Immunoregulatory Therapy in Frozen Embryo Transfer Cycle Improved Reproductive Outcomes of Women with Elevated Peripheral Blood Th1/Th2 Ratios

Shihui Meng

Reproductive Medicine Center,Peking University People's Hospital

Huan Shen (✉ rmivf@sina.com)

Reproductive Medicine Center, Peking University People,s Hospital   https://orcid.org/0000-0001-7242-7706

Research

Abstract

Backgrounds: Little observational data exist describing prednisone+ hydroxychloroquine+cyclosporine (PDN+HCQ+CsA), prednisone+ hydroxychloroquine (PDN+HCQ), prednisone (PDN) therapy for improving IVF-ET outcomes in patients with elevated peripheral Th1/Th2 ratio.

Methods: Retrospectively collected patients who was failed in IVF-ET and had elevated Th1/Th2 ratio between 1/2019 and 3/2020. Based on researches, elevated Th1/Th2 ratio was defined as equal to 10.3 or above. Patients were assigned into treatment group and control group based on whether received immunoregulatory treatment (PDN+HCQ+CsA/PDN+HCQ/PDN) during frozen transfer cycle.

Results: Forty-one patients (PDN+HCQ+CsA/PDN+HCQ/PDN=21/9/11) in treated group and 30 patients in control group were enrolled in the study. No differences were found of baseline characteristics between treated group and untreated group. Rate of live birth was higher in treated group compared with untreated patients (41.5% vs. 16.7%, P=0.026). Rate of biochemical pregnancy (56.1% vs. 40%, P=0.18), implantation (36.5% vs. 23.9%, P=0.15), clinical pregnancy (51.2% vs. 30%, P=0.0743) were higher than control group but there were no statistical significances.

Conclusions: Use of prednisone+ hydroxychloroquine+ cyclosporine or prednisone+ hydroxychloroquine, or prednisone during frozen embryo transfer cycle for patients with past implantation failure and elevated peripheral Th1/Th2 ratio improved live birth rate compared to those untreated.

Plain English Summary

Little observational data exist describing prednisone hydroxychloroquine + cyclosporine (PDN + HCQ + CsA), prednisone + hydroxychloroquine (PDN + HCQ), prednisone (PDN) therapy for improving IVF-ET outcomes in patients with elevated peripheral Th1/Th2 ratio.

We retrospectively collected patients who was failed in IVF-ET and had elevated Th1/Th2 ratio between 1/2019 and 3/2020. Based on researches, elevated Th1/Th2 ratio was defined as equal to 10.3 or above. Patients were assigned into treatment group and control group based on whether received immunoregulatory treatment (PDN + HCQ + CsA /PDN + HCQ /PDN) during frozen transfer cycle.

Forty-one patients (PDN + HCQ + CsA/PDN + HCQ/PDN = 21/9/11) in treated group and 30 patients in control group were enrolled in the study. No differences were found of baseline characteristics between treated group and untreated group. Rate of live birth was higher in treated group compared with untreated patients (41.5% vs. 16.7%, P = 0.026). Rate of biochemical pregnancy (56.1% vs. 40%, P = 0.18), implantation (36.5% vs. 23.9%, P = 0.15), clinical pregnancy (51.2% vs. 30%, P = 0.0743) were higher than control group but there were no statistical significances.

In conclusion; Use of prednisone + hydroxychloroquine + cyclosporine or prednisone + hydroxychloroquine, or prednisone during frozen embryo transfer cycle for patients with past

implantation failure and elevated peripheral Th1/Th2 ratio improved live birth rate compared to those untreated.

1. Background

Incidence of implantation failures varies from 8 to 33% in the general population [1]. When conducting IVF/ET, pregnancy is established when an embryo, which is a semi-allograft, is successfully implanted to maternal decidua with an establishment of maternal immune tolerance [2]. The balance among Th1, Th2 cytokines played an important role. Elevated levels of Th1 cells such as TNF-α, IFN-γ are associated with embryo rejections, whereas elevated Th2 cell levels such as IL-4 are associated with successful pregnancy [3]. Previous studies have showed significantly higher Th1/Th2 ratios in peripheral blood samples in multiple implantation failures in IVF cycles [4].

Immunoregulatory therapy may be effective in treating immune disturbance. Prednisone (PDN) or hydroxychloroquine (HCQ) or cyclosporine (CsA) have been proved to inhibit Th-1 cytokines secretion, increase the number of regulatory T cells and to induce maternofetal tolerance [5]. Therefore, use of immunoregulators prior to embryo transfer may improves IVF outcome. However, some studies showed adverse results [6]. This may because these studies did not target on well-selected patients with immune disturbance which may present as elevated Th1/Th2 ratio. Besides, these studies did not use combination of immunoregulatory medicines. Therefore, we conducted a retrospective cohort study to investigate the reproductive outcomes of FET (frozen embryo transfer) cycle after use of immunoregulatory therapy versus no treatment in women with previous implantation failure and elevated peripheral blood Th1/Th2 ratio.

2. Methods

2.1 Study population

This is a retrospective cohort study in which patients were enrolled in a tertiary care hospital, Peking University People's hospital between January 2019 and March 2020. The study was approved by institutional ethics review committee (2018PHB141-01). A signed informed consent form was obtained from all patients prior to prednisone treatment. We enrolled patients based on following eligibility criteria: (1) patients age<40 years, (2) underwent frozen-embryo transfer, (3) had a history of failed IVF/ET cycles, (4) had elevated peripheral blood Th1/Th2 ratio. Study patients were assessed by transvaginal ultrasound, hysterosalpingography before IVF cycle. All serum laboratory values were obtained in our laboratory as part of the clinical evaluation and treatment of the patients. Patients were excluded if they had (1) any structural lesions of uterus or hydrosalpinges, (2) any autoimmune disease, acquired or inherited thrombophilia. Patients were assigned into treatment group and control group based on whether received immunosuppressive treatment.

2.2 Analyses of the peripheral blood Th1/Th2 Cells

According to J.Y.H.Kwak-Kim et al [4], mean TNF-a/IL-4 level is 12.81±2.52 in patients with previous implantation failures, we took one standard deviation plus mean value, which is 15.33, as lower limit of elevated Th1/Th2 cell ratio. Therefore, elevated Th1/Th2 ratio was defined as equal to 15.33 or above. To evaluate the value of Th1/Th2 ratios, peripheral blood was drawn between cycle day (CD) 5 and 10 of a cycle prior to the index ART cycle. TNF-α and IL-4 concentrations in serum were measured using ELISA Kits from Biolegend (San Diego, CA, USA), according to the manufacturer's instruction. Serum was prepared by centrifugation of coagulated blood tubes at 2000g for 10min at room temperature and stored in −70°C. Samples were tested for IL-4 and TNF-α using a sandwich enzyme-linked immunosorbent assay according to the manufacturer's instructions (R&D Systems, USA). Their concentrations were calculated using standard curves and each sample was measured in duplicate.

2.3 Immunosuppressive treatment

Five mg prednisone daily was begun on the first menstrual day of FET cycle and continued until the 8 weeks of gestation. Two-hundred mg hydroxychloroquine daily was started from day one of FET cycle and continued until 8 weeks of gestation. One- hundred mg daily cyclosporine was begun on the day of embryo transfer and continued until the 8 weeks of gestation. Twenty patients with two or more times of implantation failure took prednisone, hydroxychloroquine, and cyclosporine. Cyclosporine is a strong immunosuppressant which can cause serum creatinine and urea nitrogen elevation, as well as discomforts after first contact. Animal test has shown no teratogenic risk but need further clinical verifications. After thoroughly; explaining the benefits and risks of cyclosporine, 9 patients refused to take it and therefore received prednisone and hydroxychloroquine. Twelve patients with one time of implantation failure received prednisone only.

2.4 FET procedures

All patients received standardized ovarian stimulation regimens, oocyte retrieval, and fertilization, followed by a planned frozen transferf up to two day-3 or day-5 embryos. Patients received one of following regimens based on individual situations: gonadotropin-releasing−hormone (GnRH) antagonist, GnRH-agonist long protocol, mild stimulation protocol. When at least two follicles reached 18 mm, 5,000 to 10,000 IU of hCG (Covidrel, Merck Serono) was administered and oocyte retrieval occurred 36 hours later. Luteal-phase support was started from the day of ovulation with oral dydrogesterone at a dose of 20 mg twice a day and was continued until the day of serum hCG testing. Up to two cleavage stage frozen embryos or day 5 trophoblasts were thawed and transferred, respectively, Pregnancy test will be performed 2 weeks after embryo transfer. In women with a positive hCG test, luteal phase support was continued until 10 weeks of gestation.

2.5 Outcome measurements

The primary outcome was live birth rate, which was defined as the delivery of any viable neonate who was 28 weeks of gestation or older. Secondary outcomes included biochemical pregnancy, clinical pregnancy, implantation rate and fetal outcomes.

2.6 Statistical analysis

Baseline characteristics and laboratory results were summarized for two groups utilizing descriptive statistics, including percentage, means ±standard deviation (SD), and 95% CI. For the quantitative variable, the t-test was used to compare group differences For categorical variables, the chi-square test or Mann–Whitney U test was used for group comparisons. Significance level was set at P < 0.05; all data were analyzed by SPSS 23.0 (SPSS, IBM, NYU).

3. Results

Study population

Ninety-two patients met eligibility criteria, after excluding 21 patients, 71 patients were enrolled in the final study (Fig. 1). Prednisone, hydroxychloroquine and cyclosporin was administered in 21 patients, prednisone and hydroxychloroquine was administered to 9 patients and prednisone was administered in 11 patients, resulting in 41 patients in the treated group. The rest of the 30 subjects received no immunoregulators and served as the control group. There were no significant differences in baseline demographics (Table 1). Treated group had more previous failed embryo transfer cycle (2.34 ± 1.44 vs. 1.97 ± 1.35, P = 0.27) and transferred embryos (4.44 ± 2.79 vs. 3.73 ± 2.84, P = 0.3) than control group, but there were no statistical significances. Mean Th1/Th2 ratio was 29.42 ± 12.90 in treated group and 29.71 ± 14.87 in control group, which also had no statistical significances (P = 0.086).

Table 1
Baseline characteristics of all groups

Variable	PDN + HCQ + CsA Treated (n = 21)	PDN + HCQ Treated (n = 9)	PDN Treated (n = 11)	All Treated (n = 41)	Nontreated (n = 30)	*p*-value (All Treated vs. Nontreated)
Age, y	33.67 ± 3.99	34.67 ± 4.03	36.36 ± 3.93	34.61 ± 4.05	34.57 ± 3.42	P = 0.96, t = 0.05
Body mass index (kg/m^2)	22.59 ± 2.88	23.85 ± 2.76	20.95 ± 2.36	22.42 ± 2.85	23.83 ± 4.56	P = 0.12, t = 1.57
Fertility history						
Duration of infertility, y	4.24 ± 2.19	3.05 ± 2.13	3.86 ± 2.55	3.88 ± 2.27	4.37 ± 2.99	P = 0.43, t = 0.79
Previous conception, no. (%)	7 (33.3)	6(66.7)	5(45.5)	18 (43.9)	19 (63.3)	P = 0.11, χ^2 = 2.62
Spontaneous abortion, no. (%)	5 (23.8)	4(44.4)	1(9.1)	10 (24.4)	10 (33.3)	P = 0.41, χ^2 = 0.69
Indications for IVF, no. (%)						
Unexplained infertility	8(38.1)	2(11.1)	5(45.5)	15(36.6)	11 (36.7)	P = 0.994, χ^2 = 0.00
Tubal factor	3 (14.3)	6(66.7)	2(18.2)	11(26.8)	9 (30.0)	P = 0.769, χ^2 = 0.086
Male factor	3 (14.3)	2(22.2)	1(9.1)	6(14.6)	4 (13.3)	P = 0.876, χ^2 = 0.024
Ovulatory factor	5(23.8)	2(22.2)	2(18.2)	9(22.0)	5(16.7)	P = 0.580, χ^2 = 0.306
Previous embryo transfer history						
Number of failed embryo transfer cycle (n)	3.05 ± 1.43	1.44 ± 0.88	1.18 ± 0.60	2.34 ± 1.44	1.97 ± 1.35	P = 0.27, t = 1.11

Note: Values are presented as mean ± SD or frequencies (percentage);

Th1 cell: Tumor necrosis factor alpha producing T helper cell (CD3 + /4 + /TNF-α[+]).

Th2 cell; IL-4 producing T helper cell (CD3 + /4 + /IL-4 [+]).

Variable	PDN + HCQ + CsA Treated (n = 21)	PDN + HCQ Treated (n = 9)	PDN Treated (n = 11)	All Treated (n = 41)	Nontreated (n = 30)	p-value (All Treated vs. Nontreated)
Total number of transferred embryos (n)	5.81 ± 2.79	3.89 ± 2.53	2.09 ± 1.14	4.44 ± 2.79	3.73 ± 2.84	P = 0.3, t = 1.04
Hormone tests						
Follicle-stimulating hormone, IU/liter	7.85 ± 2.18	7.34 ± 1.70	9.62 ± 5.98	8.21 ± 3.53	8.34 ± 3.77	P = 0.88, t = 0.15
Luteinizing hormone, IU/liter	3.87 ± 1.70	4.13 ± 3.21	5.86 ± 5.54	4.45 ± 3.41	4.17 ± 1.99	P = 0.68, t = 0.42
Estradiol, pg/ml	44.81 ± 17.71	44.37 ± 6.82	38.24 ± 19.78	42.94 ± 16.50	48.10 ± 30.92	P = 0.43, t = 0.80
Progesterone, nmol/L	0.64 ± 0.43	0.61 ± 0.27	0.61 ± 0.64	0.64 ± 0.43	0.58 ± 0.27	P = 0.56, t = 0.58
Total testosterone, ng/ml	1.99 ± 0.89	2.05 ± 0.52	1.25 ± 0.67	1.82 ± 0.78	1.86 ± 0.87	P = 0.85, t = 0.19
Th1/Th2 ratio	31.09 ± 14.32	31.01 ± 12.75	24.92 ± 9.76	29.42 ± 12.90	29.71 ± 14.87	P = 0.086, t = 0.932
Ovulation induction protocol						
GnRH agonists, no. (%)	7 (33.3)	3(33.3)	4(36.4)	14 (34.1)	10(33.3)	P = 0.987, Z = 0.026
GnRH antagonists, no. (%)	9 (42.9)	3(33.3)	5(45.5)	17 (41.5)	13(43.3)	
CC Mild stimulation, no. (%)	5 (23.8)	3(33.3)	2(18.2)	10 (24.4)	7(23.3)	
Estradiol level on hCG trigger day, pg/ml	2758 ± 1752	2598 ± 1456	2807 ± 1505	2740 ± 1596	2760 ± 1805	P = 0.962, t = 0.047
Progesterone level on hCG trigger day, ng/ml	1.19 ± 0.67	1.62 ± 0.72	1.79 ± 1.62	1.45 ± 1.01	1.24 ± 0.78	P = 0.896, t = 0.373
Endometrium thickness on hCG trigger day, mm	9.06 ± 2.30	8.62 ± 1.84	9.2 ± 2.10	8.99 ± 2.11	8.07 ± 1.60	P = 0.054, t = 1.964

Note: Values are presented as mean ± SD or frequencies (percentage);

Th1 cell: Tumor necrosis factor alpha producing T helper cell (CD3 + /4 + /TNF-α[+]).

Th2 cell; IL-4 producing T helper cell (CD3 + /4 + /IL-4 [+]).

Variable	PDN + HCQ + CsA Treated (n = 21)	PDN + HCQ Treated (n = 9)	PDN Treated (n = 11)	All Treated (n = 41)	Nontreated (n = 30)	*p*-value (All Treated vs. Nontreated)
No. of oocytes retrieved	12.74 ± 6.98	13.38 ± 11.02	14.75 ± 9.32	13.38 ± 8.45	13.31 ± 8.08	P = 0.973, t = 0.035
Note: Values are presented as mean ± SD or frequencies (percentage);						
Th1 cell: Tumor necrosis factor alpha producing T helper cell (CD3 + /4 + /TNF-α$^+$).						
Th2 cell; IL-4 producing T helper cell (CD3 + /4 + /IL-4 $^+$).						

There were no statistical significances of outcomes of controlled ovarian hyperstimulation. On hCG trigger day, endometrium thickness was 8.99 ± 2.11 mm in treated group higher than 8.07 ± 1.60 mm in control group, but there was no statistical significance (P = 0.054).

Live Birth And Neonatal Outcomes

Mean number of embryos transferred was higher in treated group than control group (1.8 ± 0.5 vs.1.5 ± 0.5, P = 0.02) because more patients were transferred 2 embryos in treated group. Other variables in frozen embryo transfer procedures did not have statistical significances (Table 2). Rate of live birth was 41.5% in all treated patients (17 patients), higher than 16.7% of nontreated patients (5 patients, P = 0.026). among treated group, patients treated with PDN + HCQ + CsA had highest live birth rate of 52.4% (11 patients), patients treated with 2 kinds of immunosuppressants PDN and HCQ had second highest live birth rate (33.3%, patients), while patients treated with only PDN had lowest live birth rate of 27.3% (3 patients). Treated group had more implantation rate, clinical pregnancy rate, ongoing pregnancy rate and less pregnancy loss compared with control group, but there were no significances. PDN treated group had second higher clinical pregnancy rate among treated patients (54.5%; PDN + HCQ + CsA/ PDN + HCQ = 57.1%/33.3%), but 3 patients had pregnancy loss. Therefore, PDN treated group ended up with lowest live birth rate (27.3%). No newborns had congenital abnormalities or other complications in treated group, details about newborns were shown in supplemental table 1.

Table 2
Frozen Embryo Transfer Procedures and Reproductive Outcomes

Variable	PDN + HCQ + CsA Treated (n = 21)	PDN + HCQ Treated (n = 9)	PDN Treated (n = 11)	All Treated (n = 41)	Nontreated (n = 30)	p-value (All Treated vs. Nontreated)
Regiment of endometrial preparation						
Natural cycle no. (%)	3(14.3)	1(11.1)	2(18.2)	6(14.6)	5(16.7)	P = 0.82, χ^2 = 0.06
Artificial cycle no. (%)	18(85.7)	8(88.9)	9(81.8)	35(85.4)	25(83.3)	
Endometrium thickness before embryo transfer, mm	9.0 ± 2.0	8.8 ± 2.0	9.4 ± 2.0	9.1 ± 1.9	8.8 ± 1.5	P = 0.57, t = 0.58
Type of embryo transferred						
Cleavage transfer, no. (%)	6(28.6)	4(44.4)	5(45.5)	15(36.6)	12(40.0)	P = 0.77, χ^2 = 0.09
Blastocyst transfer, no. (%)	15(71.4)	5(55.6)	6(54.5)	26(63.4)	18(60.0)	
No. of embryos transferred						
Mean	1.8 ± 0.5	1.8 ± 0.4	1.9 ± 0.3	1.8 ± 0.5	1.5 ± 0.5	P = 0.02, t = 2.35
One embryo, no./total no. (%)	6(28.6)	2(22.2)	1(9.09)	9(22.0)	14(46.7)	P = 0.77, χ^2 = 0.09
Two embryo, no./total no. (%)	15(71.4)	7(77.8)	10(90.9)	32(78.0)	16(53.3)	
No. of MGE transferred	1.4 ± 0.5	1.6 ± 0.7	1.4 ± 0.8	1.4 ± 9.1	1.2 ± 0.7	P = 0.90, t = 0.37

CC: Clomiphene citrate
MGE: morphologically good-quality embryos

Variable	PDN + HCQ + CsA Treated (n = 21)	PDN + HCQ Treated (n = 9)	PDN Treated (n = 11)	All Treated (n = 41)	Nontreated (n = 30)	p-value (All Treated vs. Nontreated)
Reproductive outcomes						
Biochemical pregnancy no. (%)	14(66.7)	3 (33.3)	6(54.5)	23(56.1)	12(40)	P = 0.18,χ^2 = 1.80
Implantation rate no./total no. (%)	17/37(45.9)	5/16(31.3)	7/21 (33.3)	27/74(36.5)	11/46(23.9)	P = 0.15, χ^2 = 2.07
Clinical pregnancy no. (%)	12(57.1)	3(33.3)	6(54.5)	21(51.2)	9(30.0)	P = 0.07, χ^2 = 0.20
Ongoing pregnancy no. (%)	11(52.4)	3(33.3)	3(27.3)	17(41.5)	5(16.7)	P = 0.03, χ^2 = 4.98
Live birth no. (%)	11(52.4)	3(33.3)	3(27.3)	17(41.5)	5(16.7)	P = 0.03, χ^2 = 4.98
Pregnancy loss among clinical pregnancies no. (%)	1(8.3)	0	3	4(19%)	4(44.4%)	P = 0.20,χ^2 = 2.08
CC: Clomiphene citrate						
MGE: morphologically good-quality embryos						

4. Discussion

In this study, we reported on data of prednisone + hydroxychloroquine + cyclosporine, prednisone + hydroxychloroquine, prednisone therapy for improving IVF-ET outcomes in patients with elevated peripheral Th1/Th2 ratio. To our knowledge, this is the first study to evaluate the efficacy of a combination of immunosuppressants to IVF-ET in this special population. Our results indicated that use of prednisone + hydroxychloroquine + cyclosporine or prednisone + hydroxychloroquine, or prednisone during frozen embryo transfer cycle for patients with elevated peripheral Th1/Th2 ratio improved live birth rate compared to those untreated.

In IVF-ET, studies found that implantation failure is associated with elevated Th1/Th2 [7]. In our study, mean Th1/Th2 (TNFα/IL-4) ratio was 29.42 ± 12.90 in treated group, which was much higher than Th1/Th2 ratio of both infertile patients (2.4 ± 0.4, n = 80) [4] and patients with IVF failures(12.81 ± 2.52, n

= 9) in other studies [8], suggesting a severer infertile condition in our population. No difference was found Th1/Th2 ratio in two groups, suggesting there may be a similar level of immune abnormality between two groups. According to a previous report, RIF patients also had an increased Th1 cytokine response during their failed ART cycle [9]. Though mean failed cycle of IVF-ET was not enough for diagnosing recurrent implantation failure (2 ± 1.21) in our study, patients may also had a similar immune disturbance which resulted in previous implantation failure.

Cytokines of Th1 and Th2 cells interfere with pregnancy through several mechanisms. Cytokines of Th1 cells such as TNF-α may activate macrophages which could attack the trophoblast, trigger processes at the maternal utero-placental blood vessels by activation of vascular endothelial cell procoagulant [10]. In contrast Th2 cytokines such as IL-4 inhibit Th1-induced tissue factor production by monocytes. prednisone can inhibit Th-1 cytokines production by T cells and of NK cells cytotoxicity [11]. In clinical trial, prednisone was usually combined with other immunoregulatory regimens to improve live birth rate of IVF cycles [12]. In our study, we combine prednisone with hydroxychloroquine or hydroxychloroquine + cyclosporine. Hydroxychloroquine has both immune-regulatory and anti-thrombotic effects [13]: it blocks the production of pro-inflammatory cytokines and inhibits platelet aggregation and secretion of arachidonic acid by activated platelets. A study showed hydroxychloroquine administration in women with RIF with a high TNF-α/IL-10 ratio significantly decreased serum level of TNF-α and significantly increased serum level of IL-10 ($p < 0.0001$) [5]. Cyclosporine A (CsA) creates cyclophilins in the cytoplasm, and forms a complex that binds to the calcineurin which prevents lymphocytes proliferation and lymphokines transcription, including TNF-α, and IFN-γ. It can also increase Th2-associated responses ($p = 0.0001$), and reduced Th1/Th2 (26.71 ± 7.32 to 18.56 ± 4.92, $p < 0.0001$) in women with recurrent pregnancy loss [14]. After treatment, rate of live birth was 52.4% in PDN + HCQ + CsA group, 33.3% in prednisone + hydroxychloroquine group, 27.3% in prednisone group, showing a trend of improvement, and combination of PDN + HCQ + CsA had most favorable outcome. However, these findings need further verification.

Population of immunoregulators need to be well-selected. Effect of single prednisolone on IVF-ET was inconclusive in other studies [15] when studies involved did not sub-analyzed patients with immune abnormality. In our study, live birth rate of prednisolone group was 27.3%, higher than untreated group (16.7%). Therefore, prednisone may be administered in patients with implantation failure presumed to be related to immune disturbance. When patients had implantation failure, Th1/Th2 should be tested. If there's an elevation, immunoregulators should be administered during embryo transfer cycle.

Safety of immunoregulatory therapy needs to be emphasized. Prednisolone has long been proved safe in pregnancy with low prednisone amounts (< 10 mg/day) [16]. The safety of hydroxychloroquine is also well established with a favorable safety profile [17]. CsA is a particularly lipophilic peptide being able to inactively traverse the placenta and enter the fetal circulation. Though drug have been found in placenta, cord blood, and the amniotic fluid [18], it has not been convincingly verified whether or not it interferes with fetus development and growth. A meta-analysis implied that CsA does not seem to be a major

human teratogen [19].Other research have also displayed that the use of CsA during pregnancy does not increase the threat for inherited defects in infants [20].

This study has limited statistical power due to small sample size. A prospective randomized study is needed in the future to exam the efficacy and safety of immunotherapy during frozen embryo transfer cycle. Besides, this results may not be generalizable to patients underwent fresh embryo transfer.

5. Conclusions

In conclusion, our results indicated that patients with elevated peripheral Th1/Th2 ratio and previous implantation failure who received prednisone + hydroxychloroquine + cyclosporine or prednisone + hydroxychloroquine or prednisone treatment at frozen embryo transfer cycle displayed a higher live birth rate compared to those untreated.

Abbreviations

PDN, prednisone; HCQ, hydroxychloroquine; CsA, cyclosporine; IVF-ET, in vitro fertilization and embryo transfer;Th1, T helper 1;Th2 T helper 2; MGE, morphologically good-quality embryos; GnRH-A, gonadotropin-releasing−hormone antagonist

Declarations

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by institutional ethics review committee of Peking University People's Hospital (2018PHB141-01). A signed informed consent form was obtained from all patients prior to prednisone treatment.

CONSENT FOR PUBLICATION

Written informed consent for publication was obtained from all patients.

AVAILABILITY OF DATA AND MATERIAL

The datasets used during the current study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

There are no conflicts of interest.

FUNDING

There is no funding of this research

AUTHORS' CONTRIBUTIONS

Drs. Shen and Meng proposed the concept and designed the study. Dr. Meng contributed to the acquisition of data. Dr. Shen supervised the data collection. Dr. Meng performed the statistics. Dr. Meng wrote the manuscript with the help of Dr Shen. Dr. Shen performed critical revision of the manuscript. All authors provided inputs for the manuscript.

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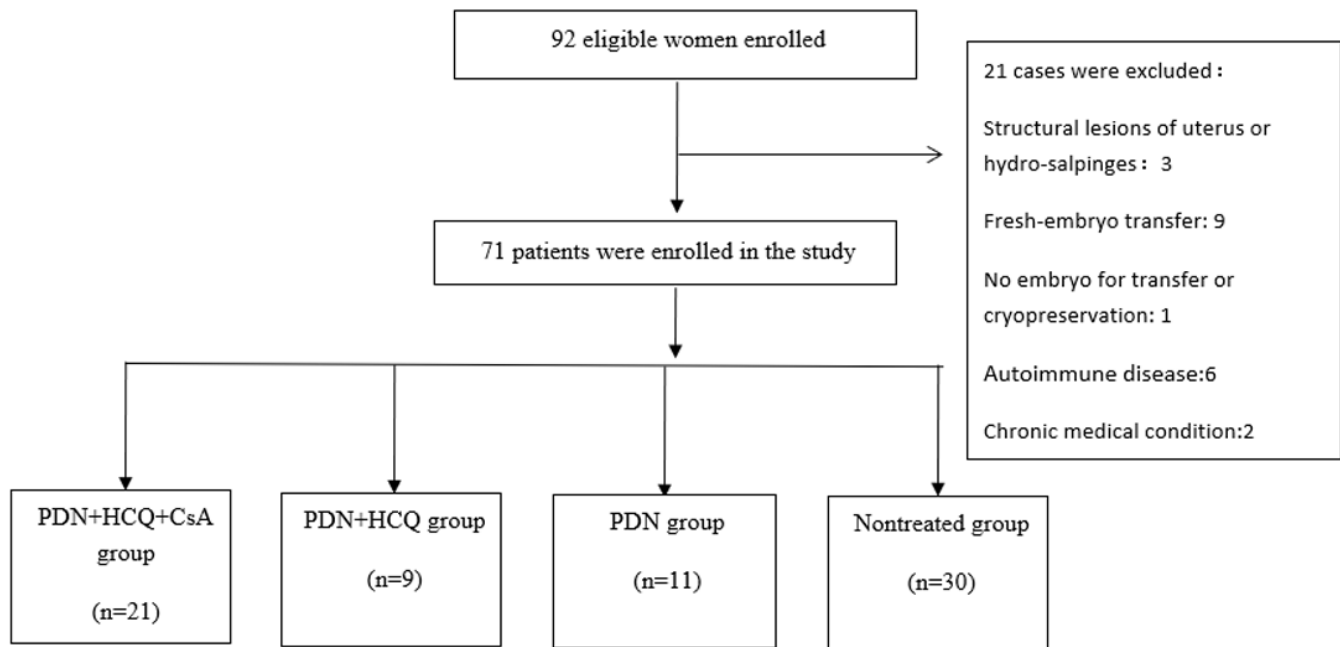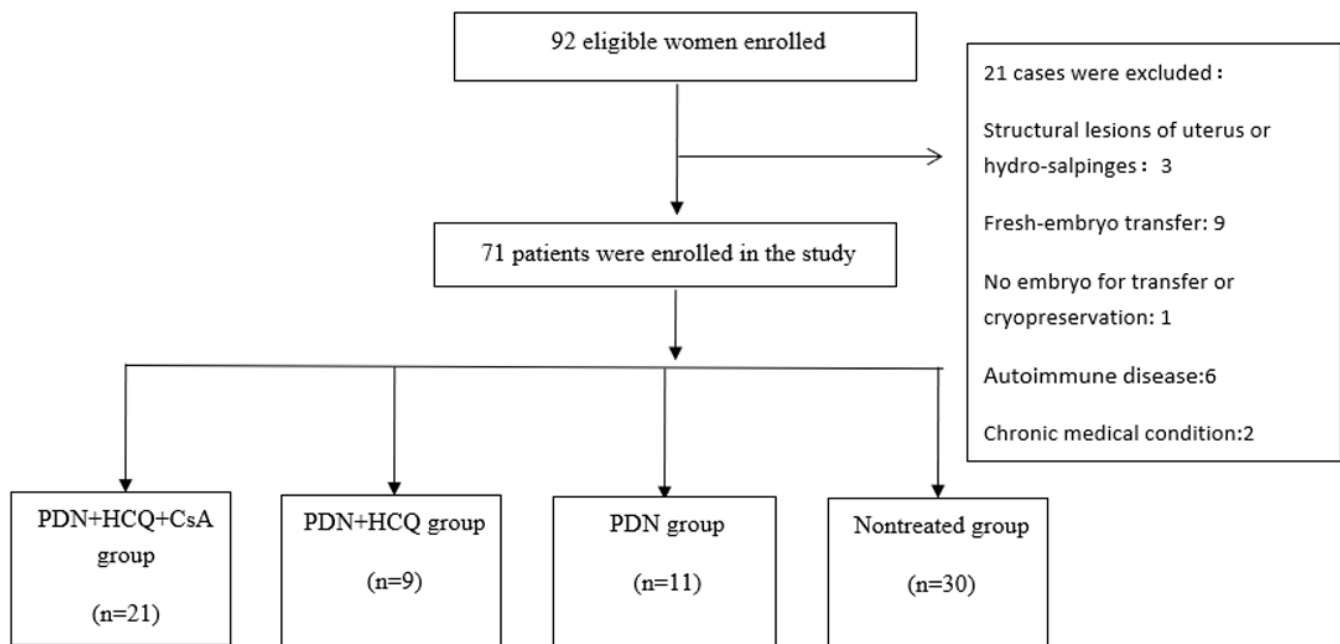
Figures

Figure 1. Disposition of Patients

Figure 1

Disposition of Patients

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