Can we modify assisted reproductive technology practice to broaden reproductive care access?

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One of the barriers to access to fertility care is the relative complexity of fertility treatments. If these can be simplified, more patients may be able to take advantage of these treatments. In this overview, we review the potential benefits of simplifying ovarian stimulation by the means of four distinct methods: 1) using mild stimulation for IVF cycles; 2) using in vitro maturation to allow for the retrieval of oocytes that are not yet fully mature yet have the potential to result in live births; 3) conducting IVF in modified natural cycles which use no exogenous FSH stimulation; and 4) allowing embryo culture to take place in a novel intravaginal incubation system. These methods are considered to be somewhat unconventional, yet they have all been shown to lead to live births. In the era of individualized patient care, these techniques present viable alternatives to standard treatment. As experience and outcome data accumulate, they may prove to be not just alternatives to standard treatment, but potentially first-line treatment choices. (Fertil Steril® 2016;105:1138–43. ©2016 by American Society for Reproductive Medicine.)

Key Words: ART, Mild stimulation, IVM, natural cycle IVF, intravaginal culture

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There are many factors that limit patient access to fertility care. In countries where insurance does not cover the treatment, cost is often a primary barrier. However, even in countries where cost is not an issue, patients frequently do not avail themselves of care they need due to its perceived complexity, physical stress, and the emotional toll that fertility treatment entails.

Many modifications of fertility treatment have been proposed over the past 30 or so years. In this article, we present an overview of four such modifications: mild stimulation for in vitro fertilization (IVF), in vitro maturation (IVM) as an alternative to stimulation, the modified natural cycle for IVF (which includes IVM), and intravaginal culture as an alternative to standard laboratory incubators and embryo culture. These techniques are as yet unconventional, but they have the potential to make IVF accessible to patients who would otherwise not be able to take advantage of this technology. This can be due to lower cost, less stress, and/or lower physical trauma to the patient.

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MILD APPROACHES IN IVF: IMPROVING ACCESS TO CARE BY REDUCING COST, BURDEN OF TREATMENT AND COMPLICATIONS

IVF history books tell us that the very first IVF pregnancy occurred after ovarian stimulation with the antiestrogen clomiphene citrate. However, that pregnancy ended in a miscarriage. Professor Bob Edwards subsequently speculated regarding the possible involvement of abnormal corpus luteum function due to ovarian stimulation for IVF. The first live birth took place in 1978 after IVF in a completely natural cycle. In subsequent years, clomiphene citrate stimulation for IVF was developed in the early 1980s in Australia and the use of exogenous gonadotropins for IVF was reported shortly thereafter in the USA. GnRH agonists were subsequently used to prevent premature luteinization due to ovarian stimulation interfering with

steroid feedback at the hypothalamic-pituitary level. In addition, oral steroid pretreatment has been widely used to schedule the IVF cycle, as reviewed by Macklon et al. (1).

It seems justified to conclude that currently used ovarian stimulation regimens have become extremely complex and expensive, with substantial concern regarding patient compliance, as well as inducing the need for frequent hospital visits and ovarian response monitoring. For example, in the Netherlands, costs associated with ovarian stimulation are equal to the cost of the IVF procedure itself.

Ovarian stimulation is used to initiate the IVF procedure with multiple oocytes, to compensate for suboptimal laboratory performance regarding oocyte fertilization, embryo development and selection, and embryo transfer and implantation. It is generally thought that a large number of oocytes is required for optimal IVF outcomes. Based on cross-sectional national data analysis, the optimal oocyte number is thought to be ≥ 15 (2). However, such analyses only assess pregnancy rates per cycle following conventional IVF in splendid isolation, disregarding other associated features such as cost, patient discomfort, and burden of treatment (giving rise to drop-outs from subsequent IVF treatment and therefore reducing cumulative pregnancy rates involving multiple IVF cycles), and risk of complications.

Overall, clinicians associate low oocyte numbers being retrieved with poor IVF outcome. Poor ovarian response to maximum stimulation represents a distinct sign of ovarian ageing (3). Under those circumstances, poor IVF outcome is due to a patient factor which is completely unrelated to ovarian stimulation per se.

We advocated more than 15 years ago (4) that the paradigm of maximum ovarian stimulation was in need of revision, based on the above-mentioned arguments. We have subsequently undertaken a series of prospective randomized studies, demonstrating that the generation of fewer oocytes after mild ovarian stimulation gives rise to improved embryo quality and implantation (5), an increased proportion of euploid embryos (6), improved embryo implantation rates at lower oocyte numbers (7), and reduced drop-out rates (8). When live birth rates per started treatment (involving multiple IVF cycles) was used as the primary end point, mild ovarian stimulation resulted in similar live birth rates (9). Mild ovarian stimulation as a realistic alternative in IVF has recently been reported by other independent investigators (10, 11). Moreover, stimulating growth of more ovarian follicles with higher doses of exogenous FSH does not result in increased live birth rates (12). Recent data suggest that fewer oocytes are required for success when mild ovarian stimulation is used (presumably 10 rather than 20 oocytes to generate a single live birth). Owing to recent improvements in cryopreservation technology, fresh transfer can be restricted to a single embryo only without sacrificing the chances of success from the entire harvest of oocytes from a single stimulation cycle.

It is generally acknowledged that great individual variability exists in ovarian response to stimulation, in relation to female age and ovarian reserve markers such as the antral follicle count and antimüllerian hormone concentrations. The challenge is to first assess what would be the optimal number of oocytes to be retrieved, which we have speculated in the past to be somewhere around five to eight (13, 14). Subsequent prospective studies should be designed with the use of different drugs and doses to develop the preferred stimulation protocol to achieve the optimal balance between IVF success, burden of treatment, complications, and cost (14, 15).

Future studies should focus on improved access to care (directly related to health economics of IVF) and reducing burden of treatment (16) in influencing overall success rates per started treatment.

IN VITRO MATURATION OF OOCYTES

In vitro maturation is a technique that differs from conventional in vitro fertilization treatment in two major ways: absence of controlled ovarian hyperstimulation; and collection of immature oocytes that are cultured in vitro until they reach the metaphase II (MII; mature) stage. Robert Edwards, the pioneer of IVF, thought that recovery of immature oocytes followed by IVM would be a potentially useful treatment for women with infertility (17, 18). IVM was first used successfully in humans in 1991 in an unstimulated donor cycle (19), and the first successful use of IVM in patients with polycystic ovary syndrome (PCOS) occurred in 1994 (20).

There is no universal protocol for performing IVM. One approach recommends administration of FSH at a dose of 100 IU/d for 3 days followed by 10,000 IU hCG, with immature oocyte pick-up 36 hours after hCG. The collected oocytes can then be classified into two groups based on their maturity level at collection. Germinal vesicle (GV) and metaphase I (MI) stage oocytes are cultured in a human tubal fluid medium that is supplemented with FSH 7.5 IU/mL, hCG 100 IU/mL, growth hormone 1 IU/mL, and 10% patient serum for 20 hours. MII-stage oocytes are inseminated on the same day. All mature oocytes are then inseminated with the use of intracytoplasmic sperm injection.

IVM has a number of advantages over conventional IVF, including safety (elimination of ovarian hyperstimulation syndrome [OHSS] in PCOS), low cost (owing to the lack of the stimulation requirement), and convenience (less patient stress, lower medication use, and fewer controls). However, there are also a number of concerns about IVM. The first of these relates to success rate. In patients with PCOS, initial reports showed a pregnancy rate of 21.9%–29.9% (21, 22). However, more recent data show that the success rate of IVM has improved, with pregnancy and delivery rates of 32%–44% and 22%–29%, respectively (23, 24). One study reported that, with single-blastocyst transfers after IVM in PCOS patients, the live birth rate could be as high as 42.4% per oocyte collection (25). The most recent analysis of IVM versus IVF in PCOS patients was a retrospective case-control study of 121 subjects who underwent 178 treatment cycles (26). The results showed no difference in clinical pregnancy rates for fresh or frozen embryo transfer (FET) cycles between the IVM and IVF groups, although the cumulative pregnancy rate was lower in the IVM group. In addition, significantly fewer live births resulted from IVM treatment for both fresh and cumulative cycles, but there was no

difference in live birth rates resulting from FET between the IVM and IVF groups (26).

In normo-ovulatory patients, the clinical pregnancy rate after IVM has been reported to be lower than that in PCOS patients (30%) (27, 28). Furthermore, there have been some concerns raised about the effects of IVM on the health of resulting offspring. For example, high rates of chromosomal abnormalities have been documented in IVM embryos, with a link between higher rates and longer periods of IVM (29, 30). In addition, IVM may induce permanent changes in the expression of imprinted genes (epigenetic changes) (31, 32). However, data for the period 1999–2004 show similar complication and malformation rates in babies born after IVM and IVF procedures (33). More recent published data also indicated a normal pregnancy course for after IVM compared with IVF cycles (34), normal growth and development in 196 babies born from IVM cycles (35), and the delivery of 1,421 healthy infants following immature oocyte retrieval and IVM (36).

In Vietnam, as well as the advantages for IVM over conventional IVF described above, IVM halves the cost per treatment cycle, increasing patient access to assisted reproductive technology (ART) treatment. IVM has been used in Vietnam since 2006, with ~2,600 cycles performed to date. Clinic report data from the start of IVM use in 2006 to the end of 2014 showed clinical pregnancy and live birth rates of 38% and 29%, respectively. Overall, 610 babies have been born from 598 pregnancies (543 at $\geq$34 weeks and 55 at <34 weeks). Mean birth weights for singletons and twins were 3,050 $\pm$ 120 g and 2,540 $\pm$ 65 g, respectively. A total of six congenital anomalies were observed: three Down syndrome pregnancies were terminated at 22 weeks, two infants had cleft palates, and one had polydactyly.

Overall, based on the available data, it would appear that IVM is a simple, convenient, and cost-effective technique that is associated with a good success rate in selected patients, such as those with PCOS. Data are also reassuring about the safety of IVM, although treatment of more patients with the use of this technique is required before definitive statements can be made in this regard.

By including IVM in the range of possible ART options, fertility specialists can provide infertility solutions to a wider range of patients, thus improving access to treatment. Awareness of barriers to the use of IVM, such as concerns about success rate, neonatal/infant health, possible insurance coverage issues, and availability of other strategies to reduce OHSS incidence, is essential. More importantly, to overcome these barriers, there need to be better training for clinicians, more research, and education for more IVF specialists about IVM. It is also important that funding is available for research into IVM and that professional societies (e.g., American Society of Reproductive Medicine) work to overcome barriers to improving fertility services and care, which is something that could be facilitated by increased use of IVM.

The goal of all ART is to help patients fulfill their most basic desires for reproduction. IVM still has some limitations, and its use may require some calculated risk taking and courageous decision making, but without this it would not have been possible to help many people to create many happy families.

MODIFIED NATURAL-CYCLE IVF

As noted previously, the first live birth achieved with the technique of IVF took place in a purely natural cycle (37). However, the inefficiency of the early IVF laboratory pushed clinicians toward controlled ovarian stimulation to increase the number of oocytes available and thus increase the efficiency of the process. As success increased, controlled ovarian stimulation became the standard of practice, and it remains so to this day. The efficiency of follicle aspiration was greatly increased by the advent of ultrasound-guided transvaginal follicle aspiration in the late 1980s. It also significantly reduced the trauma to the patient, and, therefore, it became reasonable to reinvestigate the option of IVF in a cycle without follicular stimulation (38, 39). To optimize scheduling of follicle aspiration, hCG triggering of ovulation was added to the process. Several series proved the viability of this new version of natural-cycle IVF (39–41). Embryo implantation rates were similar to those of stimulated cycles, but a lower number of embryos transferred, combined with higher cancellation rates (primarily owing to premature ovulations), led to overall lower success rates. In a review of 20 studies and 1,800 cycles (42) Pelinck et al. reported 129 ongoing pregnancies, representing 15.8% per transfer but only 7.2% per cycle. The cancellation rate ranged from 15% to 71%.

Antagonists of GnRH first became clinically available in the early 1990s. Premature ovulation could be prevented by the administration of the antagonist in the late follicular phase. This was a simple addition to stimulated cycles; however, in a natural cycle, suppression of the LH surge also inhibited further FSH secretion. For follicular development to continue, exogenous FSH had to be added back, either alone (43) or in combination with LH in an hMG preparation (44). This method eventually came to be called the "modified natural cycle." Early reports were promising (45), and most centers offering natural-cycle IVF have adopted this approach. In the largest series from a single institution (46), the pregnancy rate in women <35 years of age was 35% per transfer and 19.6% per successful aspiration. Recently, we have reported the option of using hCG (200 IU) alone to complete follicle maturation after GnRH antagonist is initiated (47), in a manner analogous to the approach in stimulated cycles (48, 49). Whereas this information is in the preliminary stages, it promises to further decrease the cost of the procedure because hCG is much less expensive than FSH-containing preparations.

One of the problems in natural-cycle IVF is that there is typically only one dominant follicle, and follicle aspiration is not 100% successful in retrieving that oocyte. If secondary follicles could also be aspirated, the overall success of the process could be increased. Therefore, IVM has the potential to increase the success of modified natural-cycle IVF (50). In cycles with controlled ovarian stimulation, there are several mature oocytes retrieved. Oocytes that are immature at the time of retrieval are typically retrieved from follicles that failed to respond to exogenous gonadotropins. Many of these follicles may already be apoptotic, and the results with IVM of these immature oocytes have been disappointing. However, in

a natural cycle, many secondary follicles have not grown because they have not experienced sufficient FSH stimulation, not necessarily because they are undergoing apoptosis. Oocytes that are retrieved from these follicles often mature after 24 hours in the laboratory and are capable of producing pregnancies (47, 50). An important component of success with the use of this approach is the embryo-endometrial synchrony. In stimulated cycles, progesterone levels are well above the luteinization threshold at the time of follicle aspiration. Therefore, embryos whose development is delayed are even further from synchrony with the endometrium. In contrast, in natural IVF cycles, progesterone levels at the time of egg retrieval are well below the luteinization threshold (51). Therefore, oocytes that are fertilized 24 hours after retrieval are still in good synchrony with the endometrium.

In summary, modified natural-cycle IVF produces embryo implantation rates that are similar to those of stimulated cycles, while being associated with lower cost and virtually no risk of OHSS. The purely natural cycle which led to the first IVF pregnancy in the world has been modified in important ways that have made it more successful: triggering of ovulation with hCG, prevention of premature ovulation with GnRH antagonists, while maintaining follicle growth with low-dose hCG, and the addition of IVM, which decreases the rate of unsuccessful follicle aspirations and increases the pregnancy potential of these cycles. The modified natural IVF cycle is a viable first choice for good-prognosis patients and a good second-line option for poor responders (47, 52).

VAGINAL INCUBATION OF EGGS AND EMBRYOS

Optimal embryo development has been shown to be influenced by subtle changes in environmental conditions, such as pH, temperature, and oxygen concentration (53). Traditional embryology laboratory incubators are complex electro-echanical devices designed to maintain precise temperature, CO_2 and O_2 environments. It is well known that these sophisticated devices can fail without warning. The temperature or gas environment can drift. Integrated digital displays may be inaccurate. For these reasons, daily quality-control checks of temperature, CO_2, and O_2 with the use of independently calibrated instruments are necessary. The possibility of abrupt catastrophic failure leads to a need for alarm systems and remote 24-7 monitoring.

The modern IVF laboratory also requires expensive air-filtration systems (54), because the embryo has no lung, kidney, or liver to filter atmospheric contaminants, including potentially embryotoxic volatile organic compounds (VOCs). As a result of the above complexity, provision of high-quality IVF laboratory services has been necessarily expensive and generally restricted to urban areas. The combination of financial burdens and geographic disparities significantly restricts access to ART (55).

Intravaginal culture (IVC) was proposed as a means to reduce the overall burden and increase access to reproductive care nearly 30 years ago (56). Initially, IVC was performed with the use of supplies intended for other uses (cryogenic vials sealed with polyethylene tubing or paraffin film). More recently, a system specifically designed for IVC was devised. This small gas-permeable plastic device (Invocell; Invo Bioscience) was approved in November 2015 by the Food and Drug Administration for the indication of human egg fertilization and subsequent embryo culture. Invocell takes advantage of the low oxygen and high CO_2 concentrations in the vaginal cavity (57). This system supplies the appropriate atmospheric environment and temperature to facilitate fertilization and early embryo development. The modern IVC technique places oocytes and sperm or inseminated eggs into the inner chamber of this simple device. The inner chamber is inserted into a protective outer plastic shell which is subsequently placed in the vaginal cavity for incubation. This procedure allows fertilization and embryo development to occur within the female reproductive tract. It has been hypothesized that some women may view this form of assisted reproduction as more "natural."

IVC procedures have been performed in a number of countries with the use of different systems. The initial case series with the use of improvised materials demonstrated the feasibility of IVC; however, the live birth rate was only 17% per egg retrieval procedure (56). Other early attempts at IVC with the use of improvised supplies demonstrated many difficulties, including observations of variations in pH of the culture medium, bacterial contamination, and loss of embryos. The Invocell device was designed to address these identified technical problems. Recent studies have used Invocell rather than improvised IVC systems (58).

The effectiveness of IVC to reduce the burden of fertility care can be determined only after its efficacy is established. Lucena et al., using a mild stimulation protocol, found that in 125 cycles, IVC yielded a live birth rate of 31.2% (59). A second nonrandomized trial of 24 patients resulted in 13 clinical pregnancies. This was similar to an internal matched IVF/ICSI control group of 74 cycles, which generated a pregnancy rate 58% (60).

A recent prospective pilot study evaluated the efficacy of extended culture in IVC. Forty patients underwent treatment with a relatively low dose but conventional stimulation (61). On the day of hCG trigger, patients were randomized to 5-day culture in either Invocell or a traditional laboratory incubator system. Live birth was achieved in 11 of 20 IVC cycles compared with 12 of 20 cycles randomized to the laboratory incubators.

Although further study is needed, IVC has the potential to remove the need for a sophisticated and costly IVF laboratory as well as reducing overall embryologist intervention (fertilization and embryo progression checks). The associated reduced capital costs and operating expenses may improve affordability and access to ART services.

SUMMARY

Since the first successful IVF cycle, great scientific and technologic advances have changed the field of reproductive medicine and have made fertility treatment a reality. But as ART became more technologically advanced, it has also become more complex. Technologic advances have led to greater success but have also been associated with greater

cost and patient inconvenience. In some cases, treatment complexity threatens to limit access to care by presenting financial barriers to treatment or by inducing patient drop-out from further medical care.

In this article, we have presented an overview of four modifications of the standard practice of IVF, including mild stimulation, IVM, natural-cycle IVF, and intravaginal embryo culture. All of these modifications have the potential to simplify IVF treatment, making it less expensive, as well as less invasive and less intimidating to patients. In the era of individualized patient care, these unconventional methods present alternatives to standard treatment. As time progresses and data are accumulated, these methods may prove to be not just viable alternatives to standard treatment, but potential first-line treatment choices.

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