Middle East Fertility Society Journal Vol. 10, No. 2, 2005

Clomiphene-acetyl cysteine combination as a new protocol to a friendly IVF cycle

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ABSTRACT

Objective: N-acetyl-cysteine (NAC) has been shown to enhance the action of clomiphene citrate in ovulation induction. The objective of this study was to examine the use of NAC with clomiphene citrate for ovarian stimulation in assisted conception as a model for "Friendly IVF"

Design: pilot study

Materials and methods: Twenty infertile patients undergoing IVF/ICSI cycles were offered NAC, 1,200 mg/day from day 3-7 of the menstrual cycle with CC (100 mg /day) starting on day 3-7. hCG (10,000 IU) was given when leading follicle(s) were ≥ 18mm followed by ICSI.

Main outcome measure(s): clinical pregnancy rate was the primary outcome and implantation rate, number of oocytes retrieved, fertilization rate were secondary outcomes

Result(s): response to CC stimulation with NAC co-treatment was evident by a number of mature follicles ranging from 2-7 at the time of hCG administration. Clinical pregnancy was achieved in 4 cycles (20%).

Conclusion(s): In this preliminary report, a potential benefit of NAC co-treatment with CC in young women undergoing IVF/ICSI cycles was demonstrated. This combination provides a cheap, effective way for ovulation induction in an IVF setting compatible with the concept of friendly IVF

Keywords: N-acetyl-cysteine; clomiphene citrate, Friendly IVF, ICSI, pregnancy

Increasing efficiency of various assisted reproduction techniques has been obtained by using more aggressive hormone stimulation protocols and improved laboratory techniques for gamete and embryos. However, this was on the expense of increased costs, high multiple pregnancy rates, and side effects of stimulation regimens. Controlled ovarian hyperstimulation in in vitro fertilization (IVF) aimed at increased oocyte and embryo numbers to compensate for a poor implantation rate per embryo. Given the fact that implantation are (30% in the majority of IVF clinics in patients below 40 years of age, it seems necessary to explore more `friendly IVF' regimens to obtain a reasonable pregnancy rate, ideally resulting in the delivery of a single, healthy child (1). The ideal IVF protocol secures a high chance of embryo transfer and accordingly a low cancellation rate, a high pregnancy rate, a low intervention level, low risk and few side-effects, low costs and practical convenience both for the patient and the clinician (2).

Natural cycle meets some of these criteria, however this was on the expense of a relatively high cancellation rate (25-75%) and a low clinical pregnancy rate per started cycle (range 0-23%), but higher per embryo transfer (range 0-30%) (3-5). The main problems with natural cycle IVF are

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spontaneous LH surge and ovulation, almost always single oocyte retrieved, low fertilization rates, high cancellation rate and low pregnancy and implantation rates. The latter problems associated with natural cycles were partially overcome by a mild stimulation yielding better chances of collecting more than one oocyte. Clomiphene citrate has been used (6).

Recently we have shown that N-acetyl cysteine (NAC) is a potent adjuvant to CC in CC resistant PCOS patients (7). NAC as an adjuvant to clomiphene citrate was more effective than placebo in those patients. Combination of CC and NAC significantly increased both ovulation rate and pregnancy rate in women with CC resistant PCOS (49.3% vs 1.3 and 21.3% vs 0% respectively). No cases of ovarian hyperstimulation syndrome were reported in the NAC group (7).

The purpose of the present study was to evaluate the efficiency of using a combination of CC and NAC in a cohort of women undergoing IVF / ICSI cycles.

MATERIALS AND METHODS

This pilot study was performed between September 2003 and July 2004. Twenty couples with previous IVF failure were invited to participate in the present study with the following inclusion criteria: age <39 years, infertility unexplained, tubal or due to severe male factor with indication for ICSI, regular menstrual cycles (± 3 days), presence of two ovaries. All patients gave written informed consent after counseling, and the study was approved by the Ethics Committee. At entry all patients received a combination of clomiphene citrate 100 mg from cycle day 3-7 and NAC (Sedico, Cairo, ARE), in a dose of 1.2 g/day orally, with timing of oocyte retrieval by human chorionic gonadotrophin (HCG) injection. All patients received one treatment cycle.

Cycle monitoring

All cycles were monitored by vaginal ultrasound on cycle day 3, and subsequently on day 9 and day 11 and thereafter as necessary. All cycles were followed until the dominant follicle was 18 mm and then HCG (Pregnyl; 10000 IU) was given followed by oocyte retrieval 35-36 h later.

Oocyte retrieval, injection and embryo culture

Oocyte retrieval was performed by ultrasound-guided transvaginal aspiration using a single-lumen needle (CCD, France) with careful flushing of each follicle. Oocytes were denuded with hyaluronidase (Vitrolife Fertility Systems, Gothenburg, Sweden) and mechanical pipetting. Mature (metaphase II) oocytes were identified by the presence of the first polar body. Only those oocytes that had extruded the first polar body (metaphase II oocytes) were microinjected.

Immediately before injection, the sperm suspension was added to a 50 µl droplet of polyvinylpyrrolidone (ICSI Scandinavia, Sweden). Oocytes were microinjected ~5 h after retrieval in microdroplets of IVF medium covered with lightweight paraffin oil. A single motile spermatozoon with apparently normal morphology was immobilized by touching its tail with the injection pipette and aspirated tail-first into the injection pipette. The sperm was microinjected into the ooplasm at the 3 o'clock position, the polar body being oriented at the 6 o'clock position.

Fertilization was assessed 24 h after injection if two pronuclei (2PN) were present and the second polar body had been extruded, then left in culture for a further 24 h.

Embryos were transferred 48-72 h after oocyte retrieval using Labotect embryo transfer catheter, (Labotect GmbH, Labor-Technik-Gottingen, Germany). Routinely, a maximum of four embryos were transferred to the patient. luteal phase support was given using oral micronised progesterone (utrogestan (two tablets bid) (October Pharma SAE, Under license of Besins International, S.A. France) .

Pregnancy was determined by serum HCG measurement on day 14-15 after transfer and clinical pregnancies were detected by presence of a gestational sac on ultrasound scans performed 6 weeks after embryo transfer. The implantation rate was defined as (the number of gestational sacs divided by the number of embryos transferred) x 100.

Table 1. Descriptive statistics for NAC-CC cycles

	Number of follicles	Number of oocytes	Number of 2PN	Number of embryos transferred
Mean	4.8	3.6	2.6	2.5
SD	1.6	1.2	1.2	0.9
Median	5	3.5	2	2
Minimum	2	2	1	1
Maximum	7	5	5	4

Data were statistically represented in terms of mean, median, minimum, maximum and standard deviation (S.D.) where appropriate.

RESULTS

Twenty women were enrolled in this pilot study with mean age of 27.3±1.9 and mean BMI of 28.1±0.8.

The mean number of follicles >14 mm on the day of HCG injection was 4.8±1.6. Only one cycle was cancelled due to premature LH surge. The mean number of oocytes retrieved was 3.6±1.2. (Table 1) Four women got pregnant (20%) [all were single ton pregnancy except one twin). No miscarriage was reported till now.

On estimating the cost of medications used, the number of NAC sachets used was 6 per day for 5 days making a total of 30 costing 27 E.P plus an average two fillings of Clomid = 18 EP, thus the total cost of medications in NAC-CC / ICSI cycle = 45 E.P (7$) which is dramatically different from the average cost of medications in the long protocol of ICSI cycle (about 2000EP) (~ 450$)

DISCUSSION

The current study has demonstrated that it is possible to obtain reasonable results using an IVF protocol with a combination of NAC-CC for ovarian stimulation. The relatively young age of the population should be borne in mind when considering the relatively high pregnancy rates in the NAC-CC/ICSI series. The concomitant administration of NAC did not influence oocyte maturity in terms of metaphase II oocytes. Ideally, the efficiency of the NAC-CC/ICSI protocol should be compared with those obtainable from the current `gold standard', the long down-regulation protocol, in a similar population. Such analyses should be considered in the future, also in terms of cost-effectiveness and clients' perceptions.

In the present study, there was no cycle cancellation. This could be due to small sample size or due to using selection potentially high responders. One point of importance is that LH surge should be tested in friendly IVF protocol. Although we did not perform such monitoring, we got high fertilization rate. Fertilization rate of 80% and an implantation rate of 14.3% obtained in the present series following NAC-CC stimulation, are higher than in unstimulated cycles (8). The proportion of unfertilized oocytes in unstimulated conventional IVF cycles in a recent study (2) was 53%. However, ICSI seems to be an efficient tool in an unstimulated or low stimulation protocol as indicated in previous studies (9).

The negative anti-estrogenic effect of clomiphene citrate on various parts of the human reproductive system (10) seems to be counteracted by the concomitant administration of NAC. Natural IVF did not achieve satisfactory results, as the pregnancy rate per cycle is 10% or below (3, 5, 11-16), but others have obtained higher rates, up to 23%. However the intensive monitoring needed is not compatible with the `friendly IVF' concept. A 20% pregnancy rate in our series is higher than previous reports using natural cycle IVF with obviously less monitoring involved.

Recently a consistent delivery rate of ~22% per started cycle has been observed during the last few years, accomplished mainly by the long down-regulation protocol and two-embryo transfers (17). Similar results were obtained with single embryo

transfer (18). Consequently, calling for a mild ovarian stimulation which provides less number of embryos and a relatively comparable pregnancy is feasible. NAC-CC thus seems to be not only compatible with the concept of 'friendly IVF' engraved by Olivennes (1) but with the growing interest in single embryo transfer as well.

In a randomized study Ingerslev and colleagues proposed that the clinical pregnancy rate per cycle using CC stimulation could be improved by reducing the rather high cycle cancellation rate. The frequency of cycle cancellation due to lack of fertilization could be less if more oocytes were harvested. They proposed adding FSH, including antagonists into the protocol or closer endocrine monitoring as alternative means of reducing the risk of cycle cancellation due to spontaneous ovulation. However, these suggestions would reduce the `friendliness' of the protocol (2).

NAC treatment has an insulin sensitizing effect and it induced a significant fall in testosterone levels and in free androgen index values (19, 20). NAC is commonly used as a safe mucolytic drug, and at higher doses it increases the cellular levels of reduced glutathione (GSH), an antioxidant, which has been shown to influence insulin receptor activity. More recently, it has also been shown to have other diverse biological effects notably; antiapoptotic (21), antioxidant (22), protection against focal ischemia (23), inhibition of phospholipid metabolism, preinflammatory cytokine release, and protease activity (24). NAC may exert the same effects at the ovarian level and these activities may be central in inducing ovulation.

In conclusion, the present study suggests that a simple NAC-CC protocol is compatible with the concept of `friendly IVF', yielding a reasonable pregnancy rate per cycle started. The results of this study should be substantiated in a larger cohort of patients.

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Received January 17, 2005; revised and accepted May 18, 2005