JRME

Journal of Reproductive Medicine and Embryology

Beyond embryos: the uncharted territory of aging endometrium and frozen embryo transfer outcomes in women aged 40 and older.

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Abstract

As the demand for assisted reproductive technologies continues to rise, frozen embryo transfer (FET) has emerged as a viable option for women seeking to conceive, particularly those over 40. However, the focus on embryo quality and selection has led to a critical oversight: the impact of endometrial aging on FET outcomes. The most controllable factor affecting FET outcomes is the patient's endometrial condition; thus, improving endometrial receptivity is a research hotspot. Currently, various endometrial preparation protocols before FET have been suggested, including natural cycles (NC), hormone replacement therapy (HRT) cycles, with or without gonadotropin-releasing hormone agonist (GnRHa) down- regulation, and ovulation induction (OI) cycles. However, until now, no consensus exists on the ideal endometrial preparation regimen before FET, especially for elderly women. the success of FET in women over 40 cannot be solely attributed to embryo quality. The endometrium, once considered a passive recipient, plays a critical role in implantation and pregnancy outcomes. For patients with advanced maternal age (AMA), we should consider the most suitable, safe, and effective endometrial preparation protocol based on the epigenetic, molecular, vascular and cellular characteristics of these patients. From the above mentioned evidence, we should be very cautious before using NC-FET in AMA, as the natural cycle is "not completely natural" nor physiologic in these subgroup of patients.

Keywords: advanced maternal age, frozen embryo transfer, endometrial preparation

JRME® Volume. 1, Issue no 4, February 2025

Debate

Introduction

As the demand for assisted reproductive technologies continues to rise, frozen embryo transfer (FET) has emerged as a viable option for women seeking to conceive, particularly those over 40. However, the focus on embryo quality and selection has led to a critical oversight: the impact of endometrial aging on FET outcomes.
In other words, while extensive research has focused on optimizing embryo quality, euploidy rates, and cryopreservation techniques, a critical gap persists: the molecular and cellular aging of the endometrium. The prevailing narrative assumes that transferring an euploid embryo into a chronologically aged uterus equates to predictable success, relegating endometrial biology to a passive backdrop. Yet, emerging evidence suggests that age-related endometrial senescence—marked by altered gene expression, immune dysregulation, and impaired decidualization—may silently undermine implantation potential. This review challenges the embryo-centric dogma, and sheds light on the uncharted territory of endometrial aging in FET, highlighting the need for a more comprehensive approach that considers both embryo and endometrial factors."

In assisted reproductive technology (ART), female age is one of the most important factors affecting the clinical outcomes (1, 2). Advanced maternal age (AMA) is defined as pregnancy at 40 years or older. Modern lifestyle has led to an increase in the age of conception. It is well known that maternal age is positively correlated with the decline of the ovarian reserve, deterioration of oocyte quality, and chromosomal abnormalities in oocytes and embryos (3, 4). However, the effect of age on endometrial function and receptivity is still under extensive research and may be an equally important factor influencing implantation rate, pregnancy rate, and the overall female fertility.

As women age, their reproductive potential declines, and this is reflected in changes that occur in the follicular phase, luteal phase, and folliculogenesis (5). Here's a summary of the changes that occur in women with AMA:

Follicular Phase

During the follicular phase, the dominant follicle grows and matures, producing estrogen and inhibin. In women with AMA, the follicular phase is often shorter, and the peak estrogen levels are lower compared to younger women (6). This is due to the decreased number and quality of follicles, leading to reduced estrogen production (7). A study published in the Journal of reproductive biology and endocrinology found that women over 40 had significantly lower peak estrogen levels and shorter follicular phases compared to women under 35 (8).

Luteal Phase

The luteal phase is the period after ovulation, during which the corpus luteum produces progesterone to prepare the uterus for implantation. In women with AMA, the luteal phase is often shorter, and progesterone levels are lower, which can affect implantation and pregnancy rates (9). A study published in Human Reproduction found that women over 40 had shorter luteal phases and lower progesterone levels compared to women under 35 (10).

Folliculogenesis

Folliculogenesis is the process by which follicles develop and mature in the ovary. In women with AMA, folliculogenesis is impaired, leading to reduced follicle numbers and quality (11). This is due to the accumulation of DNA damage and epigenetic changes in the oocytes, which affects their ability to mature and fertilize (12). A study published in fertility and sterility found that women over 40 had significantly lower follicle counts and poorer oocyte quality compared to women under 35 (13). In addition, the composition of the follicular fluid is different in AMA, as studies found several proteins are downregulated in these patients (14).

In summary, women with advanced maternal age (over 40) experience changes in the follicular phase, luteal phase, and folliculogenesis, which can affect their reproductive potential. These changes include:

- Shorter follicular phases and lower peak estrogen levels
- Shorter luteal phases and lower progesterone levels
- Impaired folliculogenesis, leading to reduced follicle numbers and quality.

Over the past few years, frozen embryo transfer (FET) has gained widespread acceptance and is now considered a good alternative to fresh embryo transfer (15). The most controllable factor affecting FET outcomes is the patient’s endometrial condition; thus, improving endometrial receptivity is a research hotspot. Currently, various endometrial preparation protocols before FET have been suggested, including natural cycles (NC), hormone

replacement therapy (HRT) cycles, with or without gonadotropin-releasing hormone agonist (GnRHa) down- regulation, and ovulation induction (OI) cycles. However, until now, no consensus exists on the ideal endometrial preparation regimen before FET, especially for elderly women.

Although modern ART has solved some reproductive problems in patients with advanced maternal age (AMA), there is still a big challenge. Studies have shown that the pregnancy rate of FET decreases with increasing age, particularly in women older than 40 years of age (3); and, only a few studies have evaluated the optimal endometrial preparation protocol in elderly patients.

In clinical practice, the HRT-FET protocol is increasingly adopted as the NC protocol may not be possible in patients with ovulatory disorders. Additionally, the HRT protocol provides better control of FET timing and transfer, which is convenient for patients and physicians (16). However, in patients with AMA, oral large-dose estradiol (E2) may cause some side effects such as nausea, vomiting, and dizziness because of their body function decline. Also, premature E2 elevation may lead to apoptosis of trophoblasts and is associated with uteroplacental insufficiency, hence further worsening of pregnancy outcomes in older women using HRT protocols (17). Moreover, since AMA is an independent risk factor of thrombotic events (18), large-dose E2 may further increase the risk of venous thrombosis (19). Stevenson et al. (20) found that oral E2 can increase the triglyceride level and its agglutinating activity, so estrogen should not be used for patients with elevated levels of triglycerides. In addition, its metabolite, estrone, can activate oncogenes which are associated with breast tumors, ovarian cysts, and uterine fibroids (21). Therefore, for endometrial preparation, estrogen should be carefully considered before use in patients with AMA.

On the other hand, the mNC FET protocol is more patient-friendly and cost-effective than other endometrial preparation FET protocols; nevertheless, there is a general belief that the ovulatory cycle of older women is not the best choice for endometrial preparation, although paradoxically they are the group of patients who could benefit most from the reduction of obstetric complications.

Liu et al. (16) compared the outcomes of three different endometrial preparation FET protocols in patients with AMA; considering the age cut-off point of AMA as 38 years. In their study, 457 patients with AMA were divided into 3 groups according to the endometrial preparation protocol; NC, OI (using Human menopausal gonadotropin), and HRT groups. There were no significant differences among the groups regarding the age (mean age was 40.49 ± 2.42, 41.32 ± 2.66, and 40.14 ± 2.83 years in NC, OI, and HRT groups respectively), basal follicle- stimulating hormone (FSH), basal luteinizing hormone (LH), body mass index (BMI), or number, developmental stage, and quality of transferred embryos. Regarding reproductive outcomes, the authors did not find significant differences among the three groups in terms of implantation rate (16.22%,14.29%, and 16.44% in the NC, OI, and HRT groups respectively), clinical pregnancy rate (CPR) (22.95%, 23.68%, and 24.58% in the NC, OI, and HRT groups respectively), abortion rate, or live birth rate (LBR) (18.03%, 15.79%, and 15.92% in the NC, OI, and HRT groups respectively). So, they suggested that in women aged 38 years or over, the endometrial preparation protocol did not affect FET outcomes.

Another retrospective cohort study by Zheng et al. (22) included 3893 NC, 11456 HRT, and 1518 GnRHa-HRT cycles of endometrial preparation before FET. The authors stratified patients by age and found that differences in pregnancy outcomes between groups were apparent in older women, but obscure in younger women. In older women (≥35 years), the ongoing pregnancy rate (OPR) and LBR were significantly higher, and the early miscarriage rate was significantly lower in NC compared to either HRT group. The decreased LBR and increased early miscarriage rate in the two HRT groups might be due to the absence of a corpus luteum (CL). On the other hand, in younger women, CPR, OPR, and LBR were all comparable between groups. So, the authors concluded that there is a remarkable priority of NC in older women and maternal age should be considered when choosing an endometrial preparation FET regimen.

Furthermore, in a single-center retrospective trial including 1096 elective frozen single- blastocyst transfer cycles, Moffa and colleagues(23) found a superiority of the mNC over the HRT for endometrial preparation in egg recipients of AMA. In their trial, there were no significant differences between both groups, the HRT and the mNC, regarding the mean recipient's age (42.2 years in the HRT group and 42.1 years in the mNC group), or donor's age. The authors found no significant differences in cancellation rates between both groups. Regarding patients aged 40-44 years, no statistically significant differences were found between both groups in CPR (50.8% and 51.1% in HRT and mNC groups respectively), or LBR (40.4% and 45.8% in HRT and mNC groups respectively). However, for

patients aged 45-50 years, CPR (44.8% and 57% in HRT and mNC groups respectively) and LBR (33.9% and 48.4% in HRT and mNC groups respectively) were significantly higher in the mNC group compared to the HRT. So, the authors recommended the mNC as the first option for endometrial preparation in egg recipients of AMA, having shown superiority over HRT in patients aged 45-50 years, with no increase in the cancellation rate.

Moreover, a retrospective study by Dong and colleagues (24) compared the outcomes of HRT and GnRHa-HRT protocols in 1264 elderly patients (aged 38 years or older) undergoing their first FET. The mean age was 40.64 ± 2.04 years in the HRT group and 40.67 ± 2.07 years in the GnRHa-HRT. Both groups had no significant differences regarding age, basal FSH, BMI, or number or stage of transferred embryos. The authors found no significant differences in CPR (33.58% vs.37.15%), or OPR (19.40% vs. 25.10%) between the GnRHa-HRT and HRT groups respectively. Also, the multivariate analysis showed no significant differences in LBR or abortion rate between the groups. So, the authors concluded that GnRHa combined with HRT did not improve the clinical outcomes of FET in AMA patients.

The impact of female age has been extensively addressed as one of the main indicators of repeated implantation failure (RIF) (25). However, few studies have focused on reproductive outcomes in older populations with RIF. Pan et al. (26) conducted a retrospective study involving 549 older patients (36–43 years) with RIF undergoing their third FET. The patients were divided into NC, HRT, and GnRHa-HRT groups of endometrial preparation. The authors found significantly higher CPR, OPR, and LBR in the GnRHa–HRT group than the other 2 groups. So they hypothesized that before FET, older patients with idiopathic RIF might benefit from GnRHa suppression compared with those without such pretreatment. Research has confirmed that leukemia inhibitory factor (LIF) signaling may be impaired in some women with RIF (27). Pituitary down-regulation with GnRHa might be involved in regulating endometrial genetic alterations in patients with RIF. GnRHa therapy may restore the endometrial secretion of implantation-related factors such as HOXA10 and LIF, which can regulate endometrial development and permit embryo implantation and decidualization (28).

However, before reaching a conclusion regarding the optimal endometrial preparation menthod in AMA the “endomerial aging” should be considered. Recentely there has been extensive reaserch aiming at eluding the charactarestics of the endometrium of aging females. A recent review by Pathare et al proved that the endometrium of women of AMA has lower implanation potentials due to epigenetic, molecular, cellular, and histological alterations that affects the endometrial receptivity (29). Women with AMA have smaller uterii with impared blood supply due to changes in the spiral arteries. This may explain why these patients have significantly thinner endometrial. These changes lead to negantive impact on the endometrial architecture which in turn will affect all key steps in implanation namely; adhesion, proliferation, apoptosis (30).

Devesa-Peiro and collegues explored the endometrial gene expression in AMA using artificial intellegence. They found dysregulation in the genes responsible for the up-regulation of the ciliary processes in the endometrium of older females (31).

In conclusion, the success of FET in women over 40 cannot be solely attributed to embryo quality. The endometrium, once considered a passive recipient, plays a critical role in implantation and pregnancy outcomes. As we move forward in the field of reproductive medicine, it is imperative that we acknowledge and address the impact of endometrial aging on FET success rates. By adopting a more holistic approach, incorporating both embryo and endometrial factors, we can improve outcomes and provide more personalized care for women seeking to conceive through FET. The time has come to venture into this uncharted territory and uncover the secrets of endometrial aging, ultimately enhancing our understanding of FET and its potential for success in women over 40."

To continue prioritizing the embryo while neglecting the endometrial microenvironment in women over 40 is to navigate fertility treatment with a map missing half its coordinates. The stakes are profound: repeated FET failures, emotional tolls, and financial burdens could be mitigated by unraveling how aging reshapes endometrial receptivity. We urge researchers, clinicians, and policymakers to recalibrate their focus, integrating endometrial biomarkers, epigenetic profiling, and personalized protocols into the FET paradigm. Only by bridging the chasm between embryology and endometrial biology can we offer women in this demographic evidence-based hope—not just iterative attempts. Let this review serve as a call to action: the uterus is not a passive vessel but a dynamic organ demanding equal scrutiny. To overlook its aging is to walk blindly into uncharted territory, leaving patients and providers alike

stranded in the fog of uncertainty. The time for holistic, age-inclusive research is now.To sum up, for patients with AMA, we should consider the most suitable, safe, and effective endometrial preparation protocol based on the epigenetic, molecular, vascular and cellular characteristics of these patients. From the above mentioned evidence, we should be very cautious before using NC-FET in AMA, as the natural cycle is “not completely natural” nor physiologic in AMA. We believe future research should focus on optimization of endometrial preparation protocols in AMA taking into consideration the age relating changes that affects implanation in them.

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