International Journal of Research and Development in Pharmacy and Life Sciences
Available online at http//www.ijrdpl.com
December - January, 2016, Vol. 5, No.1, pp 1948-1954
ISSN (P): 2393-932X, ISSN (E): 2278-0238

Research Article

COMPARATIVE STUDY ON PREGNANCY RATES FOLLOWING ADMINISTRATION OF ASPIRIN AND/OR HEPARIN FOR FEMALES UNDERGOING INTRA-UTERINE INSEMINATION OF HUSBAND SEMEN

Muhammad-Baqir M-R. Fakhrildin[1], Muhammad Oda Selman[2], Hanaa Hussein Kandooh[2*]

1. Department of Physiology, College of medicine, Jabir Ibn Hayyan Medical University, Al-Najaf Al-Ashraf.
2. Department of Clinical Reproductive Physiology, High Institute of Infertility Diagnosis & ART Al-Nahrain University.

***Corresponding author's Email:** thair_wali@yahoo.com

(Received: October 18, 2015; **Accepted:** November 22, 2015**)**

ABSTRACT

Background: Suboptimal uterine perfusion with impaired blood flow has been suggested as a possible cause of embryo implantation failure. It has been hypothesized that low-dose aspirin (LDA) and low molecular weight heparin (LMWH) may enhance uterine and ovarian blood flow and improve the results of assisted reproductive technique (ART) by increasing endometrial receptivity and blastocyst implantation.

Objectives: To evaluate the effect of LMWH and /or LDA combination on implantation and pregnancy rates in infertile patients undergoing intrauterine insemination (IUI) cycles and to compare implantation and pregnancy rates(PRs) between different treated groups: aspirin treated, non aspirin, aspirin and heparin treated group.

Patients, materials and methods: A prospective cohort study including a total of one hundred eight infertile couples participated in this study through a period from December 2013 to October 2014. Patients were divided into three groups: the first one received no treatment and served as a control, the second received LDA alone and the third one received both LDA and LMWH. All Patients underwent IUI after full history and physical examination, hormonal assay ultrasound examination, and seminal fluid analysis for husbands. Some patients had HSG examination.

Results: There was no significant difference in the pregnancy rate among the three groups enrolled in the present study. The pregnancy rates were (13.89%), (19.44 %) and (11.11%) in group of women who received neither LDA nor LMWH, group of women who received LDA and group of women who received both LDA and LMWH; respectively. Moreover, age and endometrial thickness had a significant impact on the rates of pregnancy. Sperm parameters, size of follicles, number of follicles and hormone levels showed no significant association with the PRs.

Conclusion: The addition of low molecular weight heparin and/or aspirin to treatment protocols for the women who undergo IUI fails to improve the pregnancy outcome. Age of women, duration of infertility and endometrial thickness plays a significant role in the determination of pregnancy outcome.

Keywords: ART, AI, IUI, PRs, LDA, LMWH.

INTRODUCTION

Infertility is currently defined as the failure of a couple to achieve a successful pregnancy after one year or more of regular unprotected intercourse. It is a major clinical problem with significant medical, psychosocial, and economic problems that affects approximately 15-20 % of couples[1]. Artificial insemination (AI) including IUI has been widely used as an ART to treat couples with ovulatory disturbances, mild to moderate male factor issues, unexplained infertility, coital problems and cervical hostility[2]. IUI is a satisfactory first-line choices for the assisted conception with the advantage of being shorter, less invasive, relatively simpler, less expensive, and lower morbidity than the costly and rigorous option of in vitro fertilization (IVF)[3]

Suboptimal uterine perfusion with impaired blood flow has been suggested as a possible cause of infertility by reducing endometrial receptivity, resulting in embryo implantation failure. It has been hypothesized that a LDA may enhance uterine and ovarian blood flow, and improves tissue perfusion by decreasing platelet aggregation and inhibiting vasoconstriction. This effect could improve the results of IVF by increasing endometrial receptivity and blastocyst implantation [4].

A strategy of using LMWH is now being considered as a potential therapy for all ART patients irrespective of autoimmune or thrombophilic status[5]. Their benefits include reductions in the thrombotic risks associated with exogenous gonadotrophins, optimizing implantation and trophoblast development, and giving the accumulating evidence that beneficial effects of heparin are not solely mediated via an anticoagulant properties[6]. Therefore, the aims of the present study were to evaluate the effect of LDA on implantation and PRs in infertile patients undergoing IUI cycles, to evaluate the effect of LDA and LMWH combination on implantation and PRs in infertile patients undergoing IUI cycles and finally to compare implantation and PRs between different treated groups: aspirin treated, non aspirin, aspirin and heparin treated group.

SUBJECTS, MATERIALS AND METHODS

A total of one hundred eight infertile couples participated in this study. The type of infertility was either primary (66%) or secondary (34%). The mean duration of infertility was (6.13±0.42) years with a range from 2-15 years, and the wives age ranges from (19 to 45) years. This study was conducted at the High Institute of Infertility Diagnosis and Assisted Reproductive Technology at AL-Nahrain University.

Prior to enrollment all patients signed a written consent allowing the use of their data for research purposes. Inclusion criteria were: (i) women age ≥ 19 - ≤ 45 years, (ii) Females should have one or both patent tubes, both ovaries are present, no ovarian cyst and no congenital malformation of genital tract, and (iii) no contraindications for aspirin. Exclusion criteria for both groups were: (i) Females with bilateral tubal blockage, any congenital reproductive tract malformations that interfere with conception, (ii) Husbands with sever male factor infertility which interferes with undergoing IUI according to seminal analysis parameters.

Hormonal analysis

Early follicular (cycle day 2 -3), a base line reading of FSH, LH, E2 and testosterone was done as a part of the work up. In addition, a test of the thyroid gland (TSH), as a screening of thyroid function was done[7]. Ultrasound examination was routinely performed for all the females attending infertility clinic. Early follicular U/S (CD5 - 6) was done to exclude ovarian cysts or other pathology and was repeated in the mid cycle (CD12 -14) for the detection of an antral follicle count and diameter with a measurement of endometrial thickness (ET). At least one or two dominant follicles (17 -23mm) should be recognized for a successful IUI procedure. In addition, a triple-line pattern and ET ≥8mm are intimately associated with implantation success [8]. Hysterosalpingography and / or laparoscopy for assessment of tubal patency were done for any patient in whom history or physical examination indicated an evidence of previous damage to the Fallopian tube [9].

Ovulation induction program

Ovarian stimulation was induced by either Clomiphene Citrate, and/or r-hFSH (Gonal-F®) as subcutaneous injection, or human menopausal gonadotrpins(HMG) from CD2 or 3 and the dose was adapted depending on the ovarian response as detected by a serial US examination [10]. Clomiphene citrate (50mg) twice daily was given from CD2-6 followed by rFSH or HMG for two or more injection which was adjusted depending on ovarian response[11] till follicular maturity (17-23mm) was followed by hCG administration, then insemination was performed 30- 36 hours later[12]. Clomiphene citrate (50mg) twice daily was given from CD2-6 followed by HMG (Menogon) injections intramuscularly from CD 3 through the follicular phase either daily or every other day. The female partner was prepared for IUI, which was performed in a gynecological room with a sterile technique, by a special intrauterine catheter. The patient remained in supine position for 30 minutes after the procedure. Luteal phase support started from next day for two weeks by giving the patient duphastone®10 mg twice daily [13]. Estimation of Beta human chorionic gonadotrpins (β-hCG) was carried out on day 16 after IUI for pregnancy confirmation[14].

Statistical analysis

Data were analyzed using SPSS version 16 and Microsoft Office Excel 2007. Numeric variables were expressed as mean+ SE, while nominal variables were expressed as number and percent. One way ANOVA and post hoc LSD test were used to compare between more than two groups. Moreover, Chi-square test was used to compare frequencies. P-value was regarded significant when it was less than or equal to 0.05.

RESULTS

A total of (108) infertile couples were divided into three groups of patients as follows; control group: includes 36 women who have undergone IUI and the patients received duphastone and folic acid, group1: 36 women who have undergone IUI and the patients received a LDA, folic acid and duphastone group 2: 36 women who have undergone IUI and the patients received a LDA plus LMWH- Lovenox® 2000 IU, folic acid and duphastone.

Table 1: Comparison of PRs among the three groups enrolled in the present study.

Pregnancy	Control		Group 1		Group 2		Total	
	No.	%	No.	%	No.	%	No.	%
Positive	5	13.89	7	19.44	4	11.11	16	14.81
Negative	31	86.11	29	80.56	32	88.89	92	85.19
Total	36	100.00	36	100.00	36	100.00	108	100.00
P1 "Control vs Group 1" = 0.527 Not significant								
P2 "Control vs Group 2" = 1.000* Not significant								
P3 " Group 1 vs Group 2" = 0.326 Not significant								

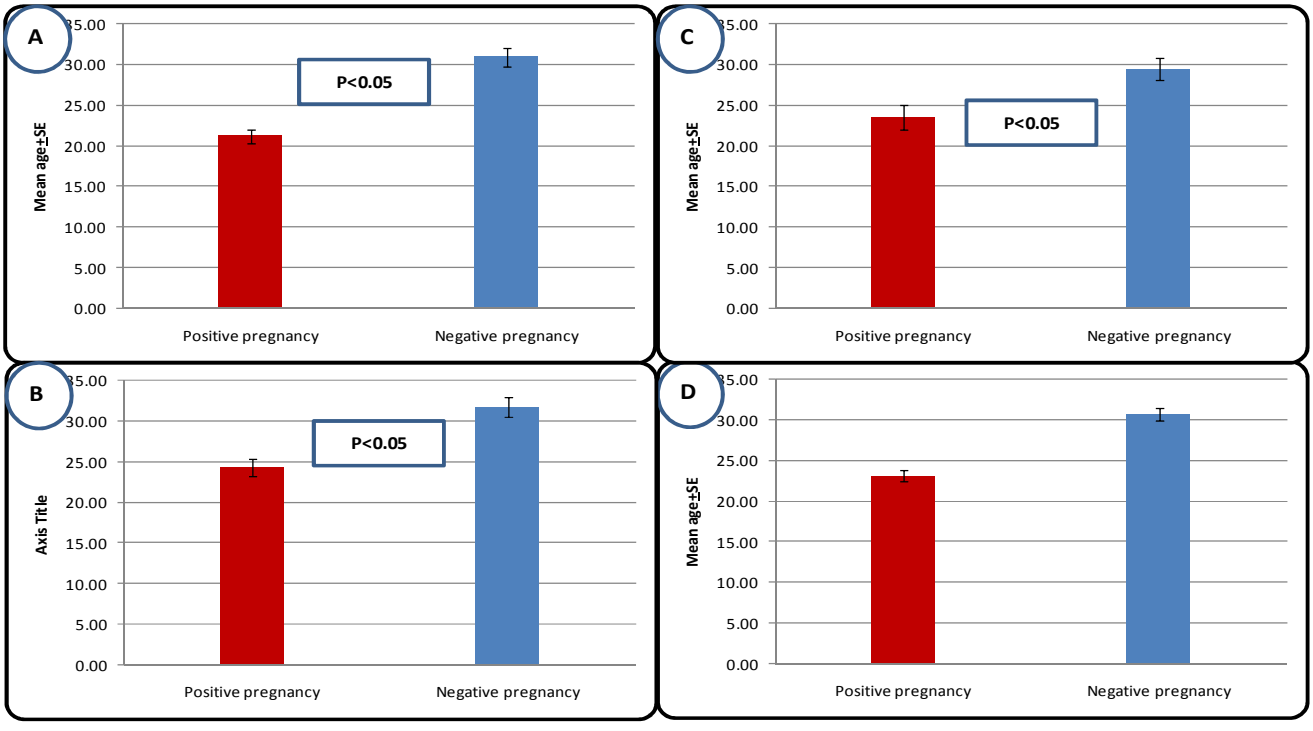

Figure1: Comparison of mean women age (in years) between those with successful pregnancy and those with pregnancy failure in control group (A), group 1 (B), group 2 (C) and in all women (D)

Table 2: Mean infertility duration in patients with successful pregnancy and those with pregnancy failure for women enrolled in the present study.

Pregnancy outcome	Control	Group 1	Group 2	All
	Mean duration	Mean duration	Mean duration	Mean duration
Positive pregnancy	3.6±0.50	5.85±0.98	4.75±1.10	4.87±0.56
Negative pregnancy	6.83±0.51	6.03±0.40	6.15±0.39	6.34±0.25
P-value	0.018	0.854	0.242	0.026

Table3: Comparison of mean follicle number, follicle size and endometrial thickness between women with successful pregnancy and women with pregnancy failure in individual groups and in all women

		Positive pregnancy (n = 5)	Negative pregnancy (n = 31)	P-value
Group	Parameter	Mean	Mean	
Control	No. of follicles	1.60±0.24	1.74±0.10	0.610
	Follicle size	19.48±0.76	19.09±1.45	0.963
	ET	9.88±0.96	8.45±0.29	0.091
		Positive pregnancy (n = 7)	Negative pregnancy (n = 29)	P-value
	Parameter	Mean	Mean	
Group 1	No. of follicles	1.71±0.18	1.76±0.13	0.874
	Follicle size	19.66±0.76	19.58±0.30	0.917
	ET	9.71±0.34	8.71±0.18	0.020
		Positive pregnancy (n = 4)	Negative pregnancy (n = 32)	P-value
	Parameter	Mean	Mean	
Group 2	No. of follicles	1.50±0.29	1.75±0.11	0.451
	Follicle size	19.50±0.58	19.78±0.46	0.906
	ET	10.00±0.87	8.55±0.19	0.022
		Positive pregnancy (n = 16)	Negative pregnancy (n = 92)	P-value
	Parameter	Mean	Mean	
Total	No. of follicles	1.63±0.13	1.75±0.06	0.448
	Follicle size	19.75±0.50	19.82±0.52	0.955
	ET	9.83±0.37	8.57±0.13	<0.001

Table 4: Relation between ovulation induction method and PRs

Ovulation Induction Method	Pregnancy						
	Positive		Negative		Total		
	No.	%	No.	%	No.	%	P-value
Clomiphene Citrate	2	8.70	21	91.30	23	100.00	0.514
rFSH(gonal-F)	2	11.76	15	88.24	17	100.00	1.000
HMG(menogon)	1	7.14	13	92.86	14	100.00	0.688
Clomiphene Citrate+ rFSH(gonal-F)	7	26.92	19	73.08	26	100.00	0.046
Clomiphene C+ HMG(menogon)	4	14.29	24	85.71	28	100.00	0.751
Total	16	14.81	92	85.19	108	100.00	---

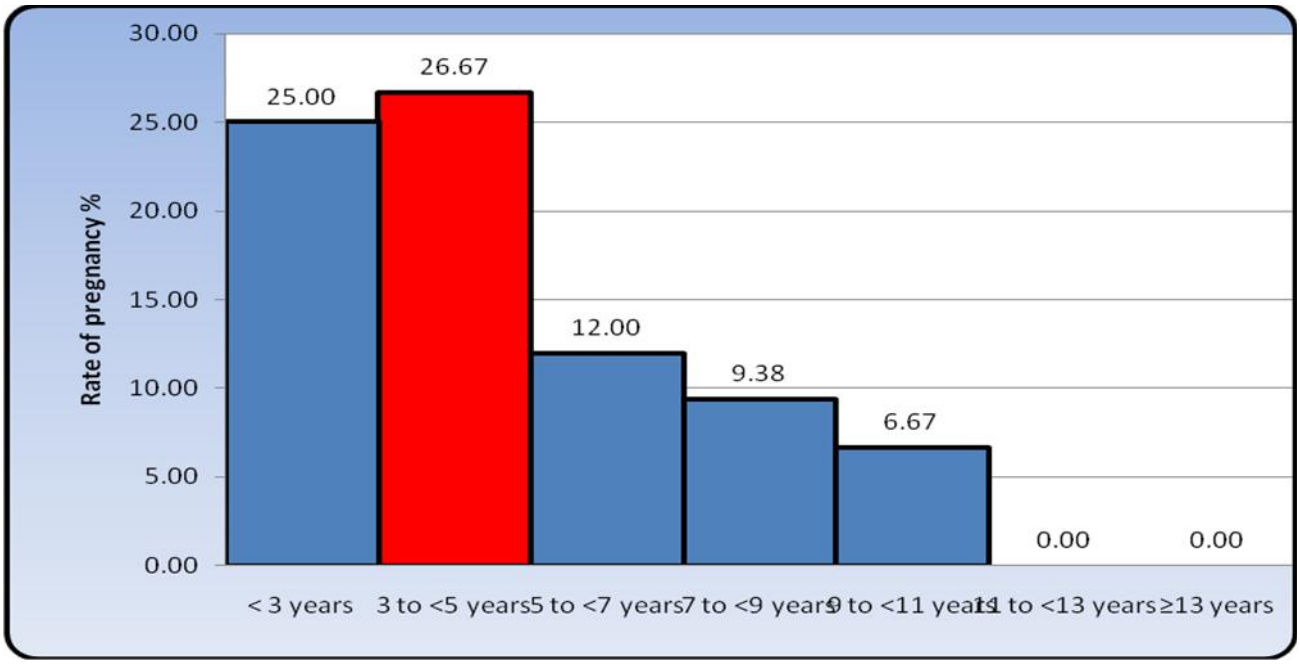

Figure 2: Pregnancy rate in relation to infertility duration

PRs in the control group was (13.89 %), while in group1, it was slightly higher (19.44 % versus 13.89 %). On the contrary, PRs were lower in group 2 than in control group (11.11% Vs 13.89 %. Unfortunately, these difference was statistically not significant (P>0.05). That is to say, the addition of LMWH and/or LDA did not alter PRs significantly. Pregnancy rates were not significantly different in group 1 in comparison to group 2 (P>0.05).Table (1).

The mean age of all women who succeeded to get pregnant was (23.13+0.71) years, while the mean age of women with implantation failure was (30.71+0.71), so the age difference was significant (P<0.05). (Figure 1). Significant difference (P<0.05) was found in the mean infertility duration between women with positive pregnancy and women with negative pregnancy and the majority of infertile women with successful pregnancy were between 3 to <5 years duration of infertility.(Figure 2) and (Table 2). Despite the minor variation in the mean follicle number and size of follicles, these differences did not reach a significant statistical level neither in individual groups nor in the total sample. Endometrial thickness was significantly higher (P value<0.05) in patients with successful pregnancy, (Table 3). In regard to ovulation induction methods, the combination of Clomiphene Citrate and rFSH had the higher PRs with a significant (P-value of 0.046), followed by combination of Clomiphene Citrate and HMG in comparison to the PRs of other groups. (Table 4).

DISCUSSION

It is well known by different studies that the age of the female partner is one of the most important predictive factors affecting IVF outcome[15] and the progressive decline in the female fecundity together with increase prevalence

of unexplained infertility in female patients of advanced age (> 35 years) attributed primarily to the detrimental impact of various aging processes on female reproductive potential[16,17]. However, successful implantation and pregnancy chance in any women is determined by many factors that can be categorized into two main groups. The first group includes factors that determine embryo quality such as number of follicles and size of follicles ovarian activity. The second group involves parameters that determine the receptive capacity of the endometrium, such as the thickness of the endometrial lining. Clearly is the relation between the above mentioned parameters and blood flow to female genital organs. That's to say the better the blood flow the better will be the endometrium receptive capacity and ovarian activity, Hoozemans DA et. al. (2004) [18].

One proposed strategy for the improvement of ART success involves the enhancement of blood flow at the implantation site with the use of LDA[19]. The daily administration of aspirin in low doses induces a shift in the balance away from thromboxane A2 and towards prostacyclin, leading to vasodilatation and increased blood perfusion. Hyper-coagulability affecting uterine blood vessels has been postulated as a possible cause of reproductive failure, such as unexplained infertility, IVF implantation failure and recurrent fetal wastage; however, there is a lot of controversial evidence to suggest an association between recurrent implantation failure, thrombophilia, infertility and potential favorable effect of anticoagulant therapy[20].

Heparin is able to alter haemostatic response to exogenous ovarian stimulation, modulate trophoblast differentiation and invasion, prevent placental thrombosis and infarction and consequently increasing implantation rate and promote the establishment and continuation of pregnancy[21]. On the other hand, a comparable amount of studies ruled out the therapeutic effectiveness of heparin, alone or in combination with LDA[22,23]. In the present study the pregnancy rate was not significantly different (P>0.05) among the three study groups; 13.89%, 19.44% and 11.11% in control, group 1 and group 2, respectively, Accordingly the use of LDA and LMWH did not improve the rate of pregnancy due to one or more of the following reasons: First of all both aspirin and LMWH did not improve uterine and ovarian blood flow significantly to make the chance for a better quality of oocytes and a better receptive endometrium[24].

The second reason may be the fact that the quality of oocyte might be affected by other vital factors than merely the quantity of blood supply to the pelvic organ and degree of tissue perfusion[25]. From results of the current study, it was concluded that the addition of low molecular weight heparin and/or aspirin to treatment protocols for the women who undergo IUI fails to improve the pregnancy outcome. Age of women, duration of infertility and endometrial thickness plays a significant role in the determination of pregnancy outcome.

REFERENCES

1. Turchi, Paolo. "Prevalence, Definition, and Classification of Infertility." Clinical Management of Male Infertility. Springer International Publishing. 2015; 5-1.
2. Ombelet, W., and J. Van Robays. "Artificial insemination history: hurdles and milestones." Facts, views & vision in Ob Gyn. 2015; 7(2): 137.
3. Ferraretti AP, Goossens V, Kupka M, et al. Assisted reproductive technology in Europe, 2009: results generated from European registers by ESHRE. Human Reprod. 2013;(28):2318–31.
4. Haapsamo M, Martikainen H, Räsänen J. Low-dose aspirin and uterine haemodynamics on the day of embryo transfer in women undergoing IVF/ICSI: a randomized, placebo-controlled, double-blind study. Human reproduction2009; (24): 861-6.
5. Levine, Alana B., and Michael D. Lockshin. "Management of Antiphospholipid Syndrome in Pregnancy." Recurrent Pregnancy Loss: Causes, Controversies, and Treatment. 2014; 175.
6. D'Ippolito, Silvia, et al. "Obstetric antiphospholipid syndrome: a recent classification for an old defined disorder." Autoimmunity reviews.2014; 13(9): 901-908
7. Hull MGR, Savage PE, Bromham DR, et al. The value of a single serum progesterone measurement in the midluteal phase as a criterion of potentially fertile cycle (ovulation) Textbook of family medicine, 2005; chp 36.
8. Martins, Wellington P. Ultrasound Imaging in Reproductive Medicine: Advances in Infertility Workup, Treatment, and ART. Ultrasound in Medicine and Biology. 2014; 40(12): 2905.
9. Dreyer, Kim, et al. Hysterosalpingo-foam sonography, a less painful procedure for tubal patency testing during fertility workup compared with (serial) hysterosalpingography: a randomized controlled trial. Fertility and sterility.2014;102(3): 821-825.
10. Prapas, Nikos, et al. Low-dose human chorionic gonadotropin during the proliferative phase may adversely affect endometrial receptivity in oocyte

recipients. Gynecological Endocrinology.2009; 25(1): 53-59.

11. Sallam HN, Makrigiannakis A, eds. Infertility and Assisted Reproduction. Cambridge: Cambridge University Press. 2008; 416-27.
12. Blasco, Víctor, et al. "Influence of follicle rupture and uterine contractions on intrauterine insemination outcome: a new predictive model. Fertility and sterility. 2014; 102(4):1034-1040.
13. Richard Palmar Dickey, clomiphene citrate for ovulation induction: Rizkbrmb, Garcia-Velasco HN, Makrigiannakis A,eds. Infertility and Assosted reproduction. Cambridge University Press. 2008; 202-219.
14. Felyyeh EH. Comparative study of ovulation induction and hormone profile using Tamoxifen, Clomiphene citrate and Dexamethasone in infertile women. Theses for Degree of High Diploma in Assisted Reproduction Technologies.2010.
15. Van Loendersloot LL, Repping S, Bossuyt PM, et al. Prediction models in in vitro fertilization; where are we? A mini review. Journal of Advanced Research. 2014; 5: 295-301.
16. Wang YA, Healy D, Black D, Sullivan EA. Age-specific success rate forwomen undertaking their first assisted reproduction technology treatment using their own oocytes in Australia, 2002–2005. Hum Reprod. 2008; 23:1633–1638.
17. Maheshwari A, Hamilton M, Bhattacharya S: Effect of female age on the diagnostic categories of infertility. Hum Reprod .2008; 23:538-542.
18. Hoozemans DA, Schats R, Lambalk CB, et al. Human embryo implantation: current knowledge and clinical implications in assisted reproductive technology. Reprod Biomed Online. 2004; 9:692–715.
19. Zhao, M., et al. "Treatment with low-dose aspirin increased the level LIF and integrin β3 expression in mice during the implantation window. Placenta. 2010; 31(12): 1101-1105.
20. Grandone, E., Villani, M., Dentali, F., et al. Low-molecular–weight heparin in pregnancies after ART-A retrospective study. Thrombosis research.2014; 134(2): 336-339.
21. Qublan H, Amarin Z, Dabbas M, et al. Low-molecular-weight heparin in the treatment of recurrent IV/ET failure and thrombophilia: A prospective randomized placebo-controlled trial. Human Reproduction. 2008; 11(4):246-53.
22. Clark P, Walker ID, Langhorne P, et al. SPIN (Scottish Pregnancy Intervention) study: a multicenter, randomized trial of low-molecular- weight heparin and low-dose aspirin in women with recurrent miscarriage. Blood. 2010;115:4162-4167.
23. Seshadri S, Sunkara SK, Khalaf Y, et al. Effect of heparin on the outcome of IVF treatment: a systematic review and meta-analysis. Reprod Biomed Online. 2012;25:572–584.
24. Bambang, K. N., Lambert, D. G., Lam, P. M., et al. Immunity and early pregnancy events: are endocannabinoids the missing link. Journal of reproductive immunology.2012; 96(1), 8-18.
25. Cavallini, Giorgio, and Gianni Paulis. "Inflammatory Infertility." Clinical Management of Male Infertility. Springer International Publishing. 2015; 105-117.

