was formed then the embryos were transferred, otherwise they were cryopreserved and the next stimulation cycle was started. Most embryo transfers were done on day 2 or day 3 of the cleavage stage of the embryos. Usually, two to three embryos were transferred. Most of the study was conducted before the onset of the new ART (Assisted reproductive treatment) regulation of India.[9] Thus, in some cases with previous failure and advanced maternal age, four embryos were transferred.

Serum beta HCG was measured 14 days after embryo transfer and ultrasound for fetal heartbeat was performed 2 weeks after the positive pregnancy test. Statistical analysis was performed appropriately using Chi square analysis.

Results

All 46 infertile females were categorized according to according to the Poseidon criteria as in Table 1. Our sample consisted of 46 women with mean age 35.8 years (range 28-46 years). Their mean AMH level was 0.9 ng/ml (range 0.17-1.8 ng/ml).

	Group 1		Group 2
Age < 35	3	Age ≥ 35	11
AMH ≥ 1.2 ng/ml	3	AMH ≥ 1.2 ng/ml	11
No. of Stim Cycles	6	No. of Stim Cycles	26
Subgp 1a: <4	2	Subgp 1a: <4	14
Subgp 1a: 4-9	4	Subgp 1a: 4-9	12
	Group 3		Group 4
Age < 35	15	Age ≥ 35	17
AMH < 1.2 ng/ml	15	AMH < 1.2 ng/ml	17

Table 1. Distribution of Patients according to the Poseidon criteria

The ovarian stimulation was done twice in 29 females and out of 46 females, 18 underwent three ovarian stimulations to pool the embryos. Table 2 shows the distribution of the patients according to the number of stimulations performed.

No. of stimulation cycles	No. of Patients
Only 1 cycle	0
2 cycles	29
3 cycles	18
Total Patients	46

Table 2. Distribution of patients according to number of stimulations performed

In all stimulated cycles, different number of oocytes were obtained by various types of ovarian stimulations. As ovarian reserve was low in all cases, number of oocytes retrieved was categorized in two categories which was assessed in Table 3.

Stimulation cycle	1st	2nd	3rd
No. of Patients	46	46	18
No. of Oocytes			
<4	34	31	10
4 to 9	12	15	8

Table 3. Distribution of oocytes in stimulation cycles

In most of the embryo transfers, two, three or four embryos in either fresh or frozen state were transferred on day 2 or day 3 of the cleavage stage or day 6 stage of the embryos. In some cases, double transfer (transfer on day 3 and day 6 in same cycle) was also done. Most of the study was conducted before the onset of the new ART regulation of India.[9] So, in some cases with previous failure and advanced maternal age, four embryos were transferred. Table 4 shows the distribution of the number of embryos transferred in all patients.

No. of Embryos Transferred	No. of Patients
1	1
2	6
3	19
4	19
0 (ET not done)	3

Table 4. Number of Embryos Transferred

The embryos were transferred in either fresh cycle or frozen cycle depending on endometrial conditions. Table 5 shows the distribution of the embryos according to the fresh or frozen stage. In two cases, embryo transfer was done twice as first attempt in both cases resulted in biochemical pregnancy. So, second transfer was done after one month gap.

Type of embryo transfer	No. of Patients
Fresh	24
Frozen	18
Fresh + Frozen	3
ET not done	3

Table 5. Distribution of embryos (Fresh/Frozen)

We had assessed the outcomes of embryo pooling cases - pregnancy rates (PR), clinical pregnancy rates (CPR) and live birth rate (LBR) from the data (Table 6). For assessing the outcomes, we considered 45 embryo transfers, as in two cases, embryo transfer was done twice in a gap of one month. In 3 cases, embryo transfer was not done out of that in two cases embryo didn't grow and in one case, all embryos were aneuploid.

Total Transfers	45
Transfers not done	03
Positive	20
Pregnancy Rate (PR)	20/45 (44.44%)
Clinical Pregnancy Rate (CPR)	16/45 (35.56%)
Live Birth Rate (LBR)	12/45 (26.67%)

Table 6. Outcome of Embryo Pooling cases

As shown in the Table 7, there was no association between the mode of transfer i.e fresh or frozen, number of embryos transferred and day of embryo transfer with the outcome.

	Outcome		P value (p<.10)
	Pregnant (N = 20)	Not Pregnant (N = 25)	
Mode of Transfer			0.8 (NS)
Fresh	10	14	
Frozen	9	9	
Fresh + Frozen	1	2	
No. of Embryos Transferred			0.79 (NS)
1	0	1	
2	3	3	
3	7	12	
4	10	9	
Day of Transfer			0.22 (NS)
Day 2	3	5	
Day 3	9	16	
Day 6	0	1	
Double Transfer (D3 + D6)	8	3	

Table 7. Results

Discussion

Low AMH is associated with lower pregnancy outcomes. A large retrospective study performed on 69,336 fresh and 15,458 frozen embryo transfer cycles demonstrated that the areas under the curve (AUC) for AMH as predictor of live birth in fresh cycles and thawed cycles were respectively, 0.631 and 0.540, suggesting that AMH alone is a weak, even if significant, age-independent predictor of live birth after ART.[10]

Oocytes may not be retrieved; embryos may not be formed and even when they are formed, they may be less in number and quality. Therefore, the suggestion of multiple cycle stimulation to form minimum of two grade A cleavage stage embryos is suggested.[11-14]

It is believed that blastocyst transfer is better than cleavage stage transfer in improving live births. This is true in most cases. However, one cannot forget that there are certain cases, especially in women where the total number of eggs is less that 5, one may not be able to extend embryos to blastocysts and there is risk of no transfer.[15] Also extending culture to blastocyst in those with good egg numbers means freezing fewer embryos so there is also a question of whether the cumulative live birth in case of blastocyst transfer is actually better than cleavage which is contested in many studies.[16,17] In our clinic we practice transferring embryos at an early stage when the total number of eggs retrieved is less than 5 which according to us gives the patient a better chance of a live birth baby.

It is widely believed and practiced that transferring fewer number of embryos leads to lower multiple pregnancy rate. While that is true it also leads to lower pregnancy rate per embryo transfer in women with advanced maternal age and women with multiple

IVF failures.[18] The Cochrane group in its 2020 analysis of the number of embryos to be transferred reiterated that elected single embryo transfer is good for only young good prognosis patients.[14] It is therefore suggested in most international guidelines that women with multiple failures and advanced age can undergo transfer of more than two embryos.[19,20] Therefore, in India where IVF is not government sponsored and is also not yet covered by any insurance plan, the couple has to totally take the burden of treatment. Therefore, in our practice to reduce the time to pregnancy and thus reducing the cost of treatment burden to the couples we transfer two embryos at the first attempt and more than two in women with advanced maternal age and repeated IVF failures.

Greco et al in 2013 evaluated the role of co-transfer of embryos derived from vitrified oocytes accumulated during previous modified natural cycles and an embryo developed from the last cycle as an alternative to repeated single embryo transfer. They concluded that the overall clinical pregnancy rate was significantly higher (34.4% vs 16%) with this protocol compared to repetitive single embryo transfers.[21]

Cobo A et al in their study stated the accumulation of oocytes from several ovarian stimulation cycles is currently possible with the aid of novel vitrification technologies. This strategy could be useful for low-responder patients, contributing to increase the inseminated cohort and creating a similar situation as in normal responders. According to the results presented herein (higher live-birth rate per patient treated, 36.4 vs 24.7), this strategy represents a successful alternative for low-responder patients, yielding comparable success rates to those in normal responders and avoiding the adverse effects of a low response.[8] Vora AV et al study is the only reported Indian study on embryo pooling to the best of our knowledge. They reported a case series of two women both of whom who had AMH of less than 1 ng/ml but more than 0.5 ng/ml. Each underwent three ovarian stimulations as in single ovarian stimulation cycles, the number of embryos were less and finally four embryos were transferred in each after pooling. Both had positive beta HCG and were continuing with single live pregnancies.[22] Lee et al in their study in 2023 concluded that oocyte pooling does not improve live birth rates. This may suggest that it is better to freeze embryos rather than oocytes.[23] Though, many studies have claimed that results with vitrified oocytes are similar to fresh oocytes.[24,25] and others have claimed that the outcome may not be at par.[26] Vitrification of oocytes is still one of the novel techniques and many laboratories and embryologists may still be in the learning curve to optimize their outcome. In our opinion, whenever possible embryos should be frozen rather than oocytes in pooling cycles to optimize outcomes.

Sadeghi et al dismissed embryo pooling as not being beneficial, giving various reasons, of which the most important one was that since in any way a single embryo is usually transferred and that can be found even if we transfer one embryo /cycle, there is no need of pooling. He had quoted, "*Regarding oocytes/embryos banking to avoid poor responders' dropout, it should be noted that the practice seems to be so selfish and a type of distraint in IVF clinics for future referral of patients*".[27] However, we need to factor in that in some cases early transfer and higher number of embryos may sometimes be beneficial therefore pooling off embryos is a better idea.[12-14] The trauma of multiple cycles of transfer and getting a negative outcome each time till we find the correct embryo for the positive outcome also needs to be factored in. The Practice Committee guidelines of ASRM 2021 clearly mentioned the sub category of women who would benefit from more than one embryo to be transferred. These were older age women and women with unfavorable prognosis.[28] Women with low ovarian reserve fit into this category. The Cochrane recommendations in 2020 also suggested that women with poor prognosis may benefit with transfer of two embryos.[14] Masschaele et al wanted to know that whether in a specific set of patients transferring more than three embryos, i.e., HLT (heavy load transfer) improved pregnancy

outcomes without significantly affecting multiple pregnancy rates. They concluded that in women more than 40, two embryos should be transferred and in women who have had failed multiple embryo transfers HLT should be considered.[20] We are not trying to propagate multiple embryo transfer but we need to identify that group of patients who will be disadvantaged by single embryo transfer.

Celik et al mentioned that though the pregnancy rate improves slightly with embryo pooling, the miscarriage rate is also high.[29] We also found that though the positive pregnancy rate was 44.44%, the clinical pregnancy rate was 35.56%. We feel the higher miscarriage rate is not because of embryo pooling per se, but higher miscarriage rate is seen in women with diminished ovarian response.[30,31]

Supporting embryo pooling in women undergoing preimplantation genetic testing of embryos, Hu et al suggested that those who do not reach enough embryos in a single stimulation cycle, pooling embryos from consecutive ovarian stimulation cycles is a promising strategy, which can render a cumulative pregnancy rate comparable to those patients who only require one stimulation cycle.[13]

As regards, which ovarian stimulation protocol is the best, we found that the number of eggs retrieved with antagonist and agonist protocol were similar. This has been collaborated by various studies that for women with diminished ovarian response no specific ovarian stimulation protocol has been proven to be better than the other.[32,33]

We have also observed that in the 3rd stimulation protocol the number of oocytes retrieved were higher in some women. This could be because of testosterone gel application which was applied in the cycle prior. Testosterone gel application has been associated with better ovarian response and this could also mean that ovaries are being sensitized to stimulation better than by previous stimulations and the consequent cycles are giving higher number of oocytes. Androgen stimulates early stages of follicular growth and increases the number of preantral and antral follicles by the proliferation of granulosa and thecal cells and reduction in granulosa cell apoptosis. It is hypothesized that positive change in microenvironment in the ovaries may lead to an increase in the number and the maturity of oocytes in poor responder group. Jeve YB et al in their metanalysis mentioned 3 trials which indicated that testosterone gel transdermal application before ovarian stimulation in poor responders significantly improved outcome.[34] This finding was further confirmed by yet another metanalysis in 2022 by Katsika ET et al.[35]

It is usually a practice to give a gap of one to two months between ovarian stimulation cycle to give the ovary some rest. However, studies have proven that even if we do back to backs cycles it does not reduce ovarian response.[36] In fact some strategies have proven that doing back-to-back cycles may increase the ovarian response to gonadotropins. Pailis M et al mentioned that immediate sequential stimulation (without an intervening menstrual cycle) in poor responders is advantageous over delayed stimulation in terms of number of aspirated oocytes and available embryos. The administration of high-dose FSH in the first cycle may benefit follicular recruitment also in the subsequent cycle. Although the effect is modest, given that each additional oocyte aspirated contributes to the outcome, it might be of significance especially in younger patients.[37] In fact, Silverberg KM et al commented that clinical pregnancies resulted significantly more often in a consecutive cycle than in an alternating cycle They concluded that consecutive cycles of ovarian stimulation with HMG are not detrimental and may, in fact, result in increased cycle fecundity compared to alternating stimulation cycles.[38]

Many studies have also proven that consecutive cycles do not harm the overall oocyte production and some protocols may in fact be better than the other. In our study the first stimulation protocol was antagonist protocol where the average oocytes yielded were 2.63, the next stimulation protocol was a long protocol where the average oocytes yielded

were 3.2 and the following was a protocol with testosterone gel application in the previous month and in that the average eggs yielded was 3.67. Ron El et al found an identical ovarian response using the same mode of stimulation in repeated cycles, and a significantly improved response with the Gonadotropin releasing hormone agonist (GnRHa)/ HMG combination compared with HMG alone in the same patient.[39]

The success of an ART program will be defined as the delivery of a healthy baby. It is well known that the treatment of poor responders is still experimental and no clear-cut modality has yet been established. The multiple treatments like various stimulation protocols, testosterone, growth hormone, Platelet rich plasma, stem cells suggests that we are yet far from the solution. The usage of donor eggs becomes the final treatment modality. Understandably many women do not want to go down that route. We argue here that the option of embryo pooling makes some women comfortable and satisfied that she has given herself the best option to have a biological child. If embryo pooling fails, one can then move on to third party reproduction. Approximately 20 % of couples after failed treatments agreed with donor egg IVF. Till date there is no study to assess the acceptance of women with poor response to go to third party reproduction after going through failed IVF cycles of self-egg stimulation.

Conclusion

We therefore conclude that embryo pooling by successive ovarian stimulation using different protocols improves the pregnancy outcome in poor responders. We believe that in this subset of patients transfer of at least two grade A cleavage stage embryos will give the maximum result. This treatment modality improves the compliance of the patient and in some cases opens up the acceptance to third party reproduction later.

Funding Statement:

No funding for the project

Disclosure Statement:

None for all authors

Attestation Statement:

- Data regarding any of the subjects in the study has not been previously published unless specified.
- Data will be made available to the editors of the journal for review or query upon request.

Data Sharing Statement:

Data can be shared as per requirement.

Trial registration:

Not applicable

Capsule:

Embryo pooling by successive ovarian stimulation using different protocols improves the pregnancy outcome in low ovarian reserve cases.

References

1. Palgamkar JB, Jindal DK, Sawkar SV et al.Anti-Mullerian Hormone Levels in Indian Women Seeking Infertility Treatment: Are Indian Women Facing Early Ovarian Senescence? *J Hum Reprod Sci.* 2021 Oct-Dec;14(4):380-385. doi: 10.4103/jhrs.jhrs_71_21. Epub 2021 Dec 31. PMID: 35197683; PMCID: PMC8812391.

2. Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L; ESHRE working group on Poor Ovarian Response Definition. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. *Hum Reprod.* 2011 Jul;26(7):1616-24. doi: 10.1093/humrep/der092. Epub 2011 Apr 19. PMID: 21505041.

3. Abu-Musa A, Haahr T, Humaidan P. Novel Physiology and Definition of Poor Ovarian Response; Clinical Recommendations. *Int J Mol Sci.* 2020 Mar 19;21(6):2110. doi: 10.3390/ijms21062110. PMID: 32204404; PMCID: PMC7139860.

4. Zhao D, Fan J, Wang P, Jiang X, Yao J, Li X. Age-specific definition of low anti-Mullerian hormone and associated pregnancy outcome in women undergoing IVF treatment. *BMC Pregnancy Childbirth.* 2021 Mar 5;21(1):186. doi: 10.1186/s12884-021-03649-0. PMID: 33673814; PMCID: PMC7936413.

5. Revelli A, Biasoni V, Gennarelli G, Canosa S, Dalmasso P, Benedetto C. IVF results in patients with very low serum AMH are significantly affected by chronological age. *J Assist Reprod Genet.* 2016 May;33(5):603-609. doi: 10.1007/s10815-016-0675-7. Epub 2016 Feb 18. PMID: 26888025; PMCID: PMC4870438.

6. Rasool S, Shah D. Fertility with early reduction of ovarian reserve: the last straw that breaks the Camel's back. *Fertil Res Pract.* 2017 Oct 11;3:15. doi: 10.1186/s40738-017-0041-1. PMID: 29046817; PMCID: PMC5637249.

7. Sunkara SK, Rittenberg V, Raine-Fenning N, Bhattacharya S, Zamora J, Coomarasamy A. Association between the number of eggs and live birth in IVF treatment: an analysis of 400 135 treatment cycles. *Hum Reprod.* 2011 Jul;26(7):1768-74. doi: 10.1093/humrep/der106. Epub 2011 May 10. PMID: 21558332.

8. Cobo A, Garrido N, Crespo J, José R, Pellicer A. Accumulation of oocytes: a new strategy for managing low-responder patients. *Reprod Biomed Online.* 2012 Apr;24(4):424-32. doi: 10.1016/j.rbmo.2011.12.012. Epub 2012 Jan 8. PMID: 22386762.

9. https://dhr.gov.in/sites/default/files/ART%20Rules%202022%20notification%20dated%2007062022%20published%20on%2008%20june.pdf

10. Tal R, Seifer DB, Wantman E, Baker V, Tal O. Antimüllerian hormone as a predictor of live birth following assisted reproduction: an analysis of 85,062 fresh and thawed cycles from the Society for Assisted Reproductive Technology Clinic Outcome Reporting System database for 2012-2013. *Fertil Steril.* 2018 Feb;109(2):258-265. doi: 10.1016/j.fertnstert.2017.10.021. Epub 2018 Jan 11. PMID: 29331235.

11. Vermey BG, Chua SJ, Zafarmand MH et al. Is there an association between oocyte number and embryo quality? A systematic review and meta-analysis. *Reprod Biomed Online.* 2019 Nov;39(5):751-763. doi: 10.1016/j.rbmo.2019.06.013. Epub 2019 Jul 3. PMID: 31540848.

12. Liu R, Zhang Q, Geng L et al. What is the optimal number of embryos to transfer for POSEIDON group 1 and group 2? A retrospective study. *J Ovarian Res.* 2024 May 31;17(1):117. doi: 10.1186/s13048-024-01443-y. PMID: 38822354; PMCID: PMC11140960.

13. Hu X, Ding C, Zhang D et al. Embryo pooling: a promising strategy for managing insufficient number of embryos in preimplantation genetic diagnosis. *Gynecol Endocrinol.* 2017 Nov;33(11):867-871. doi: 10.1080/09513590.2017.1347778. Epub 2017 Jul 6. PMID: 28682646.

14. Kamath MS, Mascarenhas M, Kirubakaran R, Bhattacharya S. Number of embryos for transfer following in vitro fertilisation or intra-cytoplasmic sperm injection. *Cochrane Database Syst Rev.* 2020

Aug 21;8(8):CD003416. doi: 10.1002/14651858.CD003416.pub5. PMID: 32827168; PMCID: PMC8094586.

15. Neblett MF 2nd, Kim T, Jones TL et al. Is there still a role for a cleavage-stage embryo transfer? *F S Rep.* 2021 Jun 29;2(3):269-274. doi: 10.1016/j.xfre.2021.06.004. PMID: 34553150; PMCID: PMC8441559.

16. Fernández-Shaw S, Cercas R, Braña C, Villas C, Pons I. Ongoing and cumulative pregnancy rate after cleavage-stage versus blastocyst-stage embryo transfer using vitrification for cryopreservation: impact of age on the results. *J Assist Reprod Genet.* 2015 Feb;32(2):177-84. doi: 10.1007/s10815-014-0387-9. Epub 2014 Nov 18. PMID: 25403438; PMCID: PMC4354180.

17. Glujovsky D, Farquhar C, Quinteiro Retamar AM, Alvarez Sedo CR, Blake D. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. *Cochrane Database Syst Rev.* 2016 Jun 30;(6):CD002118. doi: 10.1002/14651858.CD002118.pub5. Update in: Cochrane Database Syst Rev. 2022 May 19;5:CD002118. PMID: 27357126.

18. Gleicher N, Kushnir VA, Barad DH. Risks of spontaneously and IVF-conceived singleton and twin pregnancies differ, requiring reassessment of statistical premises favoring elective single embryo transfer (eSET). *Reprod Biol Endocrinol.* 2016 May 3;14(1):25. doi: 10.1186/s12958-016-0160-2. PMID: 27142226; PMCID: PMC4855800.

19. Practice Committee of the American Society for Reproductive Medicine. Electronic address: ASRM@asrm.org; Practice Committee of the Society for Assisted Reproductive Technology. Guidance on the limits to the number of embryos to transfer: a committee opinion. *Fertil Steril.* 2017 Apr;107(4):901-903. doi: 10.1016/j.fertnstert.2017.02.107. Epub 2017 Mar 11. PMID: 28292618.

20. Masschaele T, Gerris J, Vandekerckhove F, De Sutter P. Does transferring three or more embryos make sense for a well-defined population of infertility patients undergoing IVF/ICSI? *Facts Views Vis Obgyn.* 2012;4(1):51-8. PMID: 24753889; PMCID: PMC3991445.

21. Greco E, Litwicka K, Arrivi C et al. Accumulation of oocytes from a few modified natural cycles to improve IVF results: a pilot study. *J Assist Reprod Genet.* 2013 Nov;30(11):1465-70. doi: 10.1007/s10815-013-0103-1. PMID: 24077861; PMCID: PMC3879935.

22. Vora AV, Nadkarni P, Singh P N, Nadkarni V, Nadkarni A.Case reports for embryo banking - for women who want their own. *Int JReprod Contracept Obstet Gynecol* 2019;8:2926-31.

23. Lee KS, Lin MH, Hwu YM, Yang JH, Lee RK. The live birth rate of vitrified oocyte accumulation for managing diminished ovarian reserve: a retrospective cohort study. *J Ovarian Res.* 2023 Mar 3;16(1):49. doi: 10.1186/s13048-023-01128-y. PMID: 36869354; PMCID: PMC9983267.

24. Trokoudes KM, Pavlides C, Zhang X. Comparison outcome of fresh and vitrified donor oocytes in an egg-sharing donation program. *Fertil Steril.* 2011 May;95(6):1996-2000. doi: 10.1016/j.fertnstert.2011.02.035. Epub 2011 Mar 15. PMID: 21406304.

25. Domingues TS, Aquino AP, Barros B et al. Egg donation of vitrified oocytes bank produces similar pregnancy rates by blastocyst transfer when compared to fresh cycle. *J Assist Reprod Genet.* 2017 Nov;34(11):1553-1557. doi: 10.1007/s10815-017-1017-0. Epub 2017 Aug 16. PMID: 28815355; PMCID: PMC5699994.

26. Setti AS, Braga DPAF, Iaconelli A, Borges E. Fresh oocyte cycles yield improved embryo quality compared with frozen oocyte cycles in an egg-sharing donation programme. *Zygote.* 2021 Jun;29(3):234-238. doi: 10.1017/S0967199420000842. Epub 2021 Jan 18. PMID: 33455591.

27. Sadeghi MR. Oocytes/Embryos Banking: A Vague Hope for Poor Responder Women. *J Reprod Infertil.* 2018 Jul-Sep;19(3):123-124. PMID: 30167391; PMCID: PMC6104429.

28. Practice Committee of the American Society for Reproductive Medicine and the Practice

Committee for the Society for Assisted Reproductive Technologies. Electronic address: ASRM@asrm.org. Guidance on the limits to the number of embryos to transfer: a committee opinion. *Fertil Steril.* 2021 Sep;116(3):651-654. doi: 10.1016/j.fertnstert.2021.06.050. Epub 2021 Jul 28. PMID: 34330423.

29. Çelik S, Turgut NE, Cengiz Çelik D et al. The effect of the pooling method on the live birth rate in poor ovarian responders according to the Bologna criteria. *Turk J Obstet Gynecol.* 2018 Mar;15(1):39-45. doi: 10.4274/tjod.62447. Epub 2018 Mar 29. PMID: 29662715; PMCID: PMC5894535

30. McCormack CD, Leemaqz SY, Furness DL, Dekker GA, Roberts CT. Anti-Müllerian hormone levels in recurrent embryonic miscarriage patients are frequently abnormal, and may affect pregnancy outcomes. *J Obstet Gynaecol.* 2019 Jul;39(5):623-627. doi: 10.1080/01443615.2018.1552669. Epub 2019 Mar 27. PMID: 30917731.

31. Busnelli A, Somigliana E, Cirillo F, Levi-Setti PE. Is diminished ovarian reserve a risk factor for miscarriage? Results of a systematic review and meta-analysis. *Hum Reprod Update.* 2021 Oct 18;27(6):973-988. doi:10.1093/humupd/dmab018. PMID: 34254138.

32. Blumenfeld Z. What Is the Best Regimen for Ovarian Stimulation of Poor Responders in ART/IVF? *Front Endocrinol (Lausanne).* 2020 Apr 17;11:192. doi: 10.3389/fendo.2020.00192. PMID: 32362870; PMCID: PMC7180183.

33. Davis OK. IVF stimulation: protocols for poor responders. *Methods Mol Biol.* 2014;1154:329-41. doi: 10.1007/978-1-4939-0659-8_15. PMID: 24782017.

34. Jeve YB, Bhandari HM. Effective treatment protocol for poor ovarian response: A systematic review and meta-analysis. *J Hum Reprod Sci.* 2016 Apr-Jun;9(2):70-81. doi: 10.4103/0974-1208.183515. PMID: 27382230; PMCID: PMC4915289.

35. Katsika ET, Bosdou JK, Goulis DG, Grimbizis GF, Kolibianakis EM. Higher live birth rate following transdermal testosterone pretreatment in poor responders: a systematic review and meta-analysis. *Reprod Biomed Online.* 2023 Jan;46(1):81-91. doi: 10.1016/j.rbmo.2022.09.022. Epub 2022 Oct 7. PMID: 36369150.

36. Caligara C, Navarro J, Vargas G, Simón C, Pellicer A, Remohí J. The effect of repeated controlled ovarian stimulation in donors. *Hum Reprod.* 2001 Nov;16(11):2320-3. doi: 10.1093/humrep/16.11.2320. PMID: 11679512.

37. Pailis M, Sapir O, Lande Y et al. Consecutive ovarian stimulation is beneficial in patients with a poor response to high-dose follicle-stimulating hormone. *Gynecol Endocrinol.* 2021 Nov;37(11):995-999. doi: 10.1080/09513590.2021.1908991. Epub 2021 Apr 9. PMID: 33834936.

38. Silverberg KM, Klein NA, Burns WN, Schenken RS, Olive DL. Consecutive versus alternating cycles of ovarian stimulation using human menopausal gonadotrophin. *Hum Reprod.* 1992 Aug;7(7):940-4. doi: 10.1093/oxfordjournals.humrep.a137775. PMID: 1430133.

39. Ron-El R, Raziel A, Herman A et al. Ovarian response in repetitive cycles induced by menotrophin alone or combined with gonadotrophin releasing hormone analogue. *Hum Reprod.* 1990 May;5(4):427-30. doi: 10.1093/oxfordjournals.humrep.a137116. PMID: 2141846.

