Prednisolone in unexplained implantation failure: a randomized controlled clinical trial of efficacy

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Abstract

Objective: To evaluate of the effect of adding prednisolone to improve the outcomes of intracytoplasmic sperm injection (ICSI) in women with previous unexplained implantation failure.

Materials & Methods: This is a randomized controlled study performed in Fertility Care Unit in Mansoura University Hospital and private fertility care centers, Mansoura, Egypt. The study comprised of women undergoing ICSI and has history of one or more implantation failure. All women were randomly divided into two groups; the study group received prednisolone in a dose of 20 mg/day starting from the day of oocyte retrieval while the control group received no treatment. The primary outcome was clinical pregnancy rate and the secondary outcomes were implantation rate and miscarriage rate.

Results: 108 women (53 in the study group and 55 in the control group) subjected to final analysis. There was no significant difference between the study and control groups as regard the clinical pregnancy rate (45.3% vs 32.7%; P = 0.237), implantation rate (22.1% vs 15.2%; P = 0.145) and miscarriage rate (33.3% vs 33.3%; P = 1.000).

Conclusion: Administration of prednisolone in the luteal phase to women with previous unexplained implantation failure does not result in significant increase in clinical pregnancy or implantation rates.

Key Words: ICSI, Implantation failure, Prednisolone.

Introduction

The need for assisted reproductive technology (ART) is increasing, since more than 10% of couples suffer from some form of infertility. The in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) have spread throughout the world, with apparently around 5 million babies born worldwide by 2012. In just over 35 years, the field of IVF/ICSI has grown and its success has improved as measured by the increase in live birth rate per cycle initiated from less than 1% to a rate of more than 33%. For women aged < 35 years, the pregnancy rates using embryos generated from one stimulated cycle were found to be about 50-60% [1].

Many factors have been suggested as causes of failure in ICSI cycles such as poor oocyte yield by the ovary, factors related to the laboratory culture and medias and faults during embryo transfer; these factors would decrease the pregnancy rate. However, in practically successful IVF/ICSI centers with high clinical pregnancy and live birth rates, some couples suffer repeated implantation failure [2]. Implantation of the transferred embryo is the most important step determining the achievement of clinical pregnancy in IVF/ICSI cycles. Currently, the implantation rate is still not in the desired ranges mainly lower than 20-25% per embryo transfer [3].

One of the reasons of implantation failure was suggested to be related to local abnormal immunological reactions in the endometrium. Ledee-Bataille et al. found that there was local abnormal expression of various cytokines, dysregulation of interleukins 12, 15 and 18 and elevation in the level of natural killer cells [4]. Also, Inagaki et al. found that there was high IL-1β and low interferon-γ and IL-10 in patient with implantation

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failure [5]. Moreover, for implantation to occur, a complex interaction complex between the embryo and the endometrium occurred and it involves leucocytes, interleukins, growth factors stromal cells and extracellular matrix [6]. Glucocorticoids were suggested to have modulatory effect on the implantation process by affecting these factors [7-10].

In this study, we aim to find out whether cortisone administration following embryo transfer could improve pregnancy outcome in couples with previous unexplained implantation failure.

Materials & Methods

This is a prospective, randomized, controlled, parallel-group study conducted during the period from February 2012 through November 2014 in Fertility Care Unit in Mansoura University Hospital and private fertility care centers, Mansoura, Egypt. The study protocol was reviewed and approved by our Institutional Review Board. The participants of this study were recruited from couples planned for management of infertility by ICSI. Eligible participants in our study were woman aged 20-38 years with history of unexplained previous failure of one or two implantations. A written informed consent was taken from each women selected to participate before inclusion in the study. Women with any of the following criteria were excluded from the study: 1) body mass index (BMI) < 19 kg/m^2 or > 35 kg/m^2; 2) moderate or severe endometriosis; 3) hydrosalpinx; 4) uterine abnormalities; 5) uterine myoma; 6) previous uterine surgery; 7) positive results for antiphospholipid antibodies; 8) metabolic or hormonal abnormalities; or 9) hormonal therapy in the preceding 3 months.

A full, precise history was taken from each participant, and the cause of infertility was reported. Thorough clinical examination (including general, abdominal, and pelvic examination) was performed as well. The semen analysis of the husband was checked according to the WHO (2010) guidelines. The results of the previously done hysterosalpingography, laparoscopy and hysteroscopy were evaluated. Basal (day 3) serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) were assayed for each woman within 3 cycles of the scheduled ICSI cycle. A transvaginal sonography (TVS) scan was performed and any deviation from normal pelvic anatomy was looked for and reported.

The long luteal GnRHa protocol was used for COH. The GnRHa used was Triptorelin (Decapeptyl®, Ferring, Germany). Triptorelin was administered subcutaneously in a dose of 0.1 mg/day starting in the mid-luteal phase (day 21) of the preceding cycle then the dose was reduced to half the dose (0.05 mg/day) from the day of ovarian stimulation till the day of HCG administration. Ovarian stimulation using gonadotropin preparation was commenced on day 2 of the next cycle (stimulation cycle) after ensuring adequate pituitary and ovarian suppression (serum E2 < 50 pg/ml), and performing TVS scan to confirm absence of ovarian cysts and presence of endometrial thickness < 3 mm. The gonadotropin was given daily by deep intramuscular injection and the starting dose and type depended on the age of the woman, baseline FSH levels, BMI and previous trials. TVS scan was performed regularly for monitoring of follicular development (folliculometry); starting from day 8 of the cycle and repeated every 2-3 days. The dose and type of gonadotropin were ten modulated according to ovarian response.

The cycle was cancelled when poor ovarian response (< 3 follicles not reaching 18 mm correlated with serum E2 level < 400 pg/ml) was detected during follow up visits after counseling the couple regarding the success rates. The cycle was also cancelled when there was a high risk for ovarian hyperstimulation syndrome (i.e. more than 30 follicles, steep rise in serum E2, or ovarian size > 8 cm).

When there were at least 3 leading follicles > 18 mm in diameter, final oocyte maturation was induced by intramuscular administration of 10000 IU of HCG. Endometrial thickness and pattern were assessed by TVS on the day HCG administration. After HCG injection by 34-36 hours, oocyte retrieval was performed through transvaginal aspiration of follicles under TVS guidance followed by endometrial preparation for embryo transfer (ET) by giving 800 mg/day natural progesterone vaginal supplement (Cyclogest®, Actavis) + 4 mg/day estradiol oral supplement (Cyclo-Progynova® white tablets, Bayer Pharma).

On the day of oocyte retrieval, all women participating in the study were randomly divided into two groups; prednisolone group (study group) and no treatment group (control group) using a computer-generated list and was carried out by a nurse through sealed, unlabeled, opaque envelopes. The participants, caregivers, investigators and outcomes assessors were not blinded to group assignment. All women in the study group received prednisolone (Solupred®, Sanofi-Aventis) in a dose of 20 mg/day starting from the day of oocyte retrieval and continued until documentation of pregnancy while women in the control group received no treatment.

After fertilization through ICSI, 2-3 good quality embryos were transferred transcervically 3-5 days after oocyte retrieval. If there is ≤ 5 good quality cleavage-stage embryos, ET were performed on day 3 after oocyte retrieval while if there is > 5 good quality cleavage-stage embryos, embryos were left to reach

the morula or blastocyst stage and ET was performed on day 4 or 5 after oocyte retrieval. Good quality cleavage-stage embryos display stage-specific cell division, have blastomeres of fairly equal size with few to no cytoplasmic fragments. Women with no transfer of at least one good quality embryo were excluded from final analysis.

Biochemical pregnancy was documented by performing quantitative serum β-HCG assay 2 weeks after the ET and a level of ≥ 50 mIU/ml was considered positive indicator of pregnancy. Cases with positive pregnancy test were examined by TVS 24 weeks later (4-6 weeks after ET) to document clinical intrauterine pregnancy which is defined as presence of at least one intrauterine gestational sac with fetal pole and cardiac activity on TVS scan at 46 weeks after the ET. The primary outcome of this study was the clinical pregnancy rate (number of clinical pregnancies divided by the number of ET procedures) and the secondary outcomes were the implantation rate (number of gestational sacs on TVS scan at 4-6 weeks after ET divided by the number of transferred embryos) and miscarriage rate (number of miscarriages before 12 weeks divided by the number of clinical pregnancies.

Statistical analysis

The statistical analysis was performed using the IBM® SPSS® Statistics, version 20.0 for Windows. Continuous variables were expressed as mean ± standard deviation (SD) and categorical variables were expressed as frequencies and percentages. Differences among continuous variables with normal distribution were analyzed by the t-test while for continuous variables without normal distribution, non-parametric tests were used and differences were analyzed by the Mann-Whitney U-test. Differences between percentages were analyzed by the Fisher's exact test. P value ≤ 0.05 was considered statistically significant.

Results

During the period of the study, 146 women were assessed for eligibility to participate in the study and 124 of them were randomized. Of the 124 women who were randomized, outcome measures were available for 113 women as 9 women were excluded from the study due cancellation of ET. Data were analyzed from 53 women in the study group (prednisoline group) and 55 women in the control group (no treatment group) while 5 women were excluded from final analysis due to no transfer of any good quality embryo (Figure 1).

There was no significant difference between the study and control groups as regards the age, BMI, duration, type and cause of infertility, number of previous implantation failures and hormonal profile (Table 1). Also, no significant difference was between both groups as regards the total gonadotropin dose, stimulation days, number of follicles ≥ 12 mm in diameter by TVS on day of HCG administration, peak serum E2 level, number of oocytes retrieved, oocyte maturation rate, fertilization rate, percentage of good quality embryos, number of transferred embryos and number of transferred good quality embryos (Table 2).

The clinical pregnancy rate was higher in the study group than in the control group (45.3% vs 32.7%) but without significant difference (P = 0.237). Also, there was no significant difference in implantation rate between both groups though it was higher in the study group (22.1% vs 15.2%; P = 0.145). The first trimester miscarriage rate was equal in both groups (33.3% vs 33.3%; P = 1.000) (Table 2).

Figure (1): Study flow diagram:

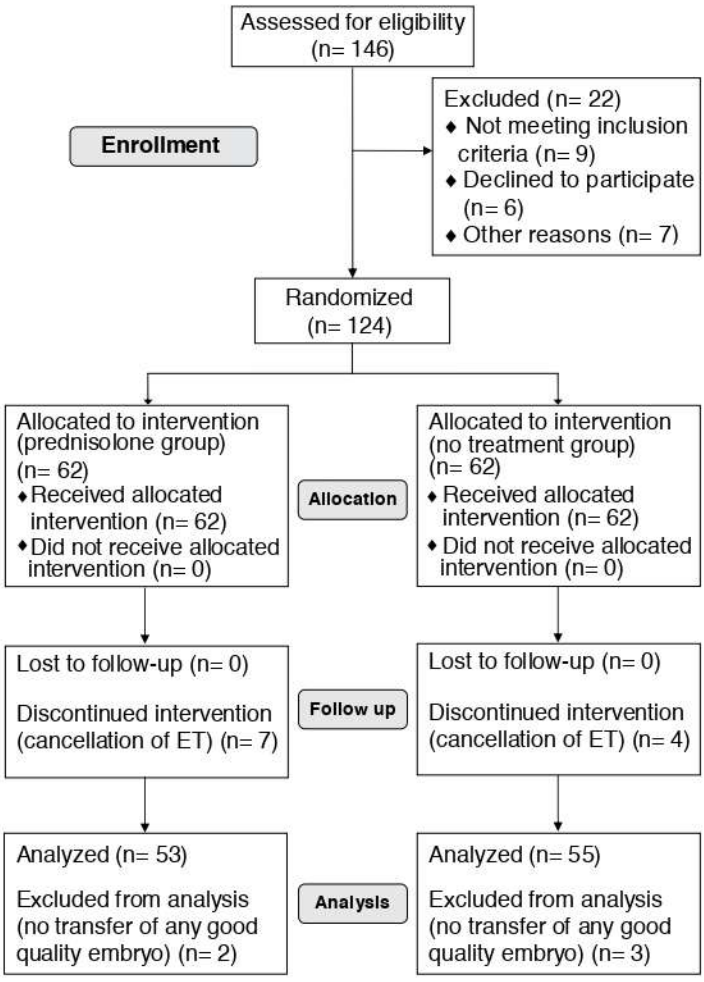

Table (1)
Demographic and clinical characteristics of the study and control groups:

		Study group (n = 53)	Control group (n = 55)	P value
Age (years) *		30.25 ± 5.51	28.96 ± 5.33	0.281
BMI (kg/m^2) *		29.77 ± 3.63	30.48 ± 3.86	0.277
Duration of infertility (years) *		7.40 ± 4.07	6.35 ± 3.70	0.169
Type of infertility †	Primary	39/53 (73.6%)	39/55 (71.0%)	0.831
	Secondary	5/53 (9.4%)	8/55 (14.5%)	0.557
	Relative	9/53 (17.0%)	8/55 (14.5%)	0.795
Cause of infertility †	Male factor	27/53 (50.9%)	29/55 (52.7%)	1.000
	PCOS	22/53 (41.5%)	19/55 (34.5%)	0.553
	Tubal factor	11/53 (20.8%)	8/55 (14.5%)	0.455
	Endometriosis	5/53 (9.4%)	7/55 (12.7%)	0.761
	Unexplained	4/53 (7.5%)	6/55 (10.9%)	0.742
Previous implantation failure †	One	23/53 (43.4%)	26/55 (47.3%)	0.704
	≥ 2	30/53 (56.6%)	29/55 (52.7%)	
Serum TSH (uIU/ml) *		1.94 ± 1.14	1.81 ± 0.77	0.851
Serum prolactin (ng/ml) *		13.12 ± 5.81	13.53 ± 6.90	0.768
Basal serum FSH (mIU/ml) *		6.48 ± 2.05	6.11 ± 1.92	0.400
Basal serum LH (mIU/ml) *		6.98 ± 5.68	6.07 ± 3.57	0.298

* Expressed as mean ± SD and P value was calculated by the Mann-Whitney U-test.
† Expressed as frequency and percentage and P value was calculated by the Fisher's exact test.

Table (2)
COH and ICSI outcomes of the study and control groups:

	Study group (n = 53)	Control group (n = 55)	P value
Total gonadotropin dose (IU) *	2829 ± 777	2690 ± 709	0.337
Stimulation days (days) *	11.62 ± 1.54	11.40 ± 1.84	0.424
Number of follicles ≥ 12 mm in diameter by TVS on day of HCG administration †	15.36 ± 5.91	17.05 ± 7.83	0.208
Peak serum E2 (pg/ml) *	3152 ± 1804	3359 ± 1686	0.312
Number of oocytes retrieved *	10.15 ± 4.05	11.56 ± 5.66	0.249
Oocyte maturation rate (%) *	84.03 ± 16.55	81.23 ± 13.34	0.157
Fertilization rate (%) *	75.35 ± 20.39	75.28 ± 18.95	0.800
Percentage of good quality embryos (%) *	67.72 ± 34.13	66.40 ± 32.45	0.678
Number of transferred embryos *	2.91 ± 0.53	2.87 ± 0.47	0.760
Number of transferred good quality embryos *	2.49 ± 0.64	2.53 ± 0.63	0.738
Clinical pregnancy rate ‡	24/53 (45.3%)	18/55 (32.7%)	0.237
Implantation rate ‡	34/154 (22.1%)	24/158 (15.2%)	0.145
Miscarriage rate ‡	8/24 (33.3%)	6/18 (33.3%)	1.000

* Expressed as mean ± SD and P value was calculated by the Mann-Whitney U-test.
† Expressed as mean ± SD and P value was calculated by the t-test.
‡ Expressed as frequency and percentage and P value was calculated by the Fisher's exact test.

Discussion

Implantation failure in IVF/ICSI cycles is a challenging and distressing subject as it represents a financial and psychological burden on infertile couples subjected to ICSI treatment. Immunosuppressive treatment has been recently used during the standard ICSI protocols to improve the implantation rate, especially in women with previous implantation failure [11]. Glucocorticoids are a class of the famous immunosuppressive drugs to be used [12, 13]. Several studies have proved the benefits of use of glucocorticoids on implantation rate in patients who had zona dissected ET (14) as cortisone decrease the uterine lymphocytes and prevent segmented neutrophils to invade and destroy the zona dissected embryos. Also, other studies revealed the beneficial effect of glucocorticoids in non micro-manipulated embryos by decreasing the release of androgen from the suprarenal gland caused by the stress of ET procedures [15-17].

In this study, which is a prospective randomized trial, infertile couples with previous ICSI treatment failure without known local pathological condition that can prevent implantation or poor ovarian response to ovarian hyperstimulation or previous in utero transfer of bad quality embryos, we tried to study the effect of administration of low dose prednisolone after oocyte retrieval on the clinical pregnancy rate. We assumed that women with previous implantation failure in spite of transfer of at least one good quality embryo can be considered to be caused by local endometrial immunological reaction.

We found increase in clinical pregnancy rate (from 35.7% to 45.3%) and implantation rate (from 15.2% to 22.1%) in patients received 20 mg of prednisolone following oocyte retrieval, a result that can be explained probably by increase in the level or the activity of uterine natural killer (NK) cells by the use of immunosuppressant as proved by previous study [13]. It was proved that NK cells and components of innate immune system are important in development of the placenta by promoting angiogenesis, trophoblastic invasion and spiral arteries remodeling [18-20]. However, that increase in the implantation rate and clinical pregnancy rate in our study group was not statistically significant. Our results agree with a previous study that concluded that use of low dose prednisolone did not improve the clinical pregnancy rate and implantation rate when used regularly in IVF/ICSI cycles for infertile couple [21]; however, this study was conducted on patients without prior implantation failure so, both groups in this study may involve patients with endometrial immunological abnormality that may lead to insignificant results.

The strength of our study comes from that it was a randomized controlled one and the allocation concealment was performed by a sealed, opaque envelopes handled by a nurse though masking was not possible because no intervention was used in the control group. Another strength point in our study lies in exclusion of patients with other possible causes of implantation failure, such as those with uterine fibroid, hydrosalpinx, uterine surgery and positive antiphospholipid antibodies. The limitations of our study are that it was conducted on a relatively small cohort and the intention to treat strategy has not been performed.

Further studies are needed on larger cohorts of women with unexplained implantation failure to confirm or refute the benefit of glucocorticoids therapy on the clinical pregnancy and implantation rates after ET. Also, the effect of other forms and different doses of glucocorticoids therapy and the ideal time for starting therapy in remain to be investigated.

Conclusion

Administration of prednisolone in the luteal phase to women with previous unexplained implantation failure does not result in significant increase in clinical pregnancy or implantation rates.

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