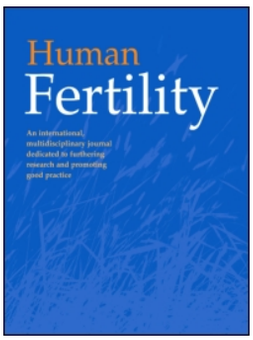

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ORIGINAL ARTICLE

Mid-luteal uterine artery Doppler indices in the prediction of pregnancy outcome in nulliparous women undergoing assisted reproduction

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ABSTRACT

Traditionally, the assessment of endometrial receptivity at transvaginal ultrasound scan has been based on the thickness and the morphological appearance of the endometrium. The objective of this study was to prospectively evaluate endometrial thickness (ET), endometrial morphology and uterine artery Doppler parameters prior to assisted reproduction treatment (ART) in the prediction of pregnancy outcome. This was a prospective cohort study. ET, morphology and uterine artery Doppler (UtAD) pulsatility index (PI) and resistance index (RI) were measured in the mid-luteal stage of the menstrual cycle ultrasonographically, timed with urinary luteinizing hormone testing. A total of 50 women were included in the analysis. The clinical pregnancy rate (CPR) per embryo transfer was 42.0% ($n = 21/50$). Twenty nine women (58.0%) had an unsuccessful outcome. There were no differences in mean ± SD endometrial thickness (ET) (10.0 ± 1.8 mm vs. 10.5 ± 2.4; $p = 0.43$), or endometrial morphology (100% ($n = 21$) vs 100% ($n = 29$); $p = 1.00$) between the pregnant and not pregnant groups. Similarly, there were no differences in mean ± SD UtAD PI (2.17 ± 0.83 vs. 2.07 ± 0.81; $p = 0.67$ or mean ± SD UtAD RI (0.84 ± 0.10 vs. 0.81 ± 0.10; $p = 0.30$). Ultrasonographic endometrial assessment did not differentiate between those who would have a subsequent clinical pregnancy.

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KEYWORDS
Ultrasound; infertility; uterine artery Doppler; endometrium; pregnancy

Introduction

Embryo implantation represents a critical step of the reproductive process (Aplin, 2000; Denker, 1993). Successful implantation requires a receptive endometrium, a normal and functional embryo and a synchronised dialogue between maternal and embryonic tissues (Simón et al., 2000).

Several non-invasive ultrasonographic markers of endometrial receptivity have been described in the prediction of successful implantation. Traditionally, the assessment of endometrial receptivity at transvaginal ultrasound scan was based on the endometrial thickness(ET) and the morphological appearance of the endometrium. More recently, estimation of uterine artery Doppler (UtAD) blood flow velocities, pulsatility index (PI) and resistance index (RI), have been added to the diagnostic workup (Hrehorcak & Nargund, 2011). With the increasing use of three-dimensional (3 D) ultrasound, the blood supply to the endometrium and subendometrial area can also be examined (Ng et al., 2006).

The relationship between UtAD PI and RI and pregnancy outcome has been the subject of many studies (Aytoz et al., 1997; Cacciatore et al., 1996; Coulam et al., 1994, 1995; El-Mazny et al., 2013; Friedler et al., 1996; Hoozemans et al., 2008; Khan et al., 2016; Kim et al., 2010; Razik et al., 2015; Schild et al., 2001; Steer et al., 1992; Tekay et al., 1996), but the data remains conflicting. Menstrual cycle stage differed between many of these studies and therefore, did not allow for an accurate comparison, as UtAD PI indices are menstrual cycle stage dependent. Maximum UtAD PI levels are found on day 1 of the cycle and decrease to a minimum prior to implantation. This is due to an increase in circulating progesterone levels towards the mid-luteal phase, leading to minimal vascular resistance and maximal uterine perfusion. UtAD PI then

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increases further until the end of the menstrual cycle (Guedes-Martins et al., 2015; Hoozemans et al., 2008). UtAD PI may be classified as low, medium or high in the ranges 0.00–1.99, 2.00–2.99 and ≥3.0 respectively (Steer et al., 1992). UtAD PI values are lower in parous women than nulliparous women (Guedes-Martins et al., 2015). The value of mid-luteal uterine artery Doppler studies in the menstrual cycle prior to assisted reproductive treatment (ART) for the prediction of pregnancy outcome has been the subject of many studies, but the data remain conflicting. The use of specific UtAD PI or RI cut-offs in assessing endometrial receptivity has been of limited value in clinical practice to date (Chien et al., 2002).

The objective of this study was therefore to evaluate endometrial thickness, morphology and uterine artery doppler indices prior to a cycle of ART, to determine their utility in differentiating between patients who will and will not become pregnant.

Materials and methods

Patient recruitment

This was a prospective cohort study approved by the Medical Research and Ethics committee of the National Maternity Hospital, Holles Street, Dublin (EC 27.2016) in October 2016. Women undergoing ART (IVF, ICSI or FET) were recruited at Merrion Fertility Clinic, Dublin between October 2016 and February 2018 inclusive. Women planning an ART cycle were offered transvaginal ultrasonographic assessment of the endometrium by a single trained researcher. Written consent was then obtained on the day of the transvaginal ultrasound scan by the study clinician. Inclusion criteria comprised the following: age <38 years, no previous pregnancy (including previous biochemical pregnancy, miscarriage or ectopic pregnancy), regular menstrual cycles (25–35 days), no steroid hormone use within the preceding three months, and a normal transvaginal ultrasound scan. Transvaginal ultrasonography was performed at a defined stage of the menstrual cycle: mid-luteal, LH + 7. Cycle stage was timed accurately with urinary luteinizing hormone (LH) testing (One Step®, Home Health UK, Herts, UK), assessed by the patient twice daily from day nine of the menstrual cycle. Matched peripheral blood was collected for serum progesterone hormone assessment. Menstrual cycle stage was confirmed by endometrial scratch biopsy and histological and morphological criteria as assessed by the department of histopathology, National Maternity Hospital, Dublin. Patients were followed prospectively and underwent a cycle of ART in the menstrual cycle following ultrasound.

Ultrasound assessment

Ultrasound assessments were performed by a single operator with experience in Doppler ultrasound to avoid inter-observer variability. A Voluson P8 ultrasound machine (GE Healthcare Technologies) with a 7.5 MHz transvaginal probe was used. Endometrial thickness (ET), endometrial morphology and uterine artery Doppler (UtAD) PI and RI were measured. Two-dimensional transvaginal ultrasonography was performed with the patient in the lithotomy position in the morning (7.30 am–8.30 am) to avoid circadian changes in blood flow (Hoozemans et al., 2008). The endometrial evaluation protocol remained unchanged throughout the study.

Endometrial thickness

Transvaginal ultrasonographic measurement of ET was performed in the mid-sagittal plane and noted in millimetres (mm).

Endometrial morphology

Endometrial ultrasonographic morphology was recorded based on a description by Grunfeld et al. (1991). This was recorded as type 1 (late proliferative: hyperechoic endometrium constituting less than 50% of the ET with a hyperechoic basalis and a hypoechoic functionalis); type 2 (early secretory: hyperechoic basalis and functionalis extending to more than 50% of the ET, but not encompassing the entire endometrial cavity); or type 3 (mid-late secretory: homogenous hyperechoic functionalis extending from the basalis to the lumen). The endometrial evaluation protocol remained unchanged throughout the study.

Uterine artery Dopplers: pulsatility index (PI) and resistance index (RI)

A sagittal image of the uterus, cervical canal and internal cervical os was obtained using colour flow mapping directed towards the lateral fornix to identify the ascending branch of the uterine artery in the para-cervical area. Each uterine artery was identified at the level of the internal cervical os as previously described by Razik et al. (2015) and Friedler et al. (1996). Pulsed wave Doppler was used with a sampling gate set at 2 mm to image the entire vessel. The

angle of insonation was set at less than 60° as previously described by Edi-Osagie et al. (2004). Three consecutive similar waveforms were obtained. Left and right uterine artery PI and RI were measured automatically, and the mean calculated and used for analysis. There was no difference in mean UtAD PI or RI between the right and left uterine arteries. UtAD PI was further classified as low, medium and high in the ranges 0.00–1.99, 2.00–2.99 and ≥3.0 respectively as previously described by Steer et al. (1992).

ART cycles

In fresh cycles, patients underwent ovarian stimulation using protocols with a combination of GnRH agonist/GnRH antagonist and recombinant FSH (Gonal F, Merck Serono (Ireland) Limited/Puregon, MSD Ireland (Human Health) Limited) or HMG (Menopur, Ferring Pharmaceuticals Ireland Limited). Embryo transfer was performed on day three or day five, depending on embryo quality. Blastocysts were graded as top, good or fair quality according to a modified Garner scale (Alpha Scientists in Reproductive Medicine & ESHRE Special Interest Group of Embryology, 2011). No more than two embryos were transferred on day three or day five. Surplus suitable blastocysts were cryopreserved. After embryo transfer, patients received luteal-phase support with 90 mg of vaginal progesterone daily (Crinone 8% (w/v), Merck Serono (Ireland) Limited). Serum β-hCG level was measured 16 days following oocyte retrieval and an ultrasound scan was performed two weeks later if the β-hCG confirmed pregnancy.

In frozen embryo transfer (FET) cycles, oral oestradiol hemihydrate 2 mg three times per day (Fematab; BGP Products Ireland Limited) or transdermal E2 (Estradot 50 mcg) was commenced on day one of the cycle for endometrial preparation. An ultrasound scan was performed after approximately 12 days and embryo transfer was scheduled when the endometrium was defined as optimal (≥ 7 mm with type 1, 2 or 3 endometrial pattern (Grunfeld et al., 1991)). Where the endometrium was deemed sub-optimal, the oestrogen dose was increased, and the patient was re-scanned three to four days later. If the endometrial thickness was sub-optimal at this stage, the cycle was cancelled. Progesterone supplementation was introduced five days prior to the day of planned embryo transfer. A natural cycle was used in some women who had ovulatory cycles and if medicated approaches had proven unsuccessful, poorly tolerated or were not optimal. In these natural cycles, an ultrasound scan was performed on day 10 of the menstrual cycle and LH urinary kit testing was commenced once daily when the lead follicle measured or was anticipated to be ≥14 mm. A natural cycle was modified by the addition of a hCG trigger of 5000 IU to time ovulation once a follicle of 17 mm was observed or anticipated in the presence of an adequate endometrium. Progesterone supplementation with 90 mg vaginal progesterone (Crinone 8% w/v, Merck Serono (Ireland) Limited) was introduced 5 days prior to the day of planned embryo transfer.

ART cycle outcomes

Successful pregnancy (pregnant) was defined as ultrasound evidence of clinical pregnancy (Zegers-Hochschild et al., 2017) and the clinical pregnancy rate was reported per embryo transferred. The ET, morphology and UtAD indices (PI and RI) were compared for the pregnant and not pregnant groups. Outcomes were analysed separately for all ART cycles, for women who underwent fresh cycles only and for women who had no previous ART cycle. Mean UtAD PI and RI were calculated and were further classified as low, medium and high in the ranges 0.00–1.99, 2.00–2.99 and ≥3.0 respectively as previously described by Steer et al. (1992). Endometrial thickness and UtAD indices were compared for women with different fertility aetiologies. Fertility aetiologies were as defined by Zegers-Hochschild et al: '*Unexplained infertility*', '*Male factor*' or '*Endometriosis*' (Zegers-Hochschild et al., 2017).

Data analysis

Statistical analysis was performed using SPSS version 23.0 software. All results are presented as mean ± SD values or as median and range, according to the distribution. Normal distribution was identified using the Kolmogorov-Smirnov test. For normally distributed variables, comparisons between two groups were carried out with Student's *t*-test, ANOVA and Mann-Whitney U test. Significance level for all analyses was set at $p < 0.05$.

Results

Patient and cycle characteristics and ART outcomes

A total of 98 women were eligible to participate in the study. Of this cohort, 55 (56.1%) underwent a transvaginal ultrasound in the cycle preceding the planned ART cycle. Of these, five (9.1%) women did

not undergo an embryo transfer in the subsequent cycle and were excluded. There was a 42.0% clinical pregnancy rate per embryo transfer ('pregnant', $n = 21/50$) and 58.0% ($n = 29/50$) had an unsuccessful outcome ('not pregnant'). The patient characteristics of the two groups are outlined in Table 1. All women were nulliparous. There were no significant differences in mean age, body mass index (BMI), duration of infertility, cycle length or serum Anti-Müllerian Hormone (AMH) between the two groups. Furthermore, there were no differences in fertility aetiology or number of previous ART cycles prior to study cycle. ART cycle characteristics are outlined in Table 2. There were no differences between the two groups.

There were no differences in mean ET or type 3 morphology between those with successful compared with unsuccessful pregnancy (10.0 mm (SD 1.8) vs. 10.5 mm (SD 2.4); $p = 0.43$, 100% ($n = 21$) vs. 100% ($n = 29$)). There were no differences in the quality of embryos transferred (Good quality embryo: 95.2 vs 89.7%; $p = 0.63$), on the day of embryo transfer (Day 3 transfer: 9.5 vs. 6.9%; $p = 1.00$) or the number of embryos transferred (Double embryo transfer: 14.3 vs. 10.3%; $p = 0.69$) between the successful and unsuccessful pregnancy groups (Table 2).

There were no differences in mean ± SD UtAD PI rates between the pregnant and not pregnant groups (2.17 ± 0.83 vs. 2.07 ± 0.81; $p = 0.67$). When analysed using PI 2.0 or 3.0 as cutoffs, there were no differences in the number of women with PI ≥ 2.0 (52.4 vs. 37.9%;p = 0.39 or ≥ 3.0 (14.3 vs. 3.4%; p = 0.30) respectively in the pregnant and not pregnant groups Furthermore, there were no differences in mean ± SD UtAD RI between the pregnant and not pregnant groups (0.84 ± 0.10 vs. 0.81 ± 0.10; $p = 0.30$) (Table 3). These findings were similar, when analysed by fresh cycles only (Table 4), women with no prior ART cycle

Table 1. Patient demographics.

	Pregnant ($n = 21$)	Not pregnant ($n = 29$)	p Value
Age (years)	34.4 ± 2.1	34.7 ± 2.2	0.63
BMI (kg/m^2)	23.4 ± 3.0	23.3 ± 2.5	0.90
Duration of infertility (months)	28.0 ± 12.7	33.9 ± 19.9	0.24
AMH (pmol/L)	21.8 ± 14.9	16.3 ± 12.8	0.17
Cycle length (days)	29.0 ± 3.0	29.0 ± 1.9	1.00
Fertility aetiology			
Male factor infertility	6 (28.6%)	8 (24.2%)	0.75
Unexplained infertility	12 (57.1%)	17 (58.6%)	1.00
Endometriosis	3 (14.3%)	4 (13.8%)	1.00
Tubal	0 (0%)	1 (3.4%)	1.00
Prior unsuccessful cycles**			
None	15 (71.4%)	15 (51.7%)	0.24
One	4 (19.0%)	9 (31.0%)	0.52
Two	2 (9.6%)	5 (17.3%)	0.68

All values reported are means ± standard deviation. All other entries are absolute values and percentages in parentheses; **Unsuccessful cycle is defined as a previous negative serum βhCG.

Table 3. Doppler PI and RI (All cycles; $n = 50$).

	Pregnant (n = 21)	Not pregnant ($n = 29$)	p Value
Mean PI	2.17 ± 0.83	2.07 ± 0.81	0.67
PI ≥ 2.0	11 (52.4%)	11 (37.9%)	0.39
PI ≥ 3.0	3 (14.3%)	1 (3.4%)	0.30
Mean RI	0.84 ± 0.10	0.81 ± 0.10	0.30
Mean ET at scratch (mm)	10.0 ± 1.8	10.5 ± 2.4	0.43
Type 3 morphology	21 (100%)	29 (100%)	1.00

Table 4. Doppler PI and RI (All fresh cycles; $n = 33$).

	Pregnant ($n = 15$)	Not pregnant ($n = 18$)	p Value
Mean PI	2.16 ± 0.85	1.92 ± 0.46	0.31
PI ≥ 2.0	6 (40.0%)	7 (38.9%)	1.00
PI ≥ 3.0	2 (13.3%)	0 (0%)	0.20
Mean RI	0.95 ± 0.39	0.83 ± 0.07	0.21
Mean ET at scratch (mm)	9.8 ± 1.4	10.3 ± 2.8	0.53

Table 5. Doppler PI and RI (all first ART cycles; $n = 30$).

	Pregnant ($n = 15$)	Not pregnant ($n = 15$)	p Value
Mean PI	2.16 ± 0.85	1.92 ± 0.46	0.34
PI ≥ 2.0	6 (40.0%)	7 (46.7%)	1.00
PI ≥ 3.0	2 (13.3%)	0 (0%)	0.48
Mean RI	0.95 ± 0.39	0.83 ± 0.07	0.25
Mean ET at scratch (mm)	9.9 ± 1.4	10.1 ± 2.8	0.81

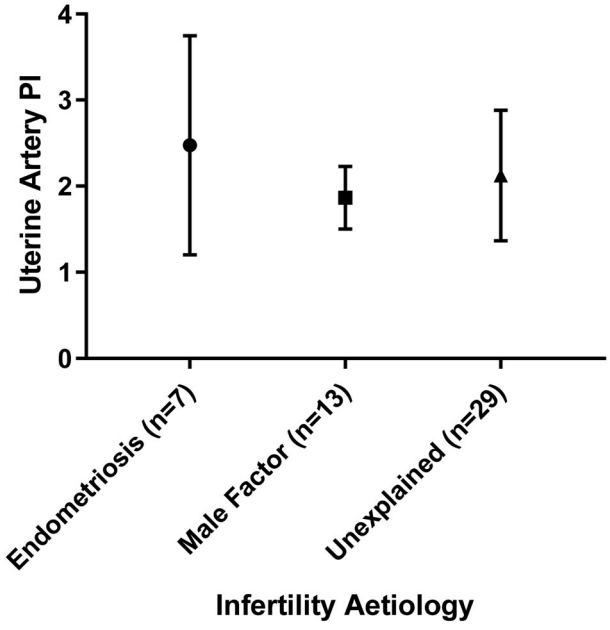
Table 2. ART characteristics.

	Pregnant ($n = 21$)	Not pregnant ($n = 29$)	p Value
Fresh cycle	15 (71.4%)	18 (62.1%)	0.56
Antagonist	4/15 (26.7%)	5/18 (27.8%)	1.00
Recombinant FSH	14/15 (93.3%)	16/18 (88.9%)	1.00
Days of stimulation (Gonadotropin)	10.8 ± 1.6	11.3 ± 1.9	0.33
Total gonadotropin dose (IU)	2482 ± 1110	2886 ± 1312	0.26
ICSI	8/15 (53.3%)	11/18 (61.1%)	0.73
Hormone Replacement Therapy FET	6/6 (100.0%)	9/11 (81.8%)	0.51
Transfer of ≥ one good quality embryo	20 (95.2%)	26 (89.7%)	0.63
Transfer of ≥ one top quality embryo	9 (42.9%)	8 (27.6%)	0.37
Double embryo transfer	3 (14.3%)	3 (10.3%)	0.69
Day 3 transfer	2 (9.5%)	2 (6.9%)	1.00
Endometrial thickness at OCR/FET schedule	9.7 ± 2.1	9.9 ± 1.8	0.72

All values reported are means ± standard deviation. All other entries are absolute values and percentages in parentheses.

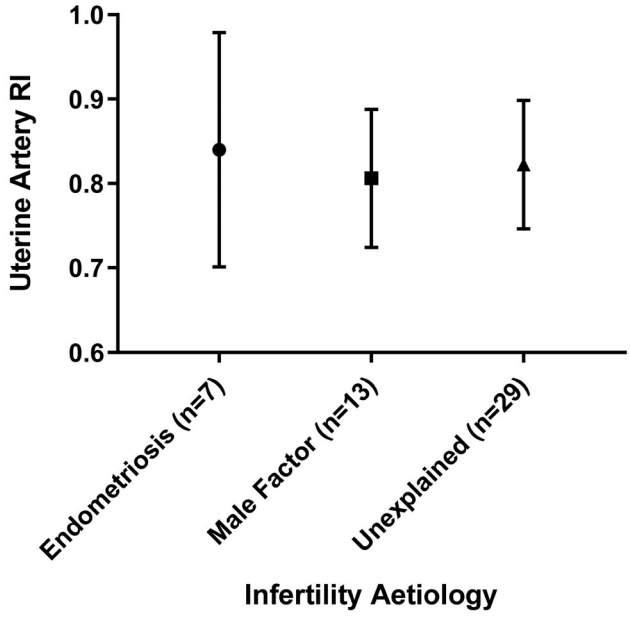
Table 6. Doppler PI and RI (unexplained infertility; $n = 29$).

	Pregnant ($n = 12$)	Not pregnant ($n = 17$)	p Value
Mean PI	2.33 ± 1.07	1.99 ± 0.46	0.26
PI ≥ 2.0	5 (41.7%)	5 (29.4%)	0.71
PI ≥ 3.0	2 (16.7%)	0 (0%)	0.19
Mean RI	0.85 ± 0.09	0.81 ± 0.07	0.19
Mean ET at scratch (mm)	10.1 ± 2.3	9.6 ± 2.2	0.57



*PI=Pulsatility Index. Values shown are mean ± standard deviation

Figure 1. Uterine artery Doppler pulsatility index by infertility aetiology.



*RI=Resistance Index. Values shown are mean ± standard deviation.

Figure 2. Mean uterine artery Doppler resistance index by infertility aetiology.

(Table 5) and those with unexplained infertility (Table 6).

When analysed by fertility aetiology (endometriosis, male factor, unexplained infertility), there were no differences in mean PI (Figure 1) or RI (Figure 2) between the groups (Table 7: PI 2.48, 1.87, 2.12; $p = 0.33$ RI 0.84, 0.81, 0.82; $p = 0.76$).

Discussion

This prospective longitudinal study of women with primary infertility found no differences in mid-luteal phase ET, endometrial morphology or UtAD PI or RI resistance index (RI) in women who had successful clinical pregnancy after a subsequent cycle of ART compared to those who did not.

To our knowledge, this is the first study performed to date assessing the association between uterine artery Dopplers and subsequent ART success in the subsequent cycle, and using urinary LH testing to accurately determine menstrual cycle stage. Cycle stage was confirmed with ovulatory serum progesterone hormone assessment and by histological dating. Compared to other conflicting studies in the literature, strict inclusion criteria were used, limiting study participation to nulliparous women with primary infertility aged less than 38 years. It is important to adopt strict inclusion criteria when studying UtAD PI indices as these are influenced by variation in several clinical parameters.

There remains debate in the literature regarding the predictive value of mid-luteal uterine artery Doppler studies in the menstrual cycle prior to ART for pregnancy outcome. For example, Hoozemans et al. (2008) assessed serial UtAD velocity parameters during IVF and ICSI cycles in the mid-luteal phase to assess for implantation failure. Eighty-three patients subsequently underwent embryo transfer of at least one embryo. They found no differences in mid-luteal UtAD PI during the ART cycle between those who had an ongoing pregnancy (1.75 ± 0.41) compared with those who did not (1.72 ± 0.43); $p = 0.94$, and concluded that there does not appear to be a place for UtAD PI assessment as a routine non-invasive marker of implantation (Hoozemans et al., 2008).

Almost all other studies published have been performed at different cycle stages during ART with conflicting results. These include UtAD assessment prior to ovulation, on the day of administration of HCG, on the day of oocyte retrieval, or on the day of embryo

Table 7. Doppler analysis by infertility aetiology ($n = 49$).

	Endometriosis ($n = 7$)	Male factor ($n = 13$)	Unexplained ($n = 29$)	p Value
PI	2.48 ± 1.27	1.87 ± 0.96	2.12 ± 0.76	0.33
RI	0.84 ± 0.14	0.81 ± 0.22	0.82 ± 0.07	0.76
ET at scratch (mm)	9.6 ± 0.9	9.6 ± 2.18	9.8 ± 2.3	0.95

There was one patient with tubal factor infertility and they were excluded from this analysis. All values in this analysis are mean ± SD.

Table 8. Published data of uterine artery doppler pulsatility indices at different ART cycle stages.

Author	Cycle stage	Number of participants	Infertility	Aetiology	Fresh/frozen	UtAD pregnant	UtAD Not pregnant	Statistically significant
Khan et al. (2016)	Day 10	200	Primary	All	Both	1.72 ± 0.43	2.27 ± 0.58	Yes
Coulam et al. (1994)	hCG trigger	100	Both	All	Frozen	2.79	3.50	Yes
Schild et al. (2001)	OCR	135	Both	All	Both	2.76 ± 0.60	2.68 ± 0.64	No
Cacciatore et al. (1996)	Embryo transfer	200	Both	All	Fresh	2.45 ± 0.54	2.66 ± 0.39	Yes
Aytoz et al. (1997)	Embryo transfer	70	Both	Male Factor	Fresh	2.33 ± 0.40	2.28 ± 0.41	No
Kim et al. (2010)	Embryo transfer	234	Primary	All	Fresh	2.14 ± 0.52	2.48 ± 0.78	No
Hoozemans et al. (2008)	Mid-luteal	83	Both	All	Fresh	1.75 ± 0.41	1.72 ± 0.43	No

transfer, and therefore this does not allow for an accurate comparison between studies (Table 8).

Strengths of our study include its prospective longitudinal study design. Transvaginal ultrasonography was performed by a single researcher. It has been shown that inter-operator variability of the PI may be as high as 11% (Friedler et al., 1996). Unlike previously published work, the menstrual cycle stage was accurately timed and confirmed using serum progesterone and by histological assessment, controlling for variation in UtAD PI due to differences in progesterone levels (Hoozemans et al., 2008). Importantly, by including nulliparous women only, the strict inclusion criteria adopted by this study, allows comparisons to be made clearly, minimising the significant clinical heterogeneity seen in previous studies. Furthermore, it is known that UtAD indices are lower in parous women than nulliparous women (Guedes-Martins et al., 2015).

A limitation of this study is the small sample size. This is mainly due to the strict inclusion criteria of the cohort, limiting the number of potential study participants. A post-hoc power calculation was performed for our results. To detect a 0.1 difference in PI between two balanced groups with 80% using a Student's t test and a type I error rate of 0.05, would require 1051 participants per group. To detect a 0.03 difference between two balanced groups with 80% using a Student's t test and a type I error rate of 0.05, would require 175 participants per group. Another limitation of this study is that the embryos were not assessed for aneuploidy.

Nevertheless, we have shown that in a highly selected cohort, ET, morphology and UtAD indices did not differentiate between those who would and would not have success in a subsequent ART cycle. Therefore, in the absence of a clear predictive effect, we believe that these investigations should not be offered routinely for the prediction of implantation. Further work could be undertaken, with larger numbers using the above power calculation, but it is likely that further research in this area focussed on molecular biomarkers of endometrial receptivity would yield more beneficial results.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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