Follicular fluid and supernatant from cultured cumulus-granulosa cells improve in vitro maturation in patients with polycystic ovarian syndrome

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Objective: To study the effectiveness of a new in vitro maturation (IVM) approach based on heterologous follicular fluid (HFF) and supernatant of cumulus-granulosa cells (CGCs) mimicking the intact follicular microenvironment to rescue immature denuded oocytes (IDOs) of patients with polycystic ovary syndrome (PCOS) whose IVM or IVF outcomes remain poor.
Design: Randomized controlled trial.
Setting: University-affiliated private center.
Patient(s): One hundred fifty-nine IDOs were obtained from 47 patients with PCOS. First, a simple IVM system (S-IVM; 40 IDOs; control group) was compared with different protocols based on the addition of autologous follicular fluid (AFF-IVM; 44 IDOs), HFF (HFF-IVM; 42 IDO), or HFF with CGC isolated from seven women without PCOS and presenting 100% in vivo oocyte maturation (HFF/CGC-IVM; 33 IDOs).
Intervention(s): None.
Main Outcome Measure(s): IVM outcomes were compared among the four groups (S-IVM, AFF-IVM, HFF-IVM, HFF/CGC-IVM); then the vitro and in vivo maturation results (from controlled ovarian stimulation of PCOS patients) were compared for each group.
Result(s): The HFF/CGC-IVM method gave the best yield of developed blastocysts per IDO compared with S-IVM, AFF-IVM, and HFF-IVM (27% vs. 2%, 2%, and 12%, respectively). The IVM rate with the HFF/CGC-IVM method was even higher than that compared with the in vivo maturation rate (79% vs. 42%), with significant improvement in the cleavage rate (71% vs. 61%).
Conclusion(s): This adapted IVM system could be used to reach an acceptable result in meiotic competence and competent metaphase II oocytes capable of developing into intact embryos after fertilization and before transfer. (Fertil Steril® 2018;110:710–9. ©2018 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: In Vitro maturation, polycystic ovary syndrome, heterologous follicular fluid, cultured cumulus granulosa cells supernatant

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Human in vitro maturation (IVM) is an approach that was developed about 50 years ago, although its use in IVF methods is still limited mainly to oncofertility treatments and female factor infertility, especially in the case of polycystic ovary syndrome (PCOS). IVM can be considered as an additional tool in IVF to mimic physiological oocyte maturation, which corresponds to resumption of the first meiotic division and progression from diplotene to metaphase II (MII). Oocyte maturation is characterized by the disappearance of the germinal vesicle (GV), chromosome condensation, spindle formation and separation of homologous chromosomes, extrusion of the first polar body, and arrest of the meiotic process at MII until fertilization (1). Successful oocyte maturation requires the completion of nuclear maturation through meiosis I and II and the maintenance of a good synchronization with cytoplasmic maturation (2, 3).

The key to IVM success goes beyond the identification of the best IVM medium and technical conditions. Therefore, the oocyte, its source, and its follicular environment should be considered as the crucial points. This could offer an acceptable explanation for the currently poor implantation rate and delivery rate per transferred embryos after IVM (about half of the rates obtained after routine IVF) (4), particularly in the case of women with PCOS (5, 6). However, other studies did not find any difference between patients with and without PCOS (7, 8).

PCOS is an ovarian disorder that affects 5%–10% of all women of reproductive age and 50% of subfertile women (9). This heterogeneous endocrine dysfunction alters the intrafollicular environment by disturbing the local paracrine/autocrine signaling (10) and the immune balance (11, 12). Therefore, women with PCOS are particularly sensitive to endocrine stimulation, although it is required for increasing the number of mature oocytes and their competence, which is regulated by several molecular pathways linked intimately to the ovarian cytokine microenvironment (13). This dysregulated ovarian response could explain at least in part (14, 15) the reduced maturation rate and quality of oocytes from women with PCOS. Indeed, follicular fluid (FF) from women with PCOS shows an increased level of VEGF, TNF, and IL-4 and -7 (12, 14, 15) and altered expression of many cytokines and growth factors, including EGF, FGF, IGF, NGF, PGE2, MMP, PA, GDF9/BMP15, IL6 (15–17).

It was previously shown that supplementing IVM medium with autologous FF (AFF) promotes oocyte maturation (18, 19) due to the presence of important cumulus-oocyte complex components, such as cytokines, growth factors, amino acids, and antioxidants (14). Moreover, coculture of immature denuded oocytes (IDOs) with cumulus-granulosa cell (CGC) monolayers or clumps, as feeder cells, can significantly improve the embryonic development yield (20–22). Therefore, in this study, we compared the maturation of IDO from stimulated patients with PCOS by using three different IVM approaches: [1] simple IVM system (S-IVM; medium alone), [2] addition of AFF (AFF-IVM), [3] addition of HFF (HFF-IVM), and [4] addition of HFF and heterologous CGC supernatant (HFF/CGC-IVM). Starting from the hypothesis that the composition of AFF from women with PCOS might be altered, we hypothesized that HFF from women with 100% in vivo oocyte maturation might supply the cytokines and growth factors required for oocyte maturation and developmental competency. Similarly, supplementation with HFF and CGC might further improve IVM efficiency because cultured CGCs can secrete factors that are absent in FF or in CGC clumps.

Furthermore, to limit the number of confounding factors, such as the oocyte source, type of ovarian stimulation, underlying infertility, and advanced maternal age, this study included a homogeneous sample of patients with PCOS younger than 40 years of age who underwent IVF using an undergoing antagonist protocol. Finally, IVM rate was also compared with the in vivo maturation results for each group.

MATERIALS AND METHODS

Ethical Standards

The study was approved by the ethics committee (Comité d'Ethique pour la Recherche Biomédicale, Faculty of Medicine and Pharmacy, University Mohammed V, Rabat, Morocco), and patients provided written informed consent after being presented with the terms and issues of the study. The authors assert that all procedures used in this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Patient Selection and Study Design

This study included 47 women with PCOS (a total of 279 collected oocytes classified based on maturation: 159 immature oocytes and 120 mature oocytes) referred to the IRIFIV center, over 2 years. PCOS was diagnosed based on the Rotterdam European Society of Human Reproduction and Embryology/American Society of Reproductive Medicine 2003 criteria (polycystic ovaries, oligo- or anovulation, and clinical or biochemical evidence of hyperandrogenism). Women with PCOS were all younger than 40 years of age (the advanced maternal age cutoff) (23). They underwent at least two previous IVF attempts and presented heterogeneous follicular cohorts with maturation rate under 50% and MII cleavage rate lower than 80%. All women received the same antagonist ovarian stimulation protocol (24) to minimize the effect of other parameters. Their partner's sperm presented normal conventional parameters, and paternal age was lower than 40 years to exclude any male factor on intracytoplasmic sperm injection (ICSI) results.

Due to the low maturation rate at the previous IVF attempts, different IVM protocols were compared: [1] S-IVM (control; n = 13 patients; 36 MII and 40 IDOs); [2], AFF-IVM (n = 14; 35 MII and 44 IDOs); [3] HFF-IVM (n = 12; 32 MII and 42 IDOs); and [4] HFF/CGC-IVM (n = 8; 24 MII and 33 IDOs). Patients were randomly assigned to one of the four IVM groups (see Fig. 1 for a schematic of the study protocol). Specifically, immature (GVs and metaphase I [MI]) oocytes and mature oocyte (MII) were retrieved, after hormonal stimulation. Immature oocytes were in vitro matured using one the four IVM protocols Then, the percentage of in vivo–matured oocytes was compared with the IVM