

# Conventional IVF versus modified natural IVF for poor ovarian reserve patients

## Abstract

**Purpose:** Women with diminished ovarian reserve have low live birth rates following assisted reproduction treatment. In this study, we investigate whether modified natural cycle IVF (MNC), involving Clomiphene Citrate and low dose gonadotrophins, can enhance clinical pregnancy rate (CPR) over conventional IVF for diminished ovarian reserve patients who prefer fresh embryo transfer.

**Methods:** We conducted a retrospective analysis to compare conventional IVF (GnRH antagonist protocol) versus MNC in the treatment of 72 patients with markedly diminished ovarian reserve.

**Results:** 46 patients had conventional IVF and 26 patients had MNC. Patients with conventional IVF had significantly more follicles: 5.5 (1–11) vs 2.5 (1–6),  $P < 0.0001$ ; more retrieved oocytes: 4 (0–8) vs 1.68 (0–5),  $P < 0.0001$ ; and more fertilized oocytes: 2.4 (0–8) vs 0.8 (0–4),  $P < 0.0001$ . No oocytes were retrieved in 5 (10.9%) patients in the conventional group, compared with 13 (50%) in the MNC group,  $P = 0.0005$ . CPR per embryo transfer in the conventional group was 43.2%, while none of the patients in the MNC group achieved pregnancy. Within the conventional group, there was no significant difference between the total FSH used in pregnant and non-pregnant patients (3129±307 IUs vs 2984±497 IUs,  $P = 0.3933$ ).

**Conclusions:** This study demonstrates that using conventional IVF in the treatment of markedly reduced ovarian reserve patients who prefer fresh embryo transfer is associated with more follicles; more retrieved and fertilized oocytes, a higher CPR and a lower chance of cycle cancellation compared with MNC.

**Keywords:** diminished ovarian reserve, modified natural cycle, conventional IVF, clomiphene citrate, fresh embryo transfer

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Mohamed Hany Mostafa,<sup>1</sup> Nganjo Kuva Endeley,<sup>1</sup> Ahmed M. H. A. M. Mostafa<sup>2</sup><sup>1</sup>Department of Obstetrics and Gynaecology, North Tees and Hartlepool Hospitals NHS Foundation Trust, UK<sup>2</sup>Medical Student, University of Cambridge, UK

**Correspondence:** Mohamed Hany Mostafa, Department of Obstetrics and Gynaecology, North Tees and Hartlepool Hospitals NHS Foundation Trust, Stockton-on-Tees, UK, Email [mohamed.mostafa@nhs.net](mailto:mohamed.mostafa@nhs.net)

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**Abbreviations:** AFC, antral follicle count; AMH, anti-mullerian hormone; CC, clomiphene citrate; CPR, clinical pregnancy rate; DOR, diminished ovarian reserve; FSH, follicle-stimulating hormone; GnRH, gonadotrophin releasing hormone; hCG, human chorionic gonadotropin; hMG, human menopausal gonadotropin; ICSI, intra cytoplasmic sperm injection; IVF, *In Vitro* fertilization; MNC, modified natural cycle IVF; POR, poor ovarian response

**Key Message:** In the treatment of patients with a markedly reduced ovarian reserve who prefer fresh embryo transfer, conventional IVF (antagonist protocol) is associated with more follicles, retrieved and fertilized oocytes, and clinical pregnancies than Modified Natural Cycle IVF. We, therefore, recommend conventional IVF over MNC in the treatment of this patient population.

## Introduction

Women of the same age can have very different responses to ovarian stimulation and have differing reproductive potential. The concept of ovarian reserve views reproductive potential as a function of the number and quality of remaining oocytes. Decreased or diminished ovarian reserve (DOR) describes women of reproductive age, having regular menses, whose response to ovarian stimulation or fecundity is reduced compared with women of a similar age.<sup>1</sup>

Patients with poor ovarian response (POR) are both challenging to treat and represent a large proportion of patients presenting with infertility. Patients with POR, often characterized by advanced maternal age, have a higher cycle cancellation rate, a higher miscarriage rate, and a significantly reduced live birth rate per cycle relative to patients without POR.<sup>2</sup>

The management of POR is highly controversial. There is currently no consensus regarding the “ideal” protocol and, as of yet, no treatment protocol has proven to be superior for this group. Conventional treatment entails controlled ovarian hyper stimulation with a long or short gonadotrophin-releasing hormone (GnRH) agonist protocol or a GnRH antagonist protocol. While this has a relatively low cancellation rate, it only achieves a low pregnancy rate with a large dose of follicle-stimulating hormone (FSH). On the other hand, minimal ovarian stimulation or natural cycles for patients with DOR have a high cancellation rate of oocyte retrieval and an extremely low pregnancy rate.<sup>3</sup> Minimal ovarian stimulation does, however, have several advantages over conventional IVF protocols. This includes less medication and fewer injections, the production of higher quality eggs, and the low cost, which makes it more repeatable.<sup>4</sup>

Teramoto and Kato reported that minimal ovarian stimulation with consecutive Clomiphene Citrate (CC) can induce follicle development

along with the inhibition of spontaneous ovulation.<sup>5</sup> In 2017, a multicentre randomized trial concluded that a mild ovarian stimulation strategy in women with poor ovarian reserve undergoing IVF leads to similar ongoing pregnancy rates as a conventional ovarian stimulation strategy.<sup>6</sup> However, minimal stimulation protocols often utilize embryo cryopreservation and frozen embryo transfer to avoid the adverse impact of CC on endometrial thickness.<sup>3</sup> Many of our patients were keen to have fresh embryo transfer to avoid the frustration of their treatment being interrupted and the risks of cryopreservation, including decreased survival of embryos and development to the blastocyst stage.<sup>7</sup> Therefore, we set out to compare minimal ovarian stimulation with conventional IVF protocols for women with DOR, using fresh embryo transfer exclusively.

## Aim of the work

In our study, we compared a conventional gonadotropin-antagonist protocol for women with poor ovarian reserve to a Modified natural IVF (MNC) protocol involving Clomiphene citrate (CC) overlapping with a low dose of gonadotropins, using only fresh embryo transfer. Our hypothesis was that using an MNC protocol with CC might enhance clinical pregnancy rates over the conventional protocol.

## Materials and methods

This study was conducted in the Assisted Reproduction Unit (ARU)-Hartlepool, between January 2015 and June 2016. We retrospectively compared the conventional IVF antagonist protocol versus MNC in the treatment of patients with a markedly reduced ovarian reserve, as defined by an AMH (Anti-Müllerian Hormone) $<5.5$  pmol/L and/or AFC (Antral Follicle Count) $\leq 7$ .<sup>8</sup> 72 patients were included in the study. 46 patients underwent conventional IVF treatment and 26 patients underwent MNC IVF.

### Controlled ovarian hyper-stimulation

We followed the conventional antagonist protocol, as outlined elsewhere,<sup>9</sup> with a baseline ultrasound scan on day one or two of the cycle to rule out ovarian cysts. We measured serum FSH and patients with FSH levels exceeding 12 mIU/mL were excluded from the study. FSH (urinary or recombinant, Menopur®, Ferring), administered by subcutaneous injection, was started on day two or three of the cycle. The FSH starting dose was calculated based on age and AMH level according to a previously developed nomogram.<sup>10</sup> The GnRH antagonist, Cetrotide® (Merck Serono), was started on day 5 at 0.25 mg per day and administered by subcutaneous injection. Cycle monitoring with ultrasound follicular tracking and Estradiol measurement was started on day 6 and was repeated every other day until the day of HCG administration.

### Modified natural cycle IVF (MNC)

Patients receiving the MNC protocol had a baseline ultrasound scan on day one or two of the cycle to rule out ovarian cysts. Clomiphene Citrate tablets (Clomid®, Sanofi-Aventis) were started on day two or three and were administered at 100 mg daily for 5 days. 75 IUs of urinary FSH and 0.25 mg of GnRH antagonist (Cetrotide) were started on day 6 with the onset of cycle monitoring. Ovarian response was monitored by ultrasound follicular tracking and measurement of Estradiol every other day until the day of HCG administration. Estradiol tablets (Progynova®, Bayer) were added to the treatment, at 2 mg three times daily (total dose of 6 mg), if the endometrial thickness measured less than 7 mm on the day 6 scan. In view of the poor ovarian reserve, all the patients agreed to undergo oocyte

retrieval if they had one or more follicles that measured 18–20 mm in diameter.

Ultrasound-guided trans-vaginal aspiration of the follicular fluid was performed, followed by either conventional IVF or intra cytoplasmic sperm injection (ICSI) according to the sperm parameters. After fertilization, all cleavage embryos were cultured to day 3–5, when fresh embryo transfer was performed.

## Statistical analysis

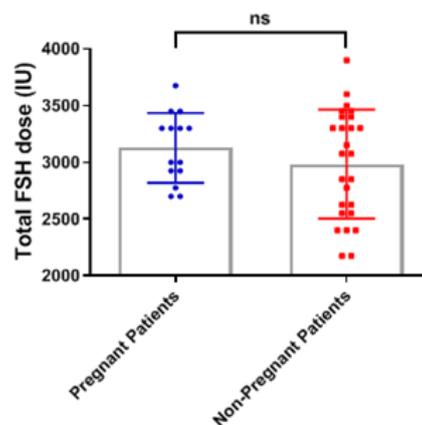
Comparisons between the two groups were made using the Mann-Whitney U-test or Fischer's exact test, as appropriate.  $P < 0.05$  was considered statistically significant. All statistics were calculated using Graph Pad Prism 8. Where appropriate, data are presented as mean  $\pm$  SD. P denotes P value, U denotes Mann-Whitney U value and RR denotes Relative Risk.

## Results

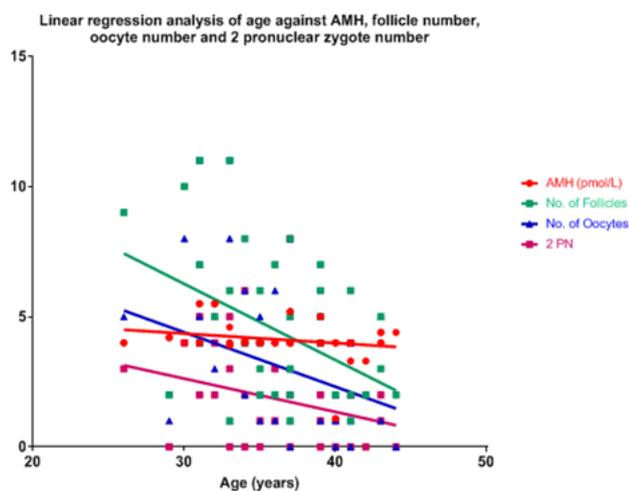
72 patients were included in the study. 46 patients had conventional IVF treatment and 26 patients had MNC IVF. There was no significant difference in the median age between the two groups: 36.5 (26–42) and 37 (29–44) respectively,  $P = 0.05$ ,  $U = 434$ . Patients with conventional IVF had a significantly larger number of follicles: 5.5 (1–11) versus 2.5 (1–6),  $P < 0.0001$ , significantly more retrieved oocytes: 4 (0–8) vs 1.68 (0–5),  $P < 0.0001$ , and significantly more fertilized oocytes: 2.4 (0–8) vs 0.8 (0–4),  $P < 0.0001$ . No oocytes were retrieved in 5 (10.9%) patients in the first group as compared to 13 (50%) in the second group,  $P = 0.0005$ ,  $RR = 1.78$ .

Clinical pregnancy rate (CPR) per embryo transfer in the first group was 43.2% while none of the patients in the second group achieved pregnancy. Within the conventional IVF treatment group, there was no significant difference between the total FSH used in pregnant and non-pregnant patients, as shown in (Graph 1) (3129  $\pm$  307 IUs vs 2984  $\pm$  497 IUs,  $P = 0.3933$ ,  $U = 151.3$ ). Linear regression analysis of all 72 patients in this study (Graph 2) revealed a decreased number of follicles, oocytes and 2 pro-nuclei (a measure of fertilization) with increasing age, despite a roughly constant AMH level among the patients. This demonstrates the adverse impact of increasing age on fertility, independent of AMH level.

Total FSH dose used in conventional IVF group



**Graph 1** Comparison of the total FSH dose (IUs) used in pregnant and non-pregnant patients within the conventional IVF group. Data is presented as mean  $\pm$  SD, with ns denoting a non-significant difference between the two groups.



**Graph 2** Linear regression analysis of age (years) against AMH level (pmol/L), follicle number, oocyte number and 2 pronuclei (2 PN) number. There are a decreased number of follicles, oocytes and 2 pronuclei with increasing age, despite a roughly constant AMH level among the 72 patients.

## Discussion

The ESHRE consensus, known as the Bologna criteria, establishes a guideline for poor ovarian reserve. However, this guideline is not universally accepted. Poor responders often present with a shortened follicular phase, which decreases the time available for follicular recruitment. In addition, lower FSH receptor expression levels in granulosa cells may also be found in this group of patients.<sup>2</sup>

Minimal stimulation usually refers to stimulation protocols that yield a maximum of five oocytes. This concept was first introduced by Corfman.<sup>11</sup> Their protocol involved using 100mg oral CC on days 3–7 of the cycle, followed by a single injection of 150 IU of intramuscular hMG on cycle day 9. Although the number of retrieved oocytes was statistically lower using this protocol, this variability did not correlate to a statistically significant difference in pregnancy rate. In this study, our results demonstrated similar findings as the number of oocytes retrieved and fertilized was statistically lower than the conventional protocol.

Clinical pregnancy rate (CPR), however, was much higher in the conventional IVF group and none of the 13 patients who had embryo transfer in the MNC group achieved pregnancy. This discrepancy between our CPR and that of Corfman et al.,<sup>11</sup> is likely due to our use of fresh embryo transfer instead of frozen embryo transfer, which was used by Corfman. CC is known to disturb endometrial growth due to its anti estrogenic effect, causing endometrial thickness to be significantly thinner in women taking CC than women not taking CC.<sup>12</sup> Corfman's use of embryo cryopreservation and frozen embryo transfer may have mitigated the adverse impact of endometrial thinning, by allowing oocytes to be obtained using CC in one cycle and transferring these oocytes in a different, hormone replacement cycle with a more developed endometrium. Our patients, however, were keen to have fresh embryo transfer to avoid the frustration of their treatment being interrupted and the risks of cryopreservation, including decreased survival of embryos and development to the blastocyst stage.<sup>7</sup> Our protocol allowed fresh embryo transfer in all cases. However, the clinical pregnancy rate was disappointingly low,

suggesting that CC compromised endometrial thickness too much. This unexpected result is rather disappointing for the patients and should be discussed prior to the start of treatment. Although one of the advantages of MNC IVF is that it can be repeated due to its relatively low cost, the frustration of failure should be factored into the equation and patients must be adequately counseled before the start of the treatment so they can make an informed decision.

One potential solution is to use a modified treatment with a half-dose or early administration of CC, which has been shown to significantly increase endometrial thickness in patients with a history of thin endometrium caused by the standard CC regimen.<sup>13</sup> Addition of sildenafil vaginal gel to CC has also been shown to increase endometrial thickness and uterine blood flow, which may improve pregnancy outcome in patients with CC failure due to thin endometrium.<sup>14</sup> Alternatively, Letrozole (aromatase inhibitor) could be used instead of CC. It has been shown to stimulate ovulation to the same extent as CC, with less endometrial thinning and fewer side effects.<sup>15</sup>

In the current study, no oocytes could be collected in half of the patients with MNC as compared to 17.9% noted by Kuroda et al.<sup>3</sup> Patients with markedly reduced ovarian reserve often suffer from oocyte collection failure during ART treatment. This is frustrating for the patients and the physician and could potentially affect the patient motivation towards treatment. Kuroda et al demonstrated that the use of CC in addition to Estradiol was effective, with a lower cancellation rate of oocyte retrieval and a higher number of retrieved oocytes. All embryos were cryo preserved and transferred in Hormone replacement cycles due to the adverse effect on implantation associated with decreased endogenous ovarian hormone levels and prolonged CC treatment,<sup>3</sup> in our study CC was used for a total of 5 days to initiate the follicle recruitment. Subsequently, a small dose of FSH was used to support the follicle growth and reduce the adverse effect on the endometrium. Estradiol was used if the endometrial thickness was less than 7 mm in the conventional IVF group. In conventional IVF treatment, only 10.9% of the patients did not have oocytes retrieved and this result is acceptable in such a complicated group of patients.

Increasing maternal age is known to increase the prevalence of sub fertility. This age-dependent loss of female fertility is widely attributed to a decline in both the quantity and quality of the oocyte/follicle pool.<sup>16</sup> We confirmed this across our patient population by showing that follicle, oocyte and 2 pro nuclei (a measure of fertilization) numbers decreased with increasing age. This trend is independent of AMH level, which was roughly constant across our patient population. This is in accordance with previous studies, which have shown that age and AMH are independent predictors of live birth.<sup>17</sup>

## Conclusions

The present study demonstrates that using conventional IVF (antagonist protocol) in the treatment of patients with a markedly reduced ovarian reserve is associated with a greater number of follicles; more retrieved and fertilized oocytes, a higher CPR and a reduced chance of cycle cancellation as compared with MNC IVF, when fresh embryo transfer is used exclusively. We would recommend the use of conventional IVF (antagonist protocol) in the treatment of patients with a markedly reduced ovarian reserve who prefer fresh embryo transfer. Patients opting for MNC treatment, should receive proper information and counseling before the start of treatment to avoid patient frustration and disappointment.

## Study limitations

The retrospective nature of the study and the relatively small number of patients are both limitations. We would recommend a larger scale prospective multicentre trial comparing different forms of minimal stimulation, modified natural cycle IVF and conventional IVF in the treatment of patients with markedly reduced ovarian reserves.

## Author contributions

MHM designed the study. MHM and NKE collected the data. MHM analyzed the data. MHM and AMHAMM wrote the manuscript. All authors have approved the final article.

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## Conflicts of interest

The author declares that there are no conflicts of interest.

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