Review Article

Safety and Efficacy of Follicular Fluid Flushing of the Endometrial Cavity on Outcomes of IVF/ICSI cycles in infertile Women: A Systematic Review and Meta-Analysis

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ABSTRACT

Objectives: To evaluate the endometrial thickness, implantation rate, and clinical pregnancy rates as results of intrauterine flushing during the ICSI cycle using granulosa cells and follicular fluid.

Methods: The Cochrane Library, MEDLINE, PubMed, Web of Science, Google Scholar, and Embase databases were thoroughly searched up till June 2024, in order to find relevant papers. Follicular fluid flushing of the endometrial cavity was employed in six randomized controlled trials to enhance IVF/ICSI cycle results.

Results: There is no statistical difference between the two cohorts as regards endometrial thickness, the summarized standardized mean difference (SMD) is 0.08 with a 95% confidence interval of (0.1 - 0.25). There is also no statistical difference between the two cohorts as regards implantation rate, the overall risk ratio is 0.95 with a 95% confidence interval of (0.69 - 1.3). And finally, there is no statistical difference between the two cohorts as regards clinical pregnancy rate, the overall risk ratio is 0.99 with a 95% confidence interval of (0.83 - 1.18). The test for overall effect for all outcomes does not show a significant effect.

Conclusion: It is crucial to stress that Follicular Fluid should be used extremely carefully, as it seems that flushing it into the endometrial cavity had no influence on endometrial thickness, clinical pregnancy or implantation rates, either favorably or unfavorably.

Key Words: Endometrial cavity, flushing, follicular fluid, implantation rate, IVF/ICSI.

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INTRODUCTION

The decrease in embryo implantation rate is one of the major barriers to assisted reproductive technology (ART)-aided infertility treatment. One arm of embryo implantation is the receptive endometrium. Although it isn't explicitly mentioned in the literature, impaired endometrial receptivity is thought to be a contributing factor in two-thirds of implantation failure instances[1]. A multitude of factors can impact the complex process of endometrial receptivity. Common gynecologic conditions that may impact endometrial receptivity and the efficacy of ART include polyps, adenomyosis, myomas, ….etc.[2].

For patients with these clinical problems, a number of therapies have been suggested to increase endometrial receptivity[3]. Endometrial receptivity array (ERA) may be used to determine the exact time of endometrial receptivity. Platelet-enriched plasma therapy (PRP), endometrial scratching and endometrial flushing with follicular fluid (FF) are possible treatment therapies for thin endometria, employed in the various studies to enhance the rate of implantation in these patients[4]. Enhancing endometrial thickness and responsiveness with a low-complication approach enhances fertility, lessens the necessity to delay embryo transfer cycles, and eases the therapeutic load on patients[5].

Vascular endothelial growth factor (VEGF), transforming growth factor (TGF) and insulin-like growth factor (IGF) are among the growth factors and cytokines found in follicular fluid, which envelops the granulosa-oocyte complex. These substances are essential for the success of natural fertilization and may have paracrine or autocrine effects on embryo implantation[6–10].

Research on flooding the uterus with follicular fluid and granulosa cells is justified by the characteristics of these substances and how they may affect fertility. During the physiological processes of ovulation, follicular fluid is released into the fallopian tube and reaches the uterus[11]. Follicle formation, maturation, and atresia are regulated by the granulosa cells that envelop the oocytes. These cells are alive, able to generate progesterone, and can be separated from the FF & can secrete progesterone for several days[12].

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Investigators intend to improve the conditions for implantation and conception by mixing these ingredients in a uterine flush. To completely comprehend the possible advantages and workings of this strategy, more investigation is necessary.

In order to assess the endometrial thickness, implantation rate, and clinical pregnancy rates as results of uterine cavity flushing with follicular fluid along with granulosa cells during the IVF/ICSI cycle, this meta-analysis was conducted.

METHODOLOGY

Plan for data Sources, and publication Search

Following the guidance of the favorable report materials for Systematic Reviews and Meta-Analysis (PRISMA) guidelines and the Cochrane guidelines, the current systematic review was conducted. The Cochrane Library, MEDLINE, PubMed, Web of Science, Google Scholar, and Embase databases were used in this search. Search terms used in the headline or abstract of English-language articles released between the database's launch and June 2024 were: "In *Vitro* Fertilization" (IVF), "Intracytoplasmic sperm injection" (ICSI), "Embryo transfer," and "Follicular Fluid", "FF", "Endometrial receptivity", and "Endometrial Flushing". Furthermore, a manual search was conducted through references found in candidate articles and reviews to find other pertinent reports.

Outcome Measures, Study Selection, and Data Extraction

The following endpoints have been documented in some of the papers: endometrial thickness (ET), rate of clinical pregnancy (CPR) and rate of implantation (IR). Studies that used quasi-experiments and randomized controlled trials (RCTs) were evaluated. Following a search using keywords of the database, Two authors independently reviewed each study's abstract. The other writer independently extracted the data using the entire text versions of the pertinent papers.

Inclusion and Exclusion Criteria for This Review

Research that met the following requirements was considered for inclusion in our review: (1) the research study was a randomized controlled trial, (2) clinically confirmed pregnancy outcomes as the endpoints; (3) the treatment involved endometrial flushing with follicular fluid at the date of oocyte retrieval; (4) the population were scheduled for IVF/ICSI; (5) the control group consisted of any other therapy, no treatment, or a placebo. Research that were self-control, case-control, case series, or cross-sectional were not included. Additionally, papers were disregarded if we could not find sufficient information about the approach or findings.

Risk of bias assessment

Using the Cochrane Risk of Bias assessment tool outlined in the Cochrane Handbook for Systematic Reviews of Interventions 5.1.0, two authors independently evaluated the quality of the included studies. Six domains comprise this tool are shown in (Figure 2). The included RCTs were rated as having "low risk," "high risk," or "unclear risk" of bias by the authors. The two writers' disagreements were settled by consensus and consultation with a senior reviewer..

Data synthesis

Using Review Manager software version 5.4 (Nordic Cochrane Centre, Cochrane Collaboration, Denmark), two authors independently conducted the meta-analysis. A senior author reconciled any differences by conversation after comparing the results' consistency. Continuous data was pooled using the standards mean difference (SMD) with 95% confidence interval (CI). For meta-analyses, we employed the Mantel-Haenszel and Inverse-Variance approaches, respectively. I-square and chi-square tests were used to measure heterogeneity; low heterogeneity was classified as I2 <30%, moderate as 30%-50%, and high as >50%. I2 test >50 and chi-square test $p<0.1$ both showed significant heterogeneity. The fixed-effects and random-effects models were used to assess the homogeneous and heterogeneous outcomes, respectively. A *p-value* of less than 0.05 was considered statistically significant.

RESULTS

Study Selection

At first, 180 articles were analyzed. The method of screening produced 102 potential matches from the databases after deleting repeats; 86 of these studies were then eliminated for further examination based on their abstract. Nine studies did not match the inclusion criteria; nine of the papers were case series, case reports, and single-arm research; one study was excluded; and one study was submitted in only abstract form and removed from the analysis because of insufficient data. Six articles in total that met the selection criteria were subsequently subjected to further examination. (Table 1) lists the essential details for each of the six studies that were examined in this evaluation. These were made available between 2006 and 2024. Over the course of the six investigations, a total of 730 women were enrolled. The enrollment and selection procedures for studies are depicted in the flowchart below (Figure 1).

Table 1: contains the essential details for each of the six research that this review examined.

Reference	no.	CPR(I)	CPR (C)	ET(I)	ET (C)	IR (I)	IR (C)	outcome	Conclusion
Hamdi K. *et al*. 2018[13]	110	30.90%	38.20%	11.52 ± 2.57	18.79 ± 3.72	11.52%	18.79%	IR,CPR	neither improves nor adversely affects the outcome
Gaafar S. *et al*. 2024[14]	60	62.50%	44.40%	9.9± 3.7	9.7± 3.5	0.00%	0.00%	CPR,ET	does not improve clinical pregnancy rate. May be in previous ICSI failure
Hashish N.*et al*. 2014[15]	100	34%	31%	10.1± 1.5	10 ±2.3	18.60%	11.30%	CPR,ET,IR	neither improved nor adversely outcome
Salama K. *et al*.2015[16]	80	35%	25%	0	0	10.50%	9.80%	IR,CPR	May improves the outcome
Berkkanoglu M. *et al*. 2006[17]	240	45.20%	51.40%	0	0	20.00%	21.20%	IR,CPR,OPR	neither improves nor adversely affects the outcomes.
Hosseini E. *et al*. 2024[18]	140	38.50%	42.90%	10.14 ± 1.53	10.16 ± 1.72	24.10%	27%	CPR,ET,IR	no effect, either positively or negatively, on clinical outcomes.

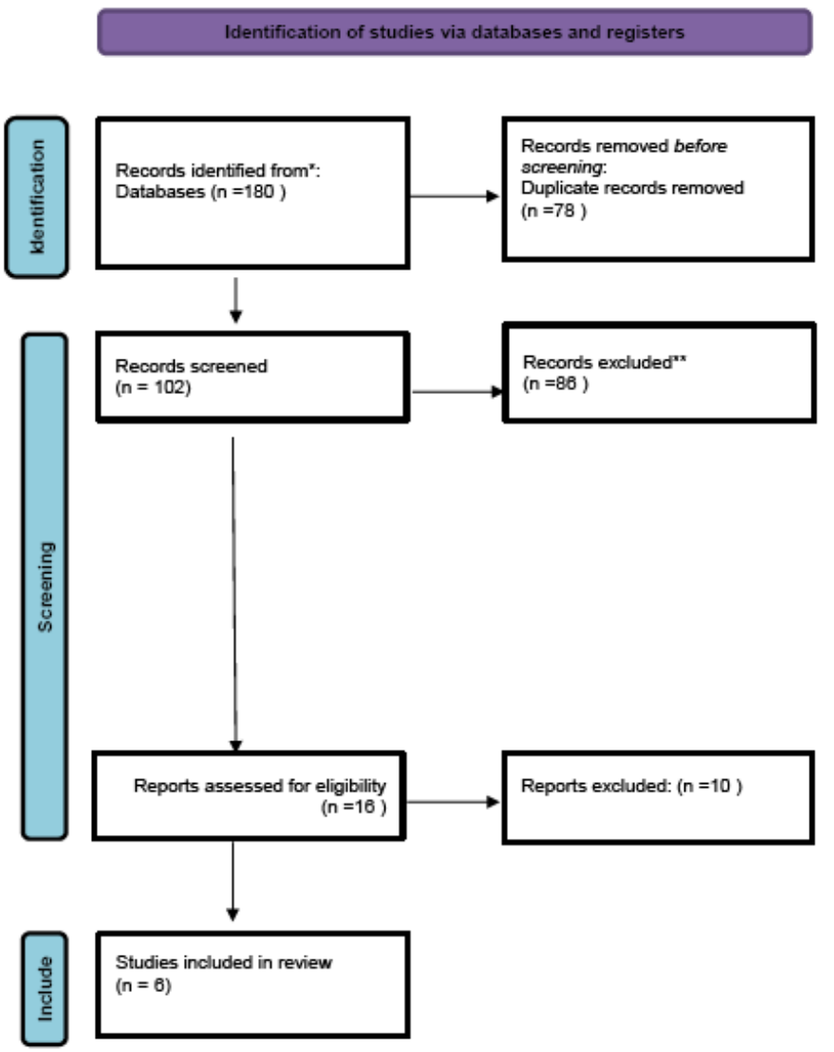

Fig. 1: PRISMA chart of Study selection

Fig. 2: Risk of bias summary for the included studies

Outcomes of the meta-analysis

Endometrial thickness

All together 4 cohorts were scrutinized with a total of 205 subjects in the Experimental cohort and 205 subjects in the Control cohort. Based on the analysis performed using random effects model with Inverse variance method to compare the standardized mean difference (SMD), there is no statistical difference between the two cohorts as regards endometrial thickness, the summarized standardized mean difference (SMD) is 0.08 with a 95% confidence interval of -0.1 - 0.25.The test for overall effect does not show a significant effect. No significant heterogeneity was observed, suggesting that the effect sizes across studies remained uniform in both scale and direction (Figure 3).

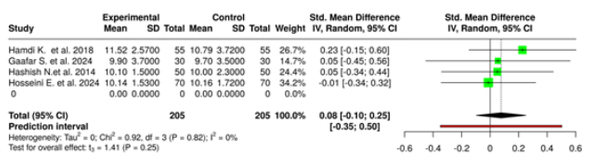

Fig. 3: Forest plot of pooled effect on endometrial thickness

Implantation rate

A total of 5 cohorts were scrutinized with a total of 365 subjects in the Experimental cohort and 365 subjects in the Control cohort. Based on the analysis performed using random effects model with Mantel-Haenszel method to compare the risk ratio, there is no statistical difference between the two cohorts as regards implantation rate, the overall risk ratio is 0.95 with a 95% confidence interval of 0.69 - 1.3. The test for overall effect does not show a significant effect. Significant heterogeneity was not observed, suggesting that the effect sizes across studies were uniform in both magnitude and direction (Figure 4).

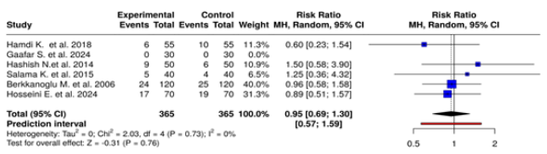

Fig. 4: Forest plot of pooled effect on implantation rate

Clinical pregnancy rate

A total of 6 trials were analyzed with a total of 365 subjects in the Experimental cohort and 365 subjects in the Control cohort. Based on the analysis performed using random effects model with Mantel-Haenszel method to compare the risk ratio, there is no statistical difference between the two cohorts as regards clinical pregnancy rate, the overall risk ratio is 0.99 with a 95% confidence interval of 0.83 - 1.18. The test for overall effect does not show a significant effect. We did not observe significant heterogeneity, signaling that the effect sizes across cohorts were consistent in both magnitude and direction (Figure 5).

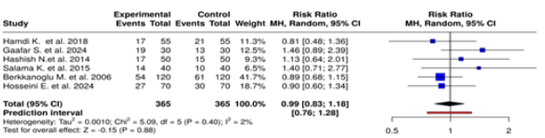

Fig. 5: Forest plot of pooled effect on clinical pregnancy rate

DISCUSSION

According to the current meta-analysis, endometrial thickness, clinical pregnancy, and implantation rates in women receiving IVF/ICSI treatment were not impacted in any way by flushing the uterine cavity with FF from mature follicles after oocyte harvest. Follicular fluid has a variety of effects on endometrial receptivity.

Some research have linked follicular fluid to the emergence of endometriosis and ovarian cancer, whereas other investigations have proposed that follicular fluid constituents can modulate gene expression, cell proliferation, and differentiation to positively impact endometrial receptivity[19]. Evidence suggests that human follicular fluid may change the receptivity of the endometrium. In this regard, a randomised controlled study (RCT) including 100 subfertile women who received ICSI was published by Hashish *et al.*[15]. They showed that improving clinical pregnancy and implantation rates was possible by injecting two milliliters of one M2 oocyte follicular fluid into the uteri of subfertile women three days before to ET. The treatment groups and the control group (no uterine flushing) did not, however, vary substantially in this increment. They proposed that follicular fluid produced from multiple mature oocytes should be injected, speculating that the lack of significant differences between the two groups was caused by insufficient follicular fluid taken from a mature oocyte.

In an RCT, Hamdi *et al.*[13] flushed the endometrium of 55 subfertile women utilizing follicular fluid from 2 to 3 follicles, as contrasted to their non-flushed counterparts. Regarding clinical or chemical pregnancy rates or implantation rates, they found no discernible differences between the treatment and the placebo groups.

Hormones, growth factors, cytokines, and immunological chemicals are among the biologically based follicular fluid components. Hormones in follicular fluid vary greatly depending on the stage of folliculogenesis[20,21]. Furthermore, there are numerous kinds of growth factors present in human follicular fluid.

It has been demonstrated that the concentrations of activin and inhibin in follicular fluid can accurately forecast the pregnancy rate and the quality of oocytes and embryos on days two or three[22]. Together with other growth factors including VEGF, LIF, TGF-α, and others, inhibitors and activin enhance endometrial receptivity and embryo implantation[23].

Before and during ovulation, the ovaries and follicular fluid produce proinflammatory cytokines. For instance, luteal granulosa cells produce IL-1β locally. High amounts of cytokines are linked to higher rates of fertilization. It has also been shown that other ILs present in follicular fluid, such as IL-2, IL-6 , increase the embryo's capacity for implantation[24,25].

All of the aforementioned elements must be present for the endometrium to become responsive. Exposure to oestrogen, progesterone, immune cells, and VEGF expressions in succession results in the formation of a receptive endometrium[26].

However, a number of research have looked into follicular fluid 's capacity to up-regulate endometrial receptivity genes because it includes a high concentration of powerful mitogen factors for cell proliferation[27]. In *vitro* research examined the impact of follicular fluid on the expression of important genes related to endometrial receptivity that have been shown to be involved in the implantation process, such as LIF and HOXA in endometrial stromal cells. All of the genes under investigation were expressed more when endometrial stromal cells were exposed to follicular fluid. However, after 72 hours of incubation with 20% follicular fluid, there were no cytotoxic consequences on endometrial stromal cells that were dependent on either time or dose[28].

On the other hand, follicular fluid is a factor that causes cancer. According to Bahar-Shany *et al*., exposure to follicular fluid caused double stranded DNA breaks and elevated the expression of genes linked to inflammation. The primary non-genetic risk indicator for ovarian cancer, ovulation, was taken into consideration[29]. Furthermore, follicular fluid -induced substantial cell proliferation in *vitro* suggested a favorable environment for endometrial cell growth. It has been demonstrated that follicular fluid derived from patients with endometriosis causes a greater proliferation of endometrial cells than follicular fluid extracted from women without the condition[30].

The impacts of follicular fluid on the endometrium and fallopian tubes can be examined using molecular and in *vitro* methods. Finding the concentration that affects implantation the most is crucial. As an alternative, separate research should be done on the immunological elements of follicular fluid and their effects on implantation. When evaluating the cost-benefit of follicular fluid in clinical practice, more factors need to be considered.

We did our best to take in account all potential confounding factors in this meta-analysis; however, some drawbacks have been emerged. Firstly , the relatively small number of studies conducted as regards this area of research. Secondly, embryo quality should be addressed to eliminate its effect on IVF outcome as a failure of clinical pregnancy could also be attributed to poor embryo quality or other factors, and not linked to aberrant endometrial receptivity.

On the other hand, strength points of our research include the novelty of this meta-analysis which nearly included all clinical trials performed regarding endometrial flushing with follicular fluid up to now. We recommend further future research including larger sample size and endometrial receptivity assay (ERA) to better express the actual effects of this intervention.

CONCLUSION

It is crucial to stress that Follicular Fluid should be used extremely carefully, as it seems that flushing follicular fluid into the endometrial cavity had no influence on endometrial thickness, clinical pregnancy or implantation rates, either favorably or unfavorably.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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