Quick Response Code

IVF Lite – A new strategy for managing poor ovarian responders

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About the Author

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ABSTRACT

Background: Previous trials have shown that neither conventional IVF nor natural cycle IVF is an effective treatment option for poor ovarian responders. However, none of the trials has examined the efficacy of accumulating embryos with serial minimal stimulation cycles, vitrifying the resulting embryos and transferring them in a remote cycle (IVF Lite protocol). Women with poor ovarian reserves, who commonly do not respond to conventional stimulation protocols, are left with few options when planning a family. The current study was undertaken to evaluate the efficacy of serial minimal stimulation in vitro fertilization (msIVF) cycles with vitrification of embryos for treatment of poor ovarian responders (PORs) as compared to conventional IVF protocols. **Materials and Methods:** This is a retrospective data analysis of PORs from June 2010 to November 2012. A total of 222 patients were included in the study. Ninety-seven patients underwent serial minimal stimulation cycles with vitrification and embryo banking (IVF Lite Group) and 125 patients underwent conventional controlled ovarian stimulation for IVF. The patients identified as PORs based on the Bologna criteria were included in the analysis. In the IVF Lite group, embryos were vitrified using Cryotec vitrfication protocol on Day 3. Once six embryos were banked with us, a frozen embryo transfer was planned. A maximum of 3 embryos were transferred. Main outcome measure was the clinical pregnancy rate defined as positive fetal heartbeat at 12 weeks of pregnancy. **Results:** There was no significant difference in the number of metaphase II (MII) oocytes retrieved between the both groups. The difference in the number of gonadotropins units required to produce one MII oocyte between the two groups was statistically highly significant: 680.4 units for the IVF Lite group and 4956.2 units for the conventional IVF group. The IVF Lite group had a higher percentage of good grade embryos. In the IVF Lite group, each patient underwent an average of 2.96 cycles of embryo accumulation before planning a frozen embryo transfer. An average of 6.2 embryos were accumulated for each patient. The clinical pregnancy rate (CPR) per embryo transfer was higher in the IVF Lite group (27.81%) than the conventional IVF group (15.15%). The CPR per patient was much higher in the IVF Lite (48.45%) than the conventional IVF group (24.0%). **Conclusion:** The results obtained in the current study demonstrate that the IVF Lite protocol consisting of ms-IVF, ACCU-VIT and rET is a very successful approach in treating poor responders. Very favorable rates of pregnancy can be achieved with IVF Lite protocol.

Key Words: Embryo accumulation, embryo vitrification, *in vitro* fertilization Lite, minimal stimulation, poor ovarian responders, poor response

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INTRODUCTION

Garcia *et al.,* first described a poor responder patient in 1983, about 30 years ago.[1] Many studies have been published since then about the potential treatment approaches for poor ovarian responders (PORs).[2-5] However, a satisfactory treatment approach to improve the outcomes for this particular group of patients has not been established. In 2011, the European Society for Human Reproduction and Embryology (ESHRE) reached a consensus on the minimal criteria required to define poor ovarian response (POR). The acronyms POR and PORs were also proposed to be entered into conventional assisted reproduction treatment terminology to define poor ovarian response and poor ovarian responders, respectively, at the same meeting by ESHRE.[6]

Minimal stimulation protocols were evolved with the intention of providing a more natural stimulation for IVF. These protocols have been shown to have many advantages over conventional ovarian stimulation protocols, the main one being production of fewer but better quality oocytes.[7-18]

Kyrou *et al.*, in a systematic review and meta-analysis of 22 randomized controlled trials in poor responders, compared different protocols. This meta-analysis concluded that none of the reviewed protocols was successful in significantly increasing the success rates for PORs.[19]

The low number of embryos available for transfer poses a great challenge in the management of PORs.[20] A potential management of poor responders is to create a sufficient pool of embryos by accumulating vitrified good-grade embryos over several minimal stimulation cycles. This would potentially make the chances of success for poor responders similar to normal responders. This management, however, is unthinkable without an outstanding vitrification program. The option of accumulating embryos has become a promising reality with the advent of vitrification technologies.

The aim of the current study was to evaluate the efficacy of serial minimal stimulation IVF (msIVF) cycles with vitrification and accumulation (ACCU-VIT) of embryos followed by a remote frozen embryo transfer for the treatment of poor responders as compared to conventional IVF protocols.

MATERIALS AND METHODS

Study population

This is a retrospective data analysis of PORs who underwent treatment at Rotunda – The Center for Human Reproduction, Mumbai, India between 1 June 2010 and 30 November 2012. The patients identified as PORs based on the Bologna criteria[6] were included in the analysis.

IVF Lite protocol

The IVF Lite protocol consists of msIVF + ACCU-VIT + rET

msIVF

Clomiphene citrate (Ovofar, MSD, India) 50 mg per day was started on day 2 or day 3 of the menstrual cycle and was continued for 10 days till maturity of follicles was established by ultrasound guidance. Maturity was said to have been achieved when the lead follicle size reached 21-22 mm; 150 IU of human menopausal gonadotropin (hMG) [HUMOG, Bharat Serums and Vaccines Ltd. (BSVL), India] was added on day 5 and continued till the desired follicular size was achieved. Gonadotropin-releasing hormone (GnRH) antagonist cetrorelix (Ciscure, Emcure, India) 0.25 mg was added to the stimulation when the lead follicle size was 18 to 19 mm in diameter. When adding cetrorelix, the dose of hMG was increased by 75/150 IU at the discretion of the treating physician. Cetrorelix was continued till the day of the human chorionic gonadotropin (hCG) trigger; 10,000 IU of hCG (HUCOG, BSVL, India) was given when the lead follicle size was 21 to 22 mm in diameter. Oocyte retrieval was performed under ultrasound guidance 32 to 34 hours after the hCG trigger.

ACCU-VIT

The retrieved eggs were fertilized using either IVF or intracytoplasmic sperm injection (ICSI) and the resulting embryos were vitrified on day 3 using vitrification (Cryotech, Japan).

Back-to-back cycles of msIVF followed by ACCU-VIT were performed till about six top-grade (grades A and B) embryos were accumulated per patient.

rET

An rET was performed when adequate embryos were accumulated. The embryos were warmed using the Cryotec (Cryotech, Japan) warming protocol. The warmed embryos were transferred on day 4, after preparing the endometrium with estradiol valerate tablets (Progynova, Zydus Healthcare, India) which was started from day 3 or 4 of the menstrual cycle after a baseline scan was done to rule out any ovarian cyst and to measure endometrial lining. Standard luteal support was started four days prior to embryo transfer and continued for 12 days. Embryo transfers were performed under transabdominal ultrasound guidance using a Wallace Sureview (Smiths Medical

International Ltd, United Kingdom) embryo transfer catheter.

Conventional Stimulation

Stimulation was started on day 2 of the menstrual cycle with an initial dose of 300 to 450 IU of biosimilar recombinant follicle-stimulating hormone (bs-recFSH) (Foligraf, BSVL, India) along with buserelin (Busag, Zydus Gynova, India) 0.25 mg daily after ruling out ovarian cysts of >10 mm on transvaginal ultrasound. Day 5 onward, the dose of bs-recFSH was adjusted according to the ovarian response seen on transvaginal ultrasound performed every two days. When the lead follicle reached a diameter of 18-19 mm 10,000 IU of hCG (HUCOG, BSVL, India) was given, and ultrasound-guided oocyte retrieval was done approximately 34 hours later. Fresh embryos were transferred on day 3. Embryo transfers were performed under transabdominal ultrasound guidance using a Wallace Sureview (Smiths Medical International Ltd, United Kingdom) embryo transfer catheter. Adequate luteal support was given.

RESULTS

A total of 97 PORs underwent treatment with IVF Lite protocol and 125 PORs underwent treatment with conventional IVF stimulation protocol.

The mean values for age, basal blood FSH, and anti-Mullerian hormone (AMH) were comparable in the IVF Lite and conventional IVF groups [Table 1]. For the IVF Lite group, a total of 97 patients underwent 287 treatment cycles. For the conventional IVF group, a total of 125 patients underwent 277 treatment cycles. The percentages of cancellation of cycles due to poor response, cycles with no oocytes retrieved, and cycles with failed fertilization were also similar in both the groups [Table 2].

It is interesting to note that there was no statistical difference in the number of metaphase II (MII) eggs in both the groups. However, the percentage of MII oocytes retrieved was 78.06% for the IVF Lite group and 47.6% for the conventional IVF group (statistically significant, $P < 0.01$) [Table 3]. The difference in the number of gonadotropin units required to produce one MII egg between the two groups was statistically highly significant: 680.4 units for the IVF Lite group and 4956.2 units for the conventional IVF group, $P < 0.05$ [Table 2].

The fertilization and cleavage rates were similar in both the groups. The average number of good-grade embryos per cycle was 1.69 in the IVF Lite group and 1.04 in the conventional IVF group [Table 3]. It is interesting to note the significant difference in the percentage of good-grade embryos between the two groups ($P < 0.01$).

Table 4 shows the clinical outcome. The number of embryos transferred per cycle was similar in both the groups (1.75 and 1.77, respectively, in IVF Lite and conventional IVF), though the number of good-grade embryos transferred was

Table 1: Basal characteristics of patients

	IVF lite	Conventional IVF	*P* value
Patients (*n*)	97	125	
Mean age (yrs)	38.20±4.31	36.17±5.07	NS
Follicle-stimulating hormone (FSH) (mIU/mL)	8.41±1.9	8.97±2.1	NS
Anti-Mullerian hormone (AMH) (ng/mL)	0.97±0.49	0.82±0.52	NS

NS: Not significant

Table 2: Summary of total stimulation cycles

	IVF Lite	Conventional IVF	*P* value
Patients (*n*)	97	125	
No. of initiated cycles	287	277	
Avg no. of initiated cycles/patient	2.96	2.22	
Dosage of gonadotropins (IU)	1646.59±950.78	11349.13±4638.86	<0.001
No. of retrieval cycles	246	221	
% canceled retrieval cycles/ initiated cycle	14.29 (41/287)	20.22 (56/277)	NS
% cycle with no oocytes retrieved/ retrieval cycle	7.32 (18/246)	8.14 (18/221)	NS
% cycle with no fertilization/ retrieval cycle	1.63 (4/246)	2.26 (5/221)	NS
Dosage of gonadotropins required/MII oocyte	680.4 (1646.59/2.42)	4956.15 (11349.59/2.29)	<0.05

IVF: *In vitro* fertilization; MII: Metaphase II; Avg: Average; NS: Not significant

Table 3: Embryology details

	IVF Lite	Conventional IVF	*P* value
No. of oocytes/retrieval cycle	3.1±1.44	4.81±2.37	NS
No. of metaphase II oocytes/retrieval cycle	2.42±0.99	2.29±1.42	NS
% of metaphase II oocytes	78.06 (2.42/3.1)	47.60 (2.29/4.81)	<0.01
Fertilization rate (%)	90.85	90.09	NS
Cleavage rate (%)	98.15	96.61	NS
No. of embryos/cycle	2.15±1.01	1.94±1.26	NS
% Good-grade embryos/cycle	80.09 (1.69/2.15)	53.60 (1.04/1.94)	<0.01

IVF: *In vitro* fertilization; NS: Not significant

higher in the IVF Lite group. Clinical pregnancy rate (CPR) per embryo transfer was 27.81% in the IVF Lite group and 15.15% in the conventional IVF group. The CPR per patient was 48.45% in the IVF Lite group which is much higher than the 24.0% CPR per patient in the conventional IVF group. It is important to note that the IVF Lite group did not have a single cycle with cancelled embryo transfer. In the conventional group, 10.41% of the patients did not have any embryo transfer.

Table 5 shows the embryological outcomes of the vitrified warmed embryos. For the IVF Lite group, an average of 6.2 embryos was accumulated per patient; 169 warming cycles were performed for 97 patients. An average of 1.88 embryos was warmed per cycle; 97.34% of embryos survived the vitrification-warming process. An average of 1.75 embryos was transferred.

Figure 1 shows the number of cycles required to accumulate a minimum of six good-grade embryos for each patient before the embryo transfers were performed. All the patients required more than one stimulation cycle. Thirty patients accumulated the required number of embryos in two cycles, and 43 patients had to undergo three stimulation cycles. There were 22 patients who required four stimulation cycles, and two patients required five stimulation cycles to accumulate adequate embryos.

Figure 2 shows the number of attempts required to achieve pregnancy in the IVF Lite group. Twenty-four patients achieved pregnancy in the first attempt of frozen embryo transfer and 14 patients achieved pregnancy in the second attempt. There were eight patients who achieved pregnancy in the third attempt of frozen embryo transfer, and one patient who required four attempts of frozen embryo transfer to be pregnant.

Figure 3 shows the number of attempts required to achieve pregnancy in the conventional IVF group. This group had all fresh embryo transfers. Nine patients achieved pregnancy in the first attempt, 15 patients achieved pregnancy in the second attempt. There were six patients who achieved pregnancy in the third attempt. No patients tried more than three attempts of stimulation cycles with the conventional IVF protocol.

Table 4: Cycle outcomes

	IVF Lite	Conventional IVF	*P* value
Patients (*n*)	97	125	
No. of transfer cycles (*n*)	169	198	
Total embryos/transfer	1.75±0.37	1.77±0.24	NS
Good-grade embryos/transfer	1.52±0.29	1.04±0.46	<0.05
Clinical pregnancy rate/ET (%)	27.81	15.15	<0.05
Clinical pregnancy rate/ patient (%)	48.45	24.00	<0.01
% cycles with canceled embryo transfers	0	10.41 (23/221)	<0.01

IVF: *In vitro* fertilization; NS: Not significant; ET: Embryo transfer

Table 5: Outcome of vitrified-warmed embryos cycles of IVF Lite group

Avg no. of embryos vitrified/patient	6.2±2.3
No. of transfer cycles	169
Average no. of embryos warmed/cycle	1.88±0.51
Average no. of embryos survived/cycle	1.83±0.42
Survival rate (%)	97.34
No. of embryos transferred	1.75±0.37

IVF: *In vitro* fertilization

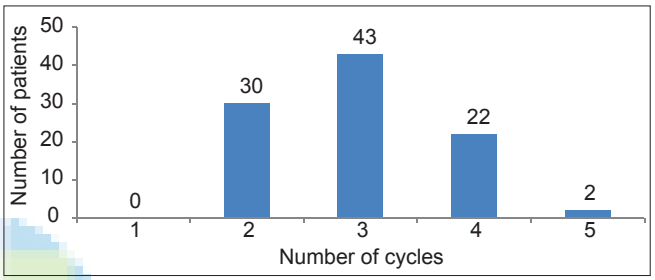

Figure 1: Number of cycles required to accumulate minimum six embryos for the IVF Lite group

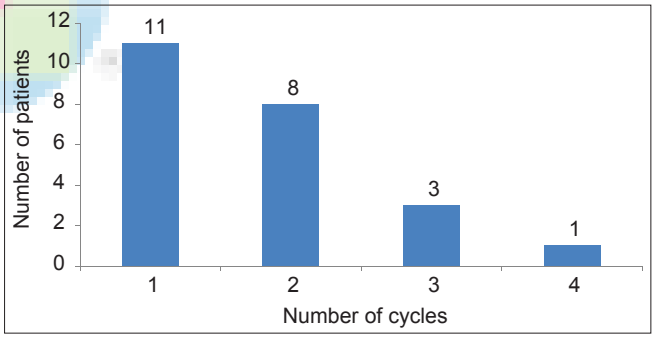

Figure 2: Number of embryo transfer cycles required to achieve a pregnancy for IVF Lite group

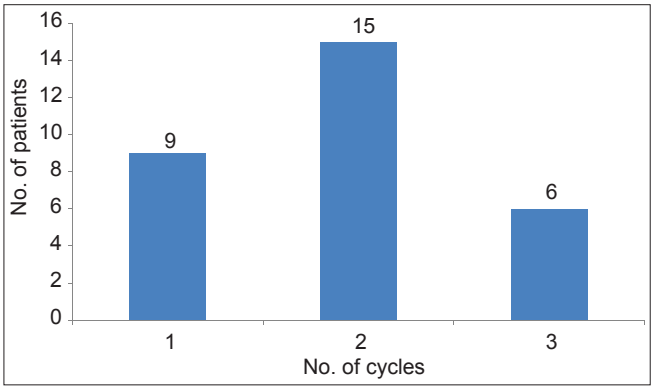

Figure 3: Number of embryo transfer cycles required to achieve a pregnancy for Conventional IVF group

DISCUSSION

POR is not a rare occurrence in ovarian stimulation. The incidence of POR is 9.24% in patients undergoing IVF treatment.[21]

Tarlatzis *et al.*, in their elegant systematic review evaluating all the existing ovarian stimulation protocols applied to poor responders, concluded that the exhausted ovarian apparatus is unable to react to any stimulation, no matter how powerful this might be.[2] The ideal stimulation for poor responders still remains a challenge, as the hypothesized diminished oocyte cohort cannot be reversed within the limits of our present capabilities.[2]

The current study was set out to assess the application of IVF Lite protocol[22] as a new strategy for managing these patients.

IVF Lite (ms-IVF + ACCU-VIT + rET)

Zhang *et al.*,[23] described a minimal stimulation protocol christened 'mini-IVF'. This protocol requires a reliable method for embryo cryopreservation such as vitrification, because of the negative impact of clomiphene citrate on the endometrium and because cryopreserved embryo transfers with this protocol have yielded much higher rates of pregnancy than fresh transfers. In this series, the patients were not denied treatment based on their day-3 FSH value or ovarian reserve.[23] Yet, acceptable rates of pregnancy were achieved (20% for fresh embryo transfers and 41% for cryopreserved embryo transfers).[24] These results strengthen the argument for a mini-IVF protocol and vitrification as an alternative to standard conventional IVF stimulation protocols.

The IVF Lite protocol similar to the 'mini-IVF' protocol[22] based on a minimal stimulation protocol including clomiphene citrate and hMG, vitrification, and cryopreserved rETs has yielded much higher pregnancy rates than fresh transfers.[25]. IVF Lite includes ACCU-VIT over a few cycles for PORs.

Unlike standard stimulation protocols, minimal stimulation protocols do not require a resting cycle between two treatment cycles. The women with poor ovarian reserves can have back-to-back consecutive cycles before their follicular reserve is depleted. It maximizes the already limited life span of the ovaries, allowing the patients to store embryos while production of oocytes is still active. This gives a very favorable pregnancy rate per time spent by the patient, which is of utmost significance in this group of patients who are in a race against time to beget their own genetic offspring.

The reliance on clomiphene citrate, with its negative impact on the endometrium, required a very reliable method of embryo cryopreservation. These are the patients who produce only one or two good-grade embryos per cycle. We have to try all that we can to ensure that we get pregnancies out of these embryos. A highly efficient vitrification program is extremely critical to minimize loss of embryo and maximize the chances of pregnancy. The study center chose the latest Cryotec vitrification method developed by Dr Masashige Kuwayama.

The vitrification method was developed to overcome the harmful effects of ice crystal formation that occurs during the slow-freezing method. Traditionally, slow-freezing protocols were used to freeze all kinds of human embryos, but clinically, satisfactory results were not obtained.[24] Furthermore, the results were not consistent. There are many papers in the literature that prove that vitrification is a more secure method of cryopreservation. Vitrification implants fewer traumas to the cells and is therefore a more effective means of cryopreservation of embryos than slow freezing.[26]

The Cryotec vitrification method (Cryotech, Japan) chosen by the study center has many advantages over the previous methods of vitrification of Dr Kuwayama.[27] Now, with major improvement in the solutions and the newly designed vitrification plates, a new method called Cryotec has been developed (Cryotech, Japan). The Cryotec vitrification solution has no added serum or synthetic serum supplements. It is a completely chemically defined solution and is stable for a year at 4-8°C. It contains trehalose instead of sucrose. This overcomes the problem of endotoxicity due to sucrose.[28] The Cryotec vitriplates have a special holder for the Cryotec; thus, the focus remains the same while washing the embryos in vitrification solution and placing them on the Cryotec.[29] The study center chose this method owing to its numerous advantages, which all cumulatively add up to an extremely high survival rate of the embryo.

The difference in the embryo transfer cancelation rate between the two groups is noteworthy. Most patients find it difficult to accept and cope with the terrible consequence of no embryo transfer.[30] Many of them turn to ovum donation, whereas many quit. In the current study, none of the patients in the conventional IVF group continued treatment beyond the third attempt, as indicated in Figure 3. The conventional IVF group showed a high cancellation rate of embryo transfer. With ACCU-VIT, as we have extremely high survival rates of embryos, there was not a

single patient who had oocytes retrieved in multiple cycles and no embryo transfer. The thought of having multiple chances of accumulating embryos at a much lower cost and trauma increases the acceptance and willingness of the patient to accumulate embryos to mimic the situation of normoresponder patients.

Interestingly, there was no significant difference in the number of MII oocytes between the two groups. The IVF Lite group had MII oocytes comparable to the conventional IVF group, with significantly less gonadotropins used. The IVF Lite group had a significantly higher percentage of good-grade embryos than the conventional IVF group. This suggests that when minimal stimulation is used, a cohort of few but better quality oocytes is obtained.

The CPR per patient was significantly higher in the IVF Lite group which confirms the efficacy of this approach for managing PORs. The CPR observed in this study for poor responder patients in the IVF Lite group is comparable to the success rate in normal responding patients.[31,32]

Many patients in the IVF Lite group were pregnant using only 2-3 of their accumulated embryos. They still have 3-4 surplus embryos stored, with the study center allowing them the opportunity to have a second child in the future if they so wished. This chance would otherwise be most likely denied to them with conventional IVF.

The small sample size and the retrospective, nonrandomized nature of the analysis are limiting factors of this study. However, the results are very encouraging. We are now looking at the cost benefits of applying this protocol to all groups of patients.

CONCLUSION

The results obtained in the current study demonstrate that the IVF Lite protocol consisting of ms-IVF, ACCU-VIT, and rET is a very successful approach in treating poor responders. Very favorable rates of pregnancy can be achieved with IVF Lite protocol, imparting a new hope to patients who would otherwise have a very small chance of having their own genetic children.

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REFERENCES

1. Garcia JE, Jones GS, Acosta AA, Wright G. HMG/hCG follicular maturation for oocytes aspiration: Phase II. 1981. Fertil Steril 1983;39:174-9.
2. Tarlatzis BC, Zepiridis L, Grimbizis G, Bontis J. Clinical management of low ovarian response to stimulation for IVF: A systematic review. Hum Reprod Update 2003;9:61-76.
3. Loutradis D, Vomvolaki E, Drakakis P. Poor responder protocols for *in-vitro* fertilization: Options and results. Curr Opin Obstet Gynecol 2008;20:374-8.
4. Kyrou D, Kolibianakis E, Venetis CA, Papanikolaou EG, Bontis J, Tarlatzis BC. How to improve the probability of pregnancy in poor responders undergoing *in vitro* fertilization: A systematic review and meta-analysis. Fertil Steril 2009;91:749-66.
5. Pandian Z, McTavish AR, Aucott L, Hamilton MP, Bhattacharya S. Intervention for poor responders to controlled ovarian hyper stimulation in IVF. Cochrane Database Syst Rev 2010;I: CD004379.
6. Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for *in vitro* fertilization: The Bologna criteria. Hum Reprod 2011;26:1616-24.
7. Baart EB, Martini E, Eijkemans MJ, Van Opstal D, Beekers NG, Verbeoff A, *et al.* Milder ovarian stimulation for *in-vitro* fertilization reduces aneuploidy in human preimplantation embryo: A randomized controlled trial. Hum Reprod 2007;22:980-8.
8. Collins J. Mild stimulation for *in vitro* fertilization: Making progress downward. Hum Reprod 2009;15:103.
9. De Jong D, Macklon NS, Fauser BC. A pilot study involving minimal ovarian stimulation for *in vitro* fertilization: Extending the 'follicle-stimulating hormone window' combined with the gonadotropin-releasing hormone antagonist cetrorelix. Fertil Steril 2008;73:1051-4.
10. Devreker F, Pogonici E, DeMaertelaer V, Revelard P, Vanden Bergh M, Englert Y. Selection of good embryos for transfer depends on embryo cohort size: Implications for the 'mild ovarian stimulation' debate. Hum Reprod 1999;14:3002-8.
11. Fauser BC, Devroey P, Yen SS, Gosden R, Crowley WF Jr, Baird DT, *et al.* Minimal ovarian stimulation: Appraisal of potential benefits and drawbacks. Hum Reprod 1999;14:2681-6.
12. Heijnen EM, Eijkemans MJ, De Klerk C, Polinder S, Beckers NG, Klinkert ER, *et al.* A mild treatment strategy for *in-vitro* fertilisation: A randomised non-inferiority trial. Lancet 2007;369:743-9.
13. Pelinck MJ, Vogel NE, Arts EG, Simons AH, Heineman MJ, Hoek A. Cumulative pregnancy rates after maximum of nine cycles of modified natural cycle IVF and analysis of patient drop-out: A cohort study. Hum Reprod 2007;22:2463-70.
14. Polinder S, Heijnen EM, Macklon NS, Habbema JD, Fauser BJ, Eijkemans MJ. Cost-effectiveness of a mild compared with a standard strategy for IVF: A randomized comparison using cumulative term live birth as the primary endpoint. Hum Reprod 2008;23:316-23.
15. Van der Gaast MH, Eijkemans MJ, Van der Net JB, Boer EJ, Burger CW, Van Leeuwen FE, *et al.* Optimum number of oocytes for a successful first IVF treatment cycle. Reprod Biomed Online 2006;13:476-80.
16. Verberg MF, Eijkemans MJ, Heijnen EM, Broekmans FJ, de Klerk C, Fauser BC, *et al.* Why do couples drop-out from IVF treatment? A prospective cohort study. Hum Reprod 2008;23:2050-5.
17. Verberg MF, Eijkemans MJ, Macklon NS, Heijnen EM, Baart EB, Hohmann FP, *et al.* The clinical significance of the retrieval of a low number of oocytes following mild ovarian stimulation for IVF: A meta-analysis. Hum Reprod Update 2009a; 15:5-12.
18. Verberg MF, Macklon NS, Nargund G, Frydman R, Devroey P, Broekmans FJ, *et al.* Mild ovarian stimulation for IVF. Hum

Reprod Update 2009b; 15:13-29.
19. Kyrou D, Kolibianakis EM, Venetis CA, Papanikolaou EG, Bontis J, Tarlatzis BC. How to improve the probability of pregnancy in poor responders undergoing in vitro fertilization: a systematic review and meta-analysis. Fertil Steril 2009;91:749-66.
20. Pellicer A, Ardiles G, Neuspiller F, Remohi J, Simon C, Musoles F. Evaluation of the ovarian reserve in young low responders with normal basal levels of follicle stimulating hormone using three-dimensional ultrasonography. Fertil Steril 1998;70:671-5.
21. Keay SD, Liversedge NH, Mathur RS, Jenkins JM. Assisted conception following poor ovarian response to gonadotrophin stimulation. Br J Obstet Gynaecol 1997;104:521-7.
22. Allahbadia GN. IVF Lite: Is this the future of assisted reproduction? J Obstet Gynecol India 2013;63:1-4.
23. Zhang J, Chang L, Sone Y, Silber S. Minimal ovarian stimulation (mini-IVF) for IVF utilizing vitrification and cryopreserved embryo transfer. Reprod Biomed Online 2010;21:485-95.
24. Glujovsky D, Riestra B, Sueldo C, Fiszbajn G, Repping S, Nodar F, *et al*. Vitrification versus slow freezing for women undergoing oocyte cryopreservation (Protocol). Issue 8. Wiley: The Cochrane Library; 2012.
25. Gandhi G, Allahbadia G, Kagalwala S, Allahbadia A, Ramesh S, Patel K, *et al.* ACCU-VIT: A new strategy for managing poor responders. Hum Reprod 2013;28:i149-206.
26. Valojerdi MR, Yazdi PE, Karimian L, Hassani F, Movaghar B. Vitrification versus slow freezing gives excellent survival, post warming embryo morphology and pregnancy outcomes for human cleaved embryos. J Assist Reprod Genet 2009;26:347-54.
27. Kuwayama M. Highly efficient vitrification for cryopreservation of human oocytes and embryos: The Cryotop method. Theriogenology 2007;67:73-80.
28. Minutoli L, Altavilla D, Bitto A, Polito F, Bellocco E, Laganà G, *et al*. The disaccharide trehalose inhibits proinflammatory phenotype activation in macrophages and prevents mortality in experimental septic shock. Shock 2007;27:91-6.
29. Kuwayama M. The Cryotec method manual book for oocyte and embryo. Japan: Cryotech Lab; 2012. p. 1.
30. Cobo A, Diaz C. Clinical application of oocyte vitrification: A systematic review and meta-analysis of randomized controlled trials. Fertil Steril 2011;96:277-85.
31. Garrido N, Bellver J, Remohi J, Simon C, Pellicer A. Cumulative live-birth rates per total number of embryos needed to reach newborn in consecutive *in vitro* fertilization (IVF) cycles: A new approach to measuring the likelihood of IVF success. Fertil Steril 2011;96:40-6.
32. Malizia BA, Hacker MR, Penzias AS. Cumulative live-birth rates after *in vitro* fertilization. N Engl J Med 2009;360: 236-43.

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