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Systematic Review (Pages: 27-36)

Effects of Immunosuppressive Treatments on Pregnancy Outcomes in Infertile Women: A Systematic Review of Clinical Trials

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Abstract

Background: Despite recent significant advances in the field of in-vitro fertilization (IVF), repeated implantation failure (RIF) remains a serious challenge for fertility. The suppression and regulation of the immune system by using immunomodulatory or immunosuppressive agents are one of the key ways to reduce RIF. The present study aimed to review the literature on the effect of immunosuppressive drugs on pregnancy outcomes, in infertile women with RIF.

Materials and Methods: In this systematic review, two independent reviewers selected related articles from online databases (Scopus, EMBASE, Cochrane Central Register of Controlled Trials, International Registry Platform for ongoing trials and clinical trails.gov, Web of Science, CINAHL, Medline, and Google Scholar search engine), without time limits from the inception restrictions up to January 2023.

Results: Fertilization, implantation, and clinical pregnancy rates were significantly higher in the prednisone + HCQ group than in the prednisone group. However, abortion rates were not different between the two groups. In a retrospective cohort study, there was no difference between the groups treated with prednisone + hydroxychloroquine + cyclosporine (PDN + HCQ + CsA), prednisone + hydroxychloroquine (PDN + HCQ), and PDN compared with untreated patients in the rate of live birth, implantation, biochemical, and clinical pregnancy ($p>0.05$). Treatment with HCQ significantly down-regulated Th17-related cytokines and function and up-regulated T-reg-related cytokines and function ($p<0.001$). Hydroxychloroquine (400 mg/orally per day) treatment significantly decreased the serum level of TNF-α and significantly increased the serum level of IL-10 ($p< 0.0001$).

Conclusion: Based on the combined results, immunoregulatory agents can be effective in fertilization, clinical pregnancy, live birth, and implantation rates. However, it was not effective in reducing abortion rates and biochemical pregnancy.

Key Words: Immunosuppressive treatment, Infertility, Pregnancy, Outcomes, Reproductive

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1- INTRODUCTION

Infertility is a condition of the male or female reproductive system and is defined as the failure to conceive after 12 months or more of regular unprotected sexual intercourse (1). Infertility affects millions of people of reproductive age worldwide and has an impact on their families and communities. Estimates suggest that approximately 48 million couples and 186 million individuals live with infertility globally (2-4). Assisted reproductive technology (ART) includes all fertility treatments where eggs or embryos are handled outside of the body. In general, ART procedures involve removing mature eggs from a woman's ovaries using a needle, combining the eggs with sperm in the laboratory, and returning the embryos to the woman's body or donating them to another woman. The primary type of ART is in-vitro fertilization (IVF) (5). ART has made considerable advancements since its inception. However, the current success rate is under 30%, and implantation failure remains a main challenge in female fertility (6). About 25-40% of natural implantations are lost, and loss of implantation occurs in 75% of pregnancies that are not even clinically recognized. Immunological factors have recently drawn the attention of scientists as the causes of reproductive failure (7).

Recurrent implantation failure means the nonachievement of clinical pregnancy after the transfer of at least four good-quality embryos in a minimum of three fresh or frozen cycles in a woman younger than 40 (8). Repeated implantation failure (RIF) or in-vitro fertilization (IVF) failure occurs when the transferred embryos fail to implant, meaning no pregnancy. RIF is defined as a lack of pregnancy after at least three times of fresh or frozen embryo transfer (FET) to the uterus (8). Immunosuppressants have been in extensive use since the late 21st century for various indications (9). At present, immunosuppressive drugs are used in the treatment of inflammatory and autoimmune diseases, as well as in transplantation (10). On the other hand, the suppression and regulation of the immune system by immunomodulatory or immunosuppressive agents are one of the key ways to reduce RIF (11, 12). Hydroxychloroquine is an antimalarial drug widely used in patients with various autoimmune diseases, particularly systemic lupus erythematosus (SLE). This drug has anti-inflammatory and immune-regulatory properties, such as inhibiting phospholipase activity, stabilizing lysosomal membranes, blocking the production of several pro-inflammatory cytokines (e.g., TNF-α, IL-17, IL-6, IFN-α, and IFN-γ), decreasing complement-dependent antigen-antibody reactions, and increased T-regulatory (T-reg) cells (13). Immunosuppressants are the first drug of choice for inflammatory and autoimmune disorders, prescribed for young and elderly patients alike. The continued use of these medications in women may have deleterious effects on fertility, pregnancy outcomes, and the unborn child (9). Despite recent significant advances in IVF, RIF remains a serious challenge in fertility. Immune cell disorders, such as the imbalance of cytokines and the Th1/Th2 ratio, have been reported as a cause of RIF (14). The present study aimed to review the literature on the effect of immunosuppressive drugs on pregnancy outcomes, in infertile women with RIF.

2- MATERIALS AND METHODS

Preferred Reporting Items for Systematic review and Meta-Analysis (PRISMA) checklist was used as a template for this review (17).

2-1. Eligibility criteria

Participants, interventions, comparators, and outcomes (PICO) was used to formulate the review objective and inclusion criteria.

Participants: Infertile women with the experience of RIF.

Interventions*:* Exposure of infertile women to any type of immunosuppressive drugs (e.g., hydroxychloroquine, cyclosporine, intravenous immunoglobulin [IVIG], and prednisone) six months before or around the time of conception.

Comparators*:* Treatment vs. control group, treatment vs. different types of treatment, before vs. after treatment.

Outcomes: Primary pregnancy outcomes: any changes in biochemical pregnancy rate, fertilization, live birth, clinical pregnancy, implantation rate, and abortion rate. Secondary outcome: other significant changes in the modulation of Th1/Th2 balance and Th17/T-reg ratio.

2-2. Included studies*:* Randomized controlled trials (RCT), randomized/nonrandomized clinical studies, and retrospective/prospective studies that assessed the effect of hydroxychloroquine on infertile women with RIF. Studies were included if they were published in English or Persian and if they included original data on infertile women's exposure to immunosuppressive drugs, published up to January 2023.

2-3. Exclusion criteria

The exclusion criteria were abstracts without the full article, articles not written in English or Persian, review articles, pilot studies, letters, editorials, short reports, case reports, and preliminary and brief studies.

2-4. Information sources

Systemic research of electronic databases: Medline (via PubMed), Cochrane Central Register of Controlled Trials (CENTRAL), International Registry Platform for ongoing trials and clinical trails.gov, EMBASE, Web of Science, Scopus, and Google Scholar search engine.

2-4. Search

Search terms were a combination of words related to fertility and pregnancy outcomes with a list of immunosuppressive drugs: (Hydroxychloroquine OR Immunosuppressive agents) AND (IRF OR Infertility OR Pregnancy OR Abortion OR Pregnancy outcomes OR Fertilization rate OR Live birth rate OR Clinical pregnancy OR Implantation rate OR Biochemical). The search was done independently and in duplication by two independent reviewers, and any disagreement between the reviews was dissolved by the supervisor. References from the primary search publications were also included in case these were missed in the search and when relevant data were missing.

2-5. Study selection

A database search was performed for possible studies. Abstracts were screened for eligible studies, full-text articles were obtained and assessed, and a final list of included studies was made. In addition to primary articles, their references were also searched for additional studies (**Figure 1**). This process was done independently and in duplication by two reviewers, and any disagreement was resolved by the third reviewer. References were organized and managed using EndNote software (version X8).

2-6. Data collection process

A researcher-made form template was developed and followed for each study. Two reviewers collected the data independently, and any discrepancies were solved by a third reviewer. The data collected from the selected studies included study design, study population (authors' names, settings, year of publication, sample size, and age group), type of intervention and comparison applied, and main results.

2-7. Risk of bias in individual studies

The final version of the Jadad scale was used for evaluating the quality of trials. The Jaded scale consists of three important items, ranging from 0 to 5 points. These items included randomization (if randomization was conducted and if it was done appropriately), blinding (if the trial was blinded and if it was done appropriately), and reporting withdrawals and dropouts (if the rate and reasons for withdrawals and dropouts were reported) (16). The quality of the retrospective studies was assessed using Newcastle-Ottawa Scale (NOS) (17) (**Table 1**). The assessment was done by two reviewers independently and in duplication, and any discrepancies were resolved by the third reviewer.

2-9. Synthesis of results

A meta-analysis was not possible due to the heterogeneity of the data.

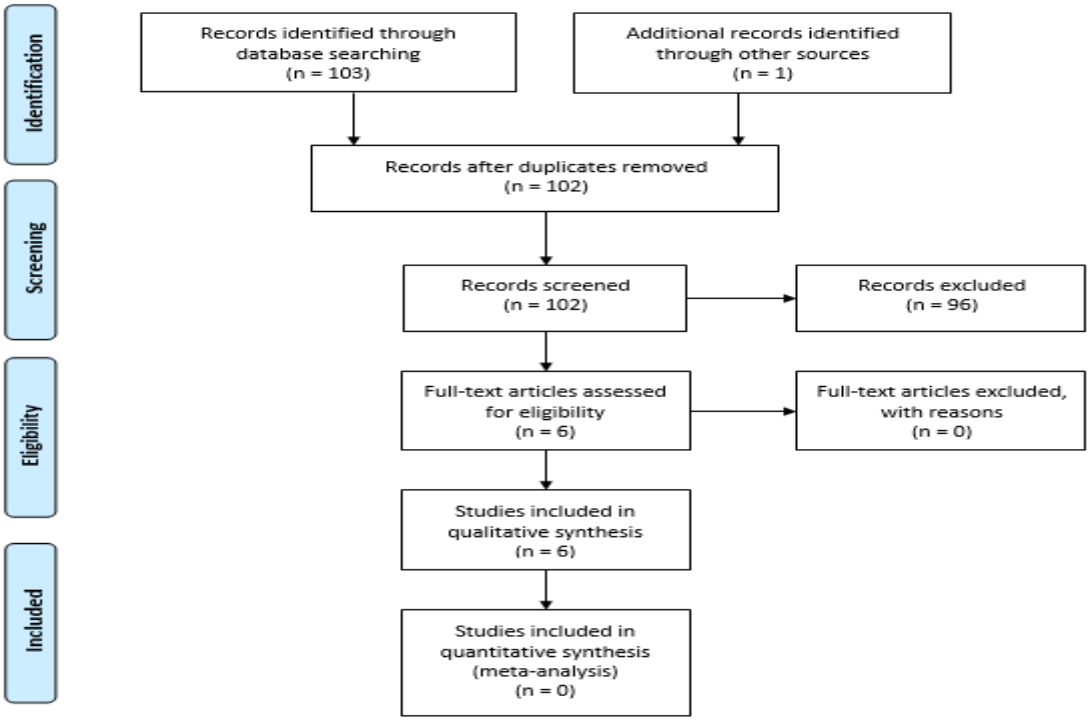

Fig.1: PRISMA Flowchart.

3- RESULTS

A total of six related studies (n=447 subjects) met the inclusion criteria for this systematic review (**Figure 1**). The main characteristics of the selected studies are summarized in **Table 1** and the following:

3-1. The effect of immunosuppressive treatment on pregnancy outcomes

Ahmadi et al. investigated the impact of IVIG on the function and frequency of T-reg and Th17 cells as two major $CD4^{+}T$ cell subclasses in pregnancy and implantation rates. The study was conducted on the RIF patients (n=72) with preconception Th1/Th2 ratio and natural killer (NK) cells frequency and activity elevation, divided into two groups: one receiving IVIG, aspirin, and enoxaparin (n=40, treatment group), and one receiving aspirin and enoxaparin without IVIG (n=32, control group). Th17, T-reg frequency, transcription factors, cytokine gene expression, and cytokine secretion were analyzed using flow cytometry, real-time PCR, and ELISA, respectively. Post-treatment examination in the IVIG group

showed a significant elevation in T-reg-related indices such as T-reg frequency (p= 0.018), Foxp3 (p<0.001), and cytokine mRNA levels (IL-10, p= 0.0058, and TGF-β, p = 0.0038). However, there was a significant difference in the case of Th17, with only a decrease in the mRNA level of RORγt (p = 0.021) (7).

In a retrospective study, Meng et al. examined women with IVF-ET failure and reported an increase in the Th1/Th2 ratio from 1/2019 to 3/2020. The findings showed an elevated Th1/Th2 ratio of ≥10.3. The patients were divided into two treatment and control groups depending on receiving or not receiving immunoregulatory treatment (PDN+HCQ+CsA/PDN+HCQ/PDN=21/9/11) in the process of the frozen transfer cycle. The research units included patients in treatment (n=41), and control (n=30) groups. There was no difference in the baseline profiles between the two groups, but higher live birth rates were found in the treatment group (41.5% vs. 16.7%, p=0.026). There was a higher rate of biochemical pregnancy (56.1% vs. 40%, p=0.18), implantation (36.5% vs. 23.9%, p=0.15), and clinical pregnancy (51.2% vs. 30%, p=0.0743) compared to the control group, but it was not statistically significant (18).

In a retrospective study, Lian et al. examined patients (n=156) who underwent IVF-ET with positive ANA and ds-DNA but without symptoms in South China from January 2010 to December 2016. Patients were treated with prednisone (7.5 mg/day) or prednisone (7.5 mg/day) + HCQ (0.2 twice a day) before and during pregnancy, and IVF-ET outcomes and complications were determined. Research units had no significant difference in demographic characteristics and reproduction-associated indices (i.e., duration of infertility, basal sex hormone, total Gn dose, E2 level on the day of HCG initiation, and the number of retrieved oocytes) between the two prednisone (n=65), and prednisone +HCQ (n=91) groups. The prednisone +HCQ group showed significantly greater fertilization (75.8% vs. 60.0%, p=0.017), implantation (29.7% vs. 15.4%, p=0.032), and clinical pregnancy rates (62.6% vs. 47.7%, p=0.028) compared to the prednisone group, as well as insignificantly lower abortion rates (7.0% vs. 12.9%). There was no relationship between the clinical pregnancy rate and ANA or ds-DNA levels. The implantation failure had an association with low C3. None of the two groups experienced complications influencing the treatment period (19).

Sadeghpour et al. conducted a clinical trial study to investigate the effects of HCQ on the level and function of Th17 and T-reg cells in women with RIF. They found no significant difference in biochemical pregnancy rates among RIF patients before and after HCQ treatment (20).

3-2. The effect of treatment with hydroxychloroquine on the modulation of Th1/Th2 balance and Th17/T-reg ratio in women with RIF

Ghasemnejad Berenji et al. examined patients (n=17) experiencing RIF for elevated TNFα/IL-10 ratios (TNFα/IL10>=30.6) before and after oral HCQ administration (400 mg/day). HCQ caused a significant reduction in the serum TNF-α level (p< 0.0001), and an elevation in the serum IL-10 level (p<0.0001). The expression of T-bet (Th1 transcription factor) was down-regulated, and that of GATA-3 (Th2 transcription factor) was up-regulated. There was a significant increase in the fluorescent immunoreactivity of IL-4 (p< 0.001), and IL-10 (p<0.05), as well as a significant decrease in the fluorescent immunoreactivity of TNFα (p<0.05), and IFN-γ (p < 0.05) in endometrial tissue following HCQ (21).

Table-1: General characteristics of the included studies (n=6).

Author/Year /Country, Reference	Study designs	Age, year, mean± SD	Sample size	Intervention (dose and duration of treatment)	Comparison (dose and duration of treatment)	Main results	Quality assessment
Ahmadi et al., 2017, Iran, (7)	Clinical trial	Intervention group:36.8 ±2.7, Control group: 37.1± 2.4	72	received IVIG, aspirin, and heparin (anoxaparin), n=40 patients	received aspirin and heparin (anoxaparin), n=32 patients	In IVIG group, there was a significant difference in Treg-related indices such as Treg frequency (p=0.0186), Foxp3 (p=0.0004), and cytokine mRNA levels (IL-10, p=0.0058 and TGF-β, p=0.0038); however, there was a significant difference in the case of Th17 only, with a decrease in the mRNA level of RORγt (p = 0.0218).	4*
Meng et al., China, 2020, (22)	Retrospective cohort	Intervention group: 34.61±4.05, Control group: 34.57±3.42	71	received (PDN+HCQ+ CsA/PDN+HCQ/ PDN), n=41	no immunoregulatory treatment, n=30	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), and clinical pregnancy (51.2% vs. 30%, p=0.0743) were higher than control group, but there were no statistical significances. It means immunoregulatory therapy improves reproductive outcomes in elevated Th1/Th2 cytokine ratio women with embryo transfer failure.	**15
Lian et al., 2018, China, (19)	Retrospective cohort	-	156	Prednisone (dose of 7.5 mg/day), n=65, or Prednisone (dose of 7.5 mg/day) + HCQ (0.2 twice a day), n=91		Fertilization rate, implantation rate and clinical pregnancy rate were significantly higher in prednisone +HCQ group than in prednisone group (75.8% vs. 60.0%, p=0.017, 29.7% vs. 15.4%, p=0.032, and 62.6% vs. 47.7%, p=0.028), respectively. Abortion rate was lower in prednisone +HCQ group, 7.0% vs. 12.9%, but it was not significant. It means combination of prednisone and HCQ may be more effective than solo treatment of prednisone for patients underwent IVF-ET.	**14
Sadeghpour et al., 2020, Iran, (20)	Clinical trial (before and after intervention)	33.6± 4.2	60	received HCQ = 400 mg daily for 16 days, n=60	-	Treatment with HCQ decrease regulated Th17 related cytokines and function and increase-regulated T-reg related cytokines and function significantly (p<0.001). RORγt and FOXP-3 expression factors were increase-regulated. The biochemical pregnancy rate was not significantly different in RIF patients before and after treatment.	*3

Ghasemnejad-Berenji et al., 2018, Iran, (21)	Clinical trial (before and after intervention)	33.6 ± 4.2	17	received HCQ= 400 mg/orally per day	-	Hydroxychloroquine administration in women with RIF with a high TNF-α/IL-10 ratio during the implantation window can decrease this ratio.	*4
Meng et al., China, 2022, (18)	Retrospective cohort study	Intervention group: 34:61± 4:05, Control group: 4:57± 3:42	71	immunoregulatory treatment during a frozen transfer cycle	-	Immunoregulatory therapy improves reproductive outcomes in elevated Th1/Th2 cytokine ratio women with embryo transfer failure.	**15

* Jadad scale, ** Newcastle-Ottawa Scale. SD: Standard deviation, IVIG: Intravenous immunoglobulin, IL-10*:* Interleukin 10, TGF- β: Transforming growth factor beta, Th17: T helper type 17, *mRNA*: messenger ribonucleic acid, PDN: Prednisone, HCQ: Hydroxychloroquine, CsA: Cyclosporine, RORγt: Retineic-acid-receptor-related orphan nuclear receptor gamma, RIF: Repeated implantation failure, Th1: T helper type 1, Th2: T helper type 2, TNF-α: Tumour necrosis factor α.

4- DISCUSSION

This systematic review aimed to explore the literature on the effect of immunosuppressive drugs on pregnancy outcomes such as fertilization, live birth, clinical pregnancy, implantation, abortion rates, and biochemical pregnancy in infertile women with the experience of RIF. Based on the gathered results, immunoregulatory agents can be effective in fertilization and live birth rates, clinical pregnancy, and implantation rate but not in reducing abortion rates and biochemical pregnancy.

Infertility is defined as a condition of the human reproductive system where the body fails to achieve pregnancy after sexual intercourse. The World Health Organization (WHO) defines infertility as a chronic health disorder that affects men and women across the world (22). In their study, Meng et al. showed that HCQ treatment in RIF patients with impaired immune cells during pregnancy influenced the Th17/T-reg ratio, increased T-reg, and reduced Th17 responses as successful pregnancy outcomes. However, there was no significant difference in pregnancy outcomes in the current research, and the results of this study should be interpreted with caution due to the small sample size (18). Based on the results, the abortion rate was lower in the prednisone +HCQ group than in the prednisone group (7.0% vs. 12.9%), but the difference was not significant (19). The live birth rate is defined as the delivery of at least one live-born infant (>24 weeks of gestation) (23). Based on the results, the rate of live birth was higher in groups treated with (PDN + HCQ + CsA), (PDN + HCQ), and PDN compared with untreated patients (41.5% vs. 16.7%, p=0.026) (22). Clinical pregnancy is defined as positive serum hCG and confirmed gestational sac on ultrasound or clinical findings of trophoblasts (24). Based on the results, clinical pregnancy rates were higher in the group treated with immunoregulatory agents than the control group (51.2% vs. 30%, p = 0.0743), but there were no statistical significances (22). Also, clinical pregnancy rates were significantly higher in the prednisone +HCQ group than in the prednisone group (62.6% vs. 47.7%, p=0.028), respectively (19). In addition, immunoregulatory treatment improved clinical pregnancy rates in patients with RIF (19). Implantation is defined as the process of the embryo attaching to the endometrial surface of the uterus and invading the epithelium, and then the maternal circulation to form the placenta (25). Based on the results, implantation rates were higher in the immunoregulatory treatment group than in the control group (36.5% vs. 23.9%, p= 0.15), but there were no statistical significances (22). Also, implantation rates were significantly

higher in the prednisone + HCQ group than in the prednisone group (29.7% vs. 15.4%, p=0.032) (19). In addition, combinations of HCQ as immunosuppressing agents with prednisone improved implantation rates compared to the PDN group (18). The fertilization rate is defined as the total number of embryos over the total number of retrieved oocytes (26). Based on the results, fertilization rates were significantly higher in the prednisone +HCQ group than in the prednisone group (75.8% vs. 60.0%, p=0.017) (19). Biochemical pregnancy is the determination of HCG levels in blood or urine along with clinical symptoms of pregnancy. Clinical pregnancy refers to the observation of the intrauterine gestational sac under ultrasound (27-29). In the study by Meng et al., the immunoregulatory treatment group showed a higher biochemical pregnancy rate (56.1% vs. 40%, p= 0.18) compared to the control group, but it was not statistically significant (16). Sadeghpour et al. found no significant difference in the biochemical pregnancy rate among RIF patients before and after HCQ treatment (20). The immunoregulatory treatment group showed a higher biochemical pregnancy rate, but it was not significant. These results should be interpreted with caution due to the small sample size (18). The pros and cons of using antimalarial agents for infertile people are debated, as long-term use or high doses can induce ocular toxicity and even irreversible retinopathy in some cases. Reportedly, the HCQ levels in cord blood are almost as high as in maternal blood. Animal research has indicated ocular drug accumulation in fetal mice (30). A systematic review reported that 789 out of 1477 infants were exposed to HCQ or chloroquine in previous studies. Overall, 563 exposed infants were followed postnatally (from less than three months to 19 years), of which 331 underwent ophthalmologic examinations during the follow-up (31). The literature review revealed a low-to-nonexistent risk of visual abnormalities in the offspring exposed to anti-malarial drugs (31). In a systematic review, patients with auto-immune disorders had no indication of any elevated risk of congenital defects, spontaneous abortions, fetal death, prematurity, and reduced live birth rate (32).

4-1. Study Limitations

The difference between the studies included in this systematic review could be attributed to different definitions of RIF across studies. Also, the difference in the prevalence of RIF in communities is 10-30% (11). The duration of infertility is a criterion for predicting pregnancy outcomes. Couples who have been infertile for less than three years have a higher odds ratio for pregnancy. Therefore, confounding factors should be carefully examined in future studies. There is a need for a prospective randomized trial in the future to investigate the efficacy and safety of immunotherapy during the frozen embryo transfer cycle. Furthermore, these results may not be generalizable to patients undergoing fresh embryo transfer.

5- CONCLUSION

HCQ treatment in RIF's patients with impaired immune cells during pregnancy influenced the Th17/T-reg ratio. Immunoregulators may be helpful, especially for those with immunological disturbance. The findings also showed that the immunoregulators could be effective in fertilization, clinical pregnancy, live birth, and implantation rates but not effective in reducing abortion rates and biochemical pregnancy. These results may not be generalizable to patients undergoing fresh embryo transfer. Also, the findings should be interpreted with caution due to the small number of studies, small sample size, and cohort type.

6- ABBREVIATIONS

ART: Assisted Reproductive Technology, IVF: In vitro fertilization, RIF: Repeated implantation failure, FET: Frozen embryo transfer, SLE: Systemic lupus erythematosus, TNF-α: Tumour necrosis factor α, IL-17: Interleukin 17 family, IL-10: Interleukin 10, IL-6: Interleukin 6, IL-4: Interleukin 4, IFN-α: Interferon-α, IFN-γ: Interferon gamma, T-reg*:* Regulatory T, IVIG:Intravenous immunoglobulin, Th1: T helper type 1, Th2: T helper type 2, Th17: T helper type 17, CD4+T cells:T helper cells, Real-time PCR: Real-time polymerase chain reaction, ELISA: enzyme linked immunosorbent assay, Foxp3: Forkhead box P3, mRNA: messenger ribonucleic acid, TGF-β: Transforming growth factor beta, RORγt: Retineic-acid-receptor-related orphan nuclear receptor gamma, IVF-ET: In vitro fertilization- embryo transfer failure, PDN: Prednisone, HCQ: Hydroxychloroquine, CsA: Cyclosporine, ANA: Antinuclear antibodies, ds-DNA: Double-Stranded DNA, GATA3: GATA Binding Protein 3, IFN-γ: Interferon-gamma, C3: Complement 3, E2: Estradiol.

7- CONFLICT OF INTEREST: None.

8- REFERENCES

1. Izadi Firoozabadi N, Ghazanfari A, Mashhadizadeh S, Sharifi T. Comparison of the effectiveness of Mentalization-based treatment and Mindfulness-Based Art Therapy on hopelessness of infertile women in Isfahan. Razi Journal of Medical Sciences.2023;30 (4).

2. Mascarenhas M, Flaxman S. R., Boerma, T., Vanderpoel, S., & Stevens, GA (2012). PLoS Medicine.9(2):1-12.

3. Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. Human reproduction. 2007;22(6):1506-12.

4. Rutstein SO, Shah IH. Infecundity, infertility, and childlessness in developing conuntries. Infecundity, infertility, and childlessness in developing conuntries, 2004. p. 56-.

5. Control CfD, Prevention. Division of Reproductive Health, National Center for Chronic Disease Prevention and Health Promotion. Pregnancy Mortality Surveillance System. 2005.

6. Somigliana E, Vigano P, Busnelli A, Paffoni A, Vegetti W, Vercellini P. Repeated implantation failure at the crossroad between statistics, clinics and over-diagnosis. Reproductive biomedicine online. 2018;36(1):32-8.

7. Ahmadi M, Abdolmohammadi-Vahid S, Ghaebi M, Aghebati-Maleki L, Dolati S, Farzadi L, et al. Regulatory T cells improve pregnancy rate in RIF patients after additional IVIG treatment. Systems biology in reproductive medicine. 2017;63(6):350-9.

8. Coughlan C, Ledger W, Wang Q, Liu F, Demirol A, Gurgan T, et al. Recurrent implantation failure: definition and management. Reproductive biomedicine online. 2014;28(1):14-38.

9. Shenuka S, Thiyagarajan T, Kumar RS. A Review on the Effect of Immunosuppressants on Fertility. Research Journal of Pharmacy and Technology. 2019;12(3):1441-47.

10. Leroy C, Rigot J-M, Leroy M, Decanter C, Le Mapihan K, Parent A-S, et al. Immunosuppressive drugs and fertility. Orphanet journal of rare diseases. 2015;10:1-15.

11. Alijotas-Reig J, Llurba E, Gris JM. Potentiating maternal immune tolerance in pregnancy: a new challenging role for regulatory T cells. Placenta. 2014;35(4):241-8.

12. Abdolmohammadi-Vahid S, Danaii S, Hamdi K, Jadidi-Niaragh F, Ahmadi M, Yousefi M. Novel immunotherapeutic approaches for treatment of infertility. Biomedicine & Pharmacotherapy. 2016;84:1449-59.

13. Ghasemnejad-Berenji H, Novin MG, Hajshafiha M, Nazarian H, Hashemi S, Ilkhanizadeh B, et al. Immunomodulatory effects of hydroxychloroquine on Th1/Th2 balance in women with repeated implantation failure. Biomedicine & Pharmacotherapy. 2018;107:1277-85.

14. Berker B, Taşkın S, Kahraman K, Taşkın EA, Atabekoğlu C, Sönmezer M. The role of low-molecular-weight heparin in recurrent implantation failure: a prospective, quasi-randomized, controlled study. Fertility and sterility. 2011;95(8):2499-502.

15. Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic

review and meta-analysis protocols 2015: elaboration and explanation. BMJ. 2015;349.

16. Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJM, Gavaghan DJ, et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary? Controlled clinical trials. 1996;17(1):1-12.

17. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Oxford; 2000.

18. Meng S, Zhang T, Li C, Zhang X, Shen H. Immunoregulatory Therapy Improves Reproductive Outcomes in Elevated Th1/Th2 Women with Embryo Transfer Failure. Biomed Res Int. 2022 Jun 26;2022:4990184.

19. Lian F, Zhou J, Wang Y, et al.

FRI0338 Hydroxychloroquine may help to improve the in vitro fertilization-embryo transfer outcomes in ana and ds-dna positive patients Annals of the Rheumatic Diseases 2018;77:705.

20. Sadeghpour S, Ghasemnejad Berenji M, Nazarian H, Ghasemnejad T, Nematollahi MH, Abroon S, et al. Effects of treatment with hydroxychloroquine on the modulation of Th17/Treg ratio and pregnancy outcomes in women with recurrent implantation failure: clinical trial. Immunopharmacology and Immunotoxicology. 2020;42(6):632-42.

21. Ghasemnejad-Berenji H, Novin MG, Hajshafiha M, Nazarian H, Hashemi S, Ilkhanizadeh B, et al. Immunomodulatory effects of hydroxychloroquine on Th1/Th2 balance in women with repeated implantation failure. 2018;107:1277-85.

22. Meng S., Shen H. Immunoregulatory therapy in frozen embryo transfer cycle improved reproductive outcomes of women with elevated peripheral blood Th1/Th2 ratios. 2020.

23. Polyzos NP, Drakopoulos P, Parra J, Pellicer A, Santos-Ribeiro S, Tournaye H, et al. Cumulative live birth rates according to the number of oocytes retrieved after the first ovarian stimulation for in vitro fertilization/intracytoplasmic sperm injection: a multicenter multinational analysis including~ 15,000 women. Fertility and sterility. 2018;110(4):661-70. e1.

24. Balaban B, Isiklar A, Ata B, Yakin K, Urman B. The effect of the embryologist/technician on fertilization rate and embryo quality following ICSI and pregnancy rate following embryo transfer procedures. Fertility and Sterility. 2007;88:S113.

25. Kim S-M, Kim J-S. A review of mechanisms of implantation. Development & reproduction. 2017;21(4):351.

26. Roustan A, Perrin J, Debals-Gonthier M, Paulmyer-Lacroix O, Agostini A, Courbiere B. Surgical diminished ovarian reserve after endometrioma cystectomy versus idiopathic DOR: comparison of in vitro fertilization outcome. Human Reproduction. 2015;30(4):840-7.

27. Zeng X, Jin S, Chen X, Qiu Y. Association between ambient air pollution and pregnancy outcomes in patients undergoing in vitro fertilization in Chengdu, China: a retrospective study. Environmental research. 2020;184:109304.

28. Al Mamari N, Al Zawawi N, Khayat S, Badeghiesh A, Son W-Y, Dahan MH. Revisiting serum β-hCG cut-off levels and pregnancy outcomes using single embryo transfer. Journal of Assisted Reproduction and Genetics. 2019;36:2307-13.

29. Wang Z, Gao Y, Zhang D, Li Y, Luo L, Xu Y. Predictive value of serum β-human chorionic gonadotropin for early pregnancy outcomes. Archives of Gynecology and Obstetrics. 2020;301:295-302.

30. Tzekov R. Ocular toxicity due to chloroquine and hydroxychloroquine: electrophysiological and visual function correlates. Documenta ophthalmologica. 2005;110:111-20.

31. Gaffar R, Pineau CA, Bernatsky S, Scott S, Vinet E. Risk of ocular anomalies in children exposed in utero to antimalarials: a systematic literature review. Arthritis care & research. 2019;71(12):1606-10.

32. Sperber K, Hom C, Chao CP, Shapiro D, Ash JJPR. Systematic review of hydroxychloroquine use in pregnant patients with autoimmune diseases. 2009;7(1):1-9.