



The Effect of Aspirin and Sildenafil on Endometrial Thickness, Oocyte Characteristic, Embryo quality and Pregnancy test in Iraqi Infertile Women Undergoing Intracytoplasmic Sperm Injection

Balsam Qahtan Mohammed ¹, Huda Ali Hussaini ², Wasan Adnan AL-Jubori ¹

¹ High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al Nahrain University, Baghdad, Iraq.

² College of Medicine, Al Nahrain University, Baghdad, Iraq.

balsam.mohammed@ierit.nahrainuniv.edu.iq

ABSTRACT

Received:
07-Sep- 2022
Accepted:
30-Sep-2022
Published:
08-Oct-2022

Infertile couples undergoing Assisted Reproductive techniques are really receptive to any intervention intended to enhancement their fertility. Successful implantation of an embryo requires a receptive endometrium, a good quality embryo, and embryo endometrial synchronization in all species. Pharmacological agents such as aspirin and sildenafil have been included in previous clinical trials to improve endometrial vascularity and perfollicular blood flow but the results were inconsistent. To evaluate the effect of oral low dose aspirin or vaginal sildenafil citrate on endometrial thickness, retrieved oocyte, degree of maturation, embryo quality and pregnancy test. This comparative prospective study was conducted on the 60 infertile women who participated in intracytoplasmic sperm injection (ICSI) cycles at the infertility clinic associated with the "High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al- Nahrain University / Baghdad/ Iraq." December 2020 to June 2022. In the present study the count of total oocytes retrieved was significantly higher in aspirin treated group in comparison to sildenafil treated group 12.5 ± 5.3 versus 9.37 ± 5.5 respectively. No significant variation in mean endometrial thickness among study groups. Regarding biochemical pregnancy test, positive results were obtained in 12 (40.0 %), 10 (33.3 %) in aspirin treated group, sildenafil treated group respectively and the difference was not significant. Aspirin treatment resulted in higher number of total retrieved oocytes, aspirin and sildenafil did not improve endometrial thickness, but they resulted in improvement of sub endometrial vascularity; however, biochemical pregnancy test was not affected by either pharmacological agent.

How to cite:

Balsam Q. Mohammed; Huda A. Hussaini; Wasan A. AL-Jubori; The Effect of Aspirin and Sildenafil on Endometrial Thickness, Oocyte Characteristic, Embryo quality and Pregnancy test in Iraqi Infertile Women Undergoing Intracytoplasmic Sperm Injection; Iraqi Journal of Embryos and Infertility Researches (IJEIR), (2022); 12 (2): 40-61.
Doi: <http://doi.org/10.28969/IJEIR.v12.i2.r4.22>

KEYWORD

ICSI, ART, Aspirin, Sildenafil citrate

1. Introduction

Infertility can be defined as a disease that can affect the lives of couples who fail to achieve pregnancy post twelve months of sexual intercourse without using any forms of contraception. Medical evaluation of infertility and providing treatment is vindicated through the clinical findings from the couples' based of case history and examination. Special attention should be provided for women over the age of 35 years (Gurunath et al., 2011 [1]) .

Types of infertility: Primary infertility can be defined as the premiant failure to conceive a child after one year of unprotected sexual intercourse. This is due to the failure in initiating a pregnancy or to maintain a pregnancy till term (WHO, 2018 [2]). Secondary infertility This condition can be defined as the failure to conceive a child or to maintain a pregnancy to term after a successful pregnancy (WHO 2018 [2]). Worldwide, a sizable percentage of couples will struggle with infertility.

For instance, 8–12% of couples worldwide reported experiencing infertility (Kumar and Singh, 2015 [3]), and it was predicted that one of every seven couples in the UK reported experiencing infertility (National Institute for Health and Clinical Excellence, 2013 [4]).

Infertility is typically brought on by an anomaly in either the male or female genital system. About 30% of cases of infertility are caused by male causes, 30% by female factors, and 10% by combination issues in both parts. The syndrome is unexplained and is reported in about 30% of cases where both males and females tested negative for an identifiable reason (WHO 2018 [2]). It might be caused by abnormalities of either the ovulation process or the uterine function, in addition to oviductal occlusions and other peritoneal factors that all together could contribute to women's infertility. Variations in the cervix of the uterus were also showed to cause a number of incidents of infertility. However, these

conditions are rarely to occur (**Lindsay and Vitrikas, 2015 [5]**) .

Aspirin's significance in helping infertile women conceive is debatable, and the research is conflicting (**RCOG 2003 [6]**). Aspirin has been shown in certain studies to be beneficial for those going ART interventions (**Rubinstein et al.1999 [7]**), contradicting findings from other studies (**Urman et al., 2000 [8]**). According to Rubinstein (1999 [7]), It is believed that aspirin can enhance the blood flow to the uterus and the ovaries and was found to block platelet cyclooxygenase, which stops the generation of thromboxane and prevents blood clots in the placental plexus. Aspirin has also been shown activity to boost levels of leukotriene, which is correlated positively with better conception. In fact, moderate concentrations of Aspirin were observed to stimulate the production of tissue interleukin-3 (**Fishman 1995 [9]**).

Sildenafil citrate enhances uterine blood flow and causes endometrial

growth brought on by estrogen (**Richter et al., 2007 [10]**). Patients with a thin endometrium can use sildenafil citrate; it works well to promote endometrial development and pregnancy outcomes (**Razieh et al., 2013 [11]; Tehraninejad et al., 2018 [12]**). However, findings those of in favor of the clinical actions of sildenafil citrate to infertility were challenged by other published studies (**Kansouh AM, El-Naggar, 2017 [13]**).

Intracytoplasmic sperm injection (ICSI) procedure includes sperm injection inside the oocyte by utilizing a special devise capable of holding and injecting the gametes by passing the zona layers and the oocyte's plasma membrane (**Simopoulou et al., 2016 [14]**). This method is considered superior to conventional IVF due to a number of reasons, such as achieving higher fertilization and pregnancy rates with ICSI. Also, live birth rates (LBRs) is irrespective to different semen parameters as most of these abnormal parameters can be bypassed by

injecting the sperm directly (**Guo et al., 2018 [15]; and Luna et al., 2011 [16]**). The technique also can be applied in infertility conditions related with unexplained infertility, high women's age, fertilization failure, PGD, oocyte in vitro maturation, and in conditions where the oocyte has been cryopreserved (**Gianaroli et al., 2012 [17]**). Oocyte maturation stage

, it is essential to remove the granulosa cells surrounding the oocyte (corona cells); this would generally help in assessing the oocyte morphologically and also would help in assessing the oocyte nuclear maturation. By utilizing light microscopy, assessing nuclear oocyte maturity occurs at the metaphase stage of meiosis II at the time of PBI and becomes observable at the perivitelline space (PVS). Meiosis II is visible by the aligned chromosomes at the metaphase stage of gametogenic. Following ovulation induction and oocyte retrievals, 85% of the collected oocytes are at MII, which shows PBI, whereas about 10% of them

are at the GV stage of the MI. Oocyte characteristics such as nuclear developmental status, meiotic spindle orientation, ooplasm appearance, zona pellucida structure, and polar body presence could all be inspected post-removal of these supporting cells (**Rienzi et al., 2012 [18]**). For this, a consensus at Istanbul developed standards and terminology for the grading of oocytes, zygotes, and embryos that could be used routinely in any IVF laboratory (**Alpha Scientists, 2011 [19]**). Endometrium in the Menstrual Cycle: during the first half of the ovarian cycle, endometrium increases in thickness increasingly in response to estrogen. These changes can be observed by Ultrasound as layered of endometrium of trilaminar or 5-line (term of preference). Post ovulation time, the endometrium thickness digresses due to changes in the sub-glandular layer of the uterus. Endometrial assessment: this is routinely performed by specialists in the field as part of an overall checkup

and diagnosis of infertility (Malhotra et al., 2017 [20]). Aspirin's significance in helping infertile women conceive is debatable; Aspirin has been shown in certain studies to be advantageous for women undergoing assisted conception, whilst others have not. One of aspirin's alleged advantages is an improvement in uterine and ovarian blood flow and inhibition of platelet cyclooxygenase, which prevents thrombosis in the placental blood supply by preventing thromboxane production. (Siristatidis et al., 2016 [21]). Sildenafil citrate enhances uterine blood circulation and causes endometrial growth prompted by estrogen hormone. It was found useful for enhancing endometrial development and pregnancy outcomes in people with a thin endometrium. However, the medication has also been shown not to influence the endometrium development (Han et al., 2013 [22]).

2. Patients and Methods

The current work has gotten ethical approval, according to the Local Medical Ethical Committee of the "High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University." Additionally, each woman who agreed to participate in the study gave written consent. Subfertile females were completely examined, including gynecological and general examination and weight (BMI); investigations were also accomplished, including baseline hormonal evaluation at the follicular phase as early as day one or two of the cycle. Transvaginal sonography, hysterosalpingography, or Hycosy were performed to evaluate the cavity of the uterus and assure patency of tube. Reserve of the ovary was assured based on assessment of antral follicle count, serum level follicular stimulating hormone, serum level of Anti-Müllerian hormone, and E2 measured at (early follicular phase). Antagonist

stimulation protocol of the ovary was applied to all enrolled infertile females .

They were categorized into two groups randomly. Patients in Group I (n = 30) received low-dose aspirin (81 mg) during controlled ovarian stimulation till the day of HCG, while those in Group II (n = 30) received sildenafil as 25 mg four times daily vaginally from the day of stopping of the cycle to the day of HCG .

Exclusion and Inclusion criteria:

Inclusion criteria: subfertile women 18-42 years old. Females underwent fresh IVF/ICSI cycles based on an antagonist protocol. Infertility causes due to female factors and male factors, combined and unexplained. Cases with previous IVF/ICSI cycles failure. FSH <12 and normal endometrial cavity

Exclusion criteria: Patient with chronic disease (such as Asthma, CVD, diabetes, hypertension, peptic ulcer, liver, or kidney disease). Patients on nitrates and alpha-blockers, Congenital uterine malformations, uterine pathology (fibroid, polyp), and

previous ovarian surgery, and Polycystic ovarian syndrome (PCOS) .

Intracytoplasmic sperm Injection (ICSI) Program Ovarian stimulation protocol:

In the current study, the flexible GnRH antagonist protocol was utilized. It comprised ovarian stimulation with recombinant FSH (r-FSH) harboring 75 IU of FSH /vial by regular subcutaneous injection with doses depended on the BMI, age of women, previous response to ovulation induction, and AFC. The r-FSH started on day two of the cycle with or without human menopausal gonadotropin (hMG) 75 IU of urinary FSH and LH /ampule, based on the requirement intramuscularly, were given. Transvaginal ultrasound was performed on the fifth day of stimulation, and a consequent scan had been done every two to three days as required. GnRH antagonist injections (Cetrorelix 0.25 mg) were initiated at a time when the follicles that are leading achieved a diameter of 13-14 mm with

multiple dose regimen, in which Cetorelix 0.25 mg has been given every day until the day of trigger, at which time at least two to 3 follicles achieved an average diameter of 18 mm (Copperman, and Benadiva 2013 [23]). The follicular growth is monitored by E2 level and trans-vaginal ultrasound until the day of trigger. Retrieval of oocytes was performed by transvaginal ultrasound-guided aspiration 34-36 hours after the injection of the trigger. Oocyte preparation and maturity evaluation: Categorization of oocytes with respect to its level of maturity was carried out following the denudation of its corona layers and cumulus. The assessment of oocyte nuclear maturity was based on the existence of “an extruded polar body (first) (Rienzi et al., 2012 [18]) .Oocyte preparation and maturity evaluation, Intracytoplasmic sperm injection procedure, Embryo Transfer, Support of Luteal Phase

Statistical analysis :

The statistical software for social sciences was used to analyze the data

(SPSS, IBM, Chicago, USA, version 23). Quantitative data were expressed as mean, standard deviation, and range, whilst qualitative data were expressed as numbers and percentages. Chi-square test was used to compare proportions between two groups, whereas an independent sample t-test was used to compare means between two groups. To find the correlation between quantitative or ordinal variables, the Pearson correlation was utilized. The significance level was set at $p \leq 0.05$.

3. Results

Comparison of Stimulation characteristics of infertile women participating in the present study among study groups is shown in (Table 1). There was no significant difference in mean endometrial thickness and mean dose of gonadotropin ($p > 0.05$) among study groups. Comparison of oocyte characteristics of infertile women participating in the present study among study groups is shown in

(Table 2). Total oocyte count was significantly higher in the aspirin-treated group in comparison with the sildenafil-treated group, 12.67 ± 5.32 versus 9.37 ± 5.57 , respectively ($p = 0.035$). However, other characteristics showed no significant difference ($p > 0.05$). Comparison of embryo characteristics of infertile women participating in the present study is shown in **(Table 3)**. There was no significant difference in grade 1 and grade 3 embryo counts and in the count of transferred embryos ($p > 0.05$). However, the count of grade 2 embryos was highest in the aspirin group, followed by the sildenafil group, and the difference was significant ($p = 0.026$). Comparison of primary outcome (pregnancy test) among study groups is shown in **(Table 4)**. Positive pregnancy test was reported in 12 (40.0 %) of women in the aspirin group, and 10 (33.3 %) of women in the sildenafil group, but the difference was insignificant from a statistical perspective. In this section, all enrolled

women were considered as a single group in order to evaluate the bi-serial correlation of pregnancy test results (positive versus negative) to other characteristics, and the results were demonstrated in tables through . Correlation of pregnancy test to endometrial thickness and dose of Gonadotropins of all enrolled infertile women is shown in **(Table 5)**. Pregnancy outcome was significantly and positively correlated to endometrial thickness ($r = 0.245$; $p = 0.021$), indicating that the greater thickness of the endometrium is correlated to better pregnancy outcome. Moreover, pregnancy outcome was significantly and positively correlated to the dose of gonadotropin ($r = 0.253$; $p = 0.016$). Correlation of pregnancy test to embryo characteristics of all enrolled infertile women is shown in **(Table 6)** in which pregnancy outcome was positively and significantly correlated to grade embryo count (good quality embryo) ($r = 0.501$; $p < 0.001$). Positive pregnancy was not correlated

to grade 2 embryo count or grade 3 embryo ($p > 0.05$); however, it was positively and significantly correlated to the count of transferred embryos ($r = 0.306$; $p = 0.019$). Number of oocytes,

MII, GV oocytes, maturity index, and fertilization rate. Serum BPA is positively correlated with MI and abnormal oocytes.

Table (1): Comparison of endometrial thickness and gonadotropins dose of infertile women among study groups

Characteristic	Aspirin group <i>n</i> = 30	Sildenafil group <i>n</i> = 30	<i>p</i>
Endometrial thickness			
Mean ±SD	8.51 ±1.06	8.98 ±1.34	0.226 O NS
Range	6.5 -9.6	7 -11	
Dose of Gonadotropin			
Mean ±SD	1615.00 ±335.83	1997.50 ±839.83	0.061 O NS
Range	1350 -2250	1125 -3850	

n: number of cases; SD: standard deviation; O: one way ANOVA; C: chi-square test; NS: not significant

Table (2): Oocytes characteristic of infertile women participating in the present study

Characteristic	Aspirin <i>n</i> = 30	Sildenafil <i>n</i> = 30	<i>P</i>
Total oocyte			
Mean ±SD	12.67 ±5.32	9.37 ±5.57	0.035 O*
Range	4 -19	3 -26	
Immature Metaphase I oocyte count			

Mean ±SD	1.50 ±1.36	2.60 ±1.24	0.117 O NS
Range	0 -4	0 -8	
Mature Metaphase II oocyte count			
Mean ±SD	6.37 ±4.21	7.77 ±4.21	0.301 O NS
Range	2 -18	3 -14	
Injected oocyte count			
Mean ±SD	7.23 ±4.22	8.77 ±4.92	0.191 O NS
Range	2 -18	3 -16	
Number of fertilized oocytes			
Mean ±SD	6.07 ±2.77	5.37 ±2.99	0.257 O NS
Range	2 -12	2 -11	

n: number of cases; SD: standard deviation; O: one way ANOVA; C: chi-square test; NS: not significant; *: significant at $p \leq 0.05$

Table (3): Embryo characteristics of infertile women participating in the present study

Characteristic	Aspirin group n = 30	Sildenafil group n = 30	p
Grade1embryo			
Median (IQR)	2 (2)	2 (2.25)	0.572 K NS
Range	0 -10	0 -6	
Grade2embryo			
Median (IQR)	2 (2.25)	1 (2.25)	0.026 K *
Range	0 -10	0 -6	
Grade3embryo			

Median (IQR)	0 (0)	0 (0)	0.903 K NS
Range	0 -5	0 -3	
Number of transferred embryos			
Median (IQR)	3 (2)	2.5 (2)	0.755 K NS
Range	2 -4	2 -4	

n: number of cases; IQR: inter-quartile range; K: Kruskal Wallis test; NS: not significant; *: significant at $p \leq 0.05$

Table (4): Comparison of pregnancy tests among study groups

Characteristic	Aspirin group <i>n</i> = 30	Sildenafil group <i>n</i> = 30	<i>p</i>
Pregnancy test			
Positive, <i>n</i> (%)	12 (40.0 %)	10 (33.3 %)	0.234 C NS
Negative, <i>n</i> (%)	18 (60.0 %)	20 (66.7 %)	

n: number of cases; C: chi-square test; NS: not significant

Table (5): Correlation of pregnancy test to Endometrial thickness and Dose of Gonadotropins

Characteristic	Pregnancy test	
	<i>R</i>	<i>P</i>
Endometrial thickness	0.245	0.021 *
Dose of gonadotropin	0.253	0.016 *

r: correlation coefficient; *: significant at $p \leq 0.05$

Table (6): Correlation of pregnancy test to embryo characteristics of all enrolled infertile women

Characteristic	Pregnancy test	
	<i>R</i>	<i>P</i>
Grade1 embryo count	0.501	<0.001 ***
Grade 2 embryo count	0.024	0.822 NS
Grade 3 embryo count	-0.042	0.692 NS
Count of transferred embryos	0.306	0.019 *

r: correlation coefficient; NS: not significant; *: significant at $p \leq 0.05$; ***: significant at $p \leq 0.001$

4. Discussion

Comparison of endometrial thickness and gonadotropin dose among study groups:

In the current study, significant variation was not observed in the mean thickness of endometrial and mean dose of gonadotropin among study categories. The lack of significant variation in the mean thickness of endometrium revealed that the use of aspirin and sildenafil did not succeed in improving this cornerstone parameter in association with ART outcome .

In one recent Iraqi study, aspirin in a low dose was shown to increase endometrial thickness in the intervention group in comparison with the placebo group (**Jamel and Abid-Alkareem, 2022 [24]**). In the study of (**Zhang et al., 2022 [25]**), there exists no substantial variation in the thickness of endometrium following treatment ($P = 0.302$), and our findings are in consistence with these results. However, in contrast to our finding, the further report demonstrated that aspirin at low doses profoundly improved the thickness of endometrium in women

undergoing ART (**Kaaja et al., 1993 [26]**). In a clinical trial that is randomized and controlled carried out by (**Davar et al., 2020 [27]**), the thickness of endometrium was less in women who received aspirin when compared to the reference category. There existed statistically profound variations between both categories ($p = 0.018$). In addition, in the study of (**Zhang et al., 2022b [28]**), no substantial enhancement in the thickness of endometrium was reported. In one meta-analysis done by (**Wang et al., 2017 [29]**), there existed no substantial enhancement in the thickness of endometrium with respect to low-dose aspirin. Therefore, the action of aspirin in improving endometrial thickness has no general agreement, and in order to reach a consensus, further experimental and clinical research work is needed .

The lack of significant difference in the dose of gonadotropin is necessary in such a case-control study to avoid bias in the results that may be attributed to

significant variations in a dose of gonadotropin received by various groups enrolled in the study. The significant impact of various doses of gonadotropin on ART outcomes has been proved by (**Friedler et al., 2016 [30]**).

In our study, sildenafil was also unable to improve endometrial thickness significantly. In accordance with our findings (**Moini et al., 2020 [31]**) showed that vaginal sildenafil did not improve endometrial thickness significantly. **Check et al. in 2004 [32]** also reported no significant improvement in endometrial thickness following administration, which is also consistent with our observation. The results of previous studies have shown that sildenafil citrate (vaginal or oral alone or with oestradiol) is significantly effective in improving endometrial thickness (**Paulus et al., 2002 [33]**; **Takasaki et al., 2010 [34]**; **Al-Assadi et al., 2012 [35]**).

Reports have demonstrated that sildenafil increases the width of the

vasculature by its action on arterial smooth muscles (Jerzak et al., 2010 [36]; Ballard et al., 1998 [37]). Sildenafil citrate is one of the inhibitors of the 5-phosphodiesterase enzyme, and it potentiated the effect of nitric oxide on arterial smooth muscle via prevention of the cGMP effect (Ballard et al., 1998 [37], Boolell et al., 1996 [38]). Based on the findings of several studies, the utilization of sildenafil at the time of the proliferative phase of the menstrual cycle makes improvement of endometrial growth and uterine blood flow (Sher and Fisch, 2002 [39], Sher and Fisch, 2000 [40]).

Comparison of oocytes characteristic of infertile enrolled women

In the present study, total oocyte count was profoundly greater in the aspirin-treated group in contrast with the sildenafil-treated group. However, other characteristics showed no significant difference. In line with our results, a meta-analysis carried out by

Wang et al. (2017 [29]) showed that among five reports (patients' total number =1061) which highlighted the count of retrieved oocytes, the findings demonstrated that the count of retrieved oocytes of the aspirin category was substantially greater than that of the no treatment of placebo categories. In addition, it has been shown that administration of low-dose aspirin has been shown to have no effect on oocyte characteristics in women undergoing ART (Frattarelli et al., 2008 [41]) .

Embryo characteristics of infertile women participating in the present study:

There was no significant difference in grade 1 and grade 3 embryo counts and in the count of transferred embryos; however, the count of grade 2 embryos was highest in the aspirin group, followed by the sildenafil group, and the difference was significant. In the study of Gizzo et al. (2014 [42]), the administration resulted in significantly lower-quality embryos in a low-dose

aspirin-treated group, and this is in line with our observation .

According to Gizzo et al. (2014 [42]), their clinical findings allow them to speculate that LDA administration may negatively affect physiological ovarian follicular growth, oocyte maturation, oocyte fertilization, and the quality of the resulting embryo through inhibition of COX activity, particularly the COX-2. The expression of COX-2 protein in humans starts at the stage of the secondary follicle and is withdrawn immediately before exposure to LH stimulation, as was initially shown by (Tokuyama et al., 2003 [43]). This finding raises the possibility that COX-2 and its metabolites are involved in follicular development, including the maturation of the oocyte cumulus complex .

Comparison of pregnancy test among study groups:

In our study, the best rate of positive pregnancy tests was reported in in aspirin group, followed by sildenafil, but the difference was

insignificant from a statistical perspective .In one previous randomized controlled clinical trial carried out by Davar et al. in 2020 [27], There was no statistically significant difference between the two groups (aspirin versus no therapy), and both the chemical and clinical pregnancy rates and the abortion rates were similar. (Madani et al. ,2019 [44]) We were reported that, in contrast to our findings, aspirin administration during ICSI cycles increased the rates of implantation, clinical pregnancy, and live births. In 2000, Hsieh and colleagues [45] found that taking aspirin increased the clinical pregnancy rate, but they select women with thin endometrium .Referring to and in line with our own research findings, reports by Cochrane reviews (Siristatidis et al., 2012 [46]; Siristatidis et al., 2016 [47]) and a meta-analysis (Khairy et al., 2007 [48]) also reported that pregnancy rates was not significantly improved with low-dose aspirin was used in ICSI cycles. Therefore, the

results of aspirin administration, based on our results and previous reports, are conflicting, and further research work is still needed to explore the exact way by which aspirin increases the pregnancy rate in some women and not in all women undergoing ICSI. The previous claim that the enhancement in endometrial blood flow and or ovarian follicle vascularity following aspirin administration would ensure better pregnancy results appears partly misleading, and there must be other unknown confounders that need to be adjusted in such case-control trials in order to reach better consensus about aspirin used in ART .

Correlation study of positive pregnancy test to other characteristics:

In the current study, pregnancy outcome was significantly and positively correlated to endometrial thickness, indicating that the greater thickness of endometrium is correlated to better pregnancy outcome. Moreover, pregnancy outcome was

significantly and positively correlated to the dose of gonadotropin. In addition, a positive correlation was reported to the count of and better-quality embryos. The higher pregnancy rate in association with greater endometrial thickness has been confirmed previously, and the claim was attributed to a better implantation rate in association with increased endometrial thickness (Xu et al., 2022 [49]).

6- Conclusion

Aspirin treatment resulted in a higher number of total retrieved oocytes. Aspirin and sildenafil did not improve endometrial thickness, but they resulted in an improvement of sub-endometrial vascularity; however, the biochemical pregnancy test was not affected by either pharmacological agent.

Acknowledgment

We would like to acknowledge the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies/ Al Nahrain University, Baghdad, Iraq.

Funding

This work received no funding .

Author Contribution

Balsam Q. Mohammed performed the study, and Huda A. Hussaini Wasan A. AL-Jubori supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The study was approved by the Ethical Approval Committee.

Financial Disclosure

There is no financial disclosure.

Ethical Clearance

The study was approved by the Ethical Approval Committee

References

[1] Gurunath, S., Pandian, Z., Anderson, R.A. and Bhattacharya, S. (2011) Defining infertility—a systematic review of prevalence studies, Human reproduction update,17(5), pp.575-588

[2] World Health Organization (WHO). International Classification of Diseases, 11th Revision (ICD-11) Geneva: WHO 2018

[3] Kumar, N. and Singh, A.K. (2015) ‘Trends of male factor infertility, an

important cause of infertility: A review of the literature,’ Journal of Human Reproductive Sciences, 8 (4), p. 191.

[4] National Collaborating Centre for Women’s and Children’s Health (UK). Fertility: Assessment and Treatment for People with Fertility Problems. London: Royal College of Obstetricians & Gynaecologists; 2013 Feb. PMID: 25340218.

[5] Lindsay, T. J., & Vitrikas, K. R. (2015). Evaluation and treatment of infertility. American Family Physician, 91 (5), 308–314.

[6] Infertility evaluation and management. Strategies for family physicians. Allison M Case Canadian Family Physician Nov 2003, 49 (11) 1465-1472.

[7] Rubinstein, M., Marazzi, A., & Polak de Fried, E. (1999). Low-dose aspirin treatment improves ovarian responsiveness, uterine and ovarian blood flow velocity, implantation, and pregnancy rates in patients undergoing in vitro fertilization: a prospective, randomized, double-blind placebo-controlled assay. Fertility and sterility, 71 (5), 825–829.

[https://doi.org/10.1016/s0015-0282\(99\) 00088-6](https://doi.org/10.1016/s0015-0282(99) 00088-6)

[8] Urman, B., Mercan, R., Alatas, C. et al. Low-Dose Aspirin Does Not Increase Implantation Rates in Patients Undergoing Intracytoplasmic Sperm

Injection: A Prospective Randomized Study. *J Assist Reprod Genet* 17, 586–590 (2000).

<https://doi.org/10.1023/A:1026491426423>

[9] 9. Fishman, S.B., 1995. A breath of life: Feminism in the American Jewish

[10] 10. Richter, K.S., Bugge, K.R., Bromer, J.G. and Levy, M.J., 2007. Relationship between endometrial thickness and embryo implantation, based on 1,294 cycles of in vitro fertilization with transfer of two blastocyst-stage embryos. *Fertility and sterility*, 87 (1), pp.53-59.

[11] Masoud Mirzaei, M.D., Nasim Namiranian, M.D., Razieh Dehghani Firouzabadi, M.D. and Gholami, S., The prevalence of infertility in 20-49 years women in Yazd, 2014-2015: A cross-sectional study

[12] Shahrokh-Tehraninejad, E, Aliani, F., Ashrafi, M., Arabipoor, A., Jahanian Sadatmahalleh, S. and Akhond, M.R., 2018. Comparison of the symptoms and localisation of endometriosis involvement according to fertility status of endometriosis patients. *Journal of Obstetrics and Gynaecology*, 38 (4), pp.536-542 .

[13] El-Naggar, H.A Rushdi, H.E., Saad, M.F., and Saeed, A.M., 2017. Association of chromosomal aberrations and semen quality in Egyptian buffalo bulls. *Egyptian*

Journal of Animal Production, 54 (2), pp.95-103.

[14] Simopoulou, M., Giannelou, P., Bakas, P., Gkoles, L., Kalampokas, T., Pantos, K., & Koutsilieris, M. (2016). Making ICSI Safer and More Effective: A Review of the Human Oocyte and ICSI Practice. *In vivo* (Athens, Greece), 30 (4), 387–400.

[15] Guo, N., Hua, X., Li, Y. F., & Jin, L. (2018). Role of ICSI in Non-male Factor Cycles as the Number of Oocytes Retrieved Decreases from Four to One. *Current medical science*, 38 (1), 131–136. <https://doi.org/10.1007/s11596-018-1856-7>

[16] Luna, M., Bigelow, C., Duke, M., Ruman, J., Sandler, B., Grunfeld, L. and Copperman, A.B., 2011. Should ICSI be recommended routinely in patients with four or fewer oocytes retrieved? *Journal of assisted reproduction and genetics*, 28 (10), pp.911-915.

[17] Gianaroli, L., Racowsky, C., Geraedts, J., Cedars, M., Makrigiannakis, A., & Lobo, R. (2012). Best practices of ASRM and ESHRE: a journey through reproductive medicine. *Human reproduction* (Oxford, England), 27 (12), 3365–3379.

<https://doi.org/10.1093/humrep/des338>

[18] Rienzi, L., Cobo, A., Paffoni, A., Scarduelli, C., Capalbo, A., Vajta, G., Remohí, J., Ragni, G., & Ubaldi, F. M. (2012). Consistent and predictable delivery rates after oocyte vitrification: an observational longitudinal cohort multicentric study. *Human reproduction* (Oxford, England), 27 (6), 1606–1612.

<https://doi.org/10.1093/humrep/des088>

[19] The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting." *Human reproduction* 26, no. 6 (2011): 1270-1283.

[20] Malhotra N, Malhotra J, Singh A, Gupta P, Malhotra N. Endometrial Receptivity and Scoring for Prediction of Implantation and Newer Markers. *J South Asian Feder Obst Gynae* 2017;9 (2):143-154.

[21] Siristatidis CS, Basios G, Pergialiotis V, Vogiatzi P. Aspirin for in vitro fertilisation. *Cochrane Database Syst Rev* 2016; 11: CD004832

[22] Han, M. K., Bach, D. S., Hagan, P. G., Yow, E., Flaherty, K. R., Toews, G. B., Anstrom, K. J., Martinez, F. J., & IPFnet Investigators (2013). Sildenafil preserves exercise capacity in patients with idiopathic pulmonary fibrosis and right-sided ventricular dysfunction. *Chest*, 143 (6), 1699–1708.

[23] Copperman AB, Benadiva C. Optimal usage of the GnRH antagonists: a review of the literature. *Reprod Biol Endocrinol*. 2013 Mar 15;11:20. Doi: 10.1186/1477-7827-11-20.

[24] Jamel, F.A.F. and Abid-Alkareem, I.H., 2022. Role of Aspirin in Secretory Phase in Pre-implantation Pregnancy. *Journal of Pharmaceutical Negative Results*, pp.1123-1127 .

[25] Zhang, X., Guan, Q., Yu, Q., Xiao, W., Chen, Z., Dong, C., Deng, S., Zhuang, Y. and Xia, Y., 2022. Estimating the effects of policies on infertility prevalence worldwide. *BMC Public Health*, 22 (1), pp.1-11 .

[26] Kaaja R, Julkunen H, Viinikka L, Ylikorkala O. Production of prostacyclin and thromboxane in lupus pregnancies: effect of a small dose of aspirin. *Obstet Gynecol*. 1993;81 (3):327-331.

[27] Davar, R., Pourmasumi, S., Mohammadi, B., & Lahijani, M. M. (2020). The effect of low-dose aspirin on the pregnancy rate in frozen-thawed embryo transfer cycles: A randomized clinical trial. *International journal of reproductive biomedicine*, 18 (9), 693–700.

[28] Zhang, X., Guo, F., Wang, Q., Bai, W., & Zhao, A. (2022b). Low-dose aspirin treatment improves endometrial receptivity in the mid-luteal phase in

unexplained recurrent implantation failure. International Journal of Gynaecology and Obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics, 156 (2), 225–230. <https://doi.org/10.1002/ijgo.13699>

[29] Wang, Liping MSa; Huang, Xiaman MD, MSb; Li, Xueli MD, MSc; Lv, Fang MD, PhDc; He, Xiao MD, MSc; Pan, Yu MD, PhDc; Wang, Li MD, PhDd; Zhang, Xiaomei MD, PhDc,*. Efficacy evaluation of low-dose aspirin in IVF/ICSI patients' evidence from 13 RCTs: A systematic review and meta-analysis. Medicine: September 2017 - Volume 96 - Issue 37 - p e7720 doi: 10.1097/MD.00000000000007720

[30] Friedler, S., Meltzer, S., Saar-Ryss, B., Rabinson, J., Lazer, T., & Liberty, G. (2016). An upper limit of gonadotropin dose in patients undergoing ART should be advocated. Gynecological endocrinology: the official journal of the International Society of Gynecological Endocrinology, 32 (12), 965–969.

[31] Moini, A., Zafarani, F., Jahangiri, N., Jahanian Sadatmahalleh, S. H., Sadeghi, M., Chehrazi, M., & Ahmadi, F. (2020). The Effect of Vaginal Sildenafil on The Outcome of Assisted Reproductive Technology Cycles in Patients with Repeated Implantation

Failures: A Randomized Placebo-Controlled Trial. International journal of fertility & sterility, 13 (4), 289–295. <https://doi.org/10.22074/ijfs.2020.5681>

[32] Check, J. H., Graziano, V., Lee, G., Nazari, A., Choe, J. K., & Dietterich, C. (2004). Neither sildenafil nor vaginal estradiol improves endometrial thickness in women with thin endometria after taking oral estradiol in graduating dosages. Clinical and experimental obstetrics & gynecology, 31 (2), 99–102.

[33] Paulus WE, Strehler E, Zhang M, Jelinkova L, El-Danasouri I, Sterzik K. Benefit of vaginal sildenafil citrate in assisted reproduction therapy. Fertil Steril. 2002;77 (4):846–847 .

[34] Takasaki A, Tamura H, Miwa I, Taketani T, Shimamura K, Sugino N. Endometrial growth and uterine blood flow: a pilot study for improving endometrial thickness in the patients with a thin endometrium. Fertil Steril. 2010;93 (6):1851–1858.

[35] Al-Assadi AF, Al-Rubaye SA, Laaiby Z. The effect of Sildenafil on endometrial characters in patients with infertility. Tikrit Medical Journal. 2012;18 (2):330–338 .

[36] Jerzak, M., Niemiec, T., Nowakowska, A., Klochowicz, M., Górski, A. and Baranowski, W., 2010. First successful pregnancy after

addition of enoxaparin to sildenafil and etanercept immunotherapy in a woman with fifteen failed IVF cycles—a case report. *American Journal of Reproductive Immunology*, 64 (2), pp.93-96 .

[37] Ballard SA, Gingell CJ, Tang K, Turner LA, Price ME, Naylor AM. Effects of sildenafil on the relaxation of human corpus cavernosum tissue in vitro and on the activities of cyclic nucleotide phosphodiesterase isozymes. *J Urol*. 1998;159 (6):2164–2171 .

[38] Boolell M, Gepi-Attee S, Gingell JC, Allen MJ. Sildenafil is a novel, effective oral therapy for male erectile dysfunction. *Br J Urol*. 1996;78 (2):257–261.

[39] Sher G, Fisch JD. Effect of vaginal sildenafil on the outcome of in vitro fertilization (IVF) after multiple IVF failures attributed to poor endometrial development. *Fertil Steril*. 2002;78 (5):1073–1076 .

[40] Sher G, Fisch JD. Vaginal sildenafil (Viagra): a preliminary report of a novel method to improve uterine artery blood flow and endometrial development in patients undergoing IVF. *Hum Reprod*. 2000;15 (4):806–809 .

[41] Frattarelli, J. L., McWilliams, G., Hill, M. J., Miller, K. A., & Scott, R. T., Jr (2008). Low-dose aspirin use does

not improve in vitro fertilization outcomes in poor responders. *Fertility and sterility*, 89 (5), 1113–1117. <https://doi.org/10.1016/j.fertnstert.2007.05.007>

[42] Gizzo, S., Capuzzo, D., Zicchina, C., Di Gangi, S., Coronella, M. L., Andrisani, A., Gangemi, M., & Nardelli, G. B. (2014). Could empirical low-dose-aspirin administration during the IVF cycle affect both the oocytes and embryo quality via COX 1-2 activity inhibition? *Journal of assisted reproduction and genetics*, 31 (3), 261–268. <https://doi.org/10.1007/s10815-014-0174-7>

[43] Tokuyama O, Nakamura Y, Musoh A, et al. Expression and distribution of cyclooxygenase-2 in the human ovary during follicular development. *Osaka City Med J*. 2003;49 (1):39–47.

[44] Madani T, Ahmadi F, Jahangiri N, Bahmanabadi A, Bagheri Lankarani N. Does low-dose aspirin improve pregnancy rate in women undergoing frozen-thawed embryo transfer cycle? A pilot double-blind, randomized, placebo-controlled trial. *J Obstet Gynaecol Res* 2019; 45: 156–163.

[45] Hsieh YY, Tsai HD, Chang CC, Lo HY, Chen CL. Low-dose aspirin for infertile women with thin endometrium receiving intrauterine insemination: a

prospective, randomized study. *J Assist Reprod Genet* 2000; 17: 174–177 .

[46] Siristatidis CS, Dodd SR, Drakeley AJ. Aspirin is not recommended for women undergoing IVF. *Hum Reprod Update* 2012; 18: 233.

[47] Siristatidis, C. S., Basios, G., Pergialiotis, V., & Vagiati, P. (2016). Aspirin for in vitro fertilisation. The Cochrane database of systematic reviews, 11 (11), CD004832. <https://doi.org/10.1002/14651858.CD004832.pub4>

[48] Khairy M, Banerjee K, El-Toukhy T, Coomarasamy A, Khalaf Y. Aspirin

in women undergoing in vitro fertilization treatment: a systematic review and meta-analysis. *Fertil Steril* 2007; 88: 822–831 .

[49] Xu, J., Zhang, S., Jin, L., Mao, Y., Shi, J., Huang, R., Han, X., Liang, X., & Zhang, C. (2022). The Effects of Endometrial Thickness on Pregnancy Outcomes of Fresh IVF/ICSI Embryo Transfer Cycles: An Analysis of Over 40,000 Cycles Among Five Reproductive Centers in China. *Frontiers in endocrinology*, 12,788706. <https://doi.org/10.3389/fendo.2021.788706>