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ORIGINAL ARTICLE *Infertility*

Thin endometrial lining: is it more prevalent in patients utilizing preimplantation genetic testing for monogenic disease (PGT-M) and related to prior hormonal contraceptive use?

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STUDY QUESTION: Is a thin endometrial lining before ovulation triggering more prevalent in patients utilizing preimplantation genetic testing for monogenic disease (PGT-M) compared to the regular IVF/ICSI population and is this associated with prior hormonal contraceptive use?

SUMMARY ANSWER: Thin (<8 mm) endometrial lining is more prevalent in PGT-M patients compared to the regular IVF/ICSI population and is associated with both longer prior hormonal contraceptive use and a shorter cessation interval of hormonal contraceptives before IVF/ICSI treatment.

WHAT IS KNOWN ALREADY: Thin endometrial lining has been associated with lower pregnancy rates in IVF/ICSI cycles and increased chances of miscarriage and low birth weight. Endometrial thinning and atrophy occur during hormonal contraceptive use. Patients utilizing PGT-M typically use hormonal contraceptives up until treatment to avoid the risk of conception of a genetically affected child. Whether this could negatively affect endometrial thickness achieved during subsequent IVF/ICSI cycles is not known.

STUDY DESIGN, SIZE, DURATION: A retrospective case control study was performed, including all PGT-M patients attending the University Medical Centre Groningen (cases), between 2009 and 2018. The control group consisted of two non-PGT IVF/ICSI patients for each PGT-M patient, matched for age and treatment period.

PARTICIPANTS/MATERIALS, SETTING, METHODS: First cycles of 122 PGT-M patients and 240 controls were included. Cessation interval of hormonal contraceptives was categorized as late cessation (cessation <1 year prior to treatment) or early cessation (>1 year prior to treatment). Endometrial thickness was routinely measured on the day of hCG triggering or 1 day prior. The prevalence of an endometrial lining <8 mm was compared between PGT-M patients and controls. Hormonal contraceptive use (both duration and cessation interval) was compared between both groups. Univariable and multivariable regression analyses were performed to identify risk factors for thin endometrial lining. In addition, cycle and pregnancy outcomes were compared within control/PGT-M groups between patients with endometrial lining > or <8 mm.

MAIN RESULTS AND THE ROLE OF CHANCE: Thin endometrial lining on the day of hCG triggering was found significantly more often in the PGT-M group, compared to controls: 32% vs 11% (mean difference 21.0%, 95% CI: 11.7, 30.3%). As expected, more patients in the PGT-M group ceased their hormonal contraception late (<1 year): 64% vs 2% in the control group (mean difference 61.9%, 95% CI: 53.0, 70.8%). Average duration of hormonal contraceptive use was 10.6 years in the PGT-M group vs 9.3 years in controls (mean difference 1.3 years, 95% CI: 0.2, 2.3 years). Multivariable logistic regression analysis identified late cessation (OR: 6.0, 95% CI: 1.9–19.2) and duration

of prior hormonal contraceptive use (OR per year increase 1.1, 95% CI: 1.0–1.2) as significant independent risk factors for a thin endometrial lining. In relation to outcome, we found a statistically significant increase in miscarriage rate in PGT-M patients with an endometrial lining <8 mm compared to those with an endometrial lining >8 mm (20.0% vs 1.7%, mean difference 18.3%, 95% CI: 2.3, 34.3%). A trend towards lower birth weight and gestation- and gender-adjusted birth weight (*z*-score) was also found in this group. No statistically significant differences were detected in pregnancy rate, live birth rate, or incidence of preterm delivery or SGA. Within the control group, no statistically significant differences were found in outcomes between patients with an endometrial lining <8 compared to an endometrial lining >8 mm.

LIMITATIONS, REASONS FOR CAUTION: The study is retrospective. Various types of hormonal contraceptives were reported which possibly exert different effects on the endometrial lining. In relation to pregnancy outcome measures, numbers were very limited; therefore, no firm conclusions should be drawn.

WIDER IMPLICATIONS OF THE FINDINGS: This study provides further insight into the role of prior hormonal contraceptive use as a possible contributor to the occurrence of thin endometrial lining during ART treatment. Future studies should provide more information on its clinical relevance, to determine whether PGT-M patients can be reassured, or should be counselled to stop hormonal contraceptive use and change to an alternative contraceptive method prior to PGT treatment.

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Key words: thin endometrial lining / endometrial thickness / endometrium / contraceptives / combined oral contraceptives / PGT / PGT-M / ICSI / IVF / ART

Introduction

Large cohort studies have shown a significant decrease in live birth rates (LBR) with a decreasing endometrial thickness (EMT) before ovulation triggering in IVF/ICSI treatments (Gao *et al.*, 2020; Mahutte *et al.*, 2022). Thin endometrial lining is also associated with an increased risk of miscarriage, low birth weight and children being small for gestational age (SGA) at birth (Yuan *et al.*, 2016; Oron *et al.*, 2018; Mouhayar *et al.*, 2019; Zhang *et al.*, 2019; Guo *et al.*, 2020; Du *et al.*, 2021; Hu *et al.*, 2021; Liu *et al.*, 2021; Martel *et al.*, 2021; Mahutte *et al.*, 2022).

The prevalence of thin endometrial lining differs among studies and various cut-off points that are used. EMT <7 mm was seen in 2.4% of cases in studies reviewed by Kasius *et al.* (2014). Other studies have reported a higher prevalence ranging from ∼3% to 8.5% (Gallos *et al.*, 2018; Holden *et al.*, 2018; Liu *et al.*, 2018; Ribeiro *et al.*, 2018). The prevalence of an EMT <8 mm is reported to occur in ∼10% (Liu *et al.*, 2021) of ART treatment cycles. The cause of insufficient thickening of the endometrium during ovarian stimulation can be iatrogenic, as in Asherman's syndrome (Senturk and Erel, 2008), but often the underlying cause is unknown. Multiple factors have been described to be associated with EMT on the day of ovulation trigger such as: age (Amir *et al.*, 2007), oocyte number, oestradiol levels (Amir *et al.*, 2007; Mahutte *et al.*, 2022), late-follicular progesterone levels (Ribeiro *et al.*, 2018), BMI (Ribeiro *et al.*, 2018), treatment protocol and type of gonadotropin used (Amir *et al.*, 2007).

In our hospital, we observed a relative high prevalence of thin endometrial lining in patients opting for preimplantation genetic testing for monogenic diseases (PGT-M). This patient group differs from the regular IVF/ICSI population in two important ways. First, they often are not subfertile. Secondly, they usually aim to avoid natural conceptions because of the risk of a genetically affected child. Therefore, they typically use contraceptive methods up till the start of the PGT-M IVF/ICSI treatment. It is well recognized that endometrial thinning occurs during the use of combined oral contraceptive (COC) and hormonal long-acting reversible contraceptives, such as the levonorgestrel intra-uterine device (IUD) or depot medroxyprogesteronacetate (DMPA). After prolonged use, endometrial atrophy develops (Anderson *et al.*, 2005; Grow and Iromloo, 2006; Dinehart *et al.*, 2020). Possibly, the thinning effect of hormonal contraceptives on the endometrium lingers longer than previously anticipated. Studies on such a possible effect are scarce. However, Talukdar et al demonstrated a relation between the prevalence of thin endometrium and a history of COC use ≥2 years after cessation in patients treated with oestradiol for frozen embryo transfer (FET) (Talukdar *et al.*, 2012).

We hypothesized that a combination of prolonged hormonal contraceptive use, and a short interval of cessation before the start of PGT-M treatment, might interfere with normal endometrial thickening during IVF/ICSI stimulation cycles, due to an extended effect of hormonal contraceptives. This is expected to be more pronounced in PGT-M patients, due to their particular history of contraceptive use, and could thereby hypothetically contribute to a suboptimal outcome of the PGT treatment.

We performed a retrospective case control study, to investigate whether thin endometrial lining is indeed more common in PGT-M patients in first treatment cycles compared to matched controls, i.e. the 'regular' IVF/ICSI population. Second, we investigated whether such a difference was associated with differences either in the total duration of previous hormonal contraceptive use, the interval between cessation of hormonal contraceptives and the start of the first IVF/ICSI cycle, or a combination of these two.

Materials and methods

Patients

A case control study was carried out in the University Medical Center Groningen (UMCG) of the first IVF/ICSI cycle of patients in the period from 2009 up till 2019.

Cases were patients undergoing their first ever ovarian stimulation cycle for PGT-M. For each PGT-M patient we included two unique

controls undergoing IVF/ICSI without PGT-M, matched by treatment period (cycle start date within 1 year) and age (same year of birth, or when not available, year of birth plus or minus 1). Inclusion criteria were as follows: first ovarian stimulation cycle ever started leading to at least one dominant follicle of ≥18-mm diameter (thus some cycles that were cancelled before oocyte retrieval were also included), age 18–43 years, long agonist protocol and, for PGT-M cases, PGT for one or two monogenic diseases and, for controls, IVF/ICSI indication being male factor (including microsurgical epididymal sperm aspiration (MESA) or testicular/epididymal sperm extraction (TESE)), tubal factor, a combination of these, or unexplained subfertility. Exclusion criteria were: a history of Asherman syndrome, intra-uterine surgery (except caesarian section or curettage), endometriosis, polycystic ovary syndrome (PCOS), oocyte donation program, uterine fibroids and/or uterine anomaly. None of the patients underwent PGT for aneuploidy (PGT-A) or for structural rearrangements (PGT-SR).

Ethical approval

Approval of the Institutional Review Board of the UMCG was requested, but waived as the study did not involve clinical research with human subjects as described in the Dutch Medical Research Involving Human Subjects Act (WMO). All included patients gave their written informed consent for IVF/ICSI treatment and the use of their data (anonymous) for scientific research purposes. To insure anonymity, names were left out of the database used for analysis, also, only year of birth was used instead of full birth dates after calculating age (in full years) at the start of treatment. PGT indications were not included in the database as rare indications might compromise anonymity. The local hospital database was consulted prior to data analysis, to ensure no patient had made formal objection to the use of their data for research purposes after previous consent.

Baseline data collection

Data on height, weight, smoking habits, obstetric history, previous ART treatment (only intra-uterine inseminations and/or modified natural cycle IVF/ICSI) and previous contraceptive use were systematically collected from patient questionnaires, which were completed at the first visit to our department. Contraceptive use was defined by two variables: total duration of use and the cessation period before start of IVF/ICSI treatment. As the interval between completion of the questionnaire and the first treatment cycle can range from ∼1 up to ∼12 months and contraceptive methods used in these intervening months was not routinely monitored, we could not consistently determine whether reported contraceptive use on first visit was continued up to treatment or not. Therefore, for most analyses, we defined the cessation period of contraception as follows: 'late cessation': cessation <1 year before first treatment cycle (including patients that used contraceptives up till first treatment), or 'early cessation': cessation >1 year before first treatment cycle.

Treatment protocol

All patients underwent a long gonadotropin-releasing hormone (GnRH) agonist protocol. GnRH agonist triptoreline (Decapeptyl© 0.1 mg, Ferring BV, The Netherlands) was started under oral contraceptive, or progesterone pre-treatment. Stimulation was performed with 100–450 IU FSH daily (Menopur©, Ferring BV; Puregon©, Merck Sharp & Dohme BV, The Netherlands; Bemfola©, Gedeon Richter Benelux, Belgium; or Fostimon©, IBSA, Italy). EMT was measured routinely on the day of hCG trigger, or 1 day before. Measurements were conducted in the midsagittal plane of the uterus, on the widest part of the endometrium. The EMT was recorded as the distance between the outer edges of the endometrial-myometrial interface, perpendicular on the length of the cavum. Ovulation triggering was performed with 5000 or 10 000 IU hCG (Pregnyl©) or 250 μg choriongonadotropin alfa (Ovitrelle©, Merck Sharp & Dohme BV) when at least three follicles reached a diameter of ≥18 mm. Serum oestradiol level measurements around ovulation triggering were not routinely performed. Oocyte retrieval was performed 36 hr after triggering. PGT-M was performed in collaboration with the Maastricht University Medical Center (MUMC); cleavage stage embryos were biopsied on Day 3 in the UMCG. Blastomeres were transported to the MUMC the same day, where analyses took place either by PCR or FISH (in some cases of X-linked disease). Results were available the next day, subsequently a Day 4 fresh embryo transfer was performed in the UMCG. In control patients, a fresh embryo transfer was performed on Day 2. First cycle data were collected from medical files (e.g. number of stimulation days, number of dominant follicles, pregnancy) and also, if performed, second cycle and third cycle EMT and start date were collected, to asses a possible 'wear out' effect of hormonal contraceptive use on EMT.

Statistical analysis

Patient baseline, cycle characteristics and outcome data were presented as mean and standard deviation when normally distributed, as median and interquartile range when not normally distributed, or as frequency and percentage in case of categorical variables. Levene's test was used to test for normality prior to comparisons of continuous variables. Differences between PGT-M and the control group were analysed with either *T*-test (normally distributed continuous variables), Mann–Whitney *U* test (not normally distributed continuous variables), or Chi-square test (categorical variables). A paired *T*-test was used for the comparison of endometrial thickness between first, second and third ovarian stimulation cycles in patients with late cessation of hormonal contraceptives and patients with early cessation.

To identify significant risk factors for a thin endometrium, first a univariable binary logistic regression analysis was performed, followed by a multivariable binary logistic regression with appropriate variables, selected based on the univariable analysis and literature.

Results

Patient inclusion

In total, 128 eligible PGT-M cases were identified, of whom six were excluded for various reasons (endometriosis n = 1, PCOS n = 3, uterine anomaly n = 1, uterine fibroids n = 1), leaving 122 cases for analysis. Initially, two unique controls were identified for each PGT-M case, but 24 were excluded for various reasons (PCOS n = 4, uterus fibroids n = 5, previous intra-uterine surgery n = 5, endometriosis n = 6, uterus

anomaly n = 4). Replacement controls could not be identified for all cases: for two patients, only one unique control could be found and, for one patient, no unique control was identified, resulting in a control group of 240 regular IVF/ICSI patients for analysis.

Baseline characteristics: PGT-M vs control group

Baseline charcteristics of both groups are described in Table I. In the control group, patients were more often nulliparous (82.5% vs 63.1) and more often had a history of previous ART treatment (60.0% vs 2.5%). PGT-M patients more often had one or more prior curettages (9.2% vs 2.1%) and more often reported prior use of hormonal contraceptives (100% vs 94.1%) The mean total duration of hormonal contraceptive use was longer in PGT-M patients (10.6 vs 9.3 years). Late cessation of hormonal contraceptives (cessation <1 year before first treatment cycle) was more often seen in the PGT-M patient group, as expected (63.8% vs 1.9%). For 70/78 patients who were categorised as late cessation, miscellaneous notes in patient files indicated that contraceptives were used up till the start of the first treatment (e.g. 'allready uses COC, no presciption necessary', data not shown).

Cycle characteristics and outcome: PGT-M vs control group

In Table II, cycle characterisics and cycle outcomes are shown for the PGT-M group and controls. Mean endometrial lining on the day of hCG trigger was significantly thinner in the PGT-M group (9.4 vs 10.4 mm). An endometrial lining <7 mm was more common in the PGT-M group (11.8% vs 2.6%). Moreover, an endometrial lining <8 mm was observed in approximately one third of the PGT-M patients (31.9%) compared to 10.9% of controls. Both in the PGT-M group and in the control group, EMT measurements were mostly performed on the day of hCG triggering (respectively, 67.2% and 68.5%, mean difference −1.3%, 95% CI −11.6%, 9.0%) (not shown). There was no statistically significant relation between day of EMT measurement and EMT (mean EMT on the day of hCG triggering: 9.9 mm, 1 day prior hCG triggering: 10.4 mm, mean difference -0.5 mm, 95% CI -1.0, 0.04).

There were no siginificant differences in cancellation rates. In the PGT-M group, two patients were cancelled due to thin endometrial lining (vs 0 in the comparison group, not statistically significant). One of these patients had an endometrial lining of 3.9 mm in the presence of 11 dominant follicles (15–25-mm diameter) with a serum oestradiol level of 19.0 nmol/l, there was a history of >10 years of

Table I Baseline characteristics.

	Controls (n = 240)	PGT-M group (n = 122)	Mean difference (95% CI)
Age (years)	30.4 ± 3.9	30.3 ± 3.9	−0.1 (−0.9, 0.8)
BMI	24.0 ± 4.0 (n = 239)	24.2 ± 3.6	0.2 (−0.6, 1.1)
Antral follicle count	12.8 ± 5.8 (n = 228)	12.6 ± 5.6 (n = 117)	−0.2 (−1.5, 1.1)
Nulliparous	**198/240 (82.5%)**	**77/122 (63.1%)**	**−19.4% (−9.6, −29.2)**
Prior curettage (1 or more)	**5/237 (2.1%)**	**11/119 (9.2%)**	**7.1% (1.6, 12.6)**
Prior Caesarean section	13/237 (5.5%)	12/114 (10.5%)	5.0% (−1.3, 11.3)
Smoking (at intake)	40/239 (16.7%)	20/122 (16.4%)	−0.3% (−8.4, 7.8)
Previous ART	**144/240 (60.0%)**	**3/122 (2.5%)**	**−57.5% (−64.3, −50.7)**
Prior contraceptive use,[1] of which	**223/237 (94.1%)**	**121/121 (100%)**	**5.9% (2.9, 8.9)**
COC use only	179/223 (80.3%)	96/121 (79.3%)	−1.0% (−9.9, 7.9)
COC use, ever[2]	213/223 (95.5%)	115/121 (95.0%)	−0.5% (−5.2, 4.2)
DMPA, ever[2]	9/223 (4.0%)	5/121 (4.1%)	0.1% (−4.3, 4.5)
Vaginal ring, ever[2]	12/223 (5.4%)	5/121 (4.1%)	−1.3% (−5.9, 3.3)
IUD			
-IUD copper, ever[2]	2/223 (0.9%)	4/121 (3.3%)	2.4% (−1.0, 5.8)
-IUD levonorgestrel, ever[2]	10/223 (4.5%)	11/121 (9.0%)	4.5% (−1.3, 10.3)
-IUD type unknown, ever[2]	**7/223 (3.1%)**	**0/121 (0%)**	**−3.1% (−5.4, −0.8)**
Duration contraceptive use (years)	**9.3 ± 4.5** (n = 190)	**10.6 ± 4.3** (n = 100)	**1.3 (0.2, 2.3)**
Late cessation of contraceptives before first ovarian stimulation treatment[3]	**4/210 (1.9%)**	**74/116 (63.8%)**	**61.9% (53.0, 70.8)**

COC, combined oral contraceptive; DMPA, depot medroxyprogesteronacetate; IUD, intra-uterine device; vaginal ring, containing etonogestrel/ethinylestradiol.
Data are presented as mean ± SD or n/N (%).
[1]Contraceptive use other than condoms or periodic abstinence.
[2]Has ever used mentioned contraceptive type (e.g. some patients used IUD and later COC, these are included in the COC group and the IUD group). In total, 52 patients used more than one type of hormonal contraceptive. For group comparisons, history including each individual contraceptive type was compared to all others.
[3]Late cessation is defined as: no cessation before treatment or cessation within 1 year preceding treatment.
For certain parameters, data were not available for each patient; the number of patients included for each comparison is given in the corresponding cells in the table.

Table II Cycle characteristics and outcomes.

	Controls (n = 240)	PGT-M group (n = 122)	Mean difference (95% CI)
Total dosage FSH (IU)	2264 ± 894 (n = 213)	2359 ± 744 (n = 122)	94.7 (−93.5, 282.8)
FSH type			Chi-square: $P = 0.4$
−rFSH (Puregon©, follitropine beta)	74/240 *(30.8%)*	36/122 *(29.5%)*	
−HMG (menopur©)	148/240 *(61.7%)*	80/122 *(65.6%)*	
−Urofollitropine (fostimon©)	8/240 *(3.3%)*	5/122 *(4.1%)*	
−Not known/other	10/240 *(4.2%)*	1/122 *(0.8%)*	
#Stimulation days	13.4 (3.8) (n = 240)	13.0 (2.7) (n = 122)	−0.4 (−1.2, 0.4)
EMT	**10.4 (2.1)** (n = 229)	**9.4 (2.5)** (n = 119)	**−1.0 (−1.5, −0.5)**
EMT <8 mm	**25/229 *(10.9%)***	**38/119 *(31.9%)***	**21.0% (11.7, 30.3)**
EMT <7 mm	**6/229*(2.6%)***	**14/119 *(11.8%)***	**9.1% (3.0, 15.4)**
EMT <6 mm	**0/229 *(0.0%)***	**9/119 *(7.6%)***	**7.6% (2.8, 12.4)**
# Follicles >14 mm[1]	7.4 (4.1) (n = 238)	8.0 (4.1) (n = 122)	0.2 (0.1, 0.3)
Oocyte retrieval	210/240 *(87.5%)*	102/122 *(83.6%)*	Chi-square: $P = 0.14$
No retrieval due to			
Hyperresponse	2/240 *(0.8%)*	4/122 *(3.3%)*	
Low response	27/240 *(11.3%)*	13/122 *(10.7%)*	
Thin endometrium	0/240 *(0.0%)*	2/122 *(1.6%)*	
Other	1/240 *(0.4%)*	1/122 *(0.8%)*	
Oocytes at oocyte retrieval	8.7 (5.2) (n = 210)	8.7 (4.8) (n = 97)	0.0 (−1.2, 1.2)
Embryo transfer/ooocyte retrieval	**196/210 *(93.3%)***	**87/102 *(85.3%)***	**Chi-square: $P = 0.02$**
No transfer due to			
No embryo available	**12/210 *(5.7%)***	**15/102 *(14.7%)***	
OHSS risk	**2/210 *(0.9%)***	**0/102 *(0%)***	
SET[2]	177/196 *(90.3%)*	74/87 (85.1%)	−5.2% (−13.8, 3.4)
hCG+	71/196 *(36.2%)*	27/87 *(31.0%)*	−5.2% (−17.0, 6.6)
Miscarriage <12 weeks per transfer	16/196 *(8.1%)*	6/87 *(6.9%)*	−1.2% (−7.8, 5.4)
Ongoing pregnancy per transfer	55/196 *(28.1%)*	21/87 *(24.1%)*	−4.0% (−15.0, 7.0)

EMT, endometrial thickness.
Data are presented as mean or n/N (%).
[1]At the day of ovulation trigger.
[2]SET: single embryo transfer, in other cases two embryos were transferred.

hormonal contraception and cessation <1 year prior treatment. The second patient cancelled for thin endometrial lining had an EMT of 5.0 mm in the presence of four dominant follicles (16–21-mm diameter), and serum oestradiol level was not determined; there was a history of >10 years of hormonal contaceptive use and cessation <1 year before treatment.

The proportion of embryo transfers per oocyte retrieval was lower in the PGT-M group, as was to be expected due to the selection effect of PGT-M. No significant differences were seen in ongoing pregnancy rates per transfer.

We performed a sensitivity analysis on the differences in endometrial thickness excluding all patients with a history of prior curettage (not shown). The initially observed differences in endometrial thickness remained present and statistically significant: EMT 9.4 mm in PGT-M patients vs 10.5 mm in controls (mean difference: −1.1 mm, 95% CI: −0.53, −1.59 mm), EMT <7 mm in 11.3% of PGT-M patients vs 2.3% in controls group (mean difference: 9,1%, 95% CI: 4.0, 14.1%), EMT <8 mm in 33.0% of PGT-M patients and 10.4% in the control group (mean difference: 22.6%, 95% CI: 14.5, 31.2%).

Baseline characteristics patient groups based on different EMT cut-off values

The baseline charachteristics for patient groups defined by different EMT cut-off values (6, 7 and 8 mm) are shown in Supplementary Table SI (PGT-M and controls taken together). For all cut-off points the duration of contraceptive use is significantly longer in the group with thin endometrial lining and late cessation is significantly more prevelant in this group (Supplementary Table SI). Other statistical significant differences found between the groups with different EMT values (<6 mm vs >6 mm, <7 mm vs >7 mm and <8 mm vs >8mm)are: relatively more PGT-M patients in thin endometrial lining groups, for EMT <6 mm; less often history of caesarian section, less often ever DMPA use, for EMT <7 mm and EMT <6 mm: less often previous ART, less often ever use of IUD (unkown type), for EMT <7 mm and

EMT <8 mm: less often use of COC only, for EMT <8 and <6 mm: more often ever levongestrel IUD use.

Risk factors of an endometrial lining <8 mm

To identify factors predicitve of a thin endometrial lining in the first treatment cycle in all patients, we first performed a univariable binary logistic regression analysis. For this analysis, we choose the cut-off point of 8 mm, as the groups of patients with an endometrial lining <7 or <6 mm were very small. We identified the following significant variables of the occurrence of a thin (<8 mm) endometrial lining: PGT-M, prior ART, duration of contraceptive use, late cessation of contraceptive use, the use of COC only and the use of a levonorgestrel IUD (Table III). Subsequent multivariable logistic regression included these variables as well as prior curettage and the number of dominant follicles, based on previous publications. In the mulit-variable analysis, only duration of prior contraception and late cessation remained significant predictors of a endometrial lining <8 mm (Table III). In addtion, we performed the same analysis including only the patients who had previously used solely COC and no other type of hormonal contraception (Supplementary Table SII). In this analysis, the multivariable regression analyses also identified duration of prior contraception and late cessation as significant predictors of a thin endometrial lining. Furthermore, in this analysis, prior curettage also was a significant positive predictor.

Comparison first, second and third cycle EMT after late cessation vs early cessation of hormonal contraceptives

To explore a possible 'washout' over time of the effect of contraceptive use, we analysed differences (paired *t*-test) between the EMT of the first, second and third treatment cycle, when available (Table IV). PGT-M and controls were combined for this analysis. In the group with late cessation, there was a significant increase in EMT from the first to the second treatment cycle (8.4–9.5 mm, $P < 0.001$). A smaller increase in EMT was seen from the second to third treatment cycle (9.5–9.7 mm) and this difference was not statistically significant. In the group with cessation of contraceptives >1 year before the first treatment, no significant differences were seen between first, second and third ovarian stimulation cycle. The time between cycles was on average ~6 months, and there were no significant differences in length of this period between groups (not shown). Information on contraceptive use in the intervening months was not available.

Thin endometrial lining and pregnancy outcome

To explore the clinical relevance of a thin endometrium in our cohort, and in the PGT-M group specifically, we analysed the pregnancies, and pregancy outcomes in relation to the EMT (Table V, baseline characteristics of groups shown in Supplementary Table SIII). In the PGT-M group, a significant higher prevelance of pregnancy loss was seen in patients with an EMT <8 mm (20.0% per transfer vs 1.7% per transfer). There were no statistical significant differences in other outcomes. A trend towards decreased birth weight and gender- and gestation-adjusted birth weight (z-score) was found in PGT-M patients with an endometrial lining <8 mm (−0.30 vs 0.50).

Table III Logistic regression analysis of baseline and cycle parameters as predictors of the occurrence of a thin (<8 mm) endometrial lining.

Determinants	Univariable analysis OR (95% CI)	Multivariable analysis OR (95% CI)[1]
PGT-M	**3.8 (2.2–6.7)**	0.78 (0.2–2.7)
Baseline		
–Age	1.0 (0.9–1.1)	
–BMI	1.0 (0.9–1.1)	
–AFC	1.0 (0.9–1.1)	
–Para ≥ 1	0.8 (0.4–1.6)	
–Prior curettage	1.7 (0.5–5.5)	2.4 (0.5–11.8)
–Prior Caesarean section	1.3 (0.5, 3.6)	
–Prior ART	**0.39 (0.2–0.7)**	0.7 (0.3–1.7)
Prior contraceptive use		
–Duration (OR per year)	**1.1 (1.1–1.2)**	**1.1 (1.0–1.2)**
–Late cessation[2]	**7.0 (3.8–12.8)**	**6.0 (1.9–19.2)**
–Type		
COC only	**0.4 (0.2–0.7)**	0.8 (0.3–2.5)
COC ever[3]	0.6 (0.1, 3.1)	
IUD copper[3]	2.2 (0.4–12.2)	
IUD levonorgestrel[3]	**6.2 (2.4–15.8)**	3.5 (0.7–17.2)
DMPA[3]	1.2 (0.3–4.3)	
Vaginal ring[3]	1.4 (0.5–4.6)	
Cycle parameters		
–Pre-treatment COC	0.5 (0.1–1.7)	
–FSH total dose/1000	1.2 (0.9–1.7)	
–FSH type		
rFSH (puregon)	1.6 (0.9–2.9)	
HMG (menopur)	0.7 (0.4–1.3)	
Urofollitropine (fostimon)	0.8 (0.2–3.8)	
–# stimulation days	1.0 (0.9–1.1)	
–# dominant follicles	1.0 (0.9–1.1)	0.9 (0.9–1.0)
–# oocytes	1.0 (0.9–1.1)	

COC, combined oral contraceptive; DMPA, depot medroxyprogesteronacetate; IUD, intra-uterine device; vaginal ring, containing etonogestrel/ethinylestradiol.
[1]On the multivariate analyses, the following variables were included: PGT-M, prior ART, duration contraception, late cessation, OC only, use of levonorgestrel IUD (based on univariable analysis) and # dominant follicles and prior curettage (based on previous publications).
[2]Cessation <1 year prior to treatment.
[3]Alone or in combination with other contraceptives, IUD copper was never reported as only contraceptive used.

Discussion

Main findings

This study reveals two main findings. The first is an increased prevalence of thin endometrial lining in PGT-M patients in first treatment cycles compared to regular IVF/ICSI controls. The second is a significant association between a thin endometrial lining and the history of hormonal contraceptive use. Both a longer duration of previous

Table IV Comparison between first, second and third cycle endometrial thickness (EMT).

	First cycle EMT vs second cycle EMT, mean (SD)	Second cycle EMT vs third cycle EMT, mean (SD)
Late cessation	**8.4 mm (2.2) vs 9.5 mm (2.1)[1]**	9.5 mm (2.3) vs 9.7 mm (2.2)
(<1 year)	n = 50	n = 30
Early cessation	10.4 mm (2.2) vs 10.5 mm (2.3)	10.6 mm (2.2) vs 11.1 mm (2.6)
(>1 year)	n = 150	n = 64

[1]$P < 0.001$.

Table V Clinical outcomes control and PGT-M patients, comparing patients based on endometrial thickness <8 or >8 mm.

Control patients	EMT <8 mm	EMT >8 mm	Mean difference (95% CI)
hCG+/ET	5/17 *(29.4%)*	63/172 *(36.7%)*	7.3% (−15.5, 30.1)
SET[1]	15/17 *(88.2%)*	156/172 *(90.7%)*	2.5% (−13.4, 18.4)
Pregnancy loss <12 weeks/ET	2/17 *(11.8%)*	15/172 *(8.7%)*	−3.1% (−19.0, 12.8)
Live birth/ET	3/17 *(17.6%)*	48/172 *(27.9%)*	10.3% (−9.0, 29.6)
Delivery <37 AD	0/3 *(0%)*	1/40 *(2.5%)*[4]	2.5% (−2.3, 7.3)
Mean birth weight, g (SD)[2]	3369 (617)	3364 (417)	−5.3 g (−525, 515)
z-score average (SD)[2]	−0.35 (1.2)	−0.03 (0.9)	0.32 (−0.8, 1.4)
SGA (≤0.10 percentile)[2]	0/3 *(0%)*	2/40 *(5%)*[4]	5% (−1.8, 11.8)
PGT-M patients	**EMT <8 mm**	**EMT >8 mm**	**Mean difference (95% CI)**
hCG+/ET	11/25 *(44.0%)*	16/60 *(26.7%)*	−17.3% (−39.7, 5.1)
SET[1]	20/25 *(80.0%)*	52/60 *(86.7%)*	6.7% (−11.2, 24.6)
Pregnancy loss <12 weeks/ET	**5/25 *(20.0%)***	**1/60 *(1.7%)***	**−18.3% (−34.3, −2.3)**
Live birth/ET	6/25 *(24.0%)*	15/60 *(25.0%)*	1% (−19.0, 21.0)
Delivery <37AD	1/6 *(16.7%)*	1/15 *(6.7%)*	−10.0% (−42.2, 22.4)
Mean birth weight (g)[3]	3171	3658	487g (−20, 993)[5]
z-score average (SD)[3]	−0.30 (1.05)	0.50 (0.9)	0.81 (−0.18, 1.79)[6]
SGA (≤0.10 percentile)[3]	1/6 *(16.7%)*	1/14 *(7.1%)*	−9.6% (−42.3, 23.1)

AD, gestational weeks; ET, embryo transfer; hCG positive (+), pregnancy test; SGA, small for gestational age.
[1]SET: single embryo transfer, in other cases two embryos were transferred.
[2]Singleton deliveries only (no twin pregnancies present in this group).
[3]Singleton deliveries only (one twin pregnancy was present in the PGT-M EMT >8 mm group).
[4]Birth weight was not available for 8 patients in the control group.
[5]$P = 0.06$.
[6]$P = 0.10$.

hormonal contraceptive use, as well as late cessation of these contraceptives before first treatment cycle were positive predictors of a thin endometrial lining. Moreover, the increased prevalence of thin endometrial lining in PGT-M patients can be explained by the typical contraceptive history in this group; on average the PGT-M patients have a longer duration of prior contraception use and shorter cessation period.

Although numbers in our study are small, when pregnancy outcomes were investigated, a trend is seen in the PGT-M group for lower (gestational age- and gender-adjusted) birth weight and increased miscarriage rate when the endometrial lining is thin (<8 mm), an association previously reported for 'regular' IVF/ICSI patients with thin endometrial lining. No differences were seen in live birth rate between PGT-M patients with or without an endometrial lining <8 mm.

The relationship between a long history of hormonal contraceptive use and a thin endometrial lining was previously reported by Talukdar *et al.* They performed a retrospective study in which patients preparing for FET with oestradiol treatment more often had an endometrial lining below 7 mm on cycle Day 10 when there was a long history (>10 years) of COC use (Talukdar *et al.*, 2012). Notably, all patients had stopped COC use ≥2 years before commencing treatment. This study supports our finding of a persistence of the thinning effects of hormonal contraceptives on the endometrium after cessation. In addition, in some studies focusing on COC pre-treatment for IVF/ICSI, a

thinner EMT has been found in COC pre-treated patients compared to no pre-treatment, although this refers to relative short periods of hormonal contraceptive use (Wei et al., 2017; Xu et al., 2019).

The reversibility of the effects of hormonal contraceptives has previously been studied, however, most studies have not focused on endometrial thickness. In general, a quick return to normal has been described for histologic aspects of the endometrium, but long-term effects have also been described, e.g. for gene-expression profiles persisting for over 1 year after cessation (Anderson et al., 2005; Dinehart et al., 2020). The biological basis for a lasting effect of hormonal contraceptives on the endometrial lining after cessation is not clear. At the time of hormonal contraceptive use, both oestrogen/progestogen combined and progestogen-only contraceptives (such as the levonorgestrel IUD) are known to cause endometrial thinning. This endometrial thinning is thought to occur through the effect of progestogens. In our study, we found a trend towards an increased incidence of thin endometrial lining after the use of levonorgestrel IUDs (containing a progestogen only) compared to other types of contraceptives. These data combined suggest that the increased incidence of thin endometrium after cessation is presumably also caused by effects of the progestogen component. Progestogens can down-regulate the oestrogen receptor after several cycles of use (Dinh et al., 2015). A reduced availability of this receptor has also been found to be associated with a thin endometrial lining in IVF cycles (Gao et al., 2019). We can speculate that a delay in oestrogen receptor upregulation after hormonal contraceptives, could be a cause of decreased thickening of the endometrial lining IVF/ICSI cycles, where oestradiol levels are relatively high and not expected to be the limiting factor. Another proposed hypothesis is suppression of endometrial stem cell proliferation due to a prolonged period of inactivity under hormonal contraceptive use (Talukdar et al., 2012). More research is necessary to elucidate underlying causal biological mechanisms.

Previous studies have also identified other factors related to endometrial thickness around ovulation triggering, such as parity, the number of dominant follicles and age (Amir et al., 2007; Ribeiro et al., 2018), which we could not corroborate in our study. The fact that we could not confirm this could be related to our relative small sample size.

In relation to clinical outcomes, a thin endometrial lining has been associated with lower chances of pregnancy in IVF/ICSI cycles (Gallos et al., 2018; Liu et al., 2018; Craciunas et al., 2019; Lv et al., 2020; Simeonov et al., 2020; Huang et al., 2021; Wen et al., 2021), though the predictive power of EMT in general on pregnancy rates remains a subject of debate (Kasius et al., 2014; Craciunas et al., 2019). Thin endometrial lining is also related to an increased risk of miscarriage, low birth weight, children being SGA at birth and obstetric complications in the general IVF/ICSI population (Yuan et al., 2016; Oron et al., 2018; Mouhayar et al., 2019; Zhang et al., 2019; Guo et al., 2020; Du et al., 2021; Hu et al., 2021; Liu et al., 2021; Martel et al., 2021). For the PGT-M subgroup specifically, this relationship has not yet been demonstrated. Although our numbers are small, we observed a statistically significant increase in miscarriage rate in PGT-M patients with an EMT of <8 mm compared to an EMT of >8 mm and a trend towards a lower birth weight and lower gender- and gestation-corrected birth weight (z-score). This could point to a similar relationship between outcome and thin endometrial lining in PGT-M patients, as is seen in the 'regular' IVF/ICSI population. However, our numbers are small and it would be inappropriate to draw firm conclusions from these data. A thin endometrial lining associated with prior contraceptive use, could be biologically (and prognostically) different from thin endometrial lining, which is related to other factors.

Residual endometrial thinning after hormonal contraceptive use might also have implications for the general population. However, most studies on return to fertility after contraceptive use are reassuring, although a slight delay of several months in return to fertility after cessation of COCs has also been described (Barnhart and Schreiber 2009, pp. 659–663; Dinehart et al., 2020, pp. 45–52).

Strengths

One of the strengths of this study is the inclusion of two controls for each PGT-M case. Controls were matched for two possible important confounders: age and treatment period, thereby avoiding any effects from changes in, e.g. laboratory procedures or medication used over time. Also, only one ovarian stimulation protocol was included in the study, as all patients underwent long downregulation with a GnRH agonist. Most studies investigating endometrial thickness have not incorporated the history of hormonal contraceptive use; one of the strengths of this study is the inclusion of these data, which enabled us not only to investigate the prevalence of thin endometrial lining in PGT-M patients but also a possible cause of the relative high prevalence.

Weaknesses

The study is retrospective. Different types of hormonal contraceptives were reported which possibly exert different effects on the endometrial lining. For COC users, we could perform separate analysis, confirming for COC the demonstrated associations between duration of use, cessation period and the occurrence of a thin endometrial lining. However, patient numbers for other types of hormonal contraceptives were too small to make separate analyses. For these types of contraceptives, further proof by other studies is necessary. History of contraceptive used was obtained from questionnaires and thus self-reported. Data on contraceptive use in the months between intake and IVF/ICSI start were noted in a free text field; these data indicated that, for the late cessation (<1 year) group, hormonal contraceptives were used up until treatment. This means we could not investigate in more detail differences between, e.g. patients with a cessation period of 0–3, 3–6, 6–9 and 9–12 months, which might be clinically relevant. Experimental studies are needed to show the effect of different terms of cessation (1–12 months) of hormonal contraceptive on endometrial thickness in subsequent IVF/ICSI cycles.

The study size was not large enough to allow strong conclusions on IVF/ICSI success rate pregnancy outcomes; further studies are needed to assess the clinical relevance the increased prevalence of an EMT <8 mm in PGT-M patients.

Also of note, in this study, the thickness of the endometrial lining in stimulated cycles and subsequent fresh embryo transfer was investigated; however, there is a worldwide trend towards trophectoderm biopsy in PGT cycles and a concomitant switch to FET only; our study does not include information of endometrial thickness of FET cycles.

Further implications

Our research contributes to the understanding of the presence of thin endometrial lining around ovulation triggering, by demonstrating that long-term hormonal contraceptive use and a short cessation interval prior to treatment, can play a role here. Especially in PGT-M patients, the history of contraceptive use might prove relevant in explaining the occurrence of a thin endometrial lining, as these patients on average have both a longer duration of use and a shorter period of cessation. Further research is necessary to determine the clinical relevance with respect to pregnancy chances and birthweight of offspring of the contraception-associated thin endometrium. We can then decide whether PGT-M patients with a thin endometrial lining can be reassured, or whether we should strive to increase endometrial thickness, possibly by advising them to stop hormonal contraceptives at an earlier stage and, e.g. switch to a copper IUD. Another question for future research would then be the optimal duration of cessation.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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Authors' roles

A.F.t.M. significantly contributed to the design and execution of the study, participated in the data analysis, critically revised the article and approved the final version. A.E.P.C. and A.H. contributed to the design of the study, critically revised the article and approved the final version. H.G. participated in the data analysis and study design, critically revised the article and approved the final version. I.H. conceived the study design, significantly contributed to the execution of the study, performed the data analysis and drafted the article.

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Conflict of interest

None declared.

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