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REVIEW

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Age-related uterine changes and its association with poor reproductive outcomes: a systematic review and meta-analysis

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Abstract

Background The decline in women's fertility becomes clinically relevant between 35–40 years old, when there is insufficient ovarian activity, and it becomes more difficult to achieve pregnancy naturally and through artificial reproductive technologies. A competent endometrium is required for establishing and maintaining a pregnancy to term, however, experts in the field underestimate the contribution of endometrial age and its impact on reproductive outcomes remains unclear.

Study design A systematic search of full-text articles available in PubMed was conducted to retrieve relevant studies published until March 2023. Search terms included: endometrium, uterus, age, aging, pregnancy, and oocyte donation. Terms related to reproductive pathologies were excluded. Eligibility criteria included original, rigorous, and accessible peer-reviewed work, published in English on the effect of age on the uterus and endometrium.

Results From 11,354 records identified, 142 studies were included for systematic review, and 59 were eligible for meta-analysis of endometrial thickness ($n=7$), pregnancy rate ($n=22$), implantation rate ($n=10$), live birth rate ($n=10$) and pregnancy loss rate ($n=11$). Studies for the meta-analysis of reproductive outcomes only included transfers of embryos from ovum donation (ovum donors < 36 years old). Age shrinks the uterus; depletes endometrial blood supply through narrow uterine veins and a progressive loss of uterine spiral arteries; disrupts endometrial architecture and cellular composition; alters hormone production, shortening menstrual cycle length and impeding endometrial progression to the secretory stage; and dysregulates key endometrial functions such as adhesion, proliferation, apoptosis, and receptivity, among others. Women over 35–40 years old had significantly thinner endometrium (MD 0.52 mm). Advanced maternal age is associated with lower odds of achieving implantation (27%) and clinical pregnancy (20%), or higher odds of experiencing pregnancy loss (44%).

Conclusion Due to the effect of age on endometrium reported in this review, managing patients with advanced maternal age may require considering the endometrial factor as a potential tissue to treat with anti-aging strategies. This review provides researchers and clinicians with an updated and in-depth summary of this topic, encouraging the development of new tailored anti-aging and preventive strategies for precision medicine in endometrial factor in infertility.

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Trial registration PROSPERO 2023 (CRD42023416947).

Keywords Uterus, Endometrium, Reproductive outcomes, Pregnancy, Implantation, Endometrial thickness, Uterine morphology, Uterine vasculature, Endometrial histology, Women's age

Introduction

Delayed motherhood due to economic, professional, and lifestyle factors is a major worldwide concern. The mean age of women when their first child is born progressively rose by one year per decade since the 1970s [1]. An age-related decline in women's fertility begins at 30 years old, becomes clinically relevant between 35–40 years of age, and is exacerbated thereafter [2–4]. To date, there is no universal definition of advanced maternal age (AMA) or international consensus on the threshold age at which women's fertility declines [5–7].

AMA was associated with reduced ovarian reserves, poor oocyte competence and quality, a higher frequency of chromosome missegregation during meiosis, and elevated blastocyst aneuploidy rates. Together, these mechanisms diminished pregnancy rates while raising miscarriage rates and the prevalence of chromosomal abnormalities in newborns [8, 9]. In this context, current clinical practice mainly focuses on declining ovarian function and oocyte quality in AMA patients, overlooking the effects of age on the uterus and especially on how changes at the endometrial level affect reproductive outcomes [10].

Successful embryo implantation is a key step for human reproduction. A competent endometrium is vital for achieving and sustaining pregnancy. In fact, endometrial factors are estimated to be responsible for 5% to 66% of implantation failures [11–15]. These large discrepancies between studies might be due to different study populations and design. For instance, [14] reported the endometrial contribution was less than 5% in patients with a mean age of 35 and a history of three unsuccessful euploid embryo transfers [14]. Alternatively, using a genome-wide functional approach, our group found that age significantly affected the endometrium when women were older than 35 [2], which suggests that the endometrium can become a therapeutic target to improve pregnancy rates in AMA patients. As endometrial age is not currently considered a clinical risk factor, the existing strategies to improve implantation rates in AMA women rely heavily on embryo selection, oocyte donation, and promoting elective oocyte cryopreservation in younger patients that plan to delay motherhood [16], despite the fact they are not always effective [17].

Molecular evidence has shown human tissues have distinct aging rates, which may differ from the individual's chronological age. In 2013, Horvath developed a molecular tool, known as Horvath's epigenetic clock, that determined the molecular age of 51 tissues and cell types by analyzing the methylation patterns of 353 CpG sites. Notably, he found that reproductive tissues, including the endometrium, age earlier than non-reproductive tissues [18].

Aging is a degenerative process characterized by a gradual decrease in cell function and progressive physiological decline [19]. Moreover, tissue aging processes drive the onset of several diseases, such as neurodegenerative diseases, cardiovascular diseases, and cancer [20]. Gaining a better understanding of aging processes, and ultimately, how to delay or reverse them is of public and clinical interest [21]. Current "rejuvenation" strategies in reproductive medicine mainly focus on ovarian pathologies [22–25]. The few advances in endometrial "rejuvenation" strategies were limited to treating pathologies, such as poor endometrial development [26, 27]. It remains to be determined whether there is a deleterious effect of age on the endometrium, and if so, whether there are biomarkers that can help identify target patients with aged endometrial tissue. The endometrial aging process [28, 29] and the effect of age on the uterus and endometrium [30, 31] were recently explored, however, there is still no consensus regarding the associated deleterious effects. To our knowledge, this is the first systematic review to comprehensively assess how age affects endometrial morphology and function in healthy women and the first meta-analysis to evaluate the effects of age on endometrial thickness and reproductive outcomes in an extensive data pool. In contrast to existing reviews [30, 31], we excluded studies performed in animal models or patients with potentially confounding uterine pathologies or low quality aneuploid embryos.

Here, we aim to summarize current literature and perform a meta-analysis to gain a better understanding of the effect of age on the uterus, particularly on endometrial morphology and function, and how these changes impact reproductive success in healthy women undergoing artificial reproductive technologies. This review provides valuable insights that can be used to develop new studies or targeted therapies, as well as inform clinical and patient decision-making.

Methods

Protocol and registration

The protocol for this systematic review was registered with the International Prospective Register of Systematic Reviews

(PROSPERO) (registration number CRD42023416947). This systematic review and meta-analysis was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [32].

Search strategy

A systematic search of full-text articles available in PubMed was conducted to identify relevant studies published before March 2023. To maximize the number of studies identified, we combined records from searches using MeSH terms and simple terms rather than identifying records manually. Searches included terms related to the endometrium, uterus, age, aging, pregnancy, and oocyte donation but excluded terms related to reproductive pathologies. The specific search queries are presented in Supplementary Table S1.

Eligibility criteria and study selection

The literature search results were exported to an Excel spreadsheet and duplicates were removed using manual methods. Titles, abstracts, and full-texts were screened independently and in quadruplicate by four authors (D.M.-G., F.S.-L., A.M.-M., and A.D.-P.) using the following eligibility criteria: original, rigorous and accessible peer-reviewed work published in English, on the effects of age on healthy human uterine morphology, endometrial function, and competence (Fig. 1). Studies were excluded if maternal age was not considered an independent study variable or was used as a criteria for applying different clinical protocols, or if age was a risk factor for pregnancy, pregnancy-associated complications (i.e., obstetric, postpartum, fetal, and general postoperative), infections, menopausal/menstrual alterations, or pathology. Studies related to gynecological pathologies and their treatments in AMA patients were also excluded, along with studies including oocytes from donors > 36 years old to minimize as far as possible potential confounding embryo factors. Questions or disagreements were discussed among six authors (D.M.-G.,

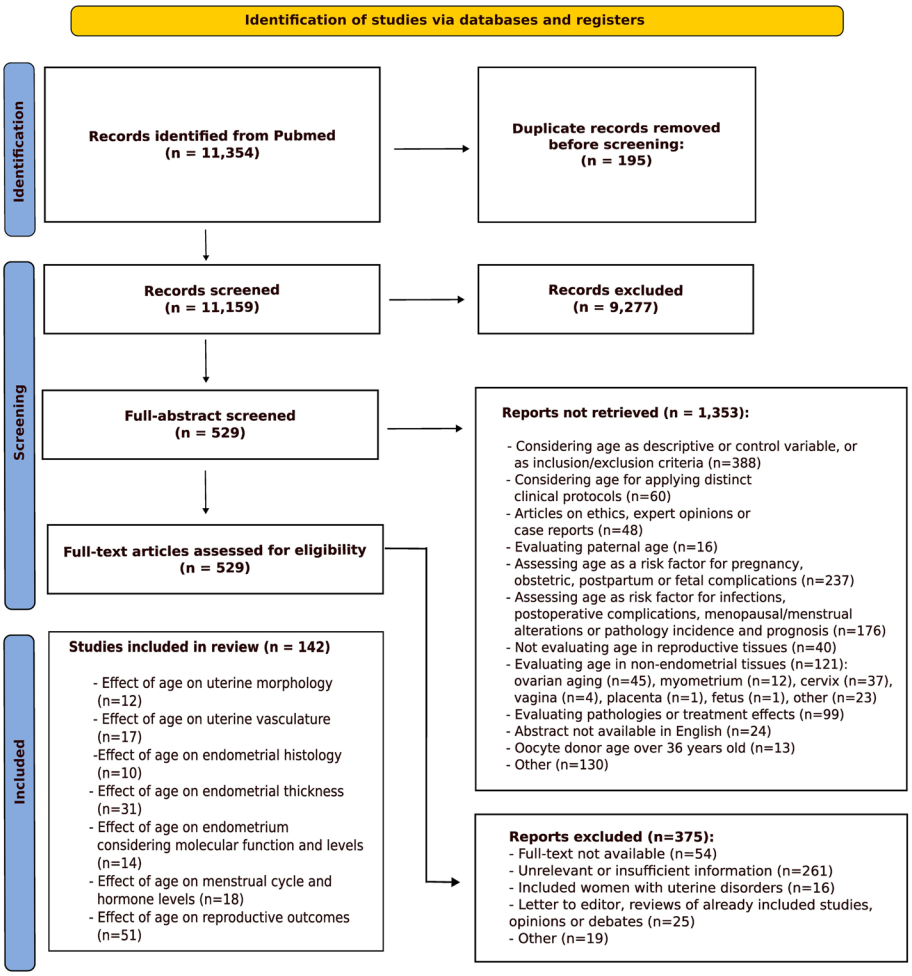

Fig. 1 Preferred reporting items for systematic reviews and meta-analysis (PRISMA) flowchart

F.S.-L., A.M.-M., A.D.-P., A.P., and P.D.-G.). The final list of included studies was approved by P.D.-G (Fig. 1).

Data extraction

Extracted data, including the patients' age, study design, sample size and main findings, uterine evaluation technique (magnetic resonance imaging, transvaginal ultrasound, and histological evaluation), IVF cycle type [natural, hormone replacement therapy (HRT), controlled ovarian stimulation (COS)], IVF cycle characteristics, fertilization method (IVF, ICSI), preimplantation genetic testing for aneuploidies (PGT-A) results, and finally, measures of endometrial thickness and reproductive outcomes following euploid embryo transfer, were compiled into a shared Excel spreadsheet and revised by P.D.-G.

Quality assessment

Four reviewers independently assessed the quality of the included studies using the study characteristics, statistical analysis performed, the significance of the results (evaluated by the *p*-value), the presence of data that corroborates the results and conclusions extracted by the authors (i.e., findings or results are supported by data collected in tables, figures or in text), the number of patients included in the statistical comparisons, and the relevance of the results. Moreover, the overall quality of the systematic review is ensured since in each key point of the process (study search and inclusion, data extraction, and quality assessment) results were cross-checked by various authors and supervised by the most experienced one. All articles that did not fulfill the required quality parameters were discarded as irrelevant information during full-text article review.

Statistical methods and meta-analysis

Meta-analyses were performed in R statistical software version 4.0.5. [33] using the *meta* R-package version 6.5.0. *p*-values ≤ 0.05 were considered statistically significant. Studies included for systematic review were selected for meta-analysis if they met the following inclusion criteria: age was evaluated as a discrete variable; sample size was greater than $n \geq 25$; there were study groups for both young and AMA patients without an age gap between groups; the mean, standard deviation, and raw data were reported (Supplementary Fig. S1). The quality of the studies included in the meta-analysis was assessed by the Newcastle-Ottawa Scale (NOS) [34]. Categorical variables were analyzed using the Mantel-Haenszel statistical method on binary outcomes and data were expressed as odds ratio (OR). For continuous variables, the inverse variance method was employed, and data were expressed as a pooled mean difference (MD). In both cases, I^2 was used to assess the heterogeneity between studies. The 95% confidence interval (CI) was calculated using the random effects model for heterogeneous data (p-value < 0.05), and the common effect model for homogeneous data (p-value > 0.05).

Results

Search results

The results of the systematic literature search are summarized in Fig. 1. A total of 11,354 studies were identified with our search queries. Following removal of duplicates (n=195), titles and abstracts were screened for eligibility based on the exclusion criteria presented in (Fig. 1) and 529 (4.7%) full-text studies were retrieved for detailed assessment. We classified studies by the effect(s) of age on the uterus, endometrium and reproductive outcomes. Finally, 142 studies were included for systematic review, including 12 (8.4%) related to uterine morphology (Table 1), 17 (11.9%) assessing endometrial and uterine vasculature (Table 2), 10 (7.0%) evaluating endometrial histology (Table 3), 31 (21.8%) measuring endometrial thickness (Table 4), 18 (12.6%) evaluating menstrual or hormonal changes (Fig. 2A and B), 14 (9.8%) investigating endometrial biomolecules (Fig. 3), and 51 (35.9%) exploring the effects on reproductive outcomes (Supplementary Table S2).

Effect of age on uterine morphology

Among the studies investigating how uterine morphology changes with age (Table 1), seven studies corroborated there is a gradual increase in uterine weight, volume and/or size, which is more pronounced during adolescence when the uterus transitions from a tubular to an inverted pear shape, but there was no consensus on the threshold age when the uterus begins to shrink [35, 39–41, 44–46]. These results contradict earlier evidence of there being no change in uterine size before menopause [43]. Of those studies reporting these rising trends, five found a further progressive reduction, although there was no consensus on the threshold age. One study reported a decrease in uterine length and volume when women reach 35–40 years [45], two reported uterine size lessened postmenopause [42, 43], and two showed uterine volume declined after 45 years of age [37, 46]. Recent evidence shows age leads to an incremental thickening of the uterine wall, particularly in the fundus [35], and a rounded shape of the uterus [40, 45]. However, when Verguts et al. took into account parity, they only found this change of shape in women who had conceived [45]. In contrast, two articles did not find a relationship between age and uterine morphologic parameters [36, 38], although one observed that there was a rising trend of uterine body lengths in older women [38]. Finally, two

Table 1 Effect of age on uterine morphology

Variable type	Age (years) / stage	Number of patients (n)	Technique	Effect of age	Summary of reported results							Reference
					UL	UW	UAP	UV	UT	UWe	US	
Discrete	15–24; 25–34; 35–45	200	MRI	Yes	-	-	-	-	↑	-	-	[35]
Continuous	≤ 50	210	TVS	No	-	*NS*	-	-	-	-	-	[36]
Discrete	45–49; 50–54; 55–59; 60–64; 65–69; 70–74	815	TVS or TAS	Yes	-	-	-	↓ (> 45)	*NS*	-	-	[37]
Continuous	NA	807	TVS	No	-	-	-	-	-	-	*NS*	[38]
Continuous	15–45	231	USG	Yes	-	-	-	-	-	-	↑	[39]
Discrete	< 25; 25–30; 30–35; ≥ 35	5,726	TVS	Yes	↑	↑	↑	-	-	-	↑	[40]
Continuous	0–40	1,418	TAS	Yes	-	-	-	↑	-	-	-	[41]
Discrete	MP stages	146	TVS	Yes	-	↓ (PostMP)	↓ (PostMP)	↓ (PostMP)	-	-	-	[42]
Discrete	PreMP vs PostMP	263	TVS	Yes No	↓ (PostMP) -	- -	- -	- -	- -	- -	- *NS*	[43]
Discrete	15–24; 25–34; 35–44; 45–54; 55–64; 65–74; ≥ 75	308	PME	Yes	-	-	-	-	-	↑ (15–54)	-	[44]
Continuous	0–91	5,466	USG	Yes	↑ (0–40) ↑ (40–91)	↑ (25–38) ↓ (38–55)	↓ (25–40) ↓ (40–55)	-	-	-	-	[45]
Continuous	17–82	72	MRI	Yes	-	-	-	↑ (17–40) ↓ (45–82)	-	-	-	[46]

The upwards (red) and downwards (blue) arrows were used to respectively showcase significant age-related increases or decreases in the evaluated parameters. If the significant changes were associated with a particular age group, the group was specified in parentheses

MP menopause, *MRI* magnetic resonance imaging, *NA* not available, *NS* not significant, *PME* post-mortem examination, *TAS* transabdominal ultrasonography, *TVS* transvaginal ultrasonography, *UAP* uterine antero-posterior length, *UL* uterine length, *US* uterine size, *USG* ultrasonography, *UT* uterine thickness, *UV* uterine volume, *UW* uterine width, *UWe* uterine weight

Table 2 Effect of age on endometrial and uterine vasculature

Variable type	Age (years) / stage	Number of patients (n)	Technique	Effect of age	Summary of reported results									Reference
					EFI	EVI	EVFI	EC[a]	USV	PI	UVD	RI	IA	
Discrete	MP stages (20–60)	486	TVS	Yes	-	-	-	-	-	-	↓ (PostMP) (41–50)	-	-	[47]
Continuous	< 60	58	CDFI	No	-	-	-	-	-	*NS*	-	-	-	[48]
Discrete	30–34; 35–39; 40–44	152	CDFI	No	-	-	-	-	-	*NS*	-	*NS*	-	[49]
Discrete	PreMP vs. PostMP	74	HBA	Yes	-	-	-	-	-	-	-	-	↑ (PostMP)	[50]
Discrete	< 31; 31–33; 34–36; > 36	79	3D-PDU	Yes	↓ (33)	↓ (33)	↓ (33)	-	-	-	-	-	-	[51]
Discrete	< 40 vs. > 40	21	TVCDU	No	-	-	-	-	-	*NS*	-	*NS*	-	[52]
Discrete	≤ 30 vs. > 30	110	TVCDU	No	-	-	-	-	-	*NS*	-	*NS*	-	[53]
Discrete	≤ 34; 35–39; ≥ 40	143	DUS	No	-	-	-	-	-	*NS*	-	-	-	[10]
Discrete	Reproductive age vs PostMP	NA	NA	Yes	-	-	-		↓ (Post MP)	-	-	-	-	[54]
Continuous	52–96	10	ICP-OES	Yes	-	-	-	↑ (≥ 70) ↓ (≥ 90)	-	-	-	-	-	[55]
Discrete	≤ 30; 31–35; 36–40; ≥ 41	638	3D-PDU	No	*NS*	*NS*	*NS*	-	-	-	-	-	-	[56]
Continuous	27–86	28	ICP-OES	Yes	-	-	-	↑ (≥ 60)	-	-	-	-	-	[57]
Discrete	≤ 35 vs. > 35	884	CDFI	Yes	-	-	-	-	-	↑ (≥ 35)	-	-	-	[58]
Discrete	< 31 vs. ≥ 31	27	3-PDA	Yes	↓ (≥ 31)	-	-	-	-	-	-	-	-	[59]
Continuous	21–40	94	DUS	No	-	-	-	-	-	*NS*	-	-	-	[60]
Discrete	< 35 vs. ≥ 35	439	USG	Yes	↓ (≥ 35)	↓ (≥ 35)	↓ (≥ 35)	-	-	-	-	↑ (≥ 35)	-	[61]
Discrete	< 25; 25–29; 30–34; ≥ 35	199	3D-PDU	Yes	*NS*	*NS*	*NS*	-	↑ (< 25) ↓ (≥ 35)	-	-	-	-	[62]

The upwards (red) and downwards (blue) arrows respectively showcase significant age-related increases or decreases in the evaluated parameters. If the significant changes were associated with a particular age group, the group was specified in parentheses

3D-PDA 3D power doppler angiography, *3D-PDU* 3D power doppler ultrasound, *CDFI* colour doppler flow imaging, *DUS* Doppler ultrasound, *EC* elements content, *EFI* endometrial flow index, *EVFI* endometrial vascular flow index, *EVI* endometrial vascular index, *HBA* histological biometric analysis, *IA* intimal area, *ICP-OES* Inductively Coupled Plasma Optical Emission spectroscopy, *MP* menopause, *NA* not available, *NS* not significant, *PI* pulsatility index, *RI* resistance index, *TVCDU* transvaginal color Doppler ultrasound, *TVS* transvaginal ultrasound, *USG* ultrasonography, *USV* uterine spiral vasculature, *UVD* uterine vein diameter

[a] Evaluated minerals included calcium, phosphorus, magnesium, and sodium

groups reported that the enlarged uterine size in premenopausal women was related to parity rather than age [36, 43]. However, these findings were contradicted by a more recent study reporting differences in the uterine morphology of nulligravida women [40].

Effect of age on endometrial and uterine vasculature

Ten of the seventeen studies evaluating the effect of age on uterine vasculature reported statistically significant changes with age, including decreased uterine blood flow, supply and poor morphology (Table 2). Notably, two studies found significant mineral accumulation (i.e., calcium, sodium and phosphorus) in the uterine arteries of older women [55, 57], while others reported altered structure and morphology of uterine vasculature with age [47, 50, 51, 54, 58, 59, 61, 62]. Specifically, age was associated with a higher intimal area and a lower medial area of uterine arteries [50]. Uterine veins dilated until 41–50 years of age, then constricted [47]. Similarly, uterine spiral arterioles volume dwindled from age 35 [54, 62].

There were contradictory results on whether functional uterine vasculature is affected by age. Three functional studies revealed reduced vascular and flow indexes with age [51, 59, 61] while two studies did not find any differences in these parameters [56, 62]. On the other hand, six studies evidenced age was not related to the resistance and/or pulsatility indexes [10, 48, 49, 52, 53, 60] while two studies found an increase of pulsatility or resistance index [58, 61].

Effect of age on endometrial histology

In general, studies evaluating changes in healthy endometrial histology with age revealed poorer endometrial histological patterns and echogenicity coupled with cellular alterations (Table 3). Three studies evaluating endometrial biopsies using Noyes' criteria [96] found no significant differences in histological patterns: one study included women undergoing HRT and biopsies collected on day 21 of the menstrual cycle; the second included women with regular menstrual cycles and biopsies collected 7–9 days after the luteinizing hormone (LH) peak; and the third included women who underwent COS and had biopsies collected the morning after hCG administration [64, 70, 96]. However, these studies all reported a decrease of glandular maturity in women over 55 years old. Similarly, there were no significant histological differences in the endometrial tissue of menopausal women assessed using Novak and Richardson's criteria [63]. Women under 35 years old presented better endometrial histology, however the criteria for this examination was not reported [72]. Notably, the two studies that found alterations in endometrial histology with age established their own criteria [69, 71] and observed less glandular epithelium in women over 37 years old [69]. In these studies, endometrial biopsies were collected on the day of the ovulation trigger or 7–9 days after natural ovulation. Finally, the postmenopausal reduction in ciliated cell numbers and alteration in non-ciliated cells indicated endometrial cellular compartments are affected by age [66].

Other histological parameters employed to evaluate the effect of age included the fractional anisotropy value and the apparent diffusion coefficient, which define the rate of local water diffusion, during different phases of the menstrual cycle. While these parameters did not significantly differ with age, the fractional anisotropy values tended to be higher in older women [65]. Age did not affect endometrial elasticity, as measured using shear wave elastography [67].

Effect of age on endometrial thickness

Among the 31 studies that compared endometrial thickness in young and AMA women (Table 4), 17 (54.8%) reported the endometrial lining gets thinner with age [42, 43, 54, 68, 73, 77, 78, 80, 84–86, 88–91, 93, 94], 11 (35.4%) found no statistical differences [10, 61, 70, 75, 76, 79, 81–83, 92, 95], one argued that thicker endometrial linings corresponded with the youngest and oldest age groups [87], and two reported the endometrium thickens with age [35, 74].

Effect of age on menstrual cycle characteristics

Eight studies (80%) corroborated that age negatively affects ovarian hormone levels (Table 5). There was evidence of significantly reduced levels of plasma oestradiol and oestradiol-17β or serum androstenedione (a precursor of active androgens and oestrogens) in menopausal women compared to menstruating women [63, 97], and diminished estrogen and progesterone levels [98]. However, a recent study reported no differences in preovulatory serum progesterone or progesterone/oestradiol ratios in women under 45 years old [99], which corroborated earlier evidence that there were no differences in endometrial oestrogen or progesterone receptor expression between menstruating and peri-menopausal women [100]. Notably, there are lower levels of oestrogen receptors in the endometrial glands of women over 51 years old [101]. Further, older women presented elevated follicle stimulating hormone (FSH) and luteinizing hormone (LH) serum levels [64, 92, 98, 102] and lower basal androgen levels in serum [103].

Eight studies reported that menstrual cycle progression is altered with age (Table 6). Earlier studies showed age led to uterine atrophy, irregular menstrual cycles [104, 105], and a progressive decline in menstrual cycle duration [98]. However, a later work found no differences in

Table 3 Effect of age on endometrial histology

Variable type	Age (years) / stage	Number of patients (n)	Technique	Effect of age	Summary of reported results		Reference
					Endometrial Histological Patterns	Others	
Discrete	PostMP	12	HBA	No	*NS*	-	[63]
Discrete	20–30 vs. 40–50	60	HBA	No	*NS* according to Noyes' criteria	-	[64]
Discrete	20–30 vs. 30- 40	29	DTI	No	-	*NS* for fractional anisotropy *NS* for apparent diffusion coefficient	[65]
Continuous	45–65	NA	SEM	Yes	-	⇩ Microvillus secretory cells (> 52) Ciliated cell number Alterations in non-ciliated cells	[66]
Continuous	25–69	56	SWE	No	-	*NS* for endometrial elasticity	[67]
NA	NA	477	TVS	No	*NS* according to Noyes' criteria	-	[68]
Continuous	22–43	50	HSC	No	Poor	⇩ Glandular epithelium (> 37)	[69]
Discrete	25–39; 40–49; 50–60	122	HBA	No	*NS* according to Noyes' criteria	⇩ Glandular maturity (> 55)	[70]
Discrete	< 41 vs. 41–45	191	USG	Yes	Poor (41–45)	-	[71]
Discrete	23–35 vs. 36–41	58	HBA	Yes	Normal (< 35)	-	[72]

Parameters with statistical differences were specified along with the corresponding age groups in brackets. The downward blue arrows indicate a significant decrease

DTI 3T diffusion tensor imaging, *HBA* histologic biometric analysis, *HSC* hysteroscopy, *MP* menopause, *NA* not available, *NS* not significant, *SEM* scanning electron microscopy, *SWE* shear wave elastography, *TVS* transvaginal ultrasonography, *US* ultrasonography

the menstrual cycle duration of older women [92, 102]. There is contradictory evidence on whether age alters the length of the follicular and luteal phases of the menstrual cycle. While most studies demonstrated that the follicular phase shortens with age [92, 98, 106], one study reported the opposite [102]. On the other hand, two studies demonstrated that age was not related to altered luteal phase length [92, 102] contradicting previous evidence by [98] describing that age lengthens the luteal phase. Finally, age was associated with an inadequate transformation to the secretory phase, impaired development of functional endometrium [72, 107], and a higher prevalence of out-of-phase endometria [104]. A summary of the age-related changes in reproductive hormone levels and menstrual cycle disturbances is depicted in Fig. 2.

Effect of age on biomolecule levels

Single experiment approaches

Among the ten studies using single experimental molecular analyses like polymerase chain reaction (PCR) or western blot, eight found that age significantly altered molecular functions of the uterus and endometrium (Fig. 3 and Table 7). Older women had reduced levels of MK167 (Marker Of Proliferation Ki-67), MCM2 (Mini-chromosome Maintenance Complex Component 2), and CCNA1 (Ciclin A1), suggesting alterations in cell cycle and proliferation [108]. E-cadherin downregulation [109] suggested adhesion is also disturbed with age, however, expression of other adhesion markers, such as MUC1 (Mucin 1), LIF (Leukaemia inhibitory factor), integrin-β3, and glycoconjugate sugar residue content and distribution (related to peri-implantation feto-maternal molecular recognition) remained stable [109–111]. Notably, older women presented lower levels of HOXA10 (Homeobox A10), a well-known marker of endometrial receptivity [112]; elevated endometrial apoptosis and senescence [113]; and higher proportion of p16-positive senescent cells in the uterine epithelium and endometrial glands [114]. Age-related metabolic disturbances include reduced glucose uptake coupled with lower cytochrome oxidase and succinate dehydrogenase activity in the endometrium [115]. Whereas age-related immune system perturbations include altered levels of chemokines, particularly CXCL12 (C-X-C Motif Chemokine Ligand

Table 4 Effect of age on endometrial thickness

Variable type	Age (years) / stage	Sample size (n)	Type of cycle	When endometrial thickness was measured	Technique	Effect of aging	Summary of reported results	Reference
Discrete	17–25; 26–35; 36–40; > 40	2,334 p	COS	hCG day	TVS	YES	⇩	[73]
Discrete	15–24; 25–34; 35–45	200 p	NA	NA	MRI	YES	⇧	[35]
Discrete	18–35 vs. 45–55	26 p	Natural	Every 1–3 days before ovulation	TVS	YES	⇧	[74]
Discrete	≤ 30; 31–35; 36–40; 41–45; 46–53	189 p	HRT	NA	TVS	NO	*NS*	[75]
Discrete	< 70 vs. ≥ 70	36 p	HRT	NA	TVS	NO	*NS*	[76]
Discrete	< 40 vs. ≥ 40	78 c	HRT	hCG day	NA	YES	⇩	[77]
Discrete	< 40 vs. ≥ 40	78 c	HRT	Donor's hCG day	TVS	YES	⇩	[78]
Discrete	21–35; 36–40; 41–49; 50–61	141 c	HRT	Days 8, 11, 15 and 21 of the cycle	TVS	NO	*NS*	[79]
Continuous	< 42	3,157 c	COS	hCG day	NA	YES	⇩	[80]
Discrete	≤ 34; 35–39; ≥ 40	143 c	HRT	2 h prior to embryo transfer	NA	NO	*NS*	[10]
Continuous	NA	1,016 p	COS	hCG day	TVS	NO	*NS*	[81]
Continuous	NA	274 p	HRT	> 12 days after E4 was administrated	TVS	NO	*NS*	[82]
Discrete	Reproductive age vs Post MP	NA	NA	NA	NA	YES	⇩ PostMP	[54]
Discrete	MP stages	146 p	Natural	Day 2 to 8 of the cycle	TVS	YES	⇩ PostMP	[42]
Continuous	20–41	120 p	NA	NA	TVS	NO	*NS*	[83]
Continuous	52–83	26 p	NA	NA	CTG	YES	⇩	[84]
Continuous	≤ 40	9,255 c	Natural or HRT	hCG day	NA	YES	⇩	[85]
Discrete	PreMP vs. PostMP	263 p	Natural	Days 4 and 8 of the cycle	TVS	YES	⇩ (PostMP)	[43]
Continuous	NA	477 p	COS	hCG day + 1	TVS	YES	⇩	[68]
Discrete	25–39; 40–49; 50–60	122 p	HRT	Day 21 of the cycle	TVS	NO	*NS*	[70]
Continuous	NA	1,111 p	Natural or HRT	Day 8 to 10 of the cycle	TVS	YES	⇩	[86]
Continuous	NA	10,165 p	HRT	Embryo transfer day	TVS	YES	⇩⇧ [a]	[87]
Discrete	< 35 vs. > 35	1,169 p	COS	NA	NA	YES	⇩	[88]
Continuous	24–40	103 p	COS	hCG day	TVS	YES	⇩	[89]

Table 4 (continued)

Variable type	Age (years) / stage	Sample size (n)	Type of cycle	When endometrial thickness was measured	Technique	Effect of aging	Summary of reported results	Reference
Discrete	≤ 35; 36–40; 41–42	2,343 p	NA	hCG day	TVS	YES	⇩	[90]
Discrete	< 35 vs. > 35	2,562 p	Natural or HRT	Daily from day 10	TVS	YES	⇩	[91]
Discrete	22–34 vs 41–46	61 p	Natural	7 days after the temperature shift	TVS	NO	*NS*	[92]
Discrete	< 35 vs. ≥ 35	439 p	Natural	Day 8 of the cycle and 2 days prior to ovulation	TVS	NO	*NS*	[61]
Continuous	Post MP	8,594 p	NA	NA	TVS	YES	⇩	[93]
Continuous	NA	783 p	COS	hCG day	TVS	YES	⇩	[94]
Continuous	< 40	6,181 p	Natural + hCG or HRT	hCG day (natural) or prior P4 initiation (HRT)	TVS	NO	*NS*	[95]

The upwards (red) and downwards (blue) arrows respectively showcase significant age-related increases or decreases in endometrial thickness. If the significant changes were associated with a particular age group, the group was specified in parentheses

c cycles, *COS* controlled ovarian stimulation, *CTG* computed tomography, *HRT* hormone replacement therapy, *MRI* magnetic resonance imaging, *MP* menopause, *NA* not available, *NS* not significant, *p* patients, *TVS* transvaginal ultrasound

[a] In this study by Shaodi et al., increased endometrial thickness was found in the youngest and oldest groups

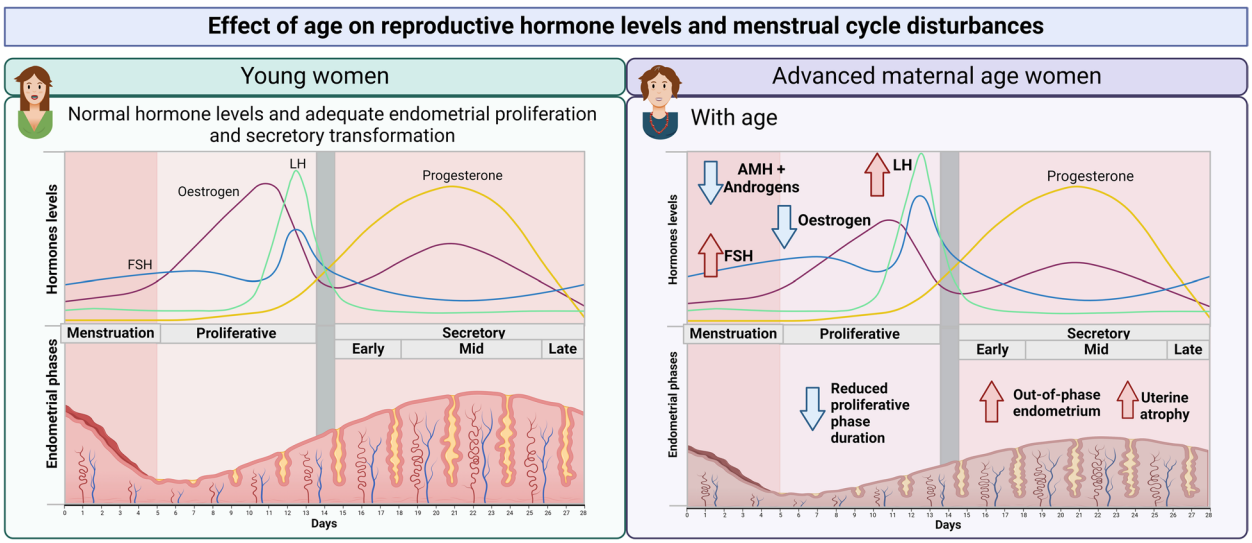

Fig. 2 Main findings of studies evaluating the effect of age on reproductive hormone levels and menstrual cycle disturbances. Comparison of hormone levels and endometrial progression in young women and those of advanced maternal age. With age, the levels of serum anti-mullerian hormone (AMH), androgen, estrogen, LH, and FSH decline. These changes collectively lead to menstrual cycle disturbances, marked by a shorter proliferative phase, higher prevalence of uterine atrophy and out-of-phase endometria. AMH, antimullerian hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; Created with BioRender.com

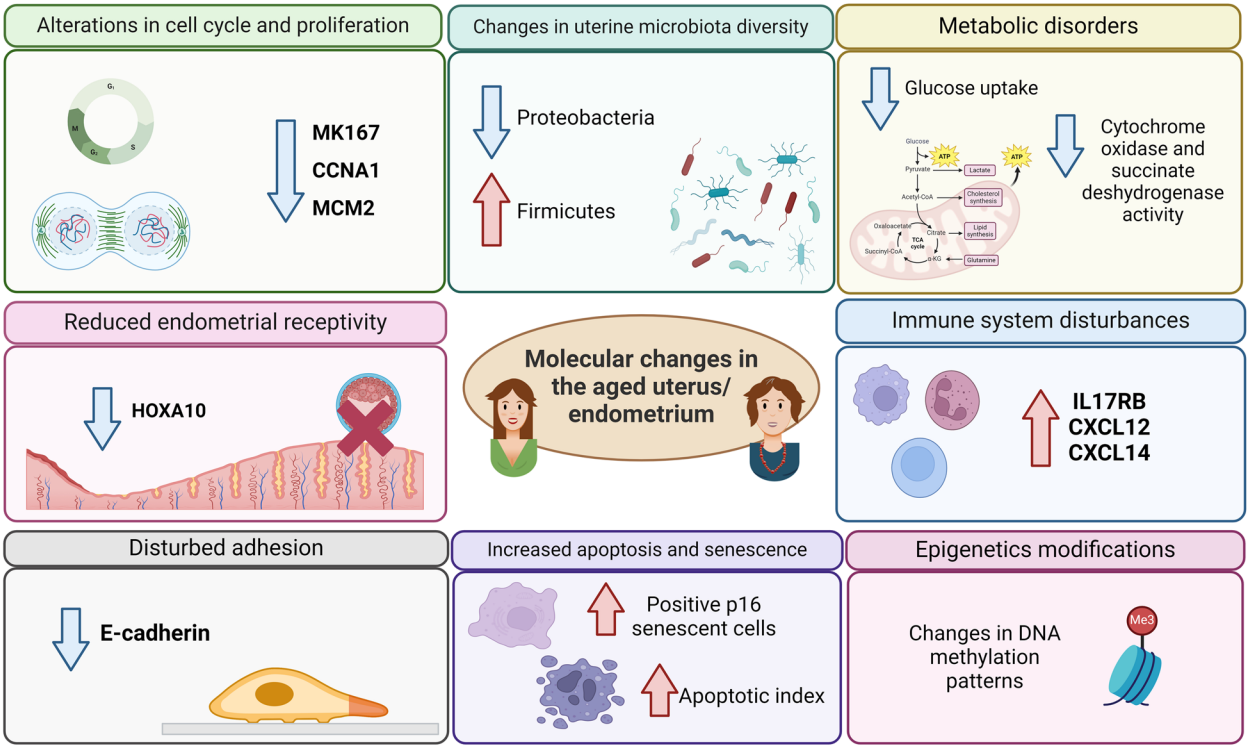

Fig. 3 Main findings of studies evaluating the effect of age on molecular mechanisms and functions of the endometrium. Summary of the main findings of studies evaluating age-related functions disruptions of the uterus or endometrium, including those reported by single and high throughput experimental approaches. MKI67 is related to regulation of chromosome segregation and mitotic nuclear division, CNNA1 is related to control of meiosis, and MCM2 is related to initiation of genome replication. CCNA1, cyclin-A1; CXCL12, CXC motif chemokine ligand 12; CXCL14, CXC motif chemokine ligand 14; HOXA10, homeobox A10 DNA-binding transcription factor; IL17RB, interleukin-17 receptor B; IL8, interleukin-8; LIF, leukemia inhibitory factor cytokine; MCM2, minichromosome maintenance complex component 2; MKI67, marker of proliferation Ki-67; MUC1, mucin 1; NS, not significant; PGR, progesterone receptor. Created with BioRender.com

12), CXCL14 (C-X-C Motif Chemokine Ligand 14), and IL17RB (Interleukin 17 Receptor B) [116]. Finally, the downregulation of the aryl hydrocarbon receptor with age suggested that this transcription factor might have a relevant role in women of reproductive age [117].

High-throughput experimental approaches

Recent omics findings confirmed that age negatively impacts uterine physiology [118] and alters uterine epigenetics [29] and microbiota [119] (Fig. 3). Transcriptomic assays of menstrual blood-derived stem cells from women of different age groups exposed a downregulation of genes related to the cell cycle, proliferation, adhesion, metabolism and growth factor receptors, along with an upregulation of genes involved in apoptosis and immune responses with age [118]. Further, analysis of multiple endometrial transcriptomic data sets revealed the endometria of older women were more susceptible to SARS-CoV-2 infection due to overexpression of target proteins, including *ACE2* (Angiotensin Converting Enzyme 2, the main target for SARS-CoV-2) [120]. On the other hand, age alters endometrial DNA methylation profiles [18]. Horvath's epigenetic clock showed a good correlation between the chronological age of women and the endometrial molecular age when the phase of menstrual cycle was taken into consideration [29], although this correlation was less accurate in young women [29]. Finally, the uterine microbiota becomes less diversified with age, resembling that of the vaginal microbiome [119]. There is an increased *Firmicutes* to *Proteobacteria* ratio in women over 40 and these changes become more evident in women over 50 [119].

Effect of age on reproductive outcomes

We identified 51 studies that investigated the effects of age on reproductive outcomes following good quality embryo transfer with autologous euploid embryos or embryos generated from oocytes derived from donors < 36 years old (Supplementary Table S2). Of these, 23 (45.1%) reported maternal age negatively impacted reproductive outcomes and 28 (54.9%) found no statistical differences.

Table 5 Effect of age on sex hormones levels

Variable type	Age or age ranges (years)/ stages	Number of total patients	Effect of age	Summary of reported results								Reference
				Endometrium	Ovaries		Plasma					
				E2 or P4 receptors	E2	P4	E1/E2	P4	Androgen levels	FSH	LH	
Discrete	PreMP vs. PostMP	40	Yes	-	-	-	↓ (Post MP)	-	-	-	-	[63]
Discrete	20–30 vs. 40–50	32	Yes	-	-	-	-	-	-	↑ (> 40)	↑ (> 40)	[64]
Discrete	Menstruating vs. PostMP	75	Yes	-	-	-	-	-	↓ (Post MP)	-	-	[97]
Discrete	Reproductive age	89	No	*NS*	-	-	-	-	-	-	-	[100]
Discrete	21–25, 26–31, 32–36, 37–45	53	Yes	-	-	-	-	-	-	↑	↑	[102]
Continuous	18–51	21	Yes	-	↓ (≥ 46)	↓ (≥ 46)	-	-	-	↑ (≥ 46)	-	[98]
Continuous	51–83	33	Yes	↓	-	-	-	-	-	-	-	[101]
Continuous	≤ 45	203	No	-	-	-	*NS*	*NS*	-	-	-	[99]
Continuous	20–45	5,278	Yes	-	-	-	-	-	↓	-	-	[103]
Discrete	22–34 vs. 41–46	61	Yes	-	-	-	-	-	-	↑ (41–46)	↑ (41–46)	[92]

The upwards (red) and downwards (blue) arrows were used to respectively showcase significant age-related increases or decreases in the evaluated parameters. If the significant changes were associated with a particular age group, the group was specified in parentheses

E2 estrogen, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *NS* not significant, *P4* progesterone, *PreMP* pre-menopause, *PostMP* post-menopause

Table 6 Effect of age on menstrual cycle

Variable type	Age range (years)	Number of patients	Effect of age	Statistically significant changes with age					Reference
				MCR	CD	FPL	LP	EPD	
NA	NA	2,054	Yes	-	-	↓	-	-	[106]
Discrete	21–25 vs. 37–45	53	Yes	-	*NS*	↑ (37–45)	*NS*	-	[102]
Continuous	18–51	21	Yes	-	↓	↓	↑	-	[98]
Discrete	< 35 vs. > 35	33	Yes	-	-	-	-	↑	[107]
Discrete	< 40 vs. > 40	36	Yes	↓ (> 40)	-	-	-	↑	[104]
Discrete	23–35 vs. 36–41	58	Yes	-	-	-	-	↑	[72]
NA	NA	NA	Yes	↓	-	-	-	-	[105]
Discrete	22–34 vs. 41–46	61	Yes	-	*NS*	↓ (41–46)	*NS*	-	[92]

The upwards (red) and downwards (blue) arrows were used to respectively showcase significant age-related increases or decreases in the evaluated parameters. If the significant changes were associated with a particular age group, the group was specified in parentheses

CD cycle duration, *EPD* endometrial phase displacement, *FPL* follicular phase length, *LP* luteal phase, *MCR* menstrual cycle regularity, *NA* not available, *NS* not significant

Meta-analysis

Endometrial thickness

Seven studies (22.5%) met the quality criteria for meta-analysis (Supplementary Fig. S1A, Supplementary Table S3A) and reported the mean endometrial thickness, measured during distinct phases of the menstrual cycle, in a total of 5,126 women. The quality assessment of the included studies is reflected in Supplementary Table S4A. The forest plot with the MDs in endometrial thickness is presented in Fig. 4. Our meta-analysis confirmed that AMA (defined here as 35–40 years old) was associated with a reduced endometrial thickness, regardless of the menstrual cycle phase (MD [95% CI] = −0.52 mm [−0.72, −0.32], $I^2 = 76\%$, p-value < 0.0001).

Reproductive outcomes

Pregnancy rate The forest plot with the calculated ORs for PR is presented in Fig. 5A. Analyzing data for 52,843 embryo transfers in donor oocyte cycles (oocyte donors < 36 years old) (34,425 in the AMA patients and 18,418 in the young patients (Supplementary Table S3B)) across 22 eligible studies (Supplementary Fig. S1B), the odds of achieving clinical pregnancy were 20% lower for AMA patients (OR 0.80; 95% CI: 0.69, 0.93; $I^2 = 55\%$, p-value = 0.0039). Age considered for AMA in pregnancy rate studies was 39–42, being 40 years old the most common cutoff age. The quality assessment of the included studies is reflected in Supplementary Table S4B.

Live birth rate The forest plot with the calculated ORs for LBR is presented in Fig. 5B and the quality assessment of the included studies is reflected in Supplementary Table S4C. Analyzing data for 26,111 clinical pregnancies (17,552 in the AMA patients and 8,559 in the young patients (Supplementary Table S3C)) across 10 eligible studies (Supplementary Fig. S1C) there was no significant decrease in the odds of achieving a live birth for AMA patients (OR 0.96, 95% CI: 0.91, 1.01, I2 = 44%, p-value = 0.0953). Age considered for AMA in live birth rate studies was 40 years old.

Implantation rate The forest plot with the calculated ORs for IR is presented in Fig. 5C. The quality assessment of the included studies is reflected in Supplementary Table S4D. Analyzing data for 69,384 embryo transfers from donor oocytes cycles (oocyte donors < 36 years old) (44,754 in the AMA patients and 24,630 in the young patients (Supplementary Table S3D)) across 10 eligible studies (Supplementary Fig. S1D) the odds of successful implantation diminished by 27% for AMA patients (OR 0.73, 95% CI: 0.57, 0.93, $I^2 = 75\%$, p-value = 0.0108). Age considered for AMA in implantation rate studies was 39–41, being 40 years old the most common cutoff age.

Table 7 Effect of age on molecular mechanisms and functions of the endometrium

Function	Comparison type	Age (years)	Sample size	Tissue	Effect of age	Statistically significant changes with age	Reference
Cell cycle and proliferation	Continuous	25-55	14	Cultured endometrium	YES	⇩ HOXA10	[112]
	Groups	18 – 38 vs 45–54	44	Endometrium and serum	YES	⇩ MKI67, MCM2, CCNA1, PGR and response to steroids (45–54)	[108]
Adhesion	Continuous	21-28	51	Endometrium	YES	⇩ E-cadherin	[109]
	Groups	< 30 vs > 40	30	Endometrium	NO	***NS*** glycoconjugate sugar residue content and distribution	[110]
	Continuous	< 40	59	Endometrium	NO	***NS*** MUC1, LIF and integrin-beta3	[111]
Apoptosis	Continuous	28-38	34	Endometrium and serum	YES	⇧ Apoptotic index	[113]
Immune system	Continuous	24-48	20	Endometrium	YES	⇧ CXCL12, CXCL14 and IL17rb ***NS*** IL8 levels	[116]
Metabolism	Groups	≤ 35; ≥ 36-45; ≥ 46	1500	Endometrium	YES	⇩ Glucose uptake ≥ 46 ⇩ Cytochrome oxidase and succinate dehydrogenase activity	[115]
Senescence	Continuous	22-48	311	Endometrium	YES	⇧ Proportion of p16-positive senescent cells	[114]
Others	Groups	25-31; 32-38; 39-45	86	Endometrium	YES	⇩ Endometrial reactivity for arylhydrocarbon receptor	[117]

The upwards (red) and downwards (blue) arrows were used to respectively showcase significant age-related increases or decreases in the evaluated parameters. If the significant changes were associated with a particular age group, the group was specified in parentheses

CCNA1 cyclin-A1, *CXCL12* CXC motif chemokine ligand 12, *CXCL14* CXC motif chemokine ligand 14, *IL17RB* interleukin-17 receptor B, *IL8* interleukin-8, *LIF* leukemia inhibitory factor cytokine, *MCM2* minichromosome maintenance complex component 2, *MKI67* marker of proliferation Ki-67, *MUC1* mucin 1, *NS* not significant, *PGR* progesterone receptor

Pregnancy loss rate The forest plot with the calculated ORs for PLR is presented in Fig. 5D. Analyzing data for 20,798 clinical pregnancies (13,421 in the AMA patients and 7,361 in the young patients (Supplementary Table S3E)) across 11 eligible studies (Supplementary Fig. S1E), the odds of miscarriage were 44% higher for AMA patients (OR 1.44, 95% CI: 1.04, 1.99, I^2 = 60%, p-value = 0.0278). The quality assessment of the included studies is reflected in Supplementary Table S4E. Age considered for AMA in pregnancy loss rate studies was 39–42, being 40 years old the most common cutoff age.

Discussion

Main findings

Overall, the results presented in this systematic review point to an influence of age in the uterus and endometrium on various levels. Contradictory evidence in qualifying literature may have been due to different study

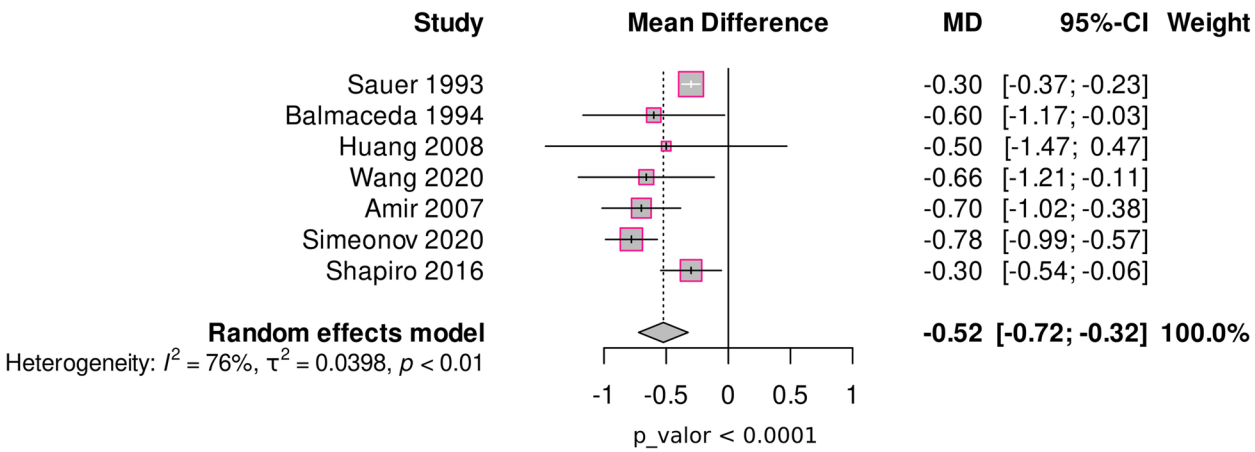

Fig. 4 Forest plot of mean difference from the meta-analysis of endometrial thickness in women with advanced maternal age. MD: mean difference; CI: confidence interval

designs, methodologies, and cohorts. Our meta-analysis showed that AMA patients had significantly reduced endometrial thickness, lower odds of a successful implantation and achieving clinical pregnancy, or higher odds of pregnancy loss, but no significant differences in the odds of achieving a live birth, compared to younger patients. Further, given all the inclusion and exclusion criteria used in these meta-analyses, we consider that only high quality studies were included; hence reinforcing the relevance of the obtained results.

The uterus is a hollow organ that acquires its inverted pear shape at the onset of puberty [121] and is implicated in gestation [122]. Overall, studies included in this review showed that the human uterus enlarges until approximately menopause, then begins shrinking [35, 37, 39–46]. A recently published study confirmed a significant relationship between age and uterine morphology [123]. This observation suggests that sex hormones are implicated in these morphological changes, since the rise and drop of their levels respectively correlate with the first and the last menstrual cycle. Supporting the hypothesis that the onset of menses promotes uterine enlargement, there is a direct relationship between serum estradiol levels and uterine length in young women between 8 and 16 years old [124]. Further, girls with Turner syndrome are treated with estrogen replacement therapy to promote uterine development and growth [125]. However, after puberty, it remains unclear whether age affects gross uterine morphology. Parity is considered to have a larger influence on uterine size than age [36, 43, 123], however, most of the studies evaluating the effect of age on uterine morphology, reviewed herein, did not consider parity. Thus, further studies would be required to confirm if uterine size is affected by age, parity, or a combination of both factors. Uterine size is important for reproductive outcomes since a minimum size is required to achieve a successful pregnancy [126].

Leading to menopause, the diminished activity of the few ovarian follicles leads to an inadequate production of inhibin B and estradiol, raising serum FSH through a loss of negative feedback to the hypothalamus [92, 98, 127]. The augmented FSH levels accelerate follicular growth, shortening the follicular phase by three to four days while maintaining the normal timeframe for ovulation and the luteal phase [92, 106, 127]. As a consequence of the perimenopausal hormonal shift and marked decline in estrogen levels, the endometrium undergoes changes that may displace the window of implantation [128] and/or lead to endometrial atrophy (i.e., thinning of the uterine lining) [129]. These age-related disturbances in endometrial development might explain the reported alterations in tissue architecture and cellular composition [69, 71]. Interestingly, studies that did not observe histological differences in the endometrium of older women employed Noyes' criteria [96], the most commonly used method for endometrial dating. This suggests that Noyes' criteria may not detect age-related alterations, adding to the established limitations of these guidelines (i.e., inter- and intra-subject variability, interobserver variability, and inability to determine fertility status) [130, 131].

Regarding the changes in endometrial cell composition with age, our search identified an early study which detected there were fewer ciliated cells and alterations in non-ciliated cells in older women, using integrated transmission and scanning electron microscopy [66]. However, our recent functional analysis showed that functions related to cilia motility and ciliogenesis are upregulated in AMA patients [2]. Similar *in silico* findings were experimentally validated by Loid and colleagues [132], confirming ample ciliated cells in the endometrial tissue of

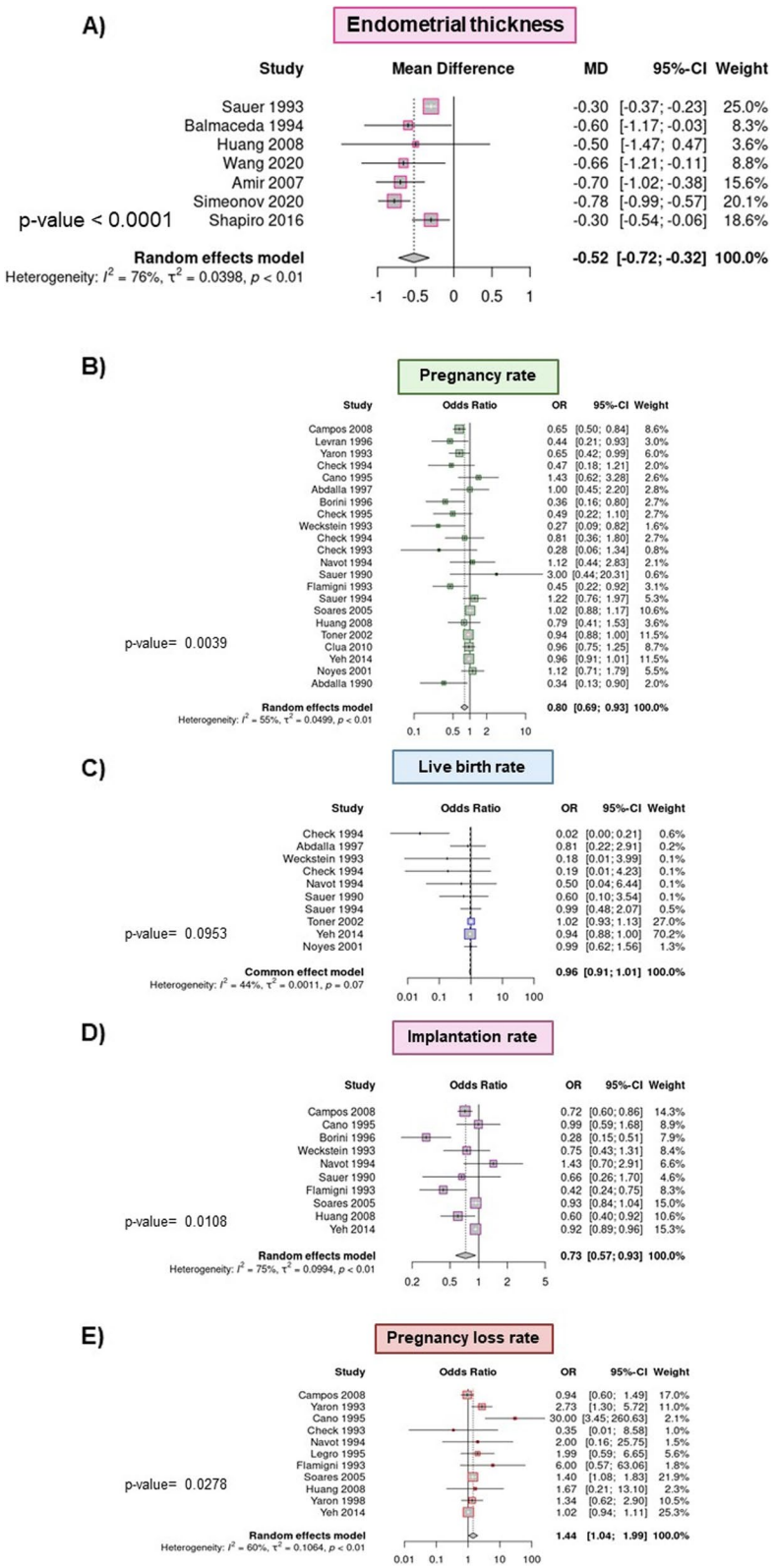

Fig. 5 Forest plots of odds ratios from the meta-analyses of reproductive outcomes in women with advanced maternal age. Forest plots for the OR of achieving (**A**) clinical pregnancy, (**B**) live birth, (**C**) implantation, or experiencing (**D**) pregnancy loss following euploid embryo transfer. OR, odds ratio; CI, confidence interval

AMA patients using immunohistochemical analysis. Ciliated cells are important for reproductive biology [133] and their abundance fluctuates throughout the menstrual cycle. The larger proportions of ciliated cells during the late proliferative phase help the embryo(s) roll over the uterine lumen while smaller proportions at the window of implantation help weaken the endometrial barrier to facilitate trophoblast invasion [133, 134]. Thus, excessive ciliated cells and cilia motility in AMA patients could hinder embryo implantation.

Uterine atrophy is characterized by a thin endometrial lining and loss of endometrial glands [135]. Apart from a good-quality embryo, a minimum endometrial thickness is required to support embryo implantation [136]. However, there is a long-standing debate on whether endometrial thickness is associated with reproductive outcomes in AMA patients. A recent single-center, retrospective, observational study demonstrated that ongoing pregnancy rates increase by 12% per millimeter of endometrial thickness for women over 35 years undergoing frozen-thawed embryo transfers [91]. Our meta-analysis confirmed the mean endometrial thickness was reduced by 0.52 mm in women 35 to 40 years old compared to women < 35 years old, suggesting that aging and its associated physiological and endocrine changes impede adequate endometrial development. Our results align with those of a previous meta-regression [137] that grouped women based on endometrial thickness (≤ 7 mm vs. > 7 mm) and found the mean age of patients with thin endometrium (≤ 7 mm) was significantly higher than that of patients with adequate endometrial thickness (> 7 mm) [137]. Given that thin endometria are characterized by poor epithelial growth, decreased vascular endothelial growth factor (VEGF) expression, and poor vascular development and blood flow [138], the age-related decline in endometrial thickness and hormonal fluctuations may also be related to changes in endometrial vasculature.

This review highlights AMA patients have vascular disturbances that decrease uterine blood supply, which may impede endometrial receptivity, and ultimately, affect reproductive outcomes following euploid embryo transfer. In the early stages of embryo implantation, active angiogenesis, confirmed by the upregulation of various angiogenesis-related factors, supports embryonic development and the establishment of pregnancy [139]. While a relationship exists between endometrial blood flow and reproductive outcomes [140, 141], previous studies used limited sample sizes and there was no consensus regarding the vascular parameters (e.g., pulsatility or resistance indexes) that may be affected by age. Our review begins to address these gaps by highlighting that AMA patients have histologic features of elevated mineral depositions in the uterine blood vessels [55, 57] that may drive uterine artery atherosclerosis. Elevated vascular calcification was recently related to a higher risk of developing atherosclerosis [142], a disease well-known to affect postmenopausal women [50]. Notably, atherosclerosis was associated with vascular stiffness and limited vasodilation [143]. As uterine vascular remodeling is essential for cyclical endometrial development, preparing for embryo implantation, placentation, and maintenance of pregnancy [144], the implications of this condition during childbearing years merit further investigation.

Studies evaluating the effect of age on the endometrium using functional genomic analyses have identified key genes and processes required for endometrial competence. Specifically, embryo implantation was hindered by dysregulated adhesion [145], immune modulation [146], glucose metabolism [147, 148], proliferation [149], apoptosis [150], and endometrial receptivity [151]. Further, our group identified 5,778 dysregulated endometrial genes in women over 35 years old, including genes essential for endometrial receptivity acquisition [*Progestagen associated endometrial protein* (*PAEP*) and *matrix metallopeptidase 26* (*MMP26*)], cell cycle arrest [*Fibroblast growth factor 2* (*FGF2*)], maintenance of telomeric length [*TERF1 interacting nuclear factor 2* (*TINF2*)], and genomic stability [*Sirtuin 1* (*SIRT1*)] [2]. Age-related declines in stromal cell proliferation and repression of crucial decidualization factors, *signal transducer and activator of transcription 3* (*STAT3*) and *bone morphogenetic protein-2* (*BMP2*), were also reported [152]. Notably, it was also reported that epigenetic endometrial molecular age was increased compared to chronological age, and that this increase was more pronounced in younger women [29]. This increase in the endometrial molecular age might be due to estrogen and/or progesterone activity, two hormones that have a key role in the endometrium [153]. In fact, it has been observed that mutations in estrogen and progesterone receptors, such as in several breast cancer types, lead to an increase of molecular age [18]. On the other hand, disrupted DNA methylation, may lead to premature endometrial aging, which hinders endometrial receptivity and embryo implantation [154]. In fact, DNA methylation of 448 sites exerted a detrimental effect on endometrial receptivity, leading to recurrent pregnancy failure [155]. Additionally, DNA methylation analysis suggests that senescent endometrial stromal cells partially contribute to decreased endometrial plasticity, which may lead to recurrent pregnancy loss [156]. This phenomenon is consistent with findings from studies presented in this review that indicate that endometrial histological patterns deteriorate with age.

Moreover, parallel to studies on the gut microbiome [157], the endometrial microbiome evolves to have a lower

biodiversity with age. A dysbiosis caused by the pathological *Proteobacterium*overgrowing the beneficial *Lactobacillus*negatively affected endometrial receptivity and reproductive outcomes [128, 158]. Taken together, epigenetic alterations, mitochondrial dysfunction, deregulated nutrient sensing, cellular senescence, and microbial dysbiosis–which are all established hallmarks of human aging–reinforce the concept of endometrial aging [19, 159, 160].

Understanding how age affects uterine morphology, histology, and cell compartments helps elucidate the impact on endometrial competence, or ability to achieve, maintain, and successfully carry a pregnancy to term. Our meta-analysis included only studies with good-quality embryos (derived from oocytes donated from women < 36 years old). This approach reduced potential confounding embryonic factors to unmask endometrial factors. Overall, we found that, compared to younger women, AMA patients had significantly lower odds of establishing and maintaining pregnancy, and tended to have lower odds of a live birth, although the latter was not statistically significant. Given the positive correlation between AMA and gestational and/or fetal complications [161], it is possible that a statistical difference was not detected due to an insufficient sample size. Overall, results obtained in our meta-analysis suggest that the detrimental effect of age on reproductive outcomes influenced only by the endometrial factor might begin around 40 years. A recent meta-analysis evaluating the endometrial receptivity of young versus AMA patients in donor oocyte cycles found a non-significant decline in the clinical pregnancy rates and an increase in PLR in AMA women, but a similar IR and LBR between AMA and young women [162]. The discrepancies between the findings reported by Zhao and colleagues [162] and those herein may be due to differences in the pooled sample sizes or the existence of uterine pathologies. However, supporting our results, this review also stablish 40 years as the age cutoff at which a worsening in the reproductive outcomes induced by the endometrial factor begins. Two other reviews published in 2023 evaluated the effect of age on reproductive outcomes. One concluded that there was no clear relationship between AMA and poor reproductive outcomes [30]. However, this group included studies where infertile patients undergoing IVF donated their oocytes to other infertile patients of advanced age, which may have confounded the study results. The second, by Wu *et al.*, also suggested there were more adverse pregnancy outcomes and pregnancy complications in older patients, but these authors mainly reviewed studies performed in animal models, which may not accurately extrapolate to humans [31].

Strengths and limitations

To our knowledge, this is the first systematic review that comprehensively evaluates how age affects the morphology and function of the endometrium, incorporating meta-analyses to discern whether AMA affects endometrial thickness and reproductive outcomes following euploid embryo transfer. Prior to this work, the scarcity of rigorous studies accounting for embryonic effects and uterine pathologies when evaluating the endometrial competence of AMA women likely contributed to the contradicting evidence. Additionally, studies that found no statistically significant differences might have been limited by different study characteristics. Not detecting age-related differences does not guarantee they do not exist, especially when other studies identified them. Despite strict inclusion criteria for meta-analysis, there was significant heterogeneity between studies, due to different study designs (prospective and retrospective), endometrium preparation protocols (replaced, natural, and stimulated), embryo states (fresh and frozen) and sample sizes. However, we controlled for the AMA cut-off age in our meta-analyses (35-40 years for endometrial thickness and 39-42 for the four reproductive outcomes). Although we excluded studies of AMA patients with uterine disorders because they might potentially mask the effects of age on endometrium, we acknowledge that the risks of these diseases increase with age. Further, these diseases have subclinical manifestations and/or are often underdiagnosed, and thus, may be present in the "healthy" patients that were included. Despite these limitations this meta-analysis provides valuable insights into the effect of age on endometrium, due to the large data pool, novel and relevant results.

Implications for future research

Studies on endometrial aging were scarce or included confounding factors that gave rise to contradicting evidence. Well-designed studies are required to elucidate if post-pubertal uterine enlargement is due to age or parity, and if there are other molecular mechanisms that disrupt endometrial histology and hinder the acquisition of endometrial competence in older women.

Implications for clinical practice

As increasingly more women delay motherhood, clinicians are faced with the challenges that accompany an aging reproductive system, including impaired ovarian and uterine function. Understanding the mechanisms of uterine aging will help older women make informed decisions knowing their likelihood of success and help clinicians guide evidence-based reproductive medicine to

improve the outcomes of assisted reproductive technologies. Identifying patients with premature uterine aging, then attempting to delay or reverse the aging processes will shift the landscape for personalized medicine and help manage AMA patients seeking assisted reproductive technologies.

Conclusion

In conclusion, this systematic review and meta-analysis highlights the existence of age-related mechanisms affecting the uterus and the endometrium, reinforcing the importance of adequate endometrial function for successful establishment and maintenance of pregnancy in AMA patients. The effect of age on the uterus and endometrium was characterized by uterine shrinkage, diminished blood supply, impaired endometrial architecture, altered cell components, thin endometrial linings, and molecular perturbations that are all directly implicated in preimplantation endometrial development and the embryo-endometrium crosstalk during and after implantation. Future studies can use these insights to develop anti-aging therapies or preventive measures for endometrial "rejuvenation" that ultimately aim to improve the reproductive outcomes of AMA patients.

Supplementary Information

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Supplementary Material 1.

Supplementary Material 2.

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Authors' contributions
D.M.-G., A.M.-M., and F.J.-S. contributed equally to this work and should be regarded as joint first authors. D.M.-G.: systematic search, study selection, data extraction, data analysis, writing – original draft, figures and tables design; A.M.-M.: systematic search, study selection, data extraction, data analysis, writing – original draft, figures and tables design; F.J.-S.: systematic search, study selection, data extraction, data analysis, writing – original draft, figures and tables design; A.D.-P.: systematic search, study selection, data extraction; P.S.-L.: data analysis, writing – original draft, figures and tables design; N.dA.-dC.: writing – original draft, figures and tables design; A.P.: conceptualization, writing—review & editing, supervision and project administration; P.D.-G.: conceptualization, methodology, data analysis, writing – review & editing, figures and tables design, supervision, project administration, funding acquisition. All authors have read and approved the final version of the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

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Competing interests
The authors declare no competing interests.

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