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The effectiveness of a low cost ovulation induction protocol in assisted reproduction. R. Mansour, M. Aboulghar, G. Serour, I. M. Fahmy, Y. Amin. The Egyptian IVF-ET Ctr, Cairo, Egypt.

Objective: The cost of ovulation induction drugs is a major limiting factor for patients undergoing assisted reproduction. The aim of this work was to study the outcome of a low cost ovulation induction protocol using clomiphene citrate in comparison to the standard long down regulation protocol.

Design: Prospective controlled.

Materials/Methods: Thirty one infertile patients undergoing ICSI who could not afford the cost of treatment drugs were chosen for this study. They received clomiphene citrate 100–150 mg/day for five days starting from day 2–3 of the cycle and 150 I.U. of hMG/day from day 6–10 then the dose was modified according to the response. GnRH antagonist 0.25 mg/day (citrolex) was started when the leading follicle reached 16 mm in mean diameter and the LH was negative. Patients undergoing ICSI during the same period of time and using long down regulation GnRH agonist long protocol were used as a control. They received 0.1 mg Decapeptyl/day starting on day 20 of the cycle till the day of hCG injection. hMG 150–300 I.U./day was started after down regulation. Selection criteria for both protocols included patients under 40 years old who were undergoing their first trial of ICSI for male factor infertility.

Results: Are shown in the table.

	Clomid/ hMGGnRH antagonist	HMG/GnRH agonistlong down regulation	P
No. of cycles	31	156	
Mean age (years ± SD)	32.8 ± 4.5	32.4 ± 4.1	NS
Mean no. of oocytes retrieved	6 ± 3	11.7 ± 6.4	S
Mean no. of 2 PN oocytes	3.6 ± 2	6.3 ± 4	S
Fertilization rate	75%	67%	NS
No. of pregnancies (%)	8(26%)	92(59%)	S
Implantation rate	13.8%	27%	S
Mean no. of hMG amps	16.8 + 7.8	36.4 + 12.4	S
Mean no. agonist/antagonist amps	2.5 + 1.3	26.7 + 2.3	S
Mean cost of medications/cycle	277.5 + 123$	482 + 114$	S

Conclusions: The use of the clomid/hMG/antagonist protocol for assisted reproduction significantly reduced the cost as compared to the standard long down regulation protocol, however the pregnancy rate was also significantly reduced to almost half. Therefore, this protocol is not a cost effective one and we do not recommend its use.

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ART: PREIMPLANTATION GENETIC DIAGNOSIS

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Preimplantation genetic diagnosis of cystic fibrosis (CF) with compound heterozgote S549R/DF508. Y. Tang, L. Krey, A. Adler, L. Chi, J. Grifo. Program for In Vitro Fertilization, Reproductive Surg and Infertility, New York Sch of Medicine, New York, NY; New York Univ Sch of Medicine, New York, NY.

Objective: To analyze simultaneously the complex cystic fibrosis mutations S549R and DF508 for preimplantation genetic diagnosis (PGD).

Design: A case report.

Materials/Methods: A couple presented for PGD in which the wife carries the S549R mutation and the husband carries the DF508 mutation. Since there was a 25% chance that a child would inherit two copies of the mutated genes, i.e., one from each of the parents, we conducted PGD to identify embryos that were free of both mutations or carried only one mutation alone. One blastomere from each embryo was biopsied on day 3 of an IVF-ICSI cycle. Two sets of specific primers (see table) were used to amplify both mutations. The mutation S549R, which is localized on exon 11 (T-G at nucleotide 1779), alters a Dra III restriction site. DF508 was identified by the heteroduplex formation.

Results: Only 5 of 11 embryos were suitable for biopsy for PGD on day 3; 4 others were only 1 cell at this time. Detectable results for both mutations were obtained for 3 embryos. Two biopsied embryos were selected for transfer on day 4; one was S549R/DF508 free and the other carried only the S549R mutation. Unfortunately, no pregnancy resulted. All of the remaining multicellular embryos were re-analyzed; a total of 17 blastomeres from 7 embryos were tested. DNA amplification was successful with 14 cells (82%) but 5 showed allele dropout (ADO). However, one 4-cell embryo accounted for 3 blastomeres with ADO.

Primer sequences used were (5′ to 3′).

S549R outside (unpublished): CF11F	AAATTGCATTTGAAATAATGGAGA
CF11R	CCACTAGCCATAAAACCCCA
Inside (Fanen et al., 1992): GCCF11	CAGATTGAGCATACTAAAAGTG
CF11	CATTTACAGCAAATGCTTGCTAG
DF508 outside (Handyside et al., 1992): 1F	GACTTCACTTCTAATGATGAT
1R	CTCTTCTAGTTGGCATGC
Inside (Handyside et al., 1992): 2F	TGGGAGAACTGGAGCCTT
2R	GCTTTGATGACGCTTCTGTAT

Conclusions: Despite the poor quality of the embryos in this PGD cycle, the foregoing data suggest that this is a useful PGD method to detect for cystic fibrosis resulting from the compound heterozgote S549R/DF508.

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Preimplantation genetic diagnosis of embryos produced from in vitro matured (IVM) oocytes. A. Ao, R. C. Chian, D. L. Blake, M. Bielanska, N. L. Dean, S. L. Tan. McGill Univ, Montreal, Canada.

Objective: To undergo preimplantation genetic diagnosis (PGD) procedure, the women partner has to undergo in vitro fertilization (IVF) to obtain sufficient number of embryos so that after the genetic test there are enough unaffected embryos left for transfer. Recently, advances have been made to treat women with PCOS-related infertility by in vitro maturation (IVM) technique without stimulating the ovaries with fertility drugs. The main objective of this study was to investigate whether enough oocytes can be retrieved from PCO patients to perform PGD on embryos produced by IVM procedure.

Design: Preimplantation genetic diagnosis was performed in embryos produced from in vitro matured oocytes in two couples with inherited genetic disorders for spinal muscular atrophy (SMA) and Duchenne muscular dystrophy (DMD).

Materials/Methods: To initiate the IVM treatment cycle, both patients received intravaginal progesterone suppositories at the dose of 200 mg for 10 days to induce withdrawal menstrual bleeding. Immature oocytes were retrieved on day 10 or day 11 of the cycle after 10,000 IU of human chorionic gonadotropin (hCG) was administered 36 hours prior to oocyte retrieval. The immature oocytes were cultured in 1 ml of maturation medium in vitro and were examined under the microscope at 12 h intervals for up to 48 h to determine the maturity, and the mature oocytes were inseminated by ICSI. Normally fertilized zygotes were then cultured until the day of the biopsy on day 3. Genetic diagnosis for spinal muscular atrophy was performed by using fluorescent based PCR assay and FISH was used for sex selection for Duchenne muscular dystrophy.

Results: a) PGD for SMA: A total of 7 immature oocytes were retrieved, 1 and 6 from right and left ovaries respectively. Five out of 7 immature oocytes were matured in vitro and 4 eggs were normally fertilized. After genetic analysis on 4 embryos, one embryo was diagnosed as normal/carrier, one embryo was diagnosed as affected and two embryos did not give any result. The embryo that was diagnosed as normal/carrier was transferred on day 4 but the patient did not become pregnant. b) PGD for DMD: A total of 8 immature oocytes were retrieved, 3 and 5 from right and left ovaries respectively. All 8 immature oocytes were matured in vitro and were normally fertilized. Two embryos were diagnosed as female when tested with three colour FISH for chromosomes X, Y and 18. The embryos diagnosed as normal/carrier female were transferred on day 4 but the patient failed to become pregnant.

Conclusions: Although neither of these two patients became pregnant, we were able to retrieve acceptable number oocytes for preimplantation genetic diagnosis. Larger number of patients needed to be treated and results