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Effect of Low Dose Aspirin Therapy on Pregnancy Rate in Women Undergoing *in vitro* Fertilization: A Randomised Controlled Trial

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ABSTRACT

Background: acetylsalicylic acid was synthesized in 1897. The product was called aspirin. In 1971, Vane described the mechanism of action of aspirin and showed that it inhibited the enzyme cyclooxygenase, thus avoiding prostaglandin (PG) synthesis. Aspirin (acetylsalicylic acid) is well known to have analgesic, anti-inflammatory and antipyretic properties. Since aspirin has been shown to increase uterine perfusion**,** it was not unreasonable to assume that aspirin administration may increase endometrial receptivity and blastocyst implantation. **Aim of the work:** this study aimed to assess the efficacy of low dose aspirin treatment in increasing the clinical pregnancy in infertile women undergoing IVF /ICSI.

Patients and methods: this study included 200 infertile women undergoing IVF/ICSI who recruited from the Infertility Clinic of Ain-Shams University, Maternity Hospital and a Private Center.

Results: there was no statistically significant difference between both groups (Aspirin and Placebo) as regard age, cumulus, MII, fertilization rate, grade 1 embryo, grade 2 embryo and total ET. There was statistically non-significant difference between both groups as regard clinical pregnancy. There was statistically non-significant difference between both groups as regard living birth. There was statistically non-significant difference between both groups as regard miscarriage.

Conclusion: in the total of 200 women were recruited. The clinical pregnancy rate of the Aspirin group showed no statistically significant difference from that of the placebo group (39% compared to 35%).

Recommendations: the use of Aspirin before IVF is not associated with a higher pregnancy rate. Therefore, no need for Aspirin to be added before IVF, However, further studies are needed to confirm our study.

Keywords: acetylsalicylic acid, follicular stimulating hormone.

INTRODUCTION

Acetylsalicylic acid was synthesized in 1897. The product was called aspirin. In 1971, Vane described the mechanism of action of aspirin and showed that it was inhibiting the enzyme cyclooxygenase, thus avoiding prostaglandin (PG) synthesis. Low-dose acetylsalicylic acid (Aspirin) irreversibly inhibits the enzyme cyclo-oxygenase in platelets, preventing the synthesis of thromboxane which is a potent vaso-constrictive agent [(1)].

Aspirin's antiplatelet activity has led to its use with investigation of a variety of disorders. The combination of low-dose aspirin and unfractionated heparin appeared to be of benefit in pregnant women with antiphospholipid antibodies and recurrent pregnancy if loss not be related to other causes. Pre-eclampsia is associated with deficient intravascular production of prostacyclin and excessive production of thromboxane. The administration of low-dose aspirin to women at risk led to a significant reduction in the likelihood of developing pre-eclampsia, preterm birth, fetal or neonatal death and small gestational age babies[(2)].

The daily administration of aspirin in low doses induced a shift in the balance away from thromboxane A2 and towards prostacyclin, leading to vasodilatation and increased blood**.** Aspirin has been found both experimentally and clinically to be cardio-protective, with few adverse effects in doses of 80–160 mg daily[(1)]**.** It has been demonstrated that low-dose of aspirin increased the weight of newborns in pregnant patients with fetal growth retardation, it was also used to prevent recurrent idiopathic fetal growth retardation and to improve placental and fetal blood flow in women with pre-eclampsia[(3)]. Low-dose aspirin may improve uterine and ovarian perfusion and it may enhance endometrial receptivity and ovarian responsiveness as well, which could result in better implantation and pregnancy rates after IVF or ICSI treatment. It has also been suggested in the literature that low-dose aspirin would lead to an increased number of oocytes in IVF/ICSI as well as a higher pregnancy rate [(4)]. Our institution at one time used aspirin routinely during IVF cycles, based on the work of studies which showed that low-dose aspirin increased implantation and pregnancy rates in women undergoing IVF[(4)]. A study was performed to determine whether aspirin inhibits the replication of human immunodeficiency virus and also whether aspirin diminishes the incidence of mortality in patients with colon cancer. Furthermore, it has been demonstrated that low-dose aspirin increased the weight of newborns in pregnant patients with fetal growth retardation; it is also used to prevent recurrent idiopathic fetal growth retardation and to improve placental and fetal blood flow in women with preeclampsia [(5)]. Since aspirin has been shown

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to increase uterine perfusion**,** it was not unreasonable to assume that aspirin administration may increase endometrial receptivity and blastocyst implantation [(5)]**.** Contrary data from **Urman** and co-investigators found no improvement in IVF outcomes with low-dose aspirin [(6)]. Subsequently, the use of aspirin was stopped in 2000. Infertility is the inability of a couple to achieve pregnancy over an average period of one year (in a woman under 35 years of age) despite adequate, regular unprotected sexual intercourse. IVF has become a technique widely utilized in assisted reproductive technology. Despite its current popularity, Success rates in IVF remain low with estimates suggesting around a twenty-eight percent live birth rate for each IVF cycle started at best. Fertilization rates have reached high success rates; however, pregnancy rates per embryo transfer are still relatively low at around 34 %[(8)].

PATIENTS AND METHODS

This was a randomized controlled clinical trial. **Study Settings**: this clinical trial was conducted at Ain-Shams University Maternity Hospital, the IVF unit and the Private Center. **Study Population:** This study included 200 infertile women whom were undergoing IVF/ICSI and they recruited from the Infertility Clinic of Ain-Shams University Maternity Hospital and a private center. **Primary outcome:** clinical pregnancy rate. **Secondary outcome**: number of oocytes, number and quality of embryos, miscarriage rate, twins rate and live birth rate. **Sample size justification**: one hundred women were required in each group to achieve an alpha error of 5% and a beta error of 1%. Thus, 100 women in each group were considered sufficient for such data types. **Inclusion criteria**: primary or secondry infertility, age less than 35 years old, No contraindications for aspirin, basal follicular stimulating hormone (FSH) level less than 10 mIU/ ml in day two of menstrual cycle, basal luteinizing hormone (LH) level less than 10 mIU/mlin day two of menstrual cycle, normal prolactin, and thyroid stimulating hormone, negative lupus anticoagulant hormone, anticardiolipin Ab IgG &IgM negative. **Exclusion criteria**: primary exclusion criteria: age more than or equal 35 years old, fibroid uterus or uterine anomalies, allergy to acetylsalicylic acid, medical disorders (e.g. heart failure, liver disease, hypertension, renal failure), patients with serum level anti Mullerian hormone of less than or equal 1 mg/ml, male factor infertility, disorders that contraindicate the use of acetylsalicylic acid, patients who were on treatment with anticoagulants or aspirin, genetic problems in the parents, refusal of participation. **Secondary exclusion criteria:** less than 2 embryos to be transferred on day 3 or day 5.

Methodology: patients were fulfilling the inclusion criteria were recruited and then an informed written consent was taken from every patient before starting the examination to confirm fulfilling all inclusion and exclusion criteria.

The study was done after approval of ethical board of Ain Shams university and an informed written consent was taken from each participant in the study.

For the statistical analysis, quantitative data were analyzed using IBM SPSS statistics (V. 24.0, IBM Corp., USA, 2016) was used for data analysis. Data were expressed as Median and Percentiles for quantitative non-parametric measures in addition to both number and percentage for categorized data.

RESULTS

Table1: comparison between Aspirin and placebo as regard (Age, cumulus, metaphase 2, fertilization rate, grade 1 embryo, grade 2 embryo and total ET)

		N	Mean	25 Perc	75 Perc	Z	P	Sig.
Age	Placebo	90	29.5	26	37	-0.005	0.996	**NS**
	Aspirin	92	31	25.25	35.75			
Cumulus	Placebo	90	12	7	20	-0.157	0.876	**NS**
	Aspirin	92	12	8	19			
Metaphase 2	Placebo	90	10	7	15	0	1	**NS**
	Aspirin	92	10	7	15			
Fertilization rate	Placebo	90	7	4	12	-0.141	0.888	**NS**
	Aspirin	92	7	4	12			
Grade 1 embryo	Placebo	90	2	2	4	-0.884	0.377	**NS**
	Aspirin	92	2	1	4			
Grade 2 embryo	Placebo	90	2	1	4	-1.198	0.231	**NS**
	Aspirin	92	3	1	6			
Number of embryo transferred	Placebo	90	2	2	3		0.888	**NS**
	Aspirin	92	2	2	3	-0.141		

This table showed no statistically significant difference between both groups as regard (Age, cumulus, MII, fertilization rate, grade 1 embryo, grade 2 embryo and total ET). **Cumulus: total number of eggs. Grade 1 embryo: embryo with no fragmentation and equal size blastomers. Grade 2 embryo: embryo with fragmentation less than 30% and un equal size blastomers.**

Table 2: comparison between placebo and aspirin as regard clinical pregnancy

			Groups		Total (n=182)
			Placebo (n=90)	Aspirin (n=92)	
Clinical pregnancy	No	Count	36	35	71
		%	40%	38%	42.3%
	Yes	Count	54	57	111
		%	60%	62%	61%
			Value	P	Sig.
Pearson Chi-Square			0.343a	0.558	NS

This table showed statistically non-significant difference between both groups as regard clinical pregnancy

Table 3: comparison between placebo and Aspirin as regard living birth

			Groups		Toal (n=182)
			Placebo (n=90)	spirin (n=92)	
Living Birth	Negative	Count	45	43	88
		%	50%	46.7%	48.4%
	Positive	Count	45	49	94
		%	50%	53.3%	51.6%
			Value	P	Sig.
Pearson Chi-Square			0.827a	0.363	NS

This table showed statistically non-significant difference between both groups as regard living birth

Table 4: comparison between placebo and Aspirin as regards miscarriage.

		Groups		Total (n=182)
		Placebo (n=90)	spirin (n=92)	
Miscarriage	Count	9	8	17
	%	10%	8.7%	9.3%

	Value	P	Sig.
Pearson Chi-Square	0.421a	0.516	NS

This table showed statistically non-significant difference between both groups as regard miscarriage

Table Table 5: comparison between placebo and Aspirin as regard Twins.

		Groups		Total (n=182)
		Placebo (n=90)	Aspirin(n=92)	
Twins	Count	11	11	22
	%	12.2%	12 %	12.1%

	Value	P	Sig.
Pearson Chi-Square	.000[a]	0.000	NS

This table showed statistically non-significant difference between both groups as regard twins

Table 6: comparison between placebo and Aspirin as regards embryo transfer day.

			Groups		Total (n=182)
			Placebo(n=90)	Aspirin(n=92)	
Embryo Transfer day	Day3	Count	29	31	67
		%	32.2%	33.7%	33.5%
	Day5	Count	61	61	131
		%	67.8%	66.3%	65.5%

	Value	P	Sig.
Pearson Chi-Square	.023[a]	.989	NS

This table showed statistically non-significant difference between both groups as regard embryo transfer day

DISCUSSION

Infertility is the inability of a couple to achieve pregnancy over an average period of one year (in women under the age of 35) or 6 months (in women above the age of 35) despite adequate, regular unprotected sexual intercourse[8]. Unexplained infertility**,** also called subfertility, is defined as failure to conceive after one year in couples with normal semen samples and no abnormality found during an infertility work-up. The principle treatments for unexplained infertility includes: expectant management, clomiphene citrate, intrauterine insemination alone or with controlled ovarian stimulation and In-vitro fertilization (IVF)**.** However, according to National Institute for Health and Care Excellence (NICE) guidelines; IVF is the recommended treatment to women with unexplained infertility who have not conceived after 2 years of regular unprotected sexual intercourse**.** The clinical pregnancy rate among women undergoing fresh embryo transfer (ET) after IVF or ICSI varies from 20 to 35% per transfer [9].

The main factors that affect the outcome of IVF and ICSI treatments are age of the women, number of oocytes retrieved, quality of the embryos, number of embryos transferred, success of embryo transfer and endometrial receptiveness [10]. Low-dose aspirin may improve uterine and ovarian perfusion also it may enhance endometrial receptivity and ovarian responsiveness as well, which could result in better implantation and pregnancy rates after IVF or ICSI treatment. It has also been suggested in the literature that low-dose aspirin would lead to an increased number of oocytes in IVF/ICSI as well as a higher pregnancy rate [4]. In our study we tried to assess the efficacy of low dose aspirin tratment in increasing the clinical pregnancy in women undergoing IVF /ICSI.

This study included 200 infertile women undergoing IVF/ICSI were assessed to join the study in which 8 cases were excluded (5 did not meet the inclusion criteria and 3 refused to participate). A total of 192 cases where divided in to 2 groups (Aspirin and placebo) with 96 cases in each group.

Group A (Aspirin group): received one tablet of acetylsalicylic acid (aspirin)^{R} as a single dose, each tablet 75 mg at home, acetylsalicylic acid were started on the first visit and it continued till confirmation of pregnancy.

Group B (Control group): this group was received placebo tablets as a single daily dose. Aspirin group with a total of 96 patients recruited to join the study, 4 cases lost the follow up (3 cases not fertilized and 1 case with difficult embryo transfer). A total of 92 cases were analyzed of which 57 cases got pregnant (62% of cases), 49 patients showed living births (53.3%), 8 patients resulted in miscarriage (8.7%) and 11 patients showed Twin pregnancy (12%). While placebo group with a total of 96 patients recruited to join the study, 6cases lost the follow up (3 cases not fertilized and 3 cases with difficult embryo transfer). A total of 90 cases were analyzed of which 54 cases got pregnant (60% of cases), 45patients showed living births (50%), 9 patients resulted in miscarriage (10%), and 11 patients showed Twin pregnancy (12.2%). The clinical pregnancy rate (1^{ry} outcome) was 62% in aspirin group compared to 60% in placebo group, So, this study showed no statistically significant difference between groups as regard clinical pregnancy rate. In this study demographic data (Age, duration of infertility) of women in both groups were similar. There were no significant differences in demographic data between two groups. Also there were no statistical differences as regards the cycle characteristics i.e. duration of induction, mean number of oocytes in MII transferred between two groups.

In results of this study the mean +/-SD age was similar in the treatment and control groups (35.9 +/- 4.2) versus 35.4 +/- 3.9 years respectively. Also the mean number of embryos transferred was identical in both groups (3.3 embryos; range 2-5). At least two good quality embryos were transferred in all patients. This study showed that the mean +/-SD number of follicles of mm on the day of Hcg administration was 19.8 +/- 7.2 versus 10.2 +/- 5.3 for the treatment and control groups respectively; which showed significantly difference.

The mean (+/- SD) number of oocytes retrieved for the treatment group was (16.2 +/- 6.7), versus (8.6 +/- 4.6) for the control group which was with significant difference. The mean (+/-SD) level of serum E2 on the day of Hcg administration was also significantly higher in the treatment group than in the control group (2, 923.8 +/- 1, 023.4) versus (1, 614.3 +/- 791.7) pg/ml, respectively.

Therefore the three indices used to determine ovarian response as Ovarian responsiveness was expressed as the number of follicles of 0.15 mm on the day of hcg administration, the number of oocytes retrieved, and serum E2 levels on the day of hcg administration were **significantly higher in the treatment group** than in the control group. So that this study demonstrated that low dose aspirin treatment **significantly improved ovarian response,** uterine and ovarian blood flow velocity in patients undergoing IVF. These results were in **disagreement with our study.** This study was a retrospective analysis of 316 consecutive IVF cycles comparing women who were treated with

low-dose aspirin versus those who did not receive aspirin treatment. Daily oral administration of aspirin 100 mg or placebo was started together with the oral contraceptive pill prior to stimulation, and was continued until confirmation of pregnancy by detection of fetal heart activity on vaginal ultrasound at 6 weeks and 3 days of amenorrhea.

Stimulation was performed with the short gonadotrophin-releasing hormone agonist protocol (Decapeptyl), 0.1 mg daily for 7 days, started on Day 5 after pill stop) and human menopausal gonadotrophin (hMG) 150–300 IU daily from Day 7 after pill stop until human chorionic gonadotrophin (hCG) administration. When at least half of the follicles were 18–20 mm in diameter, hCG 5000 IU was given subcutaneously. Oocyte retrieval took place 35 h after hCG administration. The oocytes were fertilized by routine IVF or ICSI technique. One or two embryos were transferred on Day 3 following oocyte retrieval. The number of embryos transferred was based on age of the patient and embryo quality. In this study, included 181 women (the aspirin group 93, the placebo group 88) underwent an embryo transfer.

The two groups did not differ significantly regarding age, cycle number or subfertility status. Also the use of different gonadotrophins, the variable starting dose of gonadotrophins and the use of different forms of luteal support was, due to the randomization, equally divided between the groups. Mean duration of stimulation and mean total dose of gonadotrophins were not different for the two groups. There were 31 clinical pregnancies (32%) in the aspirin group versus 30 (31%) in the placebo group.

This difference was not significant. In the aspirin group, 24 live births (including three twins) were obtained versus 27 live births (including four twins) in the placebo group. This lower live birth rate in the aspirin group (25%) versus controls (28%) is not statistically different. This differs from our study which shows higher pregnancy rate in both groups (80% and 77%).

This also differs from our study in percentage of living births which is higher in the Aspirin group (80%) compared to (77%) in the placebo group. However, living birth rate is lower in the Aspirin group (25%) compared to (28%) in the placebo group in this study. Our results confirmed that no need for Aspirin to be added before IVF as it showed no improvement in the clinical pregnancy rate. However, further studies are needed to confirm our study.

RECOMMENDATIONS

The use of Aspirin before IVF is not associated with a higher pregnancy rate. Therefore, no need for Aspirin to be added before IVF, However, further studies are needed to confirm our study.

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