Original Article

Effect of Endometrial Thickness and duration of Estrogen Supplementation on *In Vitro* Fertilization-Intracytoplasmic Sperm Injection Outcomes in Fresh Ovum/Embryo Donation Cycles

Jayesh Amrutlal Patel, Azadeh Jayesh Patel, Jwal Manish Banker[1], Sandeep I. Shah[2], Manish Banker[3]

Consultant, Nova IVF Fertility, Kankaria, Ahmedabad, [1]Third Year Resident Obs and Gynec, Shrimati Bhikhi ben Kanjibhai Shah (SBKS), Medical Institute and Research Center, Vadodara, [2]Senior Consultant, Nova IVF Fertility, Navrangpura, Ahmedabad, [3]Director, Nova IVF Fertility, Navrangpura, Ahmedabad, Gujarat, India

Abstract

Background: There is no consensus regarding optimal endometrial thickness and duration of estrogen supplementation in embryo transfer cycles, at present. **Aims:** To observe the effect of endometrial thickness and/or duration of estrogen supplementation on *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) outcomes in fresh ovum/embryo donation cycles. **Settings and Design:** This was a retrospective observational study. The study was conducted from January 2015 to November 2017. **Subjects and Methods:** Nine hundred and fifty seven fresh blastocyst transfer cycles in the recipients of oocyte/embryo donation regardless of reproductive history and diagnosis conducted at Nova IVF Fertility, Ahmedabad, Gujarat, India. Of these, 315 women had single embryo transfer (SET), while 642 had double embryo transfer (DET). Only fresh blastocysts derived from oocytes of young donors (≤30 years) and transferred in a uniform hormone replacement therapy (HRT) cycle were included. The effect of endometrial thickness and duration of estrogen on live birth rate (LBR) and other IVF/ICSI outcomes were analyzed. **Statistical Analysis:** Univariate logistic regression. **Results:** A significant improvement in LBR was noted in the recipients with each millimeter increase in endometrial thickness starting from 6 mm after transfer of either single (odds ratio [OR] = 1.3, P = 0.003) or double (OR = 1.14, P = 0.0218) blastocysts. Lower LBR was observed in recipients having SET and who received estrogen supplementation of <10 days (OR = 0.72; P = 0.02). Implantation rate and clinical pregnancy rate also improved significantly with endometrial thickness, but there was no change in clinical abortion rate and ectopic pregnancy rate. **Conclusions:** After minimizing the possible oocyte factor by including only donor oocytes and that of COH using a uniform HRT protocol, LBR improved with each millimeter increase in endometrial thickness starting from 6 mm. Shorter duration of estrogen supplementation (<10 days) reduced the chances of live birth in recipients after transfer of a single blastocyst.

Keywords: *Duration of estrogen, endometrial thickness, hormone replacement therapy, in vitro fertilization/intracytoplasmic sperm injection cycles, live birth rate, pregnancy rate*

Introduction

In the past 40 years of evolution in *in vitro* fertilization (IVF), implantation rate (IR) has increased from <5% to more than 50% per embryo

Received: 08-04-2020 **Revised:** 10-08-2020
Accepted: 22-02-2021 **Published:** 28-06-2021

Access this article online

Quick Response Code:

Website: www.jhrsonline.org

DOI: 10.4103/jhrs.JHRS_60_20

Address for correspondence: Dr. Jayesh Amrutlal Patel, Consultant, Nova IVF Fertility, 1st Floor, Radhe Arcade, Near Diwan Ballubhai School, Opposite Kankaria Zoo Gate, B/H Abad Dairy, Kankaria, Ahmedabad - 380 022, Gujarat, India.
E-mail: drjayeshpatel87@gmail.com

How to cite this article: Patel JA, Patel AJ, Banker JM, Shah SI, Banker M. Effect of endometrial thickness and duration of estrogen supplementation on *In Vitro* fertilization-intracytoplasmic sperm injection outcomes in fresh ovum/embryo donation cycles. J Hum Reprod Sci 2021;14:167-74.

transfer.[1] However, embryo implantation still remains a major rate-limiting step in the success of assisted reproductive technology (ART). It is well known that successful implantation is not guaranteed even after transferring a good quality euploid blastocyst in a well-prepared endometrial cavity. Various factors that influence implantation are the quality of the embryo, endometrial development, and its receptivity. Endometrial thickness, pattern, and blood flow have all been evaluated as noninvasive markers of endometrial receptivity and their effect on implantation and pregnancy in IVF.[2] Measurement of endometrial thickness is a simple, noninvasive, and reproducible method of assessing endometrial development and may act as an indicator for endometrial receptivity.[3]

Several studies have shown a significant correlation between pregnancy rate and endometrial thickness,[3,4] while few studies have failed to demonstrate any correlation.[5,6] Thus, there is still no consensus on the correlation between endometrial thickness and IVF outcome. Among the studies in which the correlation was found, there was no standard cutoff for thin endometrium, above which the IVF success rates increase. Recently, the systematic review and meta-analysis investigated both, the independent predictive capacity and the prognostic value of endometrial thickness on pregnancy outcomes after IVF. This study reported that the probability of clinical pregnancy for an endometrial thickness ≤7 mm was significantly lower compared with endometrial thickness >7 mm (23.3% vs. 48.1%) with an odds ratio (OR) 0.42 (95% confidence interval 0.27–0.67);[4] whereas couples in other studies reported a significant increase in pregnancy outcomes at endometrial thickness of ≥10 mm.[7,8]

Most of the studies examining the association between endometrial thickness and clinical outcome were conducted in fresh IVF/intracytoplasmic sperm injection (ICSI) cycles using self-oocytes. This may not be the ideal model to study the correlation of endometrial thickness and embryo implantation as there are adverse effects of controlled ovarian hyperstimulation (COH) on endometrium due to supraphysiological levels of estrogen.[9,10] Thus, in order to understand the association between endometrial thickness on IVF outcome, a better model is to have women, whose endometrium is prepared using standard hormone replacement therapy (HRT) protocol. Yet, another variability in the published studies is the possible impact of aneuploidy of oocytes, where women with higher age contribute to lower IR due to aueuploidy. Including only oocyte from young donors minimizes the chance of embryo aneuploidy.[11]

During endometrial preparation in donor oocyte recipient cycles, it is a common practical need to prolong the duration of estrogen treatment while waiting for the donor egg retrieval or to achieve an adequate endometrial thickness before starting progesterone. With the available evidences, there is no consensus drawn on serum estradiol levels or the ideal duration of estrogen treatment on endometrial thickness, its receptivity, and IVF outcomes.[12,13]

Hence, in our study, we aimed to find the association between endometrial thickness and the duration of estrogen supplementation on IVF/ICSI outcomes in fresh ovum/embryo donation cycles, where the endometrium was prepared by uniform HRT to minimize the influence of COH. In addition, by including only oocyte/embryo donation cycles from young donors (≤30 years), we have also tried to minimize the influence of oocyte factor on implantation.

Subjects and Methods

Study design

We conducted a retrospective observational study including 957 transfer cycles performed in the recipients of donor oocyte/embryo using a uniform HRT protocol at Nova IVF Fertility, Ahmedabad, Gujarat, India. from January 2015 to November 2017. All fresh blastocyst transfer cycles performed in recipients of donor oocytes or embryos during the above study period were included in this study, regardless of reproductive history and diagnosis. A power estimation was not done.

Study methods

All cases with embryos derived from self-oocytes and frozen thawed embryo transfer cycles in the recipients were excluded from this study. All the cases where day 3 (cleavage stage) embryos were transferred and where endometrium was prepared by any protocol other than HRT were excluded.

Hormone replacement therapy protocol used for endometrial preparation

A baseline transvaginal sonography (TVS) was performed on the 2nd day of menstruation. All patients received oral estradiol valerate tablet (*Progynova, Bayar Zydus Pharma*) 2 mg starting at a daily dose of 4 mg (in two divided doses) for 4 days and then increased to 8 mg (in two divided doses). After 6–9 days of therapy, endometrial thickness was measured by TVS in the midsagittal plane near uterine fundus. The largest thickness from one interface of the endometrial–myometrial junction to the other was measured in millimeter (mm). In case of thin endometrium (<6 mm), the duration of treatment was extended after increasing estradiol valerate to 12 mg. When endometrial thickness was ≥7 mm in general or remained ≥6 mm even with prolonged estrogen therapy,

but with a triple line pattern, progesterone administration was initiated along with the same dose of estradiol valerate from the day of donor's ovum pick up (OPU). Serum progesterone levels were checked on the day of donor OPU. HRT cycles with serum progesterone levels >0.5 ng/ml were cancelled, and all embryos were cryopreserved. Progesterone was given in the form of micronized progesterone (Miprogen, Bharat Serums And Vaccines Ltd) 800 mg/day, vaginally in two divided doses with oral dydrogesterone (Duphaston, Abbott Pharma) 20 mg/day, orally in two divided doses. Duration of estrogen supplementation was counted in days from the day of estradiol valerate initiation to progesterone supplementation initiation. Duration of estrogen supplementation varied from 6 to 23 days depending on both the availability of the oocyte donor and thickness of endometrium.

Fertilization was performed by ICSI. Quality assessment of embryos was performed daily. Blastocysts were graded using ASEBIR method as A, B, C, or D.[14] One or two blastocyst(s) were transferred into the endometrial cavity after 5 days of progesterone initiation. In majority of the recipients, Grade A or B blastocyst(s) were transferred both in single embryo transfer (SET) and double embryo transfer (DET) groups. Estradiol valerate and progesterone support were continued till serum beta human chorionic gonadotropin (β-hCG) test for pregnancy confirmation after 14 days of transfer. Serum β-hCG value of >10 IU/L was considered as positive pregnancy. TVS was performed 1 week later to confirm the number and location of the gestational sac (G-sac) and another 2 weeks later to confirm fetal cardiac activity. Pregnancies with positive β-hCG but absent G-sac on TVS were considered as biochemical pregnancies. Luteal support was continued up to 12 weeks of pregnancy.

The main outcome of this study was live birth rate (LBR). Secondary outcomes were pregnancy rate (PR), IR, clinical pregnancy rate (CPR), clinical abortion rate (CAR), and ectopic pregnancy rate (EPR). The comparative analysis of outcomes with each millimeter of endometrial thickness starting from 6 mm to more than 14 mm (range: 6 mm to 18 mm) was calculated. All the IVF outcomes were also analyzed based on days of estrogen supplementation starting with <8 days till more than 14 days (range: 6 days to 23 days). Based on the observation of these analysis, we performed subgroup analysis to compare all outcomes between the patient group who received estrogen supplementation <10 days with ≥10 days.

PR was calculated by dividing total number of positive pregnancies by total number of ET. Clinical abortion was defined as clinical intrauterine pregnancy loss before 22 weeks of gestation. IR, clinical pregnancy, ectopic pregnancy, and live birth were all defined as per ICMART, WHO glossary.[15] Each pregnant patient was referred to take standard obstetric care under an obstetrician of their choice from the beginning or after 12 weeks of gestation as we do not provide obstetric care. Follow-up of all the pregnant patients were taken from their respective obstetrician within 1 month of abortion, ectopic pregnancy, or delivery.

Statistical analysis

Univariate logistic regression analysis was used to evaluate the association of various IVF outcomes with endometrial thickness and duration of estrogen supplementation in all the cycles. Average of all the quantitative variables (e.g., female age, body mass index, endometrial thickness, duration of estrogen, and number of embryos transferred) were reported as mean ± standard deviation. $P < 0.05$ was considered to be statistically significant. The data were analyzed using R 3.5.0 software, Brisbane, Australia.

Ethical approval

Written consent of all the study subjects were taken. This study was approved by the ethical committee of CIMS Hospital, Ahmedabad, Gujarat, India.

Results

A total of 957 fresh blastocyst transfers from donor oocytes or embryos performed using HRT during our study period were included in this analysis. Out of these, 315 were SET, while 642 were DET. In our study, (1) smaller sample size and (2) higher abortion rates in SET group as compared to DET group might be responsible for lower pregnancy outcomes/LBR in SET group. Baseline characteristics and IVF outcomes in general are shown in Supplementary Table 1.

We analysed the effect of increase in each millimeter in endometrial thickness on IVF-ICSI outcomes in these recipients. A significant improvement in LBR was noted in the recipients with each millimeter increase in endometrial thickness starting from 6 mm after transfer of either single (odds ratio [OR] = 1.3, $P = 0.003$*) or double (OR = 1.14, $P = 0.028$) blastocysts. IR and CPR also improved significantly with endometrial thickness, but there was no change in CAR and EPR [Tables 1 and 2].

We also analysed the effect of increase in the duration of estrogen supplementation for endometrial preparation by each day on IVF-ICSI outcomes in these recipients, but we were unable to find any significant difference in PR, IR, CPR, LBR, CAR, and EPR with increase in the duration of estrogen supplementation. One recipient

Table 1: *In vitro* fertilization outcomes according to endometrial thickness in fresh recipients (single embryo transfer)

Endometrial thickness (mm)	Embryo transfer cycles (*n*)	Total embryos transferred	PR, *n* (%)	IR, *n* (%)	CPR, *n* (%)	BPR, *n* (%)	LBR, *n* (%)
6-7	5	5	3 (60.0)	2 (40.0)	2 (40.0)	1 (20.0)	1 (20.0)
7-8	37	37	19 (51.3)	17 (45.9)	17 (45.9)	2 (5.4)	9 (24.3)
8-9	83	83	47 (56.6)	36 (43.4)	36 (43.4)	11 (13.2)	22 (26.5)
9-10	82	82	46 (56.1)	39 (47.6)	39 (47.6)	7 (8.5)	24 (29.3)
10-11	62	62	42 (67.7)	37 (59.7)	37 (59.7)	5 (8.1)	23 (37.1)
11-12	26	26	17 (65.4)	17 (65.4)	17 (65.4)	0	10 (38.5)
12-13	11	11	8 (72.7)	8 (72.7)	8 (72.7)	0	7 (63.6)
13-14	9	9	6 (66.7)	6 (66.7)	6 (66.7)	0	5 (55.6)
>14			-	-	-	-	-

Endometrial thickness (mm)	CAR, *n* (%)	ER, *n* (%)	Average age (years)	Average DOE (days)	BMI (kg/m^2)
6-7	1 (50.0)	0	43.4±7.1	10.8±1.3	27.9±6.2
7-8	8 (47.1)	0	40.6±5.1	10.8±2.8	27.7±5.8
8-9	11 (30.3)	1 (2.8)	39.9±5.6	10.0±2.1	26.9±5.4
9-10	13 (33.3)	0	40.9±5.2	1.0±2.0	27.0±3.9
10-11	10 (27.0)	1 (2.7)	39.7±5.7	10.2±1.8	27.6±4.4
11-12	5 (29.4)	0	39.6±4.0	11.1±2.5	27.7±4.7
12-13	1 (12.5)	0	40.6±5.8	11.5±2.2	26.2±6.2
13-14	1 (16.7)	0	40.3±5.9	12.0±1.5	25.7±2.2
>14	-	-	-	-	-

PR: OR=1.2, *P*=0.04*, IR: OR=1.3, *P*=0.003*, CPR: OR=1.3, *P*=0.003*, BPR: OR=0.73, *P*=0.068, LBR: OR=1.3, *P*=0.003*; CAR: OR=0.98, *P*=0.825, ER: OR=0.95, *P*=0.92. OR=Odds ratio, PR=Pregnancy rate, IR=Implantation rate, CPR=Clinical pregnancy rate, BPR=Biochemical pregnancy rate, LBR=Live birth rate, BMI=Body mass index, CAR=Clinical abortion rate, ER=Ectopic pregnancy rate, DOE=Duration of estrogen

Table 2: *In vitro* fertilization outcomes according to endometrial thickness in fresh recipients (double embryo transfer)

Endometrial thickness (mm)	Embryo transfer cycles (*n*)	Total embryos transferred	PR, *n* (%)	IR, *n* (%)	CPR, *n* (%)	BPR, *n* (%)	LBR, *n* (%)
6-7	11	22	5 (45.5)	4 (18.2)	4 (36.4)	1 (9.1)	1 (9.1)
7-8	66	132	41 (62.1)	32 (24.2)	32 (48.5)	9 (13.6)	23 (34.8)
8-9	192	384	133 (69.3)	121 (31.5)	121 (63.0)	12 (6.2)	82 (42.7)
9-10	177	354	140 (79.1)	128 (36.1)	128 (72.3)	12 (6.8)	100 (56.5)
10-11	101	202	70 (69.3)	62 (30.7)	62 (61.4)	8 (7.9)	45 (44.6)
11-12	61	122	50 (82.0)	45 (36.9)	45 (73.8)	5 (8.2)	36 (59.0)
12-13	18	36	15 (83.3)	14 (38.9)	14 (77.8)	1 (5.6)	10 (55.6)
13-14	15	30	10 (66.7)	10 (33.3)	10 (66.7)	0	7 (46.7)
>14	1	2	0	0	0	0	0

Endometrial thickness (mm)	CAR, *n* (%)	ER, *n* (%)	Average age (years)	Average DOE (days)	BMI (kg/m^2)
6-7	2 (50.0)	0	34.8±4.7	11.9±3.8	25.0±4.6
7-8	8 (25.0)	2 (6.2)	35.6±4.1	11±3.1	27.4±6.0
8-9	25 (20.7)	4 (3.3)	35.5±4.7	10.1±2.3	27.0±5.3
9-10	19 (14.8)	2 (1.6)	35.1±4.5	10.4±2.2	27.2±4.9
10-11	8 (12.9)	0 (0.0)	35.8±4.5	10.8±2.3	26.9±5.4
11-12	5 (11.1)	2 (4.4)	35.7±5.3	10.9±2.5	27.2±5.4
12-13	3 (21.4)	0	35.1±4.4	10.9±2.1	29.8±5.3
13-14	2 (20.0)	0	32.4±4.7	11.2±2.1	23.7±3.7
>14	0	0	31.0±0	9±0	21.5±0.0

PR: OR=1.1, *P*=0.108, IR: OR=1.14, *P*=0.028*, CPR: OR=1.14, *P*=0.028*, BPR: OR=0.87, *P*=0.212, LBR: OR=1.14, *P*=0.0218*, CAR: OR=0.91, *P*=0.316, ER: OR=0.81, *P*=0.404. OR=Odds ratio, PR=Pregnancy rate, IR=Implantation rate, CPR=Clinical pregnancy rate, BPR=Biochemical pregnancy rate, LBR=Live birth rate, BMI=Body mass index, CAR=Clinical abortion rate, ER=Ectopic pregnancy rate, DOE=Duration of estrogen

with missing estrogen initiation date was excluded from this analysis [Tables 3 and 4].

However, we observed a significant decrease in LBR in fresh recipient cycles when single blastocyst

Table 3: *In vitro* fertilization outcomes according to the duration of estrogen supplementation in fresh recipients (single embryo transfer)

DOE (days)	Embryo transfer cycles (*n*)	Total embryos transferred	PR, *n* (%)	IR, *n* (%)	CPR, *n* (%)	BPR, *n* (%)	LBR, *n* (%)
<8	13	13	7 (53.8)	4 (30.8)	4 (30.8)	3 (23.1)	3 (23.1)
8	53	53	30 (56.6)	24 (45.3)	24 (45.3)	6 (11.3)	14 (26.4)
9	61	61	39 (63.9)	30 (49.2)	30 (49.2)	9 (14.8)	15 (24.6)
10	60	60	34 (56.7)	32 (53.3)	32 (53.3)	2 (3.3)	20 (33.3)
11	45	45	27 (60.0)	25 (55.6)	25 (55.6)	2 (4.4)	18 (40.0)
12	31	31	15 (48.4)	15 (48.4)	15 (48.4)	0 (0.0)	12 (38.7)
13	19	19	13 (68.4)	12 (63.2)	12 (63.2)	1 (5.3)	5 (26.3)
14	24	24	19 (79.2)	16 (66.7)	16 (66.7)	3 (12.5)	12 (50.0)
>14	9	9	4 (44.4)	4 (44.4)	4 (44.4)	0	2 (22.2)

DOE (days)	CAR, *n* (%)	ER, *n* (%)	Average age (years)	Average endometrial thickness (mm)	BMI (kg/m^2)
<8	1 (25.0)	0	36.9±6.3	9.4±0.8	27.3±3.5
8	8 (33.3)	0	40.0±4.8	9.2±1.1	27.4±5.0
9	12 (40.0)	1 (3.3)	40.7±6.1	9.1±1.1	27.3±4.8
10	10 (31.2)	1 (3.1)	40.7±4.9	9.3±1.4	26.6±4.6
11	5 (20.0)	0	40.0±4.5	9.4±1.4	26.5±5.1
12	3 (20.0)	0	38.9±5.4	9.6±1.8	26.3±5.1
13	7 (58.3)	0	41.9±5.6	9.7±1.6	29.0±3.7
14	4 (25.0)	0	41.9±4.9	9.8±1.8	27.3±4.6
>14	0	0	38.7±7.1	9.2±1.5	30.4±4.9

PR: OR=1.01, *P*=0.737, IR: OR=1.2, *P*=0.129, CPR: OR=1.2, *P*=0.129, BPR: OR=0.78, *P*=0.0304*, LBR: OR=1.1, *P*=0.112, CAR: OR=1.0, *P*=0.973, ER: OR=0.80, *P*=0.579. PR=Pregnancy rate, IR=Implantation rate, CPR=Clinical pregnancy rate, BPR=Biochemical pregnancy rate, LBR=Live birth rate, BMI=Body mass index, CAR=Clinical abortion rate, ER=Ectopic pregnancy rate, DOE=Duration of estrogen, OR=Odds ratio

Table 4: *In vitro* fertilization outcomes according to the duration of estrogen supplementation in fresh recipients (double embryo transfer)

DOE (days)	Embryo transfer cycles (*n*)	Total embryos transferred	PR, *n* (%)	IR, *n* (%)	CPR, *n* (%)	BPR, *n* (%)	LBR, *n* (%)
<8	35	70	23 (65.7)	21 (30.0)	21 (60.0)	2 (5.7)	16 (45.7)
8	100	200	66 (66.0)	62 (31.0)	62 (62.0)	4 (4.0)	40 (40.0)
9	106	212	76 (71.7)	70 (33.0)	70 (66.0)	6 (5.7)	50 (47.2)
10	129	258	91 (70.5)	78 (30.2)	78 (60.5)	13 (10.1)	54 (41.9)
11	95	190	73 (76.8)	66 (34.7)	66 (69.5)	7 (7.4)	47 (49.5)
12	54	108	42 (77.8)	37 (34.2)	37 (68.5)	5 (9.3)	32 (59.6)
13	48	96	37 (77.1)	32 (33.3)	32 (66.6)	5 (10.4)	28 (58.3)
14	27	54	20 (74.1)	19 (35.2)	19 (70.4)	1 (3.7)	15 (55.5)
>14	47	94	35 (74.5)	30 (31.9)	30 (63.8)	5 (10.6)	21 (44.7)

DOE (days)	CAR, *n* (%)	ER, *n* (%)	Average age (years)	Average endometrial thickness (mm)	BMI (kg/m^2)
<8	3 (14.3)	2 (9.5)	34.7±4.4	9.0±1.3	25.6±5.6
8	14 (22.6)	1 (1.6)	35.5±4.5	9.0±1.2	26.7±4.0
9	15 (21.4)	0 (0.0)	35.5±4.5	9.3±1.5	27.8±5.9
10	14 (17.9)	3 (3.8)	35.3±4.5	9.5±1.5	27.0±5.6
11	11 (16.7)	2 (3.0)	35.2±4.9	9.4±1.4	26.6±4.5
12	4 (10.8)	2 (5.4)	35.0±5.2	9.2±1.3	28.9±7.2
13	3 (9.4)	0	35.6±4.3	9.5±1.5	27.2±4.9
14	3 (15.8)	0	34.7±4.7	9.6±1.7	25.9±4.6
>14	5 (16.7)	0	36.3±4.7	9.1±1.5	26.8±4.9

PR: OR=1.2, *P*=0.13, IR: OR=1.02, *P*=0.441, CPR: OR=1.02, *P*=0.441, BPR: OR=1.1, *P*=0.237, LBR: OR=1.04, *P*=0.167, CAR: OR=0.96, *P*=0.405, ER: OR=0.84, *P*=0.285. PR=Pregnancy rate, IR=Implantation rate, CPR=Clinical pregnancy rate, BPR=Biochemical pregnancy rate, LBR=Live birth rate, BMI=Body mass index, CAR=Clinical abortion rate, ER=Ectopic pregnancy rate, DOE=Duration of estrogen, OR=Odds ratio

transfers were done with <10 days of estrogen priming compared with those who received ≥10 days (LBR: 25.2% vs. 36.7%; OR = 0.58, P = 0.03). Which signifies that ≥10 days of estrogen priming is helpful in achieving significant higher LBR in recipients. An increasing trend in LBR was also observed in recipients having DET but was not statistically significant (LBR: 44% vs. 49.2%; OR = 1.19, *P* = 0.47) [Supplementary Table 2].

Discussion

Most published data till date on the endometrial thickness and IVF outcomes have included both fresh and frozen ET using different protocols with self-oocytes.[3,4] Even in few studies with donor oocytes, both fresh and frozen cycles have been included. The protocol for endometrial preparation was not standardized, number of embryos transferred either not specified or not analyzed separately, and days of estrogen exposure for endometrial preparation not included.[16,17]

When self-oocytes from patients of all age groups are included to study the effect of endometrial thickness on IVF outcomes, it is difficult to analyze the effect of endometrial thickness on IVF outcomes independent of oocyte and embryo quality. In many previous publications even after logistic regression analysis, maternal age is found to be an independent negative factor in predicting IVF success rate. This trend is in line with expected physiological reproductive potential where follicular response/maturation to controlled ovarian stimulation can be predicted as a function of age.[18] Even in the meta-analysis on endometrial thickness and IVF outcomes by Kasius *et al*.,[4] they found female age and number of oocytes were the two main confounding factors in predicting IVF success rate. The effect of female age on oocyte/embryo quality and thus on IVF outcomes is also well known. Mahajan and Sharma[19] suggested that oocyte donation cycles are ideal to measure the independent effect of endometrial thickness as a parameter of endometrial receptivity as the use of the oocyte donation model reduces confounding factors related to oocyte age, embryo aneuploidy, and embryo quality. In our study, only donors of ≤30 years were included to minimize the embryo aneuploidy rates.[11]

Besides the effect of maternal age, the effect of COH on endometrium is also a confounding factor in fresh IVF cycles. In the stimulated IVF cycle, endometrium is exposed to supraphysiological levels of estrogen secreted from the multifollicular development. In 1995, Simón *et al*.[9] first demonstrated a detrimental effect on uterine receptivity of high serum estradiol concentrations particularly in high and normal responder patients. Horcajadas *et al*.[10] have proven a large degree of gene expression disturbance in the endometrium with COH and suggested the need to optimize COH protocols. Recently, Sakiner *et al*.[20] have proven that COH may have negative effects on many functions such as uterine growth, receptivity, and altered expressions of the markers. They have recognized that the expression of a majority of endometrial receptivity-related proteins is decreased, which is thought to have an adverse effect on LBRs and infertility treatment. Thus, it is important to study the effect of endometrial thickness as a marker of receptivity in an unstimulated natural cycle or where the endometrium is prepared by a standardized HRT protocol.

To avoid both the above mentioned biases in our study, we have attempted to find the effect of endometrial thickness and duration of estrogen supplementation on IVF outcomes in the fresh recipient cycles where endometrium was prepared by one standard HRT and blastocysts were prepared from oocytes young donors below 30 years. IVF outcomes are also separately analyzed after SET and DET.

Role of endometrial thickness is controversial in predicting IVF outcomes in the recipients. Some studies support,[16,21] while the others do not[22,23] support its predictive value in recipients. In our study, we found a significant improvement in the overall IVF outcomes, particularly in LBR with increase of each millimeter in endometrial thickness starting from 6 mm. Live birth is reported even with the endometrial thickness of 4 mm,[24] indirectly suggesting that the endometrial receptivity may not be associated only with the thickness. Each study included in the meta-analysis used different cutoff for thin endometrium failed to find an ideal endometrial thickness.[4] Therefore, the data of our study and of another author[17] explain the impact of each millimeter increase in endometrial thickness on IVF outcomes which helps in counseling the patients on their probability of success with their IVF treatment, thus paving way for personalized infertility management.

The correlation between EPR and CAR with endometrial thickness is also controversial. Few studies showed decreased EPR and CAR with increase in endometrial thickness,[25,26] while other showed no difference.[27] There is controversy for EPR between the fresh and frozen embryo transfer. Few studies suggest that fresh embryo transfer is associated with higher ectopic pregnancies than frozen transfer,[28,29] while others showed no difference.[30,31] However, all these evidences are from IVF treatments using self-oocytes. In our study, we have found no association between endometrial thickness and EPR or CAR in fresh recipients. Similar findings in recipient cycles were observed by Barker *et al*.[22]

Endometrial preparation in ART is easily achieved through the administration of exogenous estrogen in HRT protocol, with the best reproductive outcomes obtained within a range of 11–40 days of estrogen administration. Estrogen supplementation has to be of adequate duration as shorter estrogen replacements lead to high abortion rates, while breakthrough bleeding is common with more than 40 days of administration.[13] In our study, we found that shorter duration of estrogen (<10 days) supplementation reduces the chances of LBR in fresh recipients. This suggests that a minimal duration and level of estrogen exposure are required to sensitize endometrium at molecular level for progesterone priming and beyond the basal thickness of endometrium; this is independent of endometrial thickness. Cutoff for minimum duration of estrogen exposure below which IVF outcomes compromise also varies from 6 days[12] up to 12 days,[13] but in our study, we found a cutoff at 10 days in fresh recipients, below which the LBRs decrease significantly.

As a retrospective observational study, this study has limitations to control all the confounding factors. We included only those cycles which reached up to embryo transfers and not all initiated HRT cycles. Cancelled cycles due to high progesterone, medical reasons or endometrial factors, and frozen-thawed cycles were excluded, which may have overestimated the pregnancy outcomes. Smaller sample size in SET group as well as in lower (<7 mm thickness) and higher (>10 mm thickness) endometrial thickness groups might be responsible for bias in our study results. We observed LBR only and not twin pregnancy rate or preterm delivery rate. Hence, we would not be able to observe the effect of endometrial thickness on these parameters. Approximately 5% of cycles could not be included in the analysis due to data irregularity or missing entry.

As per our knowledge, this is the largest study done among the fresh recipients proving the independent effect of endometrial thickness and duration of estrogen therapy on IVF outcomes. By including blastocyst transfers only, we have tried to minimize heterogeneity in the study population. Our study findings may help in counseling the patients with their chance of success with embryo transfer, based on the endometrial thickness and duration of estrogen.

Conclusions

Endometrial thickness is an independent factor influencing the IVF outcomes in the recipients. After minimizing the possible oocyte factor by including only donor oocytes and that of COH using a uniform HRT protocol, LBR improved with each millimeter increase in endometrial thickness starting from 6 mm. Shorter duration of estrogen supplementation (<10 days) reduced the chances of live birth in recipients after transfer of a single blastocyst. However, there was no association between endometrial thickness with ectopic pregnancy or abortion.

Acknowledgments

We are grateful to Mr. Lalit Kumar for his continuous guidance and Dechamma for her help in statistics.

Financial support and sponsorship

Nil.

Conflicts of interest

Dr. MB is the Senior Associate Editor of JHRS but was not involved in the editorial or review process of the article.

References

1. Niederberger C, Pellicer A, Cohen J, Gardner DK, Palermo GD, O'Neill CL, *et al*. Forty years of IVF. Fertil Steril 2018;110:185-324.
2. Puerto B, Creus M, Carmona F, Civico S, Vanrell JA, Balasch J. Ultrasonography as a predictor of embryo implantation after *in vitro* fertilization: A controlled study. Fertil Steril 2003;79:1015-22.
3. Al-Ghamdi A, Coskun S, Al-Hassan S, Al-Rejjal R, Awartani K. The correlation between endometrial thickness and outcome of *in vitro* fertilization and embryo transfer (IVF-ET) outcome. Reprod Biol Endocrinol 2008;6:37.
4. Kasius A, Smit JG, Torrance HL, Eijkemans MJ, Mol BW, Opmeer BC, *et al*. Endometrial thickness and pregnancy rates after IVF: A systematic review and meta-analysis. Hum Reprod Update 2014;20:530-41.
5. Yaman C, Ebner T, Jesacher K, Sommergruber M, Radner G, Tews G. Sonographic measurement of endometrium thickness as a predictive value for pregnancy through IVF. Ultraschall Med 2002;23:256-9.
6. Weissman A, Gotlieb L, Casper RF. The detrimental effect of increased endometrial thickness on implantation and pregnancy rates and outcome in an *in vitro* fertilization program. Fertil Steril 1999;71:147-9.
7. Rinaldi L, Lisi F, Floccari A, Lisi R, Pepe G, Fishel S. Endometrial thickness as a predictor of pregnancy after *in-vitro* fertilization but not after intracytoplasmic sperm injection. Hum Reprod 1996;11:1538-41.
8. Gallos ID, Khairy M, Chu J, Rajkhowa M, Tobias A, Campbell A, *et al*. Optimal endometrial thickness to maximize live births and minimize pregnancy losses: Analysis of 25,767 fresh embryo transfers. Reprod Biomed Online 2018;37:542-8.
9. Simón C, Cano F, Valbuena D, Remohí J, Pellicer A. Implantation: Clinical evidence for a detrimental effect on uterine receptivity of high serum oestradiol concentrations in high and normal responder patients. Hum Reprod 1995;10:2432-7.
10. Horcajadas JA, Riesewijk A, Polman J, van Os R, Pellicer A, Mosselman S, *et al*. Effect of controlled ovarian hyperstimulation in IVF on endometrial gene expression profiles. Mol Hum Reprod 2004;11:195-205.
11. Kotdawala A, Patel D, Herrero J, Khajuria R, Mahajan N, Banker M. Aneuploidy screening by array comparative genomic

hybridization improves success rates of *in vitro* fertilization: A multicenter Indian study. J Hum Reprod Sci 2016;9:223-9.

12. Michalas S, Loutradis D, Drakakis P, Kallianidis K, Milingos S, Deligeoroglou E, *et al*. Human Reproduction, 1996;11:1063-66, https://doi.org/10.1093/oxfordjournals.humrep.a019297. Published: 01 May 1996.
13. Borini A, Dal Prato L, Bianchi L, Violini F, Cattoli M, Flamigni C. CLINICAL ASSISTED REPRODUCTION: Effect of duration of estradiol replacement on the outcome of oocyte donation. J Assist Reprod Genet 2001;18:187-92.
14. Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: Proceedings of an expert meeting. Hum Reprod 2011;26:1270-83.
15. Zegers FZ, Adamson GD, Dyer S, Racowsky C, de Mouzon J, Sokol R, *et al*. The International Glossary on Infertility and Fertility Care, 2017: Led by ICMART in Partnership with ASRM, ESHRE, IFFS, March of Dimes, AFS, GIERAF, ASPIRE, MEFS, REDLARA, FIGO. Fertil Steril 2017;108:393-406.
16. Noyes N, Hampton BS, Berkeley A, Licciardi F, Grifo J, Krey L. Factors useful in predicting the success of oocyte donation: A 3-year retrospective analysis. Fertil Steril 2001;76:92-7.
17. Liu KE, Hartman M, Hartman A, Luo ZC, Mahutte N. The impact of a thin endometrial lining on fresh and frozen-thaw IVF outcomes: An analysis of over 40 000 embryo transfers. Hum Reprod 2018;33:1883-8.
18. Osemwenkha AP, Osaikhuwuomwan JA. Correlation between endometrial thickness and IVF outcome in an African population. Gynecol Obstet 2012;2:119.
19. Mahajan N, Sharma S. The endometrium in assisted reproductive technology: How thin is thin? J Hum Reprod Sci 2016;9:3.
20. Sakiner L, Bulut ID, Yalcinkaya P, Elmas C. Effects of controlled ovarian hyperstimulation protocols on uterine markers. Ann Med Res 2018;25:495-98. doi: 10.5455/annalsmedres.2018.04.064
21. Gupta P, Banker M, Patel P, Joshi B. A study of recipient related predictors of success in oocyte donation program. J Hum Reprod Sci 2012;5:252-7.
22. Barker MA, Boehnlein LM, Kovacs P, Lindheim SR. Follicular and luteal phase endometrial thickness and echogenic pattern and pregnancy outcome in oocyte donation cycles. J Assist Reprod Genet 2009;26:243-9.
23. Garcia-Velasco JA, Isaza V, Caligara C, Pellicer A, Remohí J, Simón C. Factors that determine discordant outcome from shared oocytes. Fertil Steril 2003;80:54-60.
24. Check JH, Dietterich C, Check ML, Katz Y. Successful delivery despite conception with a maximal endometrial thickness of 4 mm. Clin Exp Obstet Gynecol 2003;30:93-4.
25. Yuan X, Saravelos SH, Wang Q, Xu Y, Li TC, Zhou C. Endometrial thickness as a predictor of pregnancy outcomes in 10787 fresh IVF-ICSI cycles. Reprod Biomed Online 2016;33:197-205.
26. Wu Y, Gao X, Lu X, Xi J, Jiang S, Sun Y, *et al*. Endometrial thickness affects the outcome of *in vitro* fertilization and embryo transfer in normal responders after GnRH antagonist administration. Reprod Biol Endocrinol 2014;12:96.
27. Richter KS, Bugge KR, Bromer JG, Levy MJ. Relationship between endometrial thickness and embryo implantation, based on 1,294 cycles of *in vitro* fertilization with transfer of two blastocyst-stage embryos. Fertil Steril 2007;87:53-9.
28. Fang C, Huang R, Wei LN, Jia L. Frozen-thawed day 5 blastocyst transfer is associated with a lower risk of ectopic pregnancy than day 3 transfer and fresh transfer. Fertil Steril 2015;103:655-61.
29. Perkins KM, Boulet SL, Kissin DM, Jamieson DJ, National ART Surveillance (NASS) Group. Risk of ectopic pregnancy associated with assisted reproductive technology in the United States, 2001-2011. Obstet Gynecol 2015;125:70-8.
30. Jee BC, Suh CS, Kim SH. Ectopic pregnancy rates after frozen versus fresh embryo transfer: A meta-analysis. Gynecol Obstet Invest 2009;68:53-7.
31. Decleer W, Osmanagaoglu K, Meganck G, Devroey P. Slightly lower incidence of ectopic pregnancies in frozen embryo transfer cycles versus fresh *in vitro* fertilization-embryo transfer cycles: A retrospective cohort study. Fertil Steril 2014;101:162-5.

Supplementary Table 1: Characteristics and *in vitro* fertilization outcomes of fresh recipients underwent blastocyst transfers using hormone replacement therapy cycles

Variables	Fresh recipient-SET (*n*=315), *n* (%)	Fresh recipient- DET (*n*=642), *n* (%)
Average female age (years) (mean±SD)	40.3±5.3	35.3±4.6
Average female BMI (kg/m^2) (mean±SD)	27.2±4.7	27.0±5.2
Duration of infertility (years)	8.7±8.1	6.8±5.6
Endometrial thickness	9.3±1.4	9.3±1.4
DOE (mean±SD)	10.3±2.2	10.5±2.4
Average number of embryos transferred (mean±SD)	1.0±0.5	2.0±0.5
PR	188 (59.7)	464 (72.3)
IR	162/315 (51.4)	416/1284 (32.4)
CPR	162 (51.4)	416 (64.8)
BPR	26 (8.2)	48 (7.5)
CAR	50 (30.9)	72 (17.3)
EPR	2 (0.6)	10 (1.6)
LBR	101 (32.1)	304 (47.3)

BMI=Body mass index, DOE=Duration of estrogen, PR=Pregnancy rate, IR=Implantation rate, CPR=Clinical pregnancy rate, BPR=Biochemical pregnancy rate, CAR=Clinical abortion rate, EPR=Ectopic pregnancy rate, LBR=Live birth rate, SD=Standard deviation, SET=Single embryo transfer, DET=Double embryo transfer

Supplementary Table 2: Comparing pregnancy outcomes to duration of estrogen supplementation (cut-off at 10 days of estrogen supplementation)

	Fresh recipient (SET)		
Outcomes	Days of HRT		OR and significant
	<10 (*n*=127), *n* (%)	≥10 (*n*=188), *n* (%)	
PR	76 (59.8)	112 (59.6)	OR=1.01; *P*=0.96
IR	58/127 (46.7)	104/188 (55.3)	*P*=0.09
CPR	58 (45.7)	104 (55.3)	OR=0.67; *P*=0.09
AR	21 (36.2)	29 (27.9)	OR=1.08; *P*=0.79
LBR	32 (25.2)	69 (36.7)	OR=0.58; *P*=0.03*
Ectopic	1 (0.8)	1 (0.5)	OR=1.5; *P*=0.77
	Fresh recipient (DET)		
Outcomes	Days of HRT		OR and significant
	<10 (*n*=241), *n* (%)	≥10 (*n*=400), *n* (%)	
PR	165 (68.5)	298 (74.5)	OR=0.73; *P*=0.18
IR	153/482 (31.7)	262/800 (32.7)	*P*=0.32
CPR	153 (63.5)	262 (65.5)	OR=0.79; *P*=0.32
AR	32 (20.9)	40 (15.3)	OR=0.36; *P*=0.009*
LBR	106 (44.0)	197 (49.2)	OR=1.19; *P*=0.47
Ectopic	3 (1.2)	(1.8)	

HRT=Hormone replacement therapy, SET=Single embryo transfer, DET=Double embryo transfer, OR=Odds ratio, PR=Pregnancy rate, IR=Implantation rate, CPR=Clinical pregnancy rate, AR=Assisted reproduction, LBR=Live birth rate