CHAPTER 24

How do we improve implantation rate following in-vitro maturation of oocytes?

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INTRODUCTION

Many years have passed since the in-vitro maturation (IVM) of animal oocytes was first performed by Edwards[1] in the 1960s. In-vitro maturation of human oocytes has now been technically mastered for a decade[2]. Substantial improvements in efficacy have been achieved so that the technique has really quit the research sphere and entered routine clinical use about 5 years ago.

In 2004, Chian et al.[3] estimated that more than 300 healthy infants have been born following immature oocyte retrieval and IVM. About 30–35% of infertile women with polycystic ovary syndrome (PCOS) who undergo IVM treatment achieve clinical pregnancies.

However, in spite of these encouraging results, implantation rates (typically 10–15%) tend to be lower than in conventional in-vitro fertilization (IVF) with ovarian stimulation. In our experience we also have a high miscarriage rate following establishment of clinical pregnancies. These raise concerns regarding the implantation of embryos generated from in-vitro matured oocytes. This appears to be the principal drawback of IVM.

Two main hypothesis can be put forward as an explanation:

- Embryo quality (which can be patient-determined or as a result of the process of in-vitro oocyte maturation and culture).
- Endometrial quality (which can also be patient-determined or a result of asynchronous steroid hormone preparation).

The continued spread of IVM as a real alternative to classical IVF will only be possible if it proves to have comparable outcomes. Hence, we must determine how to improve implantation rate following IVM.

POLYCYSTIC OVARY SYNDROME

As with any new technique, IVM has been tried in numerous reproductive diseases. After 10 years of experience, IVM has been successfully applied in:

- Women with PCOS[4].
- Rescue of oocytes which have failed to mature in conventional IVF stimulated cycles[5].
- Unexplained poor-quality embryos following conventional IVF[6].
- Oocyte donation[7].

- Fertility preservation prior to potentially sterilizing treatments (e.g. chemotherapy[8]).

Even though encouraging successes have been described for the last four indications, the small number of pregnancies make these arguably anecdotal. On the other hand, PCOS patients represent the large majority of women who undergo IVM and also therefore the majority of clinical pregnancies and live births follow IVM to women with PCOS.

Definition of PCOS

This reproductive syndrome is:

- One of the oldest reproductive diseases known (first described by Stein and Leventhal in 1935)[9].
- The commonest cause of anovulatory infertility (75%)[10].
- The commonest endocrinopathy among reproductive age women[11].

Its definition has been heterogeneous until relatively recently. Since 2003 and the Consensus of Rotterdam, this syndrome needs two out of three of the following criteria:

- Polycystic ovaries (PCO): ≥12 follicles/ovary measuring 2 to 9 mm and/or ovarian volume >10 ml
- Troubles of the menstrual cycle (oligo-amenorrhea)
- Hyperandrogenism (hirsutism, acne, alopecia, raised serum testosterone or androstenedione)

without other evident causes (deficiency of 21-hydroxylase, Cushing's syndrome, hyperprolactinemia, acromegaly, virilizing adrenal or ovarian neoplasms)[12].

The epidemiologic importance has often been overestimated by numerous selection biases in published studies[13]. Based on an unselected sample of American women between 18 and 35 years old, Knochenhauer et al. found this syndrome in only 4.7% of Caucasian women and 3.4% of African-American women[14].

Reproductive abnormalities

Oligo-amenorrhea and infertility

These common symptoms push many women with PCOS to consult their gynecologist. Indeed, among PCOS patients, oligomenorrhea (defined as <8 periods/year) is found in 29% to 47% of women with PCOS and total amenorrhea in 19% to 51%. Between 20% and 74% of PCOS patients also suffer from infertility[13].

Miscarriage

One author recently did cast doubt on the increased risk of miscarriage related to PCOS, suggesting a detection/selection bias[15,16]. Indeed, several reports found no association between recurrent pregnancy loss and PCOS as an independent factor[17].

Nevertheless, PCOS is accepted by many authorities as a significant risk factor for early pregnancy loss (for example, according to the Royal College of Obstetricians and Gynaecologists, amongst women with recurrent early miscarriage, PCO is found in 56%, while PCO represents only 22% in the general population). Moreover, women with PCOS seem to suffer a higher rate of early pregnancy loss (30 to 50%) compared to regularly cycling women (10 to 15%)[18]. A confounding factor (such as obesity) could explain these findings. However, most would agree that women with PCOS suffer from a high early pregnancy loss rate no matter whether it is directly or indirectly linked to the PCOS physiology.

Several mechanisms have been suggested to explain this predisposition to miscarriage: obesity (as noted above)[19], low luteal phase progesterone concentration[20], high tonic LH levels (thought to be associated with early pregnancy

loss, independently of PCOS)[18], elevated plasminogen activator inhibitor 1 (PAI 1) – related to insulin resistance which could be responsible for a thrombophilic state, and decreased serum IGFbp1 and glycodelin (which could impair placentation)[21,22].

ASSISTED REPRODUCTIVE TECHNOLOGY (ART) STRATEGY

Many treatments have proved either their safety or their efficiency in overcoming PCOS-related infertility and a balance between benefits and risks must be taken into account before determining appropriate treatment. In an editorial, Norman gave his thoughts about which treatment should be proposed for PCOS and in which order[15]. Hence, as an invasive heavy technique, IVM should not be used as a first line treatment (Figure 24.1).

Lifestyle modification (diet and exercise)

Obesity is known to impair the success of classical assisted reproduction, increasing the risk

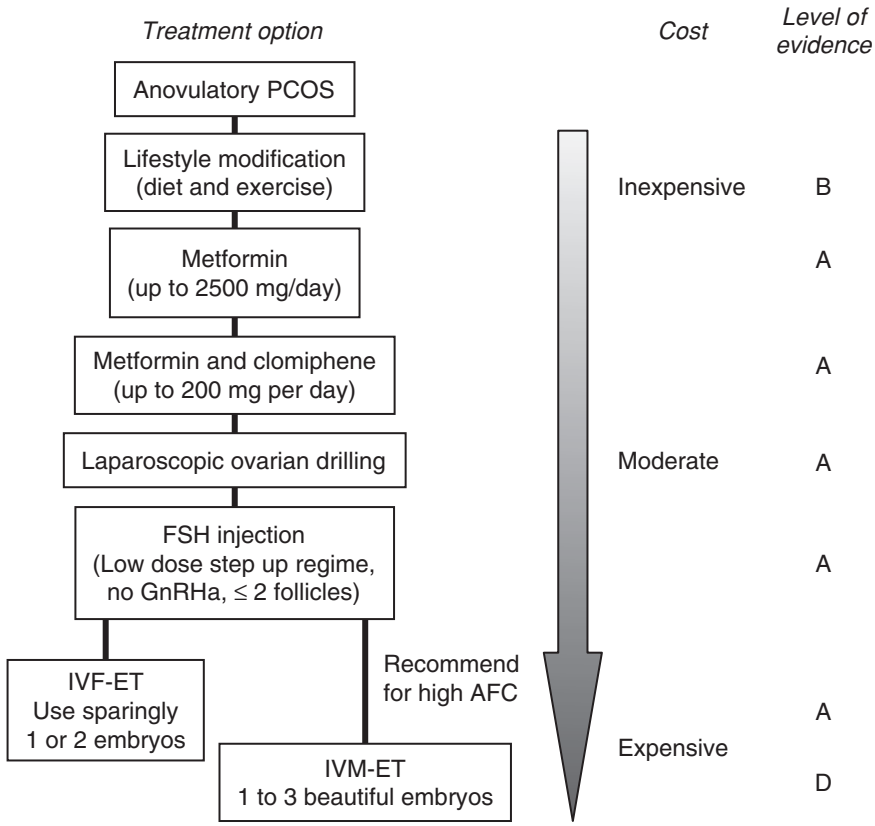

Figure 24.1 A cost-effective and evidence-based approach to the anovulatory woman with polycystic ovary syndrome (PCOS) who is seeking to become pregnant. In-vitro maturation and embryo transfer (IVM-ET) as in-vitro fertilization and embryo transfer (IVF-ET) are last lines of treatment and might depend on antral follicular count (AFC). Ovulation induction by clomiphene citrate or follicle-stimulating hormone (FSH) should be carried out with careful monitoring. Adapted from Norman[15]

of miscarriage and the gonadotropin consumption[23]. A 5–10% decrease of the body mass index (BMI) restores spontaneous reproductive function in 55–100% of women[24].

Clomiphene citrate

This is the uncontested first line of treatment for PCOS with oligo-amenorrhea and permits restoration of ovulation in 80% and a pregnancy rate of 40%[24].

Metformin

This insulin-sensitizing drug has been shown to improve clomiphene citrate efficacy on ovulation induction and pregnancy rates in infertile women with PCOS by two meta-analyses[25,26] and should be largely used as a pretreatment with clomiphene citrate. This same use could also decrease excessive follicular development during ovulation monitoring[27] and increase the biochemical pregnancy rate (pregnancy diagnosed only by hCG measurements) during conventional IVF/ICSI treatment in lean women with PCOS[28].

Gonadotropins

This efficient technique is also associated with a much increased risk of excessive follicular development – and therefore an increased risk of cycle cancellation or high-order multiple pregnancy. GnRH agonists which exacerbate this risk should be avoided in PCOS and a low-dose step-up protocol should be used[29]. For patients with very high numbers of antral follicles (AFC) in the early follicular phase, we counsel regarding the risks and benefits of ovulation induction with gonadotropins compared to the more invasive IVM.

Ovarian drilling

This old treatment is still useful and may be an alternative to IVM, although it carries the risks associated with general anesthesia and the surgical risks associated with laparoscopy. Between 43% and 84% of patients with clomiphene-resistant PCOS could achieve a pregnancy[12], especially non-obese (lean) patients and those with low testosterone and high LH levels[30]. Followed by conventional IVF, it could also increase pregnancy rate, decrease gonadotropin dose, and avoid complications, such as OHSS[31].

In-vitro fertilization

A meta-analysis of comparative studies between PCOS and non-PCOS infertile women concluded that there was an increased cancellation rate but more oocytes were retrieved at each oocyte collection. The authors concluded that the benefits (namely good response and generation of multiple good quality embryos) were balanced by the risks (namely cycle cancellation as a result of OHSS risk). The pregnancy rates were similar in both groups (around 30%)[32].

Furthermore the incidence of OHSS is higher in women with PCOS undergoing conventional IVF (10–19% versus almost 0%)[33,34]. Therefore, IVM must be discussed each time IVF is planned on a woman with PCOS.

Advantages of in-vitro maturation

Ovarian hyperstimulation syndrome

The major advantage of IVM is to avoid OHSS in women with PCOS. This complication of ovarian stimulation is known to lead to a hospitalization rate of 2%[35]. In exceptional cases, OHSS can even be fatal[36]. Because IVM does not use any ovarian stimulation, this technique cannot be complicated by OHSS. A recent review of 10 publications concluded a significant and consistent relationship between women with PCO undergoing ovarian stimulation and the development of OHSS. Indeed, when PCO was

present, the combined odds ratio of developing OHSS was 6.8 (95% CI 4.9–9.6)[37]. Although no formal relationship between AFC and the risk of OHSS has been determined, one may logically assume that the higher the AFC's, the higher the risk for OHSS exists. This may therefore be the best determinant for IVM.

Economical benefits

Because IVM does not use any ovarian stimulation, it has been suggested that there may be an economic benefit to IVM. Although this may be the case for the couple undergoing treatment, by charging set prices, most of the centers offering IVM do not take into account a likely longer oocyte retrieval and the additional laboratory work. What is acceptable for a small number of cases or proportion of the workload may not be realistic as IVM becomes more widespread. We suggest that any economic benefit is less important than initially thought.

Other advantages

The absence of ovarian stimulation makes IVM comfortable and rules out the hypothetical risk of ovarian cancer attributed to ovarian stimulation. On the other hand, oocyte retrieval is technically more demanding and may need a deeper anesthesia.

PROBLEMS ASSOCIATED WITH IN-VITRO MATURATION

Results from several centers performing IVM have been published and reviewed[38]. Cha et al.[39] recently reported their experience and the obstetric outcome in women with PCOS who conceived following IVM and further data are available in an earlier report[40]. The Family Federation of Finland recently reported a comparison of insemination with or without ICSI in their IVM program[41]. These results and ours brought up to date are presented in Table 24.1. Numerous similarities attest to the representativeness of these data. Unfortunately, we are missing data on cycle cancellation, embryo quality, and often miscarriage rate. Hence, as we feel these features are of crucial importance in the improvement of IVM outcome, we will describe our experience without being able to corroborate it.

Cycle cancellation

Since July 2002, 138 new attempts of IVM took place in 75 couples in our center. According to the consensus of Rotterdam, 60% of our women had PCOS, 21% had PCO, and 19% had no ovulatory/ovarian abnormality. Among these attempts, 11.6% were cancelled or commuted into an IVF in a natural cycle because of bleeding (2.9%) or development of a dominant follicle (8.7%). When adding logistic and male factor cancellations, in our experience, more than one patient out of five will not have her IVM as initially planned. Before considering IVM use on a large scale, solutions must be found to improve this high cancellation/delay rate.

Poor embryo quality

Among our 526 embryos obtained by IVM, 19% of them were of A or B quality, while 47% were C quality, and 34% were D quality. Because we always perform ICSI for the fertilization of IVM oocytes, we compared these results with conventional IVF/ICSI. Embryos from IVM oocytes seem different from our usual ICSI embryos in which 42% are A or B quality embryos, 35% are C quality embryos, and 23% are D quality embryos. This spread of low quality embryos obtained from IVM has repercussions on the quality of embryos transferred after IVM: 35% of embryos transferred were of A or B quality, while 54% were C quality, and 9% were D quality. With our conventional ICSI program, at embryo transfer

Table 24.1 Summary of the published outcomes of IVM. Our results are brought up to date (without frozen embryos). Those of the Montreal, Taipei, and Maria Infertility Hospital of Seoul groups are from a review by Chian et al.[38]. Those of The Family Federation of Finland can be roughly deduced from a recent article[41]. Finally, clinical data from Cha et al.[39] have been published recently, while his biological data (*) from a smaller number of patients in an older paper[40] given in brackets

ART Service		*A Beclere, Paris*	*McGill, Montreai*[38]	*Shin Kong Wu Ho-Su Memorial Hospital, Taipei*[38]	*The Family Federation of Finland, Helsinki*[41]	*Infertility Medical center of Cha, Seoul*[39,40]	*Maria Infertility Hospital Seoul*[38]	
							Embryo transfer	*Blastocyst transfer*
Cycles (*n*)		138	254	68	239	203	419	80
Oocytes retrieved	Total (*n*)	1406	3079	1528	971	3148	6860	2108
	Mean ± sd	12.1 ± 7.7	11.9 ± 6.2	22.5 ± 10.1	8.0 ± 5.2	15.5 ± 8.2	16.4 ± 7.1	26.4 ± 9.9
Maturation rate (%)		61.7	78.8	74.2	58.6	(55.3)*	73.2	77
Fertilization rate (%)		62	69.2	72.8	51.3	(75.1)*	79.0	79.3
Cleavage rate (%)		95.2	89.9	88.8		(88)*		36.3
Embryo transfer	Total (*n*)	230	865	258	287	926	1816	246
	Mean ± sd	2.4 ± 0.7	3.4 ± 0.9	3.8 ± 0.9		5.0 ± 2.1	4.3 ± 0.9	3.1 ± 0.4
Implantation rate (%)		10.9	11.1	10.5	18.5	5.5	11.6	27.2
Clinical pregnancy rate per oocyte retrieval (%)		24.5	24.0	33.8	26.6	21.9	32.7	53.8
Miscarriage rate (%)		42.3			26.5	36.8		

61% of embryos transferred are of A or B quality, 31% are C quality, and 3% are D quality. Consequently, the number of frozen embryos following IVM is very low (7.2%).

This fact could explain the low implantation rate observed in IVM. However, we must say that the quality of embryos involved in several of our clinical pregnancies was surprisingly low.

Low implantation rate

The implantation rate has always been low since the initial clinical experience with IVM. Improvements have occurred as the early practitioners reported implantation rates of around 5%[40] and most studies now report implantation rates of around 10% (Table 24.1). However, no study has been able to link this improvement to any precise practice adopted. Information about different centers' results changing is not available.

This low implantation rate (10.9% in our center when our usual implantation rate during ICSI is 27%) might either be part of the IVM technique or related to a detection bias explained by the PCOS. We will see that many convincing arguments support this low implantation rate as an intrinsic drawback of IVM rather than a PCOS characteristic.

Related to PCOS

The only argument supporting PCOS itself as a possible cause for the low implantation rate in IVM is the results from the oocyte donation program of McGill University – this center reported a 50% pregnancy rate per cycle in recipients of IVM egg donation[7]. Nevertheless, as recipients of IVM egg donation do not have PCOS (rather premature ovarian failure) and the oocyte donors do have PCO/PCOS, it is possible that implantation might be improved when the recipient does not have PCOS.

On the other hand, when comparing outcomes of 251 matched patients with and without PCOS, undergoing conventional IVF, MacDougall et al.[34] could find no difference between the implantation rates[34]. In another prospective study, Child et al[51]. compared the outcomes of 144 women with normal ovaries, PCO, and PCOS. This time, the pregnancy rate and the implantation rate were significantly lower in women with normal ovaries compared to those with PCO and PCOS[42]. These last findings do not support the implication of PCOS itself in the low implantation rate associated with IVM.

Related to the IVM technique

In another case-controlled study, Child et al.[43] compared 107 IVM cycles with 107 matched conventional IVF cycles in women with PCO. The implantation rate of IVF-derived embryos was significantly higher (17.7% vs. 9.5%) than that of IVM ($p < 0.01$). Even if more data are needed, most of the arguments support the low implantation rate as an intrinsic drawback of IVM rather than a characteristic of PCOS.

High miscarriage rate

The high frequency of early pregnancy loss in IVM is not of common knowledge. Even so, the few published figures speak for themselves, with miscarriage rates from 26.5% to 42.3%. These figures are similar to what could be the 'background' miscarriage rate of women with PCOS. However, this higher miscarriage rate is not noticed when women with PCOS undergo conventional IVF treatment[32]. On the other hand, in an oocyte donation program, Copperman et al. noted a miscarriage rate of 33% for PCOS patients compared to 6% for non-PCOS patients[44]. In this case, the high early pregnancy loss rate may be linked to PCOS.

IMPROVEMENTS IN IN-VITRO MATURATION

Patient selection

Oocytes from normo-ovulatory young women are known to show a higher developmental capability than oocytes from women with PCOS.[45] Nevertheless, Child et al.[42] compared the outcomes of 144 women with normal ovaries, PCO, and PCOS in a prospective study and found a tendency to a better maturation and fertilization rate, but a pregnancy rate and an implantation rate significantly lower in women with normal ovaries than with PCO and PCOS. Obviously, IVM seems best suited to women with PCOS.

As in every ART procedure, age is important and younger age (particularly below 35 years) is associated with a higher pregnancy and live birth rate (Smitz et al., unpublished data).

The antral follicle count (AFC) is known to be the best predictor of the number of immature oocytes retrieved and of the likelihood of clinical pregnancy following IVM[46].

Finally, over-responders during conventional ovarian stimulation for IVF can be commuted to IVM rather than cancelled. In this way, Lim[49] administered human chorionic gonadotropin (hCG) to patients undergoing IVF with a high response when the leading follicle reached 12–14 mm in diameter. IVM-ICSI was performed with a 47.1% pregnancy rate and no OHSS.

FSH and hCG priming

FSH priming may improve oocyte yield and maturational competence, although there are conflicting results[38,45]. Although the use of FSH was not thought to affect implantation, one study suggested it may do[47]. So the effect of FSH priming is still unclear and further research is needed.

On the other hand, hCG priming shows fewer conflicting results. It has been shown to hasten oocyte maturation and to increase maturation rate[38,45]. In a randomized controlled trial, comparing IVM with and without hCG priming in 20 women with PCOS, Buckett et al. noticed an increased number of oocytes retrieved with hCG priming, but no effect on the endometrium[48]. Hence, hCG priming is unlikely to affect implantation.

A combination of FSH and hCG priming does not any add benefit[49]. Most centers practice hCG priming before IVM oocyte retrieval; over 200 healthy infants have been born following hCG priming[38].

Technical improvements

In the animal model, increasing the aspiration pressure tended to reduce the proportion of oocytes with intact cumulus so that almost every center now practices immature oocyte retrieval with a reduced vacuum pressure (7.5 kPa). Further animal studies noted the deleterious effect of cooling ovaries or oocytes and suggested that a temperature higher than 30°C should always be used for oocyte culture[50].

FUTURE POSSIBLE IMPROVEMENTS

Estradiol priming

While comparing a pregnant cohort to a non-pregnant cohort following IVM, Child et al. observed a statistical significance difference between endometrial thicknesses: greater than 10 mm on the day of embryo transfer was a good predictor of pregnancy[51]. Hence, endometrial preparation is necessary and must take place in a very short period of time unless it begins before oocyte retrieval (Figure 24.2). Starting 17β-estradiol early in the cycle could permit a better endometrial preparation and may improve the cancellation rate. However, the prospective work of Russell[52] suggested that early exposure of ovaries to 17β-estradiol could impair oocyte maturation, fertilization, and cleavage. The

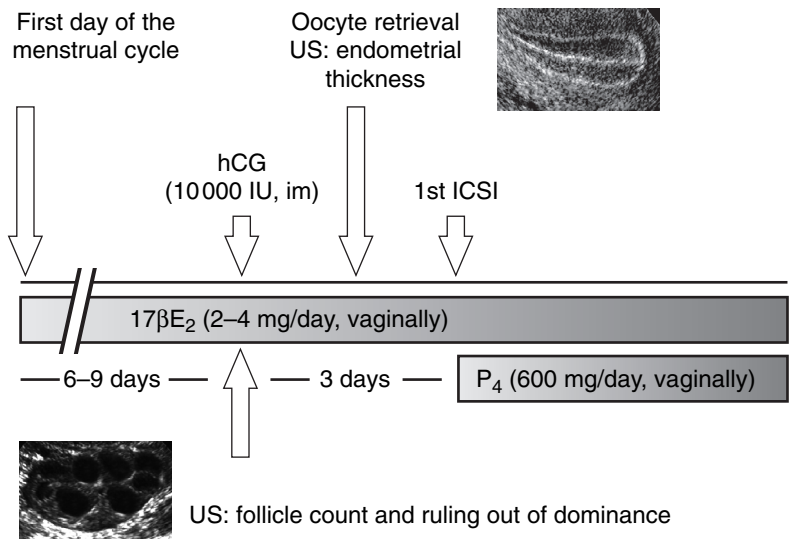

Figure 24.2 A protocol of immature oocyte retrieval and IVM designed to improve endometrial thickness. 17β-Estradiol ($17\beta E_2$) might be given from the first day of the patient's menstrual cycle. This picture represents a protocol of immature oocyte retrieval and IVM which might improve the endometrium. Ultrasound (US) is performed to rule out dominance and to measure endometrial thickness. Progesterone (P_4) is begun on the day of ICSI

global effect will depend on the improvement of the implantation rate induced by 17β-estradiol.

Metformin

This fashionable insulin-sensitizing drug has been recently tried for many aspects of ART, sometimes without any conclusive benefit[15,16]. Two observational retrospective studies with metformin alone and one with the addition of enoxaparin suggest that metformin could decrease the miscarriage rate of PCOS when continued at the beginning of the pregnancy[53,54]. On the other hand, one other observational retrospective study was unable to demonstrate this benefit[55].

Mechanisms put forward to explain this are a decrease in serum androgens, an improvement in uterine vasculature (decreasing PAI 1 and increasing IGFbp1 and glycodeline)[21], weight loss, or an improved luteal phase progesterone[20].

Metformin could be carefully assessed in PCOS patients undergoing IVM, as we have seen that the miscarriage rate is high and it could be related to endocrinologic disturbance of PCOS that may be improved by metformin.

On the other hand, we have seen that the low implantation rate observed in IVM must be an intrinsic drawback of IVM rather than a PCOS characteristic. Even if a recent study found a beneficial effect of metformin over porcine oocytes in vitro[56], it may not improve overall implantation rates.

GnRH pumps or GnRH agonists

A meta-analysis was unable to determine any significant effect of the GnRH pump when used in PCOS patients (the patient numbers were too

small)[57]. However, the pump has been shown to normalize LH levels in an observational study of 13 anovulatory PCOS women[58]. As the high miscarriage rate observed in PCOS has been linked to high LH levels, it may be possible to improve implantation rates and decrease miscarriage rates.

Blastocyst transfer

The experience of IVM in The Family Federation of Finland is novel. In this center, the use of ICSI is reserved for masculine infertility. This restrictive use of ICSI decreases the fertilization rate and the number of embryo transfers, but the clinical pregnancy rate per embryo transfer is much higher so that the clinical pregnancy rate per immature oocyte retrieval is not significantly different[41].

Blastocyst transfer shows a high implantation rate when used in conventional IVF, presumably by better selection of embryos with the greatest developmental potential. Prolonged culture may be a good selection tool for the developmental potential of embryos generated from IVM oocytes.

CONCLUSIONS

Because it avoids OHSS and because it is less expensive, IVM might become an alternative to conventional IVF. Outcomes have already improved. However, the main drawbacks remain a high cancellation rate, a low implantation rate, and a high miscarriage rate. 17β-Estradiol and metformin may be of help, but the future must go to embryo selection by prolonged (blastocyst) culture.

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