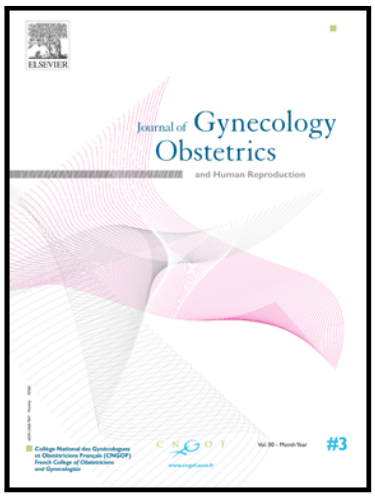

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High dose gonadotropin stimulation increases endometrial thickness but this gonadotropin induced thickening does not have an effect on implantation

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Abstract

Introduction: Endometrial thickness <8mm is related with lower pregnancy rates. This raises the question if endometrial thickness can be increased by gonadotropin stimulation to increase estradiol (E2) concentration and if such an artificial thickening of the endometrium has an effect on implantation. A model to address this question is the comparison of endometrial thickness and outcome parameters in conventional gonadotropin stimulated IVF (cIVF) compared to unstimulated natural cycle IVF (NC-IVF).

Material and methods: Retrospective study including 235 cIVF and 616 NC-IVF cycles without embryo selection and with fresh transfer on day 2 and 3 from 2015 to 2019. Endometrial and E2 measurements were included and analysed between day -4 and -2 (0 = day of aspiration). The effects of E2 on endometrial thickness, endometrial growth and the effect of endometrial thickness on implantation rates and live births were analysed.

Results: Endometrial thickness was found to be higher in cIVF compared to NC-IVF ($p < 0.001$). On day -2, the day when ovulation was triggered, mean endometrial thickness was 9.75 ± 2.05mm and 8.12 ± 1.66mm, respectively. The increase in endometrial thickness slowed down with increasing E2 concentrations (time x estradiol concentration: -0.19, p =

0.010). Implantation rates were not significantly different in cIVF and NC-IVF cycles (clinical pregnancy rate: 19.1% vs. 15.4% p = 0.2; live birth rate: 12.8% vs. 11.7%, p = 0.8).

Conclusions: Endometrial growth dynamic is different and endometrium is thicker in cIVF compared to NC-IVF. Pregnancy and live birth rates are not different. Gonadotropin induced thickening of the endometrium does not appear to improve implantation.

Keywords: Endometrial thickness, IVF, gonadotropin, natural cycle IVF, implantation rate, pregnancy rate, live birth rate

Abbreviations: cIVF, controlled gonadotropin stimulated IVF; E2, Estradiol; IVF, In vitro fertilization; NC-IVF, natural cycle IVF

Introduction

Adequate endometrial function is essential for the success of infertility treatments. Increasing estradiol (E2) levels induce proliferation of endometrial glands, stroma and endothelial cells in the follicular phase to allow the endometrium to become receptive after progesterone induced transformation.

Even though endometrium function is very complex and several endometrial factors contribute to its receptivity [1] reproductive physicians mainly rely on ultrasound parameters to evaluate the endometrium. Among various ultrasound parameters such as endometrial pattern and endometrial blood flow assessment, endometrial thickness is the easiest and most reliable parameter [2–4] and is therefore commonly being used.

The significance of endometrial thickness to achieve a pregnancy has been investigated in numerous studies. A systematic review and meta-analysis demonstrated a lower chance of pregnancy in gonadotropin stimulated IVF treatments if endometrial thickness is <8 mm [5]. This was also confirmed in unstimulated IVF treatments [6].

As the thickness of the endometrium had been confirmed as being imperative for high implantation rates, several approaches had been tested to increase endometrial thickness to achieve higher pregnancy rates.

Most studies involved frozen embryo transfers in which the effect of physiological or only slightly supra-physiological estrogen concentrations dosages were tested in different cycle regimes. The two recent Cochrane meta-analyses did not find sufficient evidence to support the use of a specific cycle regimen to prepare the endometrium for frozen embryo transfer [7,8]. For fresh embryo transfer data are less profound as the spectrum of approaches to increase endometrial thickness is limited.

One approach is not to manipulate endometrial growth at all and to allow endometrium to develop naturally as in spontaneous cycles. This approach mainly leads to monofollicular ovarian responses. Another approach is to slightly stimulate follicular growth with low dose gonadotropins. This approach also leads to monofollicular or possibly oligofollicular ovarian responses. A third approach is to stimulate endometrial growth with high dose gonadotropins, resulting mostly in polyfollicular ovarian responses.

As the first (natural cycles) and the second (low dose gonadotropins) approach has been extensively compared in frozen embryo transfers without finding a significant different in endometrial thickness and outcome [7,8], it can be assumed that both approaches are also similar regarding endometrial thickness and outcome in fresh embryo transfers.

The first (natural cycles) and the third (high dose gonadotropins) approaches however have hardly been systematically compared regarding endometrial thickness and its effect on implantation. Therefore, it is not known to which extent high dose gonadotropin stimulation has an effect on endometrial thickness and on the dynamics of endometrial growth. Furthermore, it is not known if a gonadotropin induced thickening of the endometrium by high dose gonadotropin stimulation increases pregnancy rates. The reason for the limited data is that a comparison of both strategies require treatment cycles without embryo selection and fresh transfers, a treatment combination which is usually not performed anymore.

We therefore took advantage of either the very restricted Swiss law, which did not allow embryo selection until 2017 and of the two large IVF programs in our center involving both natural cycle IVF (NC-IVF) and conventional gonadotropin stimulated IVF (cIVF). This allowed us to compare the effect of high dose gonadotropins and the associated high E2 serum concentrations on endometrial thickness, endometrial growth dynamics and on implantation rates of fresh cleavage embryos.

Materials and methods

Study population and participants

This is a retrospective, observational single center study performed between 2015 and 2019 at the University Women's Hospital of Bern. 435 women, 22-42 years of age with regular menstrual cycles (25-35 days) and basal Follicle-stimulating hormone (FSH) concentration <10IU/L undergoing NC-IVF and/or cIVF were screened. Women had been offered both kinds of therapies, but decided themselves which therapy they preferred to start with. Women without an embryo transfer, with endometriosis rASRM ≥ II (revised American Society of Reproductive Medicine classification of endometriosis: 1996, American Society for Reproductive Medicine) (as diagnosed by laparoscopy or clinical and ultrasound analysis), with fibroids as diagnosed by ultrasound or with other uterine pathology (e.g. uterine polyps, adhesions) and with sperm collection by testicular sperm extraction were excluded. Social and medical freezing-cycles, thawing cycles and cIVF cycles with poor response (≤3 oocytes) were not included.

IVF treatments

NC-IVF cycles were monitored using transvaginal ultrasound measurements of follicular diameter and endometrial thickness, together with determination of serum luteinizing hormone and E2 levels by electrochemiluminescence analysis. When the follicle diameter reached at least 18 mm and the E2 concentration was expected to be ≥700-800 pmol/L, 5000IU of Human chorionic gonadotropin (Choriomon®, IBSA Institute Biochimique SA, Lugano, Switzerland) were administrated and patients were scheduled 36 hours later for

oocyte retrieval. Oocyte pick-up was performed without anesthesia and without analgesia using 19G single lumen needles and follicles were flushed five times [9]. Embryos were transferred on day 2 to 3 as cleavage stage embryos. Luteal phase support was applied using vaginal micronized progesterone (Utrogestan®, Vifor Pharma SA, Villars-sur-Glâne, Switzerland) in patients with a short luteal phase. cIVF cycles were only analysed until 09 2017 when embryo selection was introduced in Switzerland. Before 09 2017 embryo selection was not possible as all zygotes left in culture must have been transferred as fresh embryos. Limiting the analysis to cycles with cleavage stage-embryo enabled us to compare embryos derived from NC-IVF and cIVF regarding outcome per transfer, as in case of NC-IVF the embryo transfer occurs when the embryo is in cleavage stage. cIVF involved the antagonist protocol using ovarian stimulation with gonadotropins (Merional®, IBSA Institute Biochimique SA, Lugano, Switzerland) at a dosage of 150 to 300IU and Ganirelix (Orgalutran®, MSD Merck Sharp & Dohme AG, Luzern, Switzerland) to inhibit luteinizing hormone surge. Ovulation was induced with recombinant Human chorionic gonadotropin (Ovitrelle®, Merck AG, Switzerland) 36 hours before oocyte pick-up. Oocyte pick-up was performed in the operating theatre and the embryo transfer was performed in analogy to NC-IVF two or three days after oocyte pick-up. Luteal phase support was applied using vaginal micronized progesterone (Utrogestan®, Vifor Pharma SA, Villars-sur-Glâne, Switzerland). Fertilization was performed by IVF or Intracytoplasmic Sperm Injection, depending on the sperm quality. Clinical (defined as ultrasound detection of amniotic sac) pregnancy rates and live birth rates were analysed per transferred embryo (Implantation rate). 681 endometrial thickness and 671 E2 measurements were performed on day -4 to -2 (day -2, day of ovulation trigger; day 0, day of oocyte pick up) in 616 NC-IVF cycles and in 235 c-IVF cycles. Each woman underwent 1.96 ± 1.45 IVF cycles on average.

Statistical Analysis

Cycle characteristics are presented in Table 1, stratified by treatment as number and percentage for each categorical variable. For continuous variables, we reported mean and standard deviation, median and interquartile ranges, as well as the minimum and maximum observed values. Treatment groups are compared by chi-squared and non-parametric Wilcoxon tests for categorical and continuous variables, respectively. Endometrial thickness

and E2 values measured in NC-IVF and cIVF patients at time points -4, -3 and -2 are presented in Table 2, stratified by treatment. As for baseline variables, we reported mean and standard deviation, median and interquartile ranges, as well as the minimum and maximum observed values, and compared the two treatment groups using Wilcoxon rank sum test. The effects of treatment and, more generally of E2 concentration, on the temporal evolution of endometrial thickness were investigated separately using repeated measure models adjusted for age and cause of infertility. In these models, age, treatment (or E2 concentration), time and the interaction between time and treatment (or E2 concentration) are included as fixed effects; patients and cycle within patient are declared random effects. To meet normality, assumed E2 concentrations were log transformed prior to analysis. The effect of endometrium thickness on clinical pregnancy and live births per transferred embryo were analysed using generalized estimating equation models for repeated measures, clustering on the patient level. To account for arbitrary correlation among observations within a patient, we declared an unstructured data correlation matrix and a robust variance estimator. Models were adjusted for the confounding effect of age, type of treatment and cause of infertility.

Informed written consent was obtained prior to treatment and the study was approved by the cantonal ethical committee Bern, Switzerland (KEK 2020-00634), 26.05.2020.

Results

435 women undergoing NC-IVF and/or cIVF cycle were identified. The basic characteristics of these cycles are shown in Table 1. Mean female age at the time when the cycles were performed was 35.8 ± 3.9 years for NC-IVF and 34.9 ± 4.2 for cIVF. Overall infertility factors were male factors (414 cycles, 48.6%), female factors (137 cycles, 16.1%), female and male factors (131 cycles, 15.4%) and idiopathic infertility (169 cycles, 19.9%). Age and cause of infertility were found to be slightly albeit significantly different between the two groups ($p = 0.006$ for age and 0.012 for causes of infertility).

Endometrial thickness and E2 concentrations related to day -4 to -2 are given in Table 2. Endometrial thickness increased in NC-IVF from 7.01 ± 1.42mm to 8.12 ± 1.66mm and in cIVF

from 9.34 ± 2.05mm to 9.75 ± 2.05mm between day -4 to -2 (day -2: day of HCG trigger). The temporal evolution of the endometrial thickness in NC-IVF and cIVF is shown in Figure 1. E2 concentration increased in NC-IVF from 439 ± 133pmol/L to 814 ± 220pmol/L and in cIVF from 4212 ± 3197pmol/L to 8094 ± 5366pmol/L between day -4 to -2. Endometrial thickness was higher in cIVF compared to NC-IVF ($p < 0.001$ on days -4,-3, and -2) (Figure 2, Table 2). Both, E2 concentrations and endometrial thicknesses were significantly different ($p < 0.001$) at each point between the two IVF treatments.

Looking at the dynamic of endometrial thickness over time, average endometrial thickness increased significantly over time in both treatment groups ($p < 0.001$) (Figure 1). When comparing the growth dynamics in the two groups a significant difference in endometrial growth over time was detected in NC-IVF and cIVF ($p = 0.033$), indicating a faster increase in NC-IVF cycles. The increase in thickness was estimated as 0.58mm/day [0.42, 0.73] in NC-IVF cycles and 0.22mm/day [-0.70, 0.03] in cIVF cycles between day -4 and -2. In line with this, during the three days before ovulation triggering, endometrial growth is significantly faster in cycles with low E2 concentrations (time x log (estrogen concentration): -0.19, $p = 0.010$).

Clinical pregnancy and live birth rates per transferred embryo (implantation rate) were found to be similar in NC-IVF and cIVF cycles (i.e. clinical pregnancy: 15.4% vs. 19.1%, $p = 0.2$; live birth: 11.7% vs. 12.8%, $p = 0.7$) (Table 3). We did not detect any significant effect of the endometrium thickness on either clinical pregnancy (OR 1.05, 95% CI 0.93, 1.18, $p = 0.44$) nor on live birth rates per transferred embryo (OR 1.03, 95% CI 0.9, 1.18; $p = 0.7$) after adjustment for female age, type of treatment and cause of infertility.

Discussion

Our study showed for the first time that endometrial growth dynamics are different in gonadotropin stimulated and unstimulated cycles with flattening of endometrial growth before triggering in gonadotropin stimulated cycles. We also found that endometrium is thicker in gonadotropin stimulated cycles at all time points analysed in this study. However, we could not demonstrate that the increase in endometrial thickness leads to higher pregnancy and live birth rates per transferred fresh embryo.

The strength of the study is the comparison of well-selected IVF treatment modalities such as stimulated IVF and NC-IVF, which allowed us to compare implantation and live birth rates of fresh cleavage stage embryos in treatments with different endogenous E2 productions.

The weakness of the study is its retrospective and non-randomized design requiring multiple statistical adjustments. However, even though a randomized study would be the preferred study design, such a study cannot be set up, as IVF treatments with transfer of cleavage stage embryos are not systematically performed anymore.

Our findings are, with some limitations, also relevant for other treatments such as thawing cycles. The study contributes to the so far unsolved question to which extent exposure to high estrogen serum concentrations increases endometrial thickness. It addresses the clinically relevant question if not only thick endometrium, as shown in several studies, but also if artificially thickening of the endometrium is associated with increased implantation rates.

A systematic review and meta-analysis of 22 studies found that clinical pregnancy rate is lower in gonadotropin stimulated cIVF cycles if endometrial thickness ≤7 mm compared to >7mm with a pregnancy rate of 23.3% vs 48.1% [OR 0.42 (95% CI 0.27-0.67)] [5]. Similar results were obtained in unstimulated NC-IVF cycles by von Wolff et al. [6] and Tomic et al. [10]. Von Wolff et al. [6] found in NC-IVF a lower pregnancy rate in women with an endometrial thickness of ≤7 mm compared to >7mm of 7.4% vs 30.8% (OR 5.56, 1.22-25.36) and Tomic et al. [10] described decreased pregnancy rates in women with an endometrial thickness of ≤10 mm.

Previous studies also evaluated the endometrial thickness in different IVF therapies and analysed the corresponding pregnancy rates. However, these studies were hardly suitable to study the effect of supraphysiological serum E2 concentration on endometrial thickness compared to unstimulated cycles.

Dickey et al. [2]; Gonen et al. [11]; Kovacs et al. [12] and Lenz and Lindenberg [13] compared endometrial thickness of gonadotropin stimulated IVF treatments and IVF treatments in combination with clomiphene citrate. However, as clomiphene citrate is known to reduce endometrial thickness [14] and as it has an effect on endometrial function [14,15] these studies only provide limited evidence of the effect of E2 on endometrial thickness and implantation rates. Zhang et al. [16] did not use clomiphene citrate but evaluated only IVF cycles with gonadotropin stimulation and did not include an unstimulated control group.

Randall et al. [17] did include an unstimulated control group but gonadotropin stimulated IVF cycles were combined with clomiphene citrate.

So far, only Ueno et al. [18] performed a study similar to ours and evaluated the impact of supraphysiological E2 concentration on endometrial thickness and its effect on pregnancy rates, using the model of NC-IVF and the model of gonadotropin stimulated IVF without embryo selection. They found significantly thicker endometrium in stimulated cycles compared to unstimulated cycles on the day of human chorionic gonadotropin (HCG) trigger, corresponding to day -2 in our study. Endometrial thickness was 10.6 ± 2.5 mm with E2 concentrations of 976 ± 607 pg/ml versus 8.9 ± 8 mm, with E2 concentrations of 223 ± 56 pg/ml (p=0.01). However, they did not compare implantation rates in both treatment groups.

Although the common result of a positive correlation between E2 serum concentration and endometrial thickness in the previous studies of Randall et al. [17], Zhang, et al. [16] and Bakos et al. [19], who studied 23 normal ovulatory healthy women (regression coefficient r = 0.79, p = 0.0001), it is still unknown whether the endometrium responds to ovarian hormones in a threshold or dose-response model.

The studies mentioned above of Randall et al. [17], Zhang, et al. [16] and Bakos et al. [19] demonstrated a dose-response model, where increased estradiol concentrations are associated with a thicker endometrium. According to our results, the endometrium could operate in a threshold model as the initially E2-promoted action of endometrial proliferation slowed down with increasing E2 concentration. However, in fact we cannot be certain, as we

have only examined a few cycle days and, as we may not have made use of the full growth potential of the endometrium by artificially inducing the ovulation as soon as E2 concentration was expected to be ≥700-800 pmol/L and the follicle diameter reached at least 18 mm.

We not only analysed the effect of gonadotropin stimulation on endometrial thickness but also compared the implantation rate in stimulated cycles and unstimulated controls. We confirmed that gonadotropin stimulation increased endometrial thickness, however, the gonadotropin induced thickened endometrium did not improve embryo implantation pregnancy rates. This raises several questions:

First, does our study allow any conclusion regarding the effect of gonadotropin stimulation in women with a thin endometrium? Our study did not focus on women with thin endometrium and was therefore not designed to evaluate the effect of increased E2 concentration specifically in these women.

Second, assuming that an increase of endometrial thickness by higher E2 concentrations does have a favorable effect on implantation, is the effect possibly neutralized by negative effects of very high E2 concentrations on endometrial physiology? Several studies indicate that very high E2 concentrations do alter endometrial function. Therefore, higher E2 concentrations might indeed neutralize an implantation favoring effect by negative effects on endometrial physiology.

Third, is the implantation facilitating effect possibly reduced by lower embryo quality in stimulated in cIVF? Mitter et al. have recently found in 996 cycles with the transfer of 1482 fresh embryos that embryos derived from cIVF have a lower implantation potential. NC-IVF derived embryos had a higher implantation (aOR 1.58; 95% CI 1.01-2.46; p = 0.042) and live birth rate (aOR 1.85; 95% CI 1.16-2.95; p = 0.010) than embryos derived from cIVF (unpublished results). Whether this difference is due to lower embryo quality in cIVF or due to impaired endometrial function as mentioned above is unknown. Therefore, higher E2 concentrations might also neutralize an implantation favoring effect by negative effects on embryo quality.

Fourth, is the number of patients included in our study too small to draw any conclusion of endometrial thickness on implantation rate? This is probably not the case as the number of included cycles was rather high (in total n = 851), and as the difference in implantation rate and live birth was very similar.

Accordingly, our study provides evidence that a substantial increase of endogenous E2 concentration, induced by the polyfollicular ovarian response due to gonadotropin stimulation, does lead to endometrial thickening but that this artificial increase in endometrial thickness does not facilitate implantation.

In summary,
Our study revealed that gonadotropin stimulation increases endometrial thickness, but that this gonadotropin induced thickening of the endometrium does not improve implantation. Accordingly, even though thicker endometrium is associated with higher pregnancy rates, artificial thickening is apparently not.

Author Contributions

IM and MvW contributed to conception and design of the study, to interpretation of data and prepared the manuscript.
AH contributed to the acquisition of data, MR performed the statistical analysis and PS contributed to the conception of the study.
All authors contributed to manuscript revision and approved the submitted version.

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Declaration of Competing Interest

none.

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Table 1: Cycle characteristics stratified by treatment group (NC-IVF and cIVF).

	Total (n=851)	**Natural cycle IVF (n=616)**	**Conventional IVF (n=235)**	**P-value**
Age at aspiration, years				0.006
Mean (SD)	35.6 (4.0)	35.8 (3.9)	34.9 (4.2)	
Median [IQR]	36.0 [33.0, 39.0]	36.0 [34.0, 39.0]	36.0 [32.0, 38.0]	
Range	22.0 - 42.0	22.0 - 42.0	24.0 - 42.0	
Causes of infertility, n (%)				0.012
Male factor	414 (48.6%)	297(48.2%)	117(49.8%)	
Female factor	137 (16.1%)	100 (16.2%)	37 (15.7%)	
Male and female	131 (15.4%)	83 (13.5%)	48 (20.4%)	
Idiopathic	169 (19.9%)	136 (22.1%)	33 (14.0%)	
Number of previous embryo transfers without pregnancy, n (%)				0.2

0	444 (52.2%)	316 (51.3%)	128 (54.5%)	
1-2	280 (32.9%)	213 (34.6%)	67 (28.5%)	
3-6	127 (14.9%)	87 (14.1%)	40 (17.0%)	

Table 2: Treatment characteristics on the day -4/-3/-2 (day -2 = day of ovulation trigger; day 0 = day of oocyte pick up).

	-4				**-3**				**-2**			
	Total, (n=274)	**NC-IVF[a] (n=191)**	**cIVF[b] (n=83)**	**P-value**	**Total (n=226)**	**NC-IVF[a] (n=169)**	**cIVF[b] (n=57)**	**P-value**	**Total (n=184)**	**NC-IVF[a] (n=150)**	**cIVF[b] (n=34)**	**p-value**
Endometrium (mm)				<0.001				<0.001				<0.001
Mean (SD)	7.72 (1.95)	7.01 (1.42)	9.34 (2.05)		8.12 (1.88)	7.65 (1.62)	9.53 (1.92)		8.41 (1.84)	8.12 (1.66)	9.75 (2.05)	
Median [IQR]	7.40 [6.40, 9.00]	7.00 [6.00, 7.90]	9.00 [8.00, 10.95]		8.00 [6.90, 9.10]	7.50 [6.60, 8.50]	9.20 [8.28, 10.30]		8.00 [7.05, 9.55]	8.00 [7.00, 9.10]	9.50 [8.70, 11.00]	
Range	4.00 - 16.00	4.00 - 12.10	5.10 - 16.00		3.00 - 15.20	3.00 - 15.20	5.70 - 15.00		4.50 - 16.00	4.50 - 12.10	5.90 - 16.00	
E2 (pmol/L)				<0.001				<0.001				<0.001
Mean (SD)	1’563 (2’453)	439 (133)	4’212 (3’197)		1’895 (2’634)	626 (197)	5’845 (2’796)		2’007 (3’453)	814 (220)	8’094 (5’366)	
Median [IQR]	488 [386, 1’811]	430 [348, 501]	3’639 [2’535, 5’402]		657 [540, 1255]	605 [488, 712]	5’272 [3’650, 7’427]		841 [672, 1’149]	790 [645, 976]	5’903 [4’950, 8’626]	
Range	195 - 26’167	195 - 1’136	679 - 26’167		183 - 15’597	183 - 1’339	1’487 - 15’597		286 - 23’496	286 - 1’408	3’084 - 23’496	

[a]NC-IVF: Natural Cycle IVF, [b]cIVF: controlled gonadotropin stimulated IVF

Table 3: Outcome parameters per transferred embryo.

	Total (n=699)	**Natural cycle IVF (n=332)**	**Conventional IVF (n=367)**	**p-value**
Clinical pregnancies (amniotic sacs per transferred embryo, Implantation rate) (Pregnancy rate, %)	121 (17.3%)	51 (15.4%)	70 (19.1%)	0.2
Live births (children per transferred embryo) (Live birth rate, %)	86 (12.3%)	39 (11.7%)	47 (12.8%)	0.7

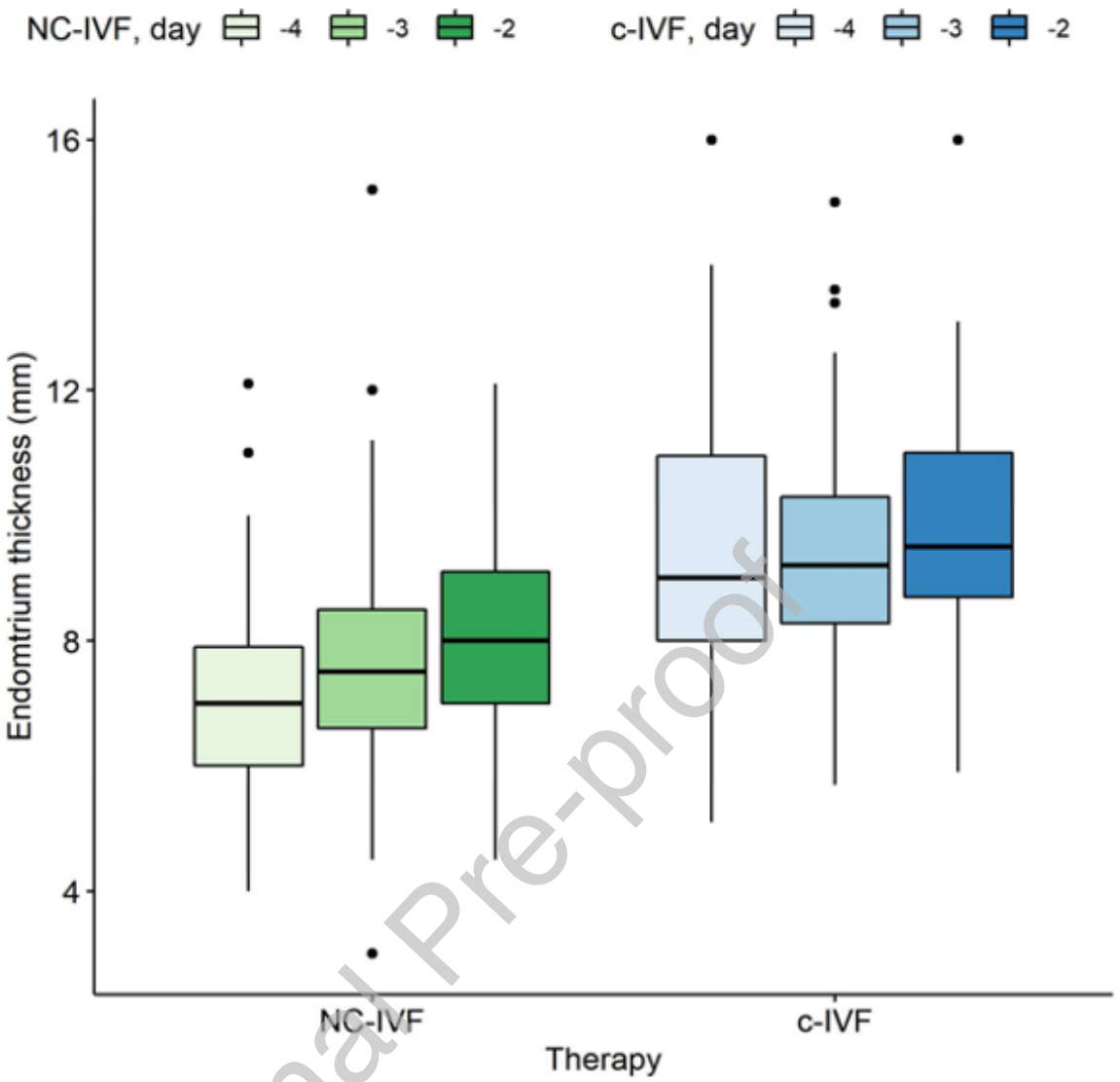

Figure 1: Temporal evolution of endometrial thickness in NC-IVF and cIVF on day -4 to -2 (day -2 = day of ovulation trigger).

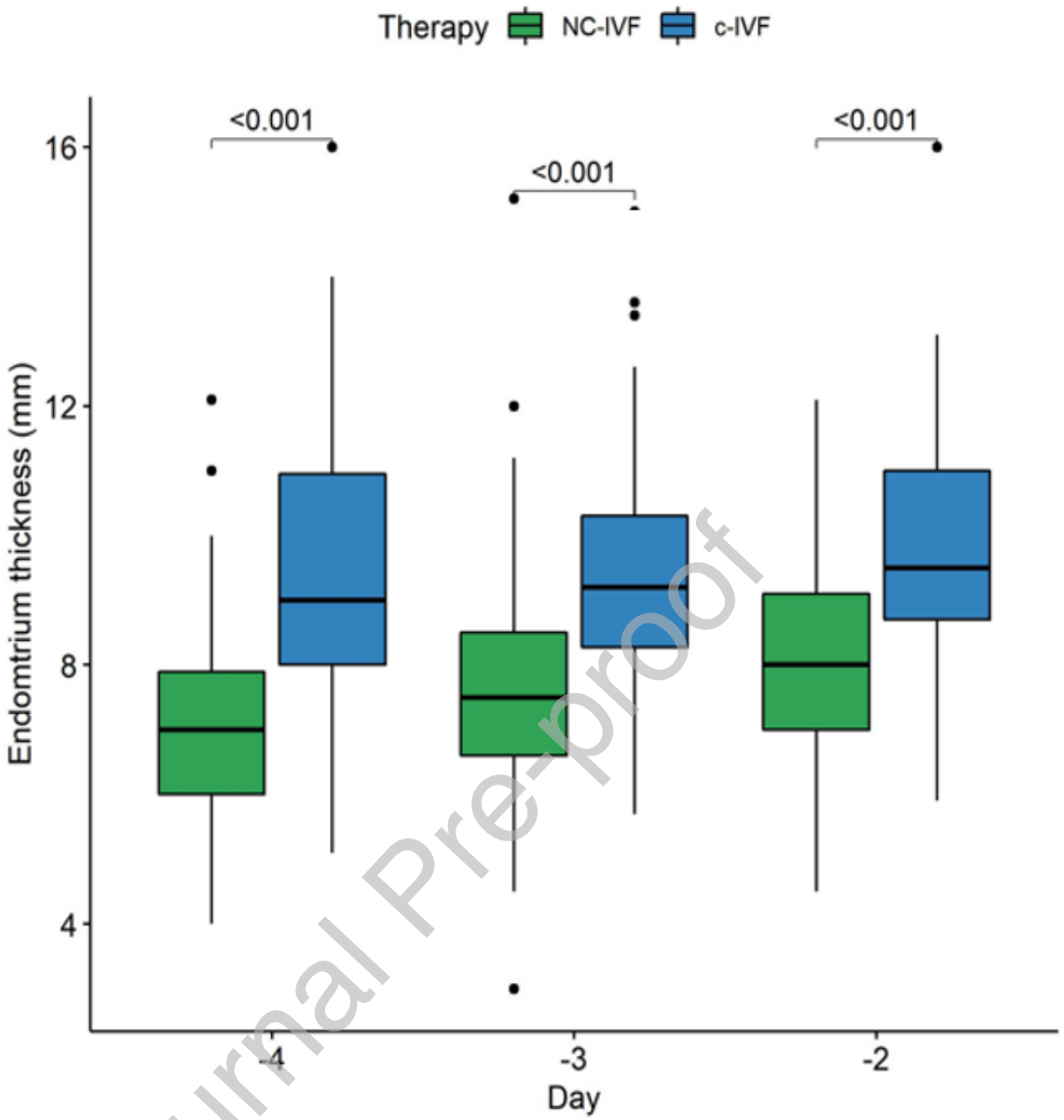

Figure 2: Endometrial thickness in NC-IVF and cIVF on day -4/-3/-2 (day -2 = day of ovulation trigger; day 0 = day of oocyte pick up).