Follicular fluid composition and reproductive outcomes of women with polycystic ovary syndrome undergoing in vitro fertilization: a systematic review

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Follicular fluid composition and reproductive outcomes of women with polycystic ovary syndrome undergoing in vitro fertilization: a systematic review

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Mariana Pereira Monteiro

Porto, junho 2021
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Abstract

Title: Follicular fluid composition and reproductive outcomes of women with polycystic ovary syndrome undergoing in vitro fertilization: a systematic review

Background and Objectives: Polycystic ovarian syndrome (PCOS) is recognized as the most prevalent endocrinopathy of reproductive-age women and one of the main causes of female infertility. Moreover, these women have more complications and poorer in vitro fertilization (IVF) outcomes. Hence, PCOS may jeopardizes oocyte quality and development competence. The aim of the current review is to find correlations between follicular fluid (FF) profiles of these women and their reproductive outcomes to identify potential biomarkers and targets to tailor therapy.

Methods: An electronic search in PubMed and Web of Science databases was conducted until March 2021. Only observational studies and randomized controlled trials (RCTs) assessing the correlation between FF composition and IVF outcomes of PCOS women and normo-ovulatory women were included. The Newcastle-Ottawa Scale (for cohort and case-control studies), the Joanna Briggs Institute Critical Appraisal Tool (for cross-sectional studies) and the Cochrane Risk of Bias tool (for RCTs) were used to access the risk of bias of the included studies.

Results: There are significant differences in the concentrations of biomolecules in FF of women with PCOS when compared to those of normo-ovulatory women, such as growth factors, cytokines, antioxidants and prooxidants, carbohydrate metabolism factors, advanced glycation end products, miRNAs. Furthermore, there are several significant associations between these factors’ levels in FF and less favourable outcomes in this group of patients.

Discussion: These findings strengthen the idea that FF can be a powerful tool to find good predictors of IVF outcomes. However, each FF factor was assessed by a very small number of studies and, in most of them, the sample size was small, potentially leading to non-significant differences and inconsistent findings. In addition, the success of the IVF outcomes depends on several factors that were not considered. Therefore the findings need to be interpreted with caution.

Keywords: Follicular fluid, polycystic ovary syndrome, oocyte quality, in vitro fertilization outcomes, biomarkers
Resumo

Título: Composição do líquido folicular e resultados reprodutivos de mulheres com síndrome do ovário poli quilístico submetidas a fertilização in vitro: uma revisão sistemática

Contexto e Objetivos: A Síndrome do Ovário Poliquístico (SOP) é a endocrinopatia mais frequente nas mulheres na idade reprodutiva e uma das principais causas de infertilidade. Além disso, estas mulheres têm mais complicações e resultados da fertilização in vitro (FIV) menos favoráveis. Assim, pensa-se que esta síndrome possa comprometer a qualidade dos ovócitos e a sua capacidade de desenvolver funções reprodutivas. O objetivo desta revisão é encontrar correlações entre os perfis do líquido folicular destas mulheres e os seus resultados reprodutivos com o intuito de identificar potenciais biomarcadores e alvos para individualizar o tratamento.

Métodos: A pesquisa foi realizada nas bases de dados PubMed e Web of Science até Março de 2021. Foram incluídos apenas os estudos observacionais e os ensaios controlados aleatorizados que avaliam a correlação entre a composição do líquido folicular e os resultados da FIV de mulheres com SOP e mulheres com ciclos menstruais regulares. A escala de NewCastle-Ottawa (para estudos de coorte e caso-controlo), a ferramenta de avaliação crítica do Joanna Briggs Institute (para estudos transversais) e a ferramenta para avaliação do risco de viés da Cochrane (para ensaios controlados aleatorizados) foram usadas para avaliar o risco de viés dos estudos incluídos.

Resultados: Existem diferenças significativas nas concentrações de biomoléculas no líquido folicular das mulheres com PCOS quando comparadas com mulheres com ciclos menstruais regulares, tais como fatores de crescimento, citocinas, antioxidantes e agentes oxidantes, fatores do metabolismo dos carboidratos, produtos finais da glicosilação avançada, microRNAs. Além disso, existem várias associações significativas entre os níveis desses fatores no líquido folicular e resultados menos favoráveis neste grupo de doentes.

Discussão: Estes achados reforçam a ideia de que o líquido folicular poder ser uma poderosa ferramenta para encontrar bons preditores dos resultados da FIV. No entanto, cada fator do LF foi estudado por um número muito reduzido de estudos ev na maioria deles, o tamanho da amostra era reduzido, levando potencialmente a diferenças não significativas e a achados inconsistentes. Adicionalmente, o sucesso dos resultados da FIV depende de vários fatores que não foram considerados. Portanto, os achados devem ser interpretados com precaução.

Palavras-chave: Líquido folicular, síndrome do ovário poliquístico, qualidade ovocitária, resultados de fertilização in vitro, biomarcadores
List of Abbreviations

COS  Controlled Ovarian Stimulation
FF   Follicular Fluid
GnRH Gonadotropin-Releasing Hormone
ICSI Intracytoplasmic Sperm Injection
IVF-ET In Vitro Fertilization-Embryo Transfer
JBI Joanna Briggs Institute
MII  Metaphase II
NOS  Newcastle-Ottawa Scale
OHSS Ovarian Hyperstimulation Syndrome
PCOS Polycystic Ovarian Syndrome
RCT Randomized Controlled Trial
RoB 2 Risk of Bias 2 (Cochrane Risk of Bias tool for Randomized Trials)
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1. Introduction

1.1. Rationale

Polycystic ovarian syndrome (PCOS) is acknowledged as the most common endocrinopathy among reproductive-age women, with a worldwide prevalence of 1.8-15%, depending on ethnic background and diagnostic criteria used\(^1\). Over the years, three distinct groups of diagnostic criteria for PCOS in adults have been proposed, with no universal consensus on which criteria should be used. The present review integrates articles that used the 2003 Rotterdam consensus for PCOS\(^2\), which requires the presence of two of the following three criteria: oligo- or anovulation, clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries, resulting in four possible phenotypes. Besides, other disorders presenting clinical features that resemble PCOS presentation (e.g., congenital adrenal hyperplasia, androgen-secreting tumours, Cushing’s syndrome) must be excluded. These cardinal features go hand in hand with multiple comorbidities, such as visceral obesity, insulin resistance, dyslipidemia, pre-diabetes and infertility\(^2,3\).

In fact, PCOS is one of the main causes of female infertility, accounting for about 80% of anovulatory infertility\(^3\). Indeed, oligo-anovulatory ovarian dysfunction is pointed out as the central reason for subfertility in PCOS women\(^4,5\).

According to the recommendations in the international evidence-based guidelines (International Evidence-Based Guidelines for the Assessment and Management of Polycystic Ovary Syndrome, 2018), after the failure of first- and second-line pharmacological therapies for ovulation induction (i.e., letrozole, clomiphene citrate, metformin, gonadotrophins, used alone or combined), in vitro fertilization (IVF) can be proposed to PCOS women with anovulatory infertility as a third-line therapy. For IVF in PCOS women, a GnRH antagonist is the preferred protocol to conduct a controlled ovarian stimulation (COS), due to the lower incidence of ovarian hyperstimulation syndrome (OHSS), which this patient population harbour an higher risk\(^6\).

Recent findings\(^7\) have revealed that hospital admissions for IVF treatments were almost nine times more frequent among women with PCOS when compared to non-PCOS women, while the total number of pregnancies was lower. Besides, PCOS women were more likely to have spontaneous miscarriages, preterm deliveries and stillbirths. Thus, although women with PCOS undergoing ovarian stimulation for IVF usually produce a high number of oocytes, the reproductive outcomes are often poorer\(^8-12\).
Subclinical factors related to oocyte quality and development competence\textsuperscript{13} as well as endometrial competence\textsuperscript{14} have been proposed as potentially implicated in these phenomena, irrespective of ovulatory dysfunction alone. Hence, it has been hypothesized that PCOS jeopardizes the follicular microenvironment, precluding the oocyte from performing its physiological functions\textsuperscript{4,15}.

Follicular fluid (FF) is produced in growing secondary follicles and consists of a plasma exudate and secretory factors from follicular cells, i.e., granulosa and theca cells, that surround the maturing oocyte\textsuperscript{15}. Follicular fluid composition can be assessed after oocyte retrieval during IVF treatment. In the past few years, researchers have shown differences in the concentrations of biomolecules in the FF of women with PCOS when compared to those of healthy women\textsuperscript{4,16,17}.

Although all the efforts, how each molecule potentially creates an adverse milieu for the developing oocyte and affects the reproductive outcomes of PCOS women have not yet been fully elucidated\textsuperscript{4,15}. Notwithstanding, as a consequence of the intimate contact of the FF with the cumulus-oocyte complex, FF’s profiling as a source of molecular predictors of oocyte quality and IVF outcomes, tailor therapy and, ultimately, improved fertility outcomes of women with PCOS holds great potential\textsuperscript{15,18}.

1.2. Objectives

In this sense, the aim of the current review was to summarize the available evidence on how FF profiles of PCOS women undergoing IVF correlates with reproductive outcomes. The ultimate goal is to identify potential biomarkers of oocyte competence and, subsequently, predictors of reproductive outcomes as well as potential targets for new therapies in this particular group of patients.
2. Methods

2.1. Protocol and Registration

The present systematic review was conducted in accordance with Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) 2020 statement\(^1\).

The protocol was registered at inception on February 16th, 2021 in the international database of prospectively registered systematic reviews (PROSPERO)\(^2\) and can be accessed with the registration number CRD42021237734 (in Annex 1). All amendments made after registration will be recorded in PROSPERO.

2.2. Eligibility Criteria

The eligibility criteria used to develop search strategy and select relevant studies were based on PICOS (Population, Intervention, Control intervention, Outcome, Study design) framework as follows:

Population

(1) Non-interventional study
- Study group: women with polycystic ovarian syndrome (PCOS) diagnosed using Rotterdam consensus criteria of PCOS, irrespective of phenotype, undergoing IVF, including intracytoplasmic sperm injection (ICSI) and embryo transfer (IVF-ET), using their own oocytes, irrespective of the ovarian stimulation protocol used.
- Control group: normal ovulatory women (without PCOS) with other infertility factor, undergoing in vitro fertilization (including intracytoplasmic sperm injection) and embryo transfer, irrespective of the ovarian stimulation protocol used.

(2) Interventional study
- Study and control groups: both PCOS women undergoing IVF-ET.

Intervention

(1) Non-interventional study: none.

(2) Interventional study: treatment with drugs and/or supplements.

Control intervention

(1) Non-interventional study: not applicable.

(2) Interventional study: placebo treatment.
Outcome

- FF components levels (after the oocyte retrieval).
- IVF outcomes: number of oocytes retrieved, number of metaphase II (MII) oocytes retrieved, oocyte quality, fertilization rate, number of good quality embryos, biochemical and/or clinical pregnancy rates.
- Correlations between aforementioned outcomes.

Study design

- Observational studies (cohort, case-control and cross-sectional studies) and randomized controlled trials.
- Studies performed in humans, irrespective of ethnicity, with no restrictions on sample size.
- Articles written in English or Portuguese.

The exclusion criteria were:

- Articles in languages other than English and Portuguese.
- Studies whose full text could not be accessed.
- Case reports and case series.
- Grey literature (conference proceedings and meeting abstracts).
- Studies conducted in other species.
- Studies that did not follow the Rotterdam criteria for PCOS (e.g., polycystic ovary morphology as the only feature present, secondary causes of hyperandrogenism not excluded).
- Studies that did not mention study participants’ diagnostic and inclusion criteria.
- Studies that pooled women with PCOS and women with infertility factors related to impaired ovarian function (e.g., ovulation disorders, endometriosis).
- Studies that chose women with impaired ovarian function or response as the control group.
- Studies that analysed serum and/or plasma or embryo culture medium or follicular cells instead of FF.
- Studies that did not assess any of the required IVF outcomes, including those of women who have not undergone IVF, i.e., assisted reproductive techniques other than IVF or oophorectomy or other procedures.
- Studies that did not assess the relationship between FF composition and IVF outcomes.
- Studies designed to investigate the effects of environmental contaminant exposure on follicular cells function or FF composition or reproductive outcomes.
- Reviews and theoretical papers (although not included during the study selection process, some were ultimately used to complete the information from included studies).

2.3. Information Sources and Search Strategy

An electronic search in PubMed and Web of Science databases was conducted for reports published up to March 31st, 2021, using a search strategy based on the following concepts: polycystic ovarian syndrome, follicular fluid, in vitro fertilization and reproductive outcomes related to oocyte-embryo developmental capacities. Medical Subject Headings (MeSH) in PubMed and synonyms were used to outspread the search. Firstly, search terms related to the same concept were combined with the “OR” Boolean operator. Secondly, the results of each concept were integrated using the “AND” Boolean operator. No restrictions regarding language and publication year or other filters were used. Some reports retrieved were available ahead of publication. The detailed search strategy for each database is present in Annex 2. A hand-search of reference lists was not conducted.

2.4. Selection Process

Results from both databases were exported to a reference management software (EndNote X9). After eliminating duplicates, study selection process occurred in two phases, to wit, abstract analysis and then full text’s analysis of potential eligible studies retrieved in the first phase. Authors were contacted by email whenever full text of articles were not available in databases or supplemental tables or figures could not be accessed. No response was obtained in 4 out of the 7 contacts made. The fulfilment of at least one exclusion criterion implied the exclusion of the concerned study. Finally, the selected for inclusion studies were assessed for risk of bias. All analyses were independently performed by two researchers (IA and EVF). Disagreements were mainly resolved by authors’ agreement.

2.5. Data Collection and Management

Data from each original study included was independently extracted and checked in a cross-over manner by two researchers (IA and EVF). For each study the information retrieved included: first author’s name, publication year, study design, randomization type if applicable and aim of the study, participants characteristics, eligibility criteria, sample size of each study group, intervention - ovarian stimulation protocol used, IVF and/or ICSI, drug or supplement used if
applicable, and aforementioned outcomes. The information gathered was then summarized in evidence tables and in a narrative manner due to heterogeneity in data.

2.6. Study Risk of Bias Assessment

The Newcastle-Ottawa Scale (NOS) was used for assessing the quality of non-randomized studies (i.e., cohort and case-control studies) by awarding stars in each domain (i.e., selection, comparability, outcome/exposure). According to Agency for Healthcare Research and Quality (AHRQ) standards, a “good” quality score implies 3 or 4 stars in selection domain and 1 or 2 stars in comparability domain and 2 or 3 stars in outcome/exposure domain, a “fair” quality score implies 2 and 1/2 and 2/3 stars in the three domains and a “poor” quality score implies 0/1 or 0 or 0/1 star in the three domains.

In the Joanna Briggs Institute (JBI) Critical Appraisal Checklist for Analytical Cross-Sectional Studies, each study was assessed against 8 criteria and rated as “yes”, “no”, “unclear” or “not applicable”. In the absence of a universal consensus, a study was judged to be at low risk of bias when it met 4 “yes” out of the 8 criteria.

For non-observational studies, it was used the Cochrane Risk of Bias tool for Randomized Trials (RoB 2 tool). Each study was assessed against 3 to 7 criteria of 5 domains and its overall risk of bias (i.e., “low”, “some concerns”, “high”) was calculated based on the risk of bias of each domain established by an algorithm.

Only studies of “good” or “fair” quality or judged to be at low risk of bias were included in the review.
3. Results

3.1. Study Selection

The initial literature search retrieved 435 papers without duplicates, of which 67 articles met the full eligibility criteria for inclusion in the systematic review. Several papers were excluded for more than one reason. The flowchart of search and selection process for studies included in the review is outlined in Figure 1.

![Flowchart of search and selection process for studies included in the review, adapted from PRISMA 2020 flow diagram. FF = Follicular Fluid, IVF-ET = In Vitro Fertilization – Embryo Transfer, PCOS = Polycystic Ovarian Syndrome.](image)

3.2. Risk of Bias in Studies

The quality assessment of the prospective cohort studies, according to NOS, is outlined in Table III (Annex 3). In accordance with NOS guidelines, statements of non-significant differences between groups are not enough to established comparability, wherefore, except for two studies, most of the studies failed to perform or report confounder adjustments. Nevertheless, all studies were rated as of “good” quality.

The quality assessment of the case-control studies is presented in Table IV (Annex 4). A similar concern regarding the comparability domain was found in these studies. For that reason, one
study did not receive any points in that domain. Thus, it was rated as of “poor” quality and
excluded. Moreover, two studies failed to report the recruitment time period.

Table V (Annex 5) shows the risk of bias assessment of the cross-sectional studies, according to
JBI tool. None of the studies were sufficiently informative about whether the outcomes were
measured in a valid and reliable way. As all scored at least 4 “yes”, none were excluded.

The risk of bias assessment of the randomized controlled trials is summarized in Table VII (Annex
6). A more detailed table (Table VII) can be found in Annex 7. None of the RCTs were excluded.

It is noteworthy that the risk of bias of 34 eligible studies was not assessed, therefore only the
remaining 31 studies were included.
### 3.3. Main Characteristics of the Select Studies

Table 1. Main characteristics of included RCTs. Studies are listed in chronological order.

<table>
<thead>
<tr>
<th>First Author Publication Year</th>
<th>Study Design Randomization Type</th>
<th>Population</th>
<th>Intervention</th>
<th>IVF/ICSI Treatment Ovarian Induction</th>
<th>Outcome Measures</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onalan, 2005&lt;sup&gt;21&lt;/sup&gt;</td>
<td>RCT Computer-generated randomization in blocks</td>
<td>28 PCOS women (Rotterdam criteria) Exclusion criteria: hormonal medications or insulin-lowering agents within 3 months  - Treatment group (n=14): age 31.8±5.3, BMI 24 (20.9-28)  - Control group (n=14): age 30.8±3.4, BMI 24 (20-31.25) 12 non-PCOS women (male infertility): age 31.8±2.8, BMI 25.3 (18.7-27.5)</td>
<td>Treatment group: 1700 mg/day (BMI &lt; 28) or 2550 mg/day (BMI &gt; 28) metformin  Control group: placebo 8-week (before their first IVF-ICSI cycle)  Both groups: without any changes in their usual eating habits</td>
<td>ICSI GnRH long agonist protocol, rFSH (GONAL-f&lt;sup&gt;®&lt;/sup&gt;)</td>
<td>Serum and FF sFas and sFasL levels FF estradiol levels GC DNA fragmentation (apoptosis of luteinized GCs)  IVF cycle characteristics and outcomes: peak E2, AFC, oocytes retrieved (n), MI and MII oocytes (n), fertilization rate (%), embryos transferred (n), biochemical and clinical pregnancy rate (%)</td>
<td>Serum sFas levels were lower* in PCOS group (vs. male factor group) Metformin treated group had higher* serum sFas levels and lower* FF sFasL levels compared to placebo group  Luteinized GC DNA fragmentation was not observed in metformin treated group  IVF outcomes were similar between metformin and control groups</td>
</tr>
<tr>
<td>Kim, 2011&lt;sup&gt;22&lt;/sup&gt;</td>
<td>RCT No information regarding randomization type</td>
<td>86 PCOS women (Rotterdam criteria) Exclusion criteria: other infertility factors, thyroid, liver or kidney diseases  Treatment group (n=43): age 32.0±2.7, BMI 22.6±2.6  Control group (n=43): age 31.4±3.1, BMI 22.4±2.6</td>
<td>Treatment group: 30 mg/day pioglitazone  Control group: placebo (from the starting day of oral contraceptive to the day of hCG injection)</td>
<td>IVF GnRH antagonist protocol, rFSH (Puregon&lt;sup&gt;®&lt;/sup&gt;)</td>
<td>FF TNF-α and IL-6 levels IVF cycle characteristics and outcomes: total gonadotropin dose, duration of stimulation, peak E2, follicles on hCG day (n), oocytes retrieved (n), MI oocytes (n), fertilization rate (%), grade 1 or 2 embryos (n), clinical pregnancy rate/cycle initiated (%), multiple pregnancy rate/clinical</td>
<td>FF TNF-α and IL-6 levels were lower* in pioglitazone group  Total dose and days of gonadotropin, n. of retrieved and MII oocytes were lower* in pioglitazone group  Clinical pregnancy and live birth rates were higher and incidence of OHSS was lower in pioglitazone group</td>
</tr>
<tr>
<td>Cheraghi, 2014</td>
<td>RCT</td>
<td>No information regarding randomization type</td>
<td>80 PCOS women (Rotterdam criteria) Exclusion criteria: hypersensitivity to MET or NAC, infertility factors other than anovulation, male infertility, pelvic organic pathologies, congenital adrenal hyperplasia, thyroid dysfunction, Cushing’s syndrome, hyperprolactinemia, androgen-secreting neoplasia, diabetes mellitus, consumption of medications affecting carbohydrate metabolism and hormonal analogues other than progesterone 2 months prior to enrolment in the study, severe hepatic or kidney disease, abnormal semen parameters Treatment groups (n=45): - MET group (n=15): age 28.07±3.41, BMI 27.9±3.1 - NAC group (n=15): age 29.67±3.35, BMI 27.7±4.5</td>
<td>Treatment groups: - MET group: 1500 mg/day metformin - NAC group: 1800 mg/day N-acetylcysteine - MET+NAC group: 1500 mg/day metformin + 1800 mg/day N-acetylcysteine Control group: placebo (oral rehydration solution) 6-week (from the 3rd day of previous cycle until the day of oocyte aspiration) All groups: without any changes in their normal physical activity and diet</td>
<td>ICSI GnRH long agonist protocol, rFSH (GONAL-f®)</td>
<td>FF insulin, LH, TT, E2, leptin, AMH and MDA levels Distribution of oocyte abnormalities regarding study group IVF outcomes: retrieved oocytes (n), immature (GV+MI) oocytes (n), MII oocytes (n), MII oocytes with MS (n), cleaved embryos (n), grade I-III embryos (n), embryos transferred (n), OHSS (%), endometrial thickness, clinical pregnancy (%)</td>
</tr>
<tr>
<td>Study</td>
<td>Design</td>
<td>Randomization</td>
<td>Number &amp; Eligibility Criteria</td>
<td>Exclusion Criteria</td>
<td>Treatment</td>
<td>Control</td>
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</tr>
<tr>
<td>Piomboni, 2014&lt;sup&gt;a&lt;/sup&gt;</td>
<td>RCT</td>
<td>Computer-generated randomization</td>
<td>68 PCOS women (Rotterdam criteria)</td>
<td>Age, BMI</td>
<td>DCI group: 500 mg bid DCI + gonadotrophins Metformin group: 850 mg bid metformin + gonadotrophins Untreated group: gonadotrophins 12-week treatment (prior to COS protocol)</td>
<td>IVF/ICSI GnRH antagonist protocol, rFSH (GONAL-f®)</td>
</tr>
<tr>
<td>Fatemi, 2017&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Double-blind RCT</td>
<td>Block randomization</td>
<td>105 (15 lost to follow-up) PCOS women (Rotterdam criteria)</td>
<td>Heart diseases, liver or kidney deficiencies, known cases of endometriosis, uterine anomaly or hydrosalpinx, retinitis pigmentosa, vitamin K deficiency, consumption of vitamin or antioxidant supplementations in the last 3 months before the trial, male factor infertility</td>
<td>Treatment group: 400 mg/day vitamin E + 50,000 IU/1 in 2 weeks vitamin D&lt;sub&gt;3&lt;/sub&gt; Control group: placebo Both groups: strict diet program 8-week (starting 2 weeks prior to COCP intake until hCG administration)</td>
<td>ICSI GnRH agonist long protocol, rFSH (GONAL-f®)</td>
</tr>
<tr>
<td>Sene, 201925</td>
<td>Double-blind RCT Permutated block randomization</td>
<td>50 PCOS women (Rotterdam criteria) Exclusion criteria: other metabolic diseases (e.g., diabetes), BMI&gt;35, allergy to myo-inositol, hormonal medications for at least 3 months before the trial, male factor infertility Treatment group (n=25): age 31.3±4.1, BMI 25.26±5.2 Control group (n=25): age 29.78±4.5, BMI 26.2±4.52</td>
<td>Treatment group: 4 g/day myo-inositol + 400 mg/day folic acid Control group: 400 mg/day folic acid Starting 4 weeks prior to COS protocol until the day of ovum pick up</td>
<td>IVF GnRH antagonist protocol, rFSH (GONAL-f®)</td>
<td>FF ROS and TAC levels Gene expression of PGK1, RGS2 and CDC42 in mural granulosa cells IVF cycle characteristics and outcomes: total gonadotropin dose, duration of stimulation, estrogen and progesterone levels on triggering day, retrieved oocytes (n), MII oocytes (%), fertilized rate (%), grade 1, 2 and 3 embryos (%), cumulative pregnancy rate (%)</td>
<td>Myo-inositol + folic acid, compared to folic acid alone, did not have a significant effect on FF ROS and TAC levels of PCOS patients Myo-inositol improved* the percentage of MII oocytes, fertilization rate and embryo quality, even though there were non-statistically differences between groups regarding the number of retrieved oocytes The cumulative pregnancy rate was not significantly higher in the myo-inositol group Gene expression of PGK1, RGS2 and CDC42 was higher* in the myo-inositol group</td>
</tr>
</tbody>
</table>

* indicates statistical significance result

bid = “bis in die” (twice a day), BMI = body mass index, CDC42 = cell division cycle 42, COCP = combined oral contraceptive pill, COS = controlled ovarian stimulation, DCI = D-chiro-inositol, FF = follicular fluid, FR = fertilization rate, GCs = granulosa cells, GnRH = gonadotropin-releasing hormone, GV = germinal vesical, hCG = human chorian gonadotropin, ICSI = intracytoplasmic sperm injection, IVF = in vitro fertilization, LH = luteinizing hormone, MDA = malondialdehyde, MET = metformin, MI = metaphase I, MII = metaphase II, n = number, NAC = N-acetylcysteine, OHSS = ovarian hyperstimulation syndrome.
Table II. Main characteristics of included observational studies. Studies are divided by design and listed in chronological order.

<table>
<thead>
<tr>
<th>First Author</th>
<th>Publication Year</th>
<th>Study Design</th>
<th>Population</th>
<th>IVF/ICSI Treatment Ovarian Induction</th>
<th>Outcome Measures</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Savchev, 2010²⁶</td>
<td>26</td>
<td>Prospective cohort study</td>
<td>6 PCOS women (Rotterdam criteria) Age 27.6 (25-31), BMI 29.48 (17.3-50.5) Other groups: - Unexplained subfertility - Tubal disease - Male infertility</td>
<td>ICSI GnRH agonist or antagonist protocols, rFSH</td>
<td>FF VEGF₁₂₁, VEGF₁₆₅ and sFlt-1 levels IVF outcomes: oocytes retrieved (n), fertilization rate (%), embryos transferred (n) Effects of PCOS, age and BMI Correlations between FF parameters and IVF outcomes</td>
<td>Higher FF VEGF₁₆₅ levels were associated* with the PCOS diagnosis, BMI &gt; 30 and age &gt; 40 years Lower FF VEGF₁₆₅ levels were associated* with the presence of a clinical pregnancy No correlations were found between FF parameters (VEGF₁₂₁ and sFlt-1 levels) and patient characteristics or IVF outcomes</td>
</tr>
<tr>
<td>Chattopadhayay, 2010²⁷</td>
<td>27</td>
<td>Prospective cohort study</td>
<td>35 PCOS women (Rotterdam criteria) Age 29.9±±2.04, BMI 23.2±±1.23</td>
<td>ICSI GnRH agonist protocol, rFSH (GONAL-f®)</td>
<td>FF ROS, LPO and TAC levels (and correlations between them) IVF outcomes: presence or absence of meiotic spindle in oocytes (%), fertilization rate (%), grade 1 and 2 embryo (%), pregnancy rate (%), miscarriage rate (%) Comparison of oxidative stress markers levels, FR, n. of good quality embryos and clinical pregnancy rate</td>
<td>FF ROS and LPO levels were higher* and TAC levels lower* in PCOS group (vs. controls) FR, n. of good quality embryos and clinical pregnancy rate were lower and miscarriage rate higher in PCOS group</td>
</tr>
</tbody>
</table>
grade 1 and 2 embryo formation between oocytes with and without MS from each group

FF ROS and LPO levels were higher* and TAC levels lower* in oocytes without MS (vs. oocytes with MS) from both PCOS and control groups FR and proportion of good quality embryos were lower* in oocytes without MS (vs. oocytes with MS) among PCOS women

Rajani, 2012* Prospective cohort study 48 PCOS women (Rotterdam criteria) Age 32.9±3.3, BMI 23.25±1.23 Another group: 56 women with endometriosis Age 33.4±3.53, BMI 21.4±2.6 63 normo-ovulatory women with tubal block (salpingectomy for ectopic pregnancy or proximal tubal obstruction or fimbrial occlusion) Age 32.7±3.8, BMI 20.8±1.45 Control group: gross hydrosalpingeal changes, dense pelvic adhesions, endometriosis, pelvic inflammatory disease, tuberculosis ICSI GnRH agonist protocol, rFSH (GONAL-f®) FF ROS levels IVF outcomes: retrieved oocytes (n), MII oocytes (%), presence or absence of meiotic spindle in oocytes (%), fertilization rate (%), grade 1 and 2 embryo (%) endometrial thickness, pregnancy rate (%) Comparison of ROS levels, FR, grade 1 and 2 embryo formation between oocytes with and without MS from each group FF ROS levels were higher* in PCOS group (vs. controls) N. of oocytes retrieved was higher* in PCOS group, but n. of MII oocytes and those with MS were lower* in this group FR, n. of good quality embryos and pregnancy rate were lower in PCOS group FF ROS levels were higher* in oocytes without MS (vs. oocytes with MS) from both PCOS and control groups FR and proportion of good quality embryos were
| Sahin, 2013<sup>28</sup> | Prospective cohort study | 21 PCOS women (Rotterdam criteria)  
- Age 29.33±0.89, BMI 24.47±0.73  
- 38 normo-ovulatory non-PCOS women  
- Age 31.68±0.80, BMI 24.37±0.53 | No information | ICSI/GnRH agonist protocol, rFSH (Puregon® or GONAL-f®)  
Serum and FF HGF levels  
Expression of c-Met in granulosa cells  
IVF cycle characteristics and outcomes: duration of induction, total FSH dose, oocytes retrieved (n), MII oocytes (n), fertilization rate (%), clinical pregnancy rate (%)  
FF HGF levels and expression of c-MET in GCs in unfertilized and fertilized oocytes and in grade 1 and 2-4 embryos | Serum and FF HGF levels and c-Met expression in GCs were similar between groups  
IVF outcomes were comparable between groups (except the n. of mature oocytes which was greater* in PCOS group)  
c-Met expression was higher* in GCs of fertilized oocytes (vs. non-fertilized oocytes)  
FF HGF level was higher* in the grade 1 embryos (vs. grades 2-4 embryos) |
| Tal, 2014<sup>29</sup> | Prospective cohort study | 14 PCOS women (Rotterdam criteria)  
- male factor (n=7)  
- ovulatory dysfunction (n=4)  
- tubal factor (n=3)  
- Age 30.1±4.4, BMI 25.5±5.6  
- 14 normo-ovulatory non-PCOS women  
- male factor (n=9)  
- tubal factor (n=5)  
- Age 30.8±3.7, BMI 24.9±3.9  
- PCOS group: secondary causes of androgen excess and anovulation  
Both groups: diminished ovarian reserve, endometriosis, hormonal treatment | IVF/ICSI  
GnRH agonist (n=8 for each group) or GnRH antagonist (n=6 for each group) protocols, combination of rFSH and HMG | Serum AMH levels  
Serum PIGF and sFlt-1 levels (on day 3, hCG day and retrieval day)  
FF PIGF and sFlt-1 levels IVF outcomes: oocytes retrieved (n), fertilization rate (%), pregnancy rate (%)  
Correlation between FF PIGF, sFlt-1 levels and age, serum AMH, IVF outcomes | Serum PIGF and sFlt-1 levels were similar between groups  
FF PIGF levels were increased* whereas sFlt-1 levels were decreased* in PCOS group (vs. controls), so FF PIGF bioavailability was greater* in this group |
<table>
<thead>
<tr>
<th>Study</th>
<th>Study Design</th>
<th>Participants</th>
<th>Main Diagnoses</th>
<th>IVF Protocol</th>
<th>FF Parameters</th>
<th>Other Parameters</th>
<th>Correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wang, 2016&lt;sup&gt;60&lt;/sup&gt;</td>
<td>Prospective cohort study</td>
<td>39 PCOS women (Rotterdam criteria) Age 27.67±3.263, BMI 21.049±2.169</td>
<td>Diabetes, chronic kidney disease, chronic metabolic diseases, endometriosis</td>
<td>IVF GnRH agonist protocol, rFSH (GONAL-f®)</td>
<td>FF sRAGE, VEGF, TNF-α, IL-6 and CRP levels</td>
<td>FF PIGF positively correlated* with n. of oocytes retrieved and serum AMH and negatively* with age FF PIGF and sFlt-1 did not correlate with fertilization rate or clinical pregnancy rate</td>
<td>FF sRAGE levels were lower* whereas FF VEGF, TNF-α, IL-6 and CRP levels were higher* in PCOS group (vs. controls) FF sRAGE inversely correlated* with total gonadotropin dose and FF VEGF, TNF-α, IL-6 and CRP levels</td>
</tr>
<tr>
<td>Tola, 2017&lt;sup&gt;31&lt;/sup&gt;</td>
<td>Prospective cohort study</td>
<td>21 PCOS women (Rotterdam criteria) Age 29.23±3.67, BMI 27.87±5.90</td>
<td>PCOS group: secondary causes of androgen excess and anovulation Control group: azoospermia and severe oligoasthenospermia Both groups: diminished ovarian reserve or endometriosis, systemic diseases, abnormal prolactin</td>
<td>ICSI GnRH antagonist protocol, recombinant and/or urinary FSH</td>
<td>FF ADAMTS-1 and aggrecan levels</td>
<td>FF ADAMTS-1 and aggrecan* levels were increased in PCOS group (vs. controls) Higher FF levels of ADAMTS-1 were related to increased implantation in PCOS group Positive predictor effect of FF ADAMTS-1 and</td>
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<tr>
<td>Study</td>
<td>Design/Methodology</td>
<td>Participants</td>
<td>Characteristics</td>
<td>Interventions</td>
<td>Outcomes</td>
<td>FF Parameters</td>
<td>Correlations</td>
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<tr>
<td>Garg, 2017</td>
<td>Prospective cohort study</td>
<td>12 PCOS women (Rotterdam criteria)</td>
<td>Age 31.5 (29.2-34.5), BMI 24.5 (22.5-33.7)</td>
<td>13 normo-ovulatory non-PCOS women</td>
<td>Age 35.0 (31.0-37.5), BMI 25.0 (21.5-29.0)</td>
<td>Diminished ovarian reserve, hormonal medications (other than the IVF medications), hypothyroidism, hyperprolactinemia, adrenal dysfunctions</td>
<td>IVF/ICSI GnRH antagonist protocol, highly purified human menopausal gonadotropin (Menopur®) or rFSH (Follistim® or GONAL-f®)</td>
</tr>
<tr>
<td>Chen, 2017</td>
<td>Prospective cohort study</td>
<td>59 PCOS women (Rotterdam criteria)</td>
<td>Age 29.1±3.5, BMI 22.2±3.2</td>
<td>120 normo-ovulatory non-PCOS women (tubal or male factors)</td>
<td>Age 30.3±3.9, BMI 21.4±2.9</td>
<td>&gt; 38 years, serum FSH levels &gt; 12 IU/L, history of ovarian surgery, ovarian cyst or tumour, hydrosalpinx, endometriosis, endocrine or systemic diseases</td>
<td>IVF/ICSI PCOS group: GnRH agonist (n=30) or GnRH antagonist (n=29) protocols (randomly assigned according to their admission time), rFSH (GONAL-f®) (both groups) Control group: GnRH agonist protocol, rFSH (GONAL-f®)</td>
</tr>
</tbody>
</table>
Patients by infertility factor (PCOS vs. control) and protocol (agonist vs. antagonist in PCOS patients) Associations of peak 2 and FF AMH levels with IVF outcomes

Butler, 2019[^4] Prospective cohort study 29 PCOS women (Rotterdam criteria) Age 30.9±4.8, BMI 26.0±3.8 30 normo-ovulatory non-PCOS women Age 32.6±4.7, BMI 25.5±3.6 < 20 or > 45 years, diabetes, renal or liver insufficiency, non-alcoholic fatty liver disease, acute or chronic infections, systematic inflammatory diseases, known immunological disease, non-classical 21-hydroxylase deficiency, hyperprolactinemia, Cushing’s disease, androgen-secreting tumours, medications other than folic acid and study medications (in the last 9 months prior to COS) IVF GnRH antagonist protocol, rFSH (Merional® or GONAL-f®)

Expression of miRNA in FF IVF outcomes: endometrium thickness (at oocyte retrieval), oocytes retrieved (n), fertilization rate (%), cleavage and blastocyst stages embryos (n), G3D3 embryos (n), clinical pregnancy (n)

Correlation between miRNA and demographic/biochemical data, IVF outcomes

Of 176 miRNA detected in FF, 29 differed* between groups

The top 7 of these were miR-381-3p, miR-199b-5p, miR-93-3p, miR-361-3p, miR-127-3p, miR-382-5p, miR-425-3p miR-382-5p positively correlated* with age and negatively* with FAI, miR-199b-5p negatively correlated* with AMH and miR-93-3p positively correlated* with CRP in PCOS group

N. of oocytes retrieved and fertilization rate were higher* in

[^4]: Butler, 2019
| Fabjan, 2020<sup>15</sup> | Prospective cohort study | 36 PCOS women (Rotterdam criteria)  
Age 30.8 (29.4-32.2), BMI 24.5 (23-27)  
Other groups:  
- 72 women with endometriosis  
Age 33.8 (33.1-34.5), BMI 21.65 (21.2-22.5)  
- 41 women with tubal factor  
Age 32.3 (31-33.5), BMI 22.45 (21.2-24) | 48 healthy women (male infertility)  
Age 31.62 (30.5-32.7), BMI 21.45 (21.4-23.3) | No information | IVF/ICSI  
Combination of GnRH analogues and gonadotrophins | FF AMH, 8-IP, 8-OHdG and TAS levels  
Relation between FF oxidative stress markers and IVF outcomes (mature and fertilized oocytes) in PCOS group  
All FF oxidative stress markers were lower* in PCOS group (vs. healthy group)  
FF AMH levels were similar between groups  
In PCOS patients, FF 8-OHdG was the only good predictor of obtaining a mature oocyte and of successful fertilization |
| Gao, 2021<sup>36</sup> | Prospective cohort study | 32 PCOS women (Rotterdam criteria)  
Age 28.88±3.44, BMI 22.2±3.04  
28 normo-ovulatory non-PCOS women (tubal factors)  
Age 29.54±3.54, BMI 20.19±2.47 | Positive test for thyroid autoantibodies, thyroid disease, Cushing’s syndrome or increased cortisol levels, obesity, diabetes, smoking | IVF  
GnRH agonist protocol, rFSH (GONAL-f®) | Serum TSH levels (on day 3 of the previous non-treatment cycle)  
FF TSH levels  
TSHR expression level and cAMP levels in granulosa cells  
IVF outcomes: oocyte maturation rate (%), fertilization rate (%), cleavage rate (%), high quality embryo rate (%), pregnancy rate (%)  
Correlation between TSH levels (in serum and FF) and IVF outcomes  
Serum and FF TSH levels and cAMP levels in GCs were higher* in PCOS group (vs. controls)  
TSHR was upregulated* in PCOS group  
Oocyte maturation and fertilization rates were lower* in PCOS group  
Serum and FF TSH levels were negatively correlated* with oocyte maturation and fertilization rates in PCOS group |
<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Characteristics</th>
<th>Treatment</th>
<th>Outcomes</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li, 2007</td>
<td>CCS</td>
<td>31 PCOS women (Rotterdam criteria) &lt;br&gt; Age 28 (22-35), BMI 23.42 (19.81-30.62)</td>
<td>PCOS group: possible ovarian tumours, congenital adrenal hyperplasia, androgen-secreting tumours, Cushing's syndrome &lt;br&gt; Both groups: male infertility</td>
<td>IVF GnRH agonist protocol, rFSH (GONAL-f®)</td>
<td>Serum and FF leptin levels mRNA expression of leptin and two isoforms of Ob-R in GCs p-STAT3 and SOCS3 protein expression in GCs IVF outcomes: oocytes retrieved (n), FR (%), good quality embryo (%), implantation rate (%), pregnancy rate (%)</td>
</tr>
<tr>
<td>Kahyaoglu, 2014</td>
<td>CCS</td>
<td>22 non-obese and non-hyperandrogenic PCOS women (Rotterdam criteria) &lt;br&gt; Age 27.6±0.9, BMI 26.1±1.3</td>
<td>PCOS group: biochemical and/or clinical hyperandrogenism, BMI &gt; 30 kg/m² &lt;br&gt; Both groups: severe male infertility</td>
<td>IVF GnRH agonist long protocol, rFSH (Puregon® or GONAL-f®)</td>
<td>Serum and FF G-CSF levels Leukocyte and neutrophil counts, neutrophil/leukocyte ratio (%) IVF cycle characteristics and outcomes: duration</td>
</tr>
</tbody>
</table>

*Denotes statistical significance.
| Selenium, 2014 | CCS | 20 PCOS women (Rotterdam criteria) Age 31.2±1.10, BMI 31.3±3.2 | 20 normo-ovulatory non-PCOS women (tubal factor) Age 29.4±2.1, BMI 29.2±3.6 | Hyperprolactinemia, hypothyroidism, Cushing’s syndrome, androgen-secreting neoplasms, associated endometriosis, current cigarette smokers, diabetes mellitus, cardiovascular diseases (e.g., hypertension), male factor infertility | ICSI GnRH agonist long protocol, rFSH (GONAL-f®) | Serum SOD activity (on the day of oocyte retrieval) FF SOD activity Copper/zinc-SOD mRNA expression in FF cells IVF cycle characteristics and outcomes: E2 on the hCG day, fertilization rate (%), embryo quality (n), transferred embryo (n), pregnancy (n) FF SOD activity and SOD mRNA expression in FF cells between fertilized and non-fertilized oocytes SOD mRNA expression in FF cells between fertilized and non-fertilized oocytes | FF SOD activity was lower* in PCOS group (vs. controls) FR was lower in PCOS FF SOD activity was similar between fertilized and non-fertilized oocytes in both groups SOD activity in FF had no effects on embryo quality or pregnancy rate after ICSI SOD mRNA expression in FF cells was lower* in PCOS SOD mRNA expression in FF cells was similar between fertilized and non-fertilized oocytes
Liu, 2019<sup>40</sup>  CCS  261 PCOS women (Rotterdam criteria)  Age 29.80±3.46, BMI < 25 (n=106), 25-30 (n=98), > 30 (n=57)  217 normo-ovulatory non-PCOS women (tubal factors)  Age 32.00 (25-34), BMI < 25 (n=147), 25-30 (n=60), > 30 (n=10)  Control group: abnormal basic endocrine indexes, endometriosis, adenomyosis  Both groups: male infertility  ICSI PCOS group: GnRH agonist long protocol, FSH and rFSH  Correlations of SHBG rs6259 and rs727428 with risk factors in PCOS  Correlations of SHBG polymorphisms with IVF outcome  Risk factors for IVF outcome  The incidence of PCOS was higher in SHBG rs6259 GA+AA carriers (vs. SHBG rs6259 GA+AA carriers), so A allele is a risk factor for PCOS  N. of retrieved oocytes, embryo count and FR were decreased in SHBG rs6259 GA+AA carriers (vs. SHBG rs6259 TT carriers)  Abortion rate, incidence of OHSS, serum and FF testosterone levels were elevated in SHBG rs6259 GA+AA carriers (vs. SHBG rs6259 TT carriers)  Risk factors for IVF outcome were serum testosterone and progesterone and GA+AA genotype of rs6259

Cross-Sectional Studies

<p>| Nafiye, 2010&lt;sup&gt;41&lt;/sup&gt; | CSS | 36 non-obese and non-hyperandrogenic PCOS women (Rotterdam criteria)  Age 29.61±5.12, BMI 26.30±2.88 | 2 groups: 23 women with infertile male partners  Age 28.43±6.01, BMI 24.76±3.28  38 women with unexplained infertility  PCOS group: congenital adrenal hyperplasia, Cushing's syndrome  Both groups: hyperandrogenism, obesity, systemic diseases, endocrinopathies | 400 μg/day of preconceptional folic acid  IVF GnRH agonist protocol, rFSH (GONAL-F®) | Serum levels of T, A, 17OHP, DHEA, glucose, insulin, Hcy and HOMA-IR  FF levels of T, A, 17OHP DHEA, glucose, insulin and Hcy  IVF outcomes: follicle (n), retrieved and MII oocytes (n), embryo (n), | The incidence of PCOS was higher in PCOS women (Rotterdam criteria)  Serum insulin, HOMA-IR and Hcy levels were higher* in PCOS group  But FF parameters and clinical pregnancy rate were similar between the three groups |
| Yilmaz, 2012&lt;sup&gt;42&lt;/sup&gt; | CSS | 16 non-obese and non-hyperandrogenic PCOS women (Rotterdam criteria) Age 29.38±4.84, BMI 24.52±6.38 | 2 groups: 19 women with infertile male partners Age 28.81±3.28, BMI 25.35±4.88 19 women with unexplained infertility Age 29.05±6.85, BMI 23.46±3.48 | No information | IVF GnRH agonist protocol, rFSH (GONAL™) | FF AMH levels IVF cycle characteristics and outcomes: total gonadotropin dose, stimulation duration, retrieved oocytes (n), MII oocytes (n), 2PN (n), embryo (n) Correlations between FF AMH levels and IVF outcomes | FF AMH levels were similar between the three groups as well as between pregnant and non-pregnant groups FF AMH levels negatively correlated* with FSH (day 3) and gonadotropin dose and positively correlated* with oocyte, 2PN and embryo counts in PCOS group |
| Yilmaz, 2016&lt;sup&gt;40&lt;/sup&gt; | CSS | 22 PCOS women (Rotterdam criteria) Age 30.00 (25-34), BMI 24.68±2.86 | 41 non-PCOS women (male infertility) Age 32.00 (25-34), BMI 23.78±1.37 | History of systemic or endocrinological disease, smoking | IVF GnRH agonist protocol, rFSH (GONAL™) | FF TAC levels IVF cycle characteristics and outcomes: total gonadotropin dose, peak E2, endometrial thickness, retrieved oocytes (n), MII oocytes (n), fertilized rate (%), clinical pregnancy rate (%) Correlations between demographical, clinical variables and TAC levels | FF TAC levels, fertilization and clinical pregnancy rates were similar between groups N. of MII oocytes were higher* in PCOS group FF TAC was positively correlated* with clinical pregnancy rate in the whole group |
| Kudsy, 2016&lt;sup&gt;41&lt;/sup&gt; | CSS | 40 PCOS women (Rotterdam criteria) Age 28.6±4.3, BMI 27.19±5.70 | 40 normo-ovulatory non-PCOS women (male infertility) &gt; 38 years, low ovarian reserve, any pathology may affect IVF outcome (e.g., endometriosis, | IVF/ICSI GnRH long agonist protocol, rFSH (GONAL™) or HMG (Menogon™) | Serum and FF VEGF levels IVF outcomes: retrieved oocyte (n), MII oocyte (n and %), fertilized oocyte (n), embryos (n), FR (%) | Serum and FF VEGF levels were higher* in PCOS group vs. controls |</p>
<table>
<thead>
<tr>
<th>CSS</th>
<th>80 non-obese and non-hyperandrogenic PCOS women (Rotterdam criteria) Age 27.63±4.10, BMI 26.25±4.08</th>
<th>80 normo-ovulatory non-PCOS women (male infertility) Age 28.93±4.65, BMI 24.99±3.43</th>
<th>Evidence of clinical hyperandrogenemia, BMI &gt; 30 kg/m², diminished ovarian reserves, history of ovarian surgery, autoimmune diseases</th>
<th>ICSI GnRH agonist protocol, rFSH (GONAL-f® or Puregon®)</th>
<th>Serum and FF Sfrp-5 levels IVF outcomes: retrieved and MII oocytes (n), FR (%), embryo quality (%), clinical pregnancy rate (%), live birth rate (%)</th>
<th>Serum and FF Sfrp-5 levels, n, of retrieved and MII oocytes were higher* in PCOS group All other IVF outcomes were similar between groups Serum and FF Sfrp-5 levels positively correlated* with fasting insulin and negatively correlated* with inflammatory</th>
</tr>
</thead>
</table>

Inal, 2018

Age 28.4±5.9, BMI 26.09±4.29

uterine fibroids, hydrosalpinx, polyps

Correlation between VEGF levels and IVF outcomes Levels of VEGF in pregnant and non-pregnant women

Both serum and FF VEGF levels were not significantly correlated with IVF outcomes Serum VEGF levels were similar between pregnant and non-pregnant groups in both PCOS and control groups FF VEGF levels were lower* in pregnant group (vs. non-pregnant group) in both PCOS and control groups FF VEGF levels had a predictive value* of pregnancy in both groups

Evidence of clinical hyperandrogenemia, BMI > 30 kg/m², diminished ovarian reserves, history of ovarian surgery, autoimmune diseases

ICSI GnRH agonist protocol, rFSH (GONAL-f® or Puregon®)
<table>
<thead>
<tr>
<th>Study (Year)</th>
<th>Design</th>
<th>Control Group</th>
<th>Case Group</th>
<th>Association</th>
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<tbody>
<tr>
<td>Liu, 2018&lt;sup&gt;45&lt;/sup&gt;</td>
<td>CSS</td>
<td>43 PCOS women (Rotterdam criteria) Age 28.0 (23.0-33.0), BMI 22.6 (20.2-24.0)</td>
<td>32 normo-ovulatory non-PCOS women (tubal infertility) Age 27.0 (25.0-30.0), BMI 20.3 (19.0-23.5)</td>
<td>Control group: family history of PCOS Both groups: medical history of hypertension, diabetes, hyperprolactinemia, thyroid disease, Cushing’s syndrome, congenital adrenal hyperplasia, using insulin-sensitizing drugs, oral contraceptives, corticosteroids, anti-androgens or GnRH agonists/antagonists, undergone unilateral ovariectomy IVF/ICSI GnRH antagonist protocol Association between FF FGF13 levels and FF FGF21, LH, FSH, TT, FAI, E2, P4, IL-6 levels in both groups IVF outcomes: oocytes retrieved (n), MII oocytes rate (%), FR (%), high-quality embryo rate (%) Association between FF FGF13 levels and oocyte competence/IVF outcomes in both groups Higher FF TT levels were more prevalent among PCOS patients with higher FF FGF13 levels (vs. without higher FF FGF13 levels) FF TT levels were positively correlated&lt;sup&gt;<em>&lt;/sup&gt; with FF FGF13 levels in PCOS patients MII oocyte rate was negatively associated&lt;sup&gt;</em>&lt;/sup&gt; with FF FGF13 levels in PCOS patients</td>
</tr>
<tr>
<td>Tola, 2018&lt;sup&gt;12&lt;/sup&gt;</td>
<td>CSS</td>
<td>22 PCOS women (Rotterdam criteria) Age 28.5±3.6, BMI 27.9±5.9</td>
<td>20 normo-ovulatory non-PCOS women (tubal factors: salpingectomy for ectopic pregnancy or proximal tubal obstruction or fimbrial occlusion) Age 30.6±4.4, BMI 25.9±4.4</td>
<td>Control group: hydrosalpinx, severe pelvic adhesions, endometriosis, pelvic inflammatory disease Both groups: secondary causes of hyperandrogenism, endocrinopathy (hypo/hyperthyroidism, abnormal prolactin levels), any surgical procedure on the ICSI GnRH antagonist protocol, recombinant and/or urinary FSH FF thiol/disulphide parameters IVF outcomes: retrieved oocytes (n), GV/MII oocytes (n), fertilization rate (%), embryo count (n), clinical pregnancy rate (%) FF native thiol levels and native thiol/total thiol ratio were lower* in PCOS group FF disulphide levels, disulphide/native thiol and disulphide/total thiol ratios were higher* in PCOS group N. of oocytes retrieved were...</td>
</tr>
</tbody>
</table>
ovaries and uterus, smoking and alcohol consumption, male infertility

higher* in PCOS group, but MII oocyte and embryo counts were similar between groups FR among PCOS women was positively correlated* with FF native thiol levels Native thiol levels are positive predictors of the FR in this group

Artimani, 2018\textsuperscript{11}  CSS  21 PCOS women (Rotterdam criteria) Age 28.75±4.9, BMI 28.22±5.3  21 normo-ovulatory non-PCOS women (tubal obstruction or male infertility) Age 28.55±4.7, BMI 29.22±4.5  Adrenal dysfunction, hyperprolactinemia, congenital hyperplasia, thyroid disorders, tobacco smoking, any systemic disease  ICSI GnRH agonist long protocol, rFSH (GONAL-f®)  FF thiol groups, TAC, MDA, TOS, TNF-α, IL-6, IL-8 and IL-10 levels IVF cycle characteristics and outcomes: total rFSH dose, retrieved oocytes (n), MII oocytes (n), fertilization rate (%), embryo count (n), clinical pregnancy rate (%) Correlations between FF oxidative stress markers and cytokines levels  FF antioxidants levels were reduced* and pro-oxidants levels were increased* in PCOS group FF TNF-α, IL-6 and IL-8 levels were higher* and IL-10 was lower* in PCOS group TNF-α was positively correlated* with pro-oxidants and negatively correlated* with antioxidants A positive correlation* was found between IL-6 and MDA and between IL-10 and TAC levels
IL-8 and IL-10 were inversely correlated* with TAC and TOS levels, respectively. N. of oocytes retrieved were higher* in PCOS group. IVF outcomes were similar between groups.

| Age (years), BMI (kg/m²) | IL-8 and IL-10 were inversely correlated* with TAC and TOS levels, respectively. N. of oocytes retrieved were higher* in PCOS group. IVF outcomes were similar between groups. |

* indicates statistical significance result.

A = androstenedione, ADAMTS-1 = a disintegrin-like and metalloproteinase with thrombospondin type motifs 1, AFC = antral follicle count, AMH = antimüllerian hormone, BMI = body mass index, cAMP = cyclic adenosine monophosphate, CCS = case-control study, c-Met = mesenchymal epithelial transition factor (hepatocyte growth factor receptor), CML = N-carboxymethyl-lysine, hs-CRP = high sensitivity c-reactive protein, CSS = cross-sectional study, DHEA = dehydroepiandrosterone, E2 = oestradiol, FAI = free androgen index, FF = follicular fluid, FGF13 = fibroblast growth factor 13, FGF21 = fibroblast growth factor 21, FR = fertilization rate, G-CSF = granulocyte colony-stimulating factor, GnRH = gonadotropin-releasing hormone, GCs = granulosa cells, G3D3 = top quality embryos on day 3, GV = germinal vesicle, Hcy = homocysteine, HGF = hepatocyte growth factor, HMG = human menopausal gonadotrophins, HOMA-IR = homeostasis model assessment estimate of insulin resistance, ICSI = intracytoplasmic sperm injection, IL = interleukin, 8-IP = 8-isoprostane, IVF = in vitro fertilization, LH = luteinizing hormone, LPO = lipid peroxidation, MDA = malondialdehyde, MI = metaphase I, MII = metaphase II, miRNA = micro ribonucleic acid, mRNA = messenger ribonucleic acid, MS = meiotic spindle, n = number, Ob-R = leptin receptor, 8-OHdG = 8-hydroxy-2'-deoxyguanosine, 17OHP = 17-OH progesterone, P4 = progesterone, PCOS = polycystic ovarian syndrome, PIGF = placental growth factor, 2PN = two pronuclei (fertilized oocytes), p-STAT3 = signal transducer and activator of transcription 3, rFSH = recombinant follicle stimulating hormone, ROS = reactive oxygen species, sFlt-1 = soluble Fms-like tyrosine kinase-1, Sfrp-5 = secreted frizzle-related protein-5, SHBG = sex hormone-binding globulin, SOCS3 = suppressor of cytokine signalling 3, SOD = superoxide dismutase, sRAGE = soluble receptor for advanced glycation end products, T = testosterone, TAC = total antioxidant capacity, TAS = total antioxidant status, TNF-α = tumour necrosis factor α, TOS = total oxidant status, TSH = thyroid stimulating hormone, TSHR = thyroid stimulating hormone receptor, TT = total testosterone, VEGF = vascular endothelial growth factor.
4. Discussion

4.1. Interpretation

The physiological balance between pro-oxidants and antioxidants is necessary in order to ensure that some reproductive processes (i.e., folliculogenesis, oogenesis and ovulation) occur normally\(^{12,46}\). Over the years, oxidative stress has been linked to female infertility. Therefore, a possible association between oxidative stress and PCOS-related infertility has been hypothesised through its potential deleterious effects on the microenvironment for the developing oocyte\(^{46}\).

Overall, most of the included studies\(^ {9,11,12,27,39}\) showed a statistically significant tendency for PCOS women have higher levels of pro-oxidant markers (i.e., ROS, MDA, TOS and LPO) and lower antioxidant agents (i.e., TAC, free thiols groups and SOD activity) in the FF. These findings suggest a disruption of the pro-oxidant-antioxidant homeostasis, leading to a deviation towards OS in FF of these women.

Moreover, although without statistical significance in most studies\(^ {9,11,27,39}\), the number of MII oocytes, fertilization rate, pregnancy rate, embryo counts and quality tended to be lower and miscarriage rate higher in PCOS women, despite the significantly higher number of oocytes retrieved in these women. In addition, Rajani et al. (2012)\(^ {9}\) found a significantly lower proportion of MII oocytes with meiotic spindle in PCOS group.

In what concerns the potential relationship between FF profile and IVF outcomes of PCOS women, not significant difference in the FF SOD activity between fertilized and non-fertilized oocytes in both PCOS and control groups was found\(^ {39}\). Also, FF TAC was positively correlated with clinical pregnancy rate in the whole group, composed of PCOS women and women with male factor infertility, but not in PCOS group alone\(^ {10}\). However, FF ROS\(^ {9,27}\) and LPO\(^ {27}\) were significantly higher and TAC\(^ {27}\) significantly lower in oocytes lacking MS both in PCOS and control groups. Besides, fertilization rate and proportion of good quality embryos were significantly higher in oocytes with MS among PCOS women\(^ {9,27}\). Thus, the presence or absence of MS as well as the oxidative stress markers levels in the FF are potential candidates for predicting oocyte competence and, to some extent, IVF outcomes of PCOS women\(^ {9,27}\). Furthermore, a positive predictive effect of native thiol levels in FF on fertilization rate of PCOS women was identified\(^ {12}\).

Along with OS, inflammation has been linked to PCOS. In fact, ROS can increase inflammatory cytokines and both phenomena are directly associated with insulin resistance and
hyperandrogenism that characterize this syndrome\textsuperscript{11,47}. Thus, the role of inflammation in oocyte quality and other reproductive outcomes was also investigated. Regarding the concentration of cytokines in FF of PCOS women, levels of inflammatory cytokines (i.e., IL-6, IL-8 and TNF-\(\alpha\)) were significantly higher in contrast to concentration an anti-inflammatory cytokine (i.e., IL-10) that was significantly lower\textsuperscript{11}. Besides, inflammatory cytokines were positively correlated with pro-oxidant agents and negatively correlated with antioxidants. While, as expected, inversely correlations were found in what concerns the anti-inflammatory cytokine\textsuperscript{11}.

Based on the above, some researchers have investigated the effects of antioxidant agents on the follicular fluid oxidative stress parameters and IVF outcomes of women with PCOS. RCTs studying insulin-sensitizers\textsuperscript{8,25} revealed an improvement of the number of MII oocytes (myo-inositol) and quality (DCI or metformin), fertilization rate and embryo quality (myo-inositol), but not of the cumulative pregnancy rate. Similarly to myo-inositol, combined vitamin E and D\textsubscript{3} supplementation\textsuperscript{24} (with both anti-inflammatory and antioxidant properties) improved the number of MII oocytes, fertilization rate and embryo quality, but, in contrast to the insulin-sensitizer, these vitamins were demonstrated to have a positive effect on implantation and pregnancy rates.

Also, although DCI and metformin increased free thiol groups in FF proteins\textsuperscript{8} both myo-inositol and vitamins supplementation\textsuperscript{24} did not have a significant effect on FF oxidants and antioxidants levels nor showed a significant correlation between oxidative stress markers and IVF outcomes. Interestingly, these findings suggest that the improvements attributed to these agents are not explained by an underlying antioxidant mechanism\textsuperscript{24}. Finally, it is noteworthy that DCI, contrary to metformin, was not associated with adverse effects, leading to a potentially higher adherence rate\textsuperscript{8}.

\textbf{4.2. Limitations}

The present review has some limitations worth noting. About methodological limitations, despite the efforts to outspread the search using MeSH and synonyms, several factors may have restricted the number of relevant studies included such as the use of two databases only, the non-performance of the hand-search of included studies’ reference lists, the fact that full text of two eligible studies could not be found and the exclusion of grey literature. Additionally, the exclusion of studies published in languages other than English or Portuguese may have left out relevant additional data and introduced bias. Finally, regarding the risk of bias in individual studies, the main concerns were related to the fact that most of the studies failed to perform or report confounder adjustments.
The study group was composed of PCOS women diagnosed using Rotterdam criteria, irrespective of phenotype, comorbidities, ethnicity and even lifestyle (e.g., smoking habits), comprising a rather heterogeneous range of patients. In addition, some of them had another concomitant infertility factor (e.g., male factor). It is therefore necessary to be cautious when extrapolating the results, which may not represent the reality of all PCOS women.

Moreover, the studies were heterogeneous respective to reproductive interventions which included different assisted reproductive techniques (conventional IVF or ICSI due to underlying male factor infertility), controlled ovarian stimulation protocols and type of gonadotropin formulation as well as different treatment durations, within the same study and between studies, which may have had influence on the results.

Also, there was a limited number of studies assessing each follicular fluid factor and, beyond that, the sample size was small in most studies, which may have led to non-significant differences between study and control groups as well as inconsistent findings.

As the success of the IVF outcomes depends on several factors (other than oocyte competence) that have not been considered (e.g., endometrial receptivity), the findings and possible associations should be interpreted with caution.
5. Conclusion

5.1. Implications for Practice

FF is easily collected during oocyte retrieval and seems to be an optimal source for predictors of reproductive outcomes as well as a promising tool to fine-tuning therapy on an individual basis. However, the data included in the current review does not yet have power to draw stronger conclusions and propose a mechanism from which we can use FF as a therapeutical tool.

5.2. Implications for Research

Larger, high quality, prospective cohort studies and RCTs using a larger sample size are needed to achieve statistically significant differences between groups and potential replicate the findings present on Section 3.4. Furthermore, it would be interesting to study subgroups of PCOS population with different body mass index, androgen status, comorbidities as well as without other infertility factors, such as low endometrial receptivity or male infertility factor. Besides, further trials with different combination of drugs and supplements and doses should be undertaken to investigate new pharmacological weapons and to explore a possible dose effect.
References


35. Fabjan T, Vrtacnik-Bokal E, Virant-Klun I, Bedenk J, Kumer K, Osredkar J. Antimullerian Hormone and Oxidative Stress Biomarkers as Predictors of Successful Pregnancy in


47. González F. Inflammation in Polycystic Ovary Syndrome: underpinning of insulin resistance and ovarian dysfunction. (1878-5867 (Electronic)).

Annexes

Annex 1 – PROSPERO Protocol

Citation

Review question
How does follicular fluid composition of women with polycystic ovary syndrome compared with women with normal ovarian function and other infertility factor undergoing in vitro fertilization impacts reproductive outcomes?

Searches

Only articles in English and Portuguese will be considered for the purpose of this review. Articles with no abstract or that cannot be accessed in full will be excluded.

Gray literature will not be sought.

Types of study to be included
Inclusion criteria: observational studies (cohort and case-control studies) and randomised controlled trials; studies performed in humans, irrespective of race, with no restrictions on sample size; studies must include at least one group composed by women with normal ovarian function and another group composed with reproductive-aged women with PCOS diagnosis.

Exclusion criteria: studies conducted in other species; studies that do not assess any of the required outcomes; studies that did not mention study participants’ inclusion and exclusion criteria.

Condition or domain being studied
Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age and one of the main causes of female infertility. Women with PCOS undergoing in vitro fertilization (IVF) treatment are typically characterized by producing an increased number of oocytes, although they are often of poor quality, leading to poorer reproductive outcomes. Hence, it is possible that this condition causes the disruption of the sustainable follicular microenvironment that is crucial for oocyte maturation and embryonic developmental competence. Because of the intimate contact with the cumulus-oocyte complex, follicular fluid (FF) can be an essential lead to understand these phenomena, tailor therapy and, therefore, improving fertility of women with PCOS.

Participants/population
Inclusion criteria: Women with polycystic ovary syndrome (as diagnosed using Rotterdam consensus criteria of PCOS), irrespective of their phenotype, undergoing in vitro fertilization using their own oocytes, irrespective of the ovarian stimulation protocol used.

Exclusion criteria: Women undergoing assisted reproductive techniques other than IVF, IVF with oocyte donation or male infertility factor.

intervention(s), exposure(s)
Follicular fluid composition can be assessed after oocyte retrieval during IVF treatment. FF’s profiling can be used as a source for biochemical predictors of IVF outcomes and, therefore, potential biomarkers of oocyte and embryo quality.
Comparator(s)/control
Non-PCOS patients, including women with normal ovarian function and other infertility factors undergoing IVF, as the control group.

Main outcome(s)
To assess the relationship between differences in the concentrations of biomolecules in the follicular fluid (reactive oxygen species, hormones, lipid species, cytokines, growth factors, metalloproteases, advanced glycation end products, miRNAs and other microenvironment factors) and IVF reproductive outcomes (number of oocytes retrieved, metaphase II oocytes retrieved ratio, oocyte quality, fertilization rate, two pronuclei embryos ratio, pregnancy rate) of women with PCOS when compared to those of non-PCOS women undergoing IVF.

Measures of effect
Narrative synthesis to assess differences in the follicular fluid profiles of infertile women with polycystic ovary syndrome, as well as the underlying pathophysiological mechanisms associated with abnormal folliculogenesis and disordered oogenesis in order to find potential biomarkers of oocyte competence.

Additional outcome(s)
None.

Measures of effect
Not applicable.

Data extraction (selection and coding)
All records retrieved by electronic searching will be exported to EndNote Software X6 Version. Two reviewers (IA and EVF) will independently screen the papers retrieved from database searches for inclusion. Titles and abstracts will be firstly checked for eligibility and full texts in the next stage. The process will occur in close consultation with a third reviewer (MPM) that will resolve disagreements, blinded for the previous assessments.

Data will be extracted by two reviewers (IA, EVF) and cross-checked. The reviewers will extract information about the study (study’s name, first author’s name, country of origin, study design, year of publication and eligibility criteria), participants/study groups characteristics (PCOS diagnosis criteria, control group’s infertility factor, sample size in each group, mean age and BMI in each group), intervention (ovarian stimulation protocol), follicular fluid composition assessed and outcomes of interest (described in “main outcome(s)”). Data obtained will be entered into Microsoft Excel Software.

Risk of bias (quality) assessment
Cochrane RoB Tool for Randomized Controlled Trial. Cochrane Risk of Bias. Cambridge Quality Checklist. JADAD score. Newcastle-Ottawa Scale for Non-Randomized Study (cohort and case-control studies). This will be performed by three reviewers and a discussion will be held to reach consensus in cases of discrepancy. Only studies judged to be at least of moderate quality will be included.

Strategy for data synthesis
This review will summarize in a narrative manner due to heterogeneity in data and to better synthesise differences in the follicular fluid profiles and their relationship with IVF reproductive outcomes in order to clarify the role of follicular fluid constituents in clinical decision-making in the management of PCOS-associated infertility.

Analysis of subgroups or subsets
In literature, oligoovulatory ovarian dysfunction is referred to be the feature that explains subfertility in PCOS. However, more recent evidence shows that many women with PCOS are infertile, despite spontaneous or induced ovulations, leading to the hypothesis that there may be subclinical factors such as oocyte quality and oocyte competence that reduce the reproductive potential of these women. Therefore, follicular fluid analysis can be very helpful clarifying these phenomena.

Contact details for further information
Emidio Vale-Fernandes
Organisational affiliation of the review
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Review team members and their organisational affiliations
Dr Emídio Vale-Fernandes, Centre for Medically Assisted Procreation, Centro Materno Infantil do Norte Dr Alcino Aroso (CMIN) - Centro Hospitalar Universitário do Porto (CHUP); Unit for Multidisciplinary Research in Biomedicine (UMIB), Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto; Portugal
Professor Mariana P Monteiro, Unit for Multidisciplinary Research in Biomedicine (UMIB), Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto; Portugal
Professor Maroço G Alves, Unit for Multidisciplinary Research in Biomedicine (UMIB), Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto; Portugal
Miss Inês C Alberqaria, Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto; Portugal

Type and method of review
Narrative synthesis, Prevention, Prognostic, Systematic review

Anticipated or actual start date
20 February 2021

Anticipated completion date
31 May 2021

Funding sources/sponsors
None.

Grant number(s)
State the funder, grant or award number and the date of award

Conflicts of interest

Language
English

Country
Portugal

Stage of review
Review Ongoing

Subject index terms status
Subject indexing assigned by CRD

Subject index terms
MeSH headings have not been applied to this record

Date of registration in PROSPERO
19 March 2021

Date of first submission
10 February 2021

Stage of review at time of this submission
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The record owner confirms that the information they have supplied for this submission is accurate and complete and they understand that deliberate provision of inaccurate information or omission of data may be construed as scientific misconduct.

The record owner confirms that they will update the status of the review when it is completed and will add publication details in due course.

**Versions**
- 16 March 2021
- 19 March 2021
Annex 2 – PubMed and Web of Science Search Queries

PubMed Query

((("Polycystic Ovarian Syndrome" OR "Polycystic Ovary Syndrome" OR "Polycystic Ovary Syndrome 1" OR "Polycystic Ovaries" OR "PCOS" OR "PCO" OR "PCO1" OR "Stein-Leventhal Syndrome" OR "Polycystic Ovary Disease" OR "Polycystic Ovarian Disease" OR "PCOD" OR "Sclerocystic Ovarian Degeneration" OR "Sclerocystic Ovary Syndrome" OR "Sclerocystic Ovarian Disease" OR "Polycystic Ovary Syndrome"[Mesh]) AND ("Follicular Fluid" OR "Antral Fluid" OR "Liquor Folliculi" OR "Follicular Fluid"[Mesh]) AND ("In Vitro Fertilization" OR "In Vitro Fertilisation" OR "IVF" OR "Fertilization in Vitro" OR "Controlled Ovarian Stimulation" OR "Controlled Ovarian Hyperstimulation" OR Stimulated Cycle* OR "Fertilization in Vitro"[Mesh])) AND (Outcome* OR Folliculogenesis OR Ovocyte OR "Oocyte Quality" OR "Oocyte Competence" OR "Oocyte Maturation" OR Embryo OR "Embryo Quality" OR "Ovarian Follicle"[Mesh] OR "Oogenesis"[Mesh] OR "Fertilization"[Mesh] OR "Embryonic Development"[Mesh] OR "Pregnancy"[Mesh] OR "Fertility"[Mesh]))

Details:


Translations

Stimulated: "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]

Ovocyte: "oocytes"[MeSH Terms] OR "ovocytes"[All Fields] OR "ovocyte"[All Fields] OR "ovocytes"[All Fields]
Embryo: "embryo's"[All Fields] OR "embryoes"[All Fields] OR "embryonic structures"[MeSH Terms] OR ("embryonic"[All Fields] AND "structures"[All Fields]) OR "embryonic structures"[All Fields] OR "embryo"[All Fields] OR "embryos"[All Fields]

Web of Science Query

TS=("Polycystic Ovar* Syndrome" OR "Polycystic Ovary Syndrome 1" OR "Polycystic Ovaries" OR "PCOS" OR "PCO" OR "PCO1" OR "Stein-Leventhal Syndrome" OR "Polycystic Ovar* Disease" OR "PCOD" OR "Sclerocystic Ovar* Degen*ration" OR "Sclerocystic Ovar* Syndrome" OR "Sclerocystic Ovar* Disease") AND TS=("Follicular Fluid" OR "Antral Fluid" OR "Liquor Folliculi") AND TS=("In Vitro Fertilization" OR "IVF" OR "Fertilization in Vitro" OR "Intracytoplasmic Sperm Injection*" OR "ICSI" OR "Controlled Ovarian Stimulation" OR "Controlled Ovarian Hyperstimulation" OR "Stimulated Cycle*") AND TS=("Outcome*" OR "Follic*" OR "Oocyte*" OR "Ovocyte*" OR "Oocyte Quality" OR "Oocyte Competence" OR "Oocyte Maturation" OR "Oogenesis" OR "Embryo*" OR "Development*" OR "Embryo Quality" OR "Fertilization" OR "Pregnancy*" OR "Fertility" OR "Fecundability")

Índices=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC Tempo estipulado=Todos os anos
## Annex 3 – Newcastle-Ottawa Quality Assessment of Prospective Cohort Studies

Table III. Newcastle-Ottawa Quality Assessment of Prospective Cohort Studies

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## Annex 4 – Newcastle-Ottawa Quality Assessment of Case-Control Studies

### Table IV. Newcastle-Ottawa Quality Assessment of Case-Control Studies

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<th>Definition of controls</th>
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- means no stars
### Annex 5 – Joanna Briggs Institute Risk of Bias Assessment of Cross-Sectional Studies

#### Table V. Joanna Briggs Institute Risk of Bias Assessment of Cross-Sectional Studies

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</table>

Y, N, U and NA mean “Yes”, “No”, “Unclear” and “Not Applicable” respectively
## Annex 6 – Risk of Bias Assessment of Randomized Controlled Trials using a Cochrane Tool (RoB 2)

### Table VI. Risk of Bias Assessment of Randomized Controlled Trials using a Cochrane Tool (RoB 2)

<table>
<thead>
<tr>
<th>First Author, Publication Year</th>
<th>Domain 1</th>
<th>Domain 2</th>
<th>Domain 3</th>
<th>Domain 4</th>
<th>Domain 5</th>
<th>Overall risk of bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onalan, 2005(^1)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Kim, 2011</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Piomboni, 2014</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cheraghi, 2016</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fatemi, 2017</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sene, 2019</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+, ?, and - mean “Low risk of bias”, “Some concerns” and “High risk of bias” respectively
Annex 7 – Risk of Bias Assessment of Randomized Controlled Trials using a Cochrane Tool (RoB 2) (detailed version)

Table VII. Risk of Bias Assessment of Randomized Controlled Trials using a Cochrane Tool (RoB 2) (detailed version)

<table>
<thead>
<tr>
<th>First Author, Publication Year</th>
<th>Domain 1</th>
<th>Domain 2</th>
<th>Domain 3</th>
<th>Domain 4</th>
<th>Domain 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.1</td>
<td>1.2</td>
<td>1.3</td>
<td>2.1</td>
<td>2.2</td>
</tr>
<tr>
<td>Onalan, 2005</td>
<td>Y</td>
<td>PY</td>
<td>N</td>
<td>N</td>
<td>PN</td>
</tr>
<tr>
<td>Kim, 2011</td>
<td>Y</td>
<td>PY</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Piomboni, 2014</td>
<td>Y</td>
<td>PY</td>
<td>N</td>
<td>N</td>
<td>NI</td>
</tr>
<tr>
<td>Cheraghi, 2016</td>
<td>Y</td>
<td>PY</td>
<td>N</td>
<td>N</td>
<td>PN</td>
</tr>
<tr>
<td>Fatemi, 2017</td>
<td>Y</td>
<td>PY</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Sene, 2019</td>
<td>Y</td>
<td>PY</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

Domain 1 – Randomization process: 1.1. Was the allocation sequence random? 1.2. Was the allocation sequence concealed until participants were enrolled and assigned to interventions? 1.3. Did baseline differences between intervention groups suggest a problem with the randomization process?

Domain 2 – Deviations from intended interventions: 2.1. Were participants aware of their assigned intervention during the trial? 2.2. Were carers and people delivering the interventions aware of participants’ assigned intervention during the trial? 2.3. If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the trial context? 2.4. If Y/PY to 2.3: Were these deviations likely to have affected the outcome? 2.5. If Y/PY/NI to 2.4: Were these deviations from intended intervention balanced between groups? 2.6. Was an appropriate analysis used to estimate the effect of assignment to intervention? 2.7. If N/PN/NI to 2.6: Was there potential for a substantial impact (on the result) of the failure to analyse participants in the group to which they were randomized?

Domain 3 – Missing outcome data: 3.1. Were data for this outcome available for all, or nearly all, participants randomized? 3.2. If N/PN/NI to 3.1: Is there evidence that the result was not biased by missing outcome data? 3.3. If N/PN to 3.2: Could missingness in the outcome depend on its true value? 3.4. If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?

Domain 4 – Measurement of the outcome: 4.1. Was the method of measuring the outcome inappropriate? 4.2. Could measurement or ascertainment of the outcome have differed between intervention groups? 4.3. If N/PN/NI to 4.1 and 4.2: Were outcome assessors aware of the intervention received by study participants? 4.4. If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received? 4.5. If Y/PY to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?

Domain 5 – Selection of the reported result: 5.1. Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis? 5.2. Is the numerical result being assessed likely to have been selected, on the basis of the results, from multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain? 5.3. Is the numerical result being assessed likely to have been selected, on the basis of the results, from multiple eligible analyses of the data?

Y, PY, N, PN and NA mean “Yes”, “Probably Yes”, “No”, “Probably No”, “No Information” and “Not Applicable” respectively