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Do women with severely diminished ovarian reserve undergoing modified natural cycles benefit from earlier trigger at smaller follicle size?

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Running head: Trigger in severely diminished ovarian reserve

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Contribution

What are the novel findings of this work?

In patients with severely diminished ovarian reserve, oocyte maturation may be accelerated and triggering at conventional follicle sizes may place patients at increased risk of premature ovulation. Smaller follicle sizes at trigger in this population may preserve retrieval outcomes while reducing the risk of premature ovulation.

What are the clinical implications of this work?

To reduce the risk of premature ovulation, women with a severely diminished ovarian reserve might benefit from triggering at a smaller follicle size.

Abstract

Objective: Would trigger and oocyte collection at smaller follicle sizes decrease the risk of premature ovulation while maintaining the reproductive potential of oocytes in women with severely diminished ovarian reserve in modified natural cycle IVF?

Methods: Retrospective cohort study including women who had at least one unsuccessful cycle (due to no response) of conventional ovarian stimulation with a high dosage of gonadotropins and subsequently underwent a modified natural cycle with a solitary growing follicle (i.e., only one follicle above >10mm at the time of trigger). The association between follicle size at trigger and various cycle outcomes was tested with regression analyses.

Results: A total of 160 cycles from 110 patients were included in the analysis. Oocyte pick-up (OPU) was performed in 153 cycles, 7 cycles were canceled due to premature ovulation. Patients who received their trigger shot at smaller follicle sizes (≤15mm) had significantly lower premature ovulation and thus higher OPU rates (98.3% vs. 94.0%, adjusted OR: 8.55, 95% CI: 1.30 – 172.2, P=0.048) compared to those who received it at larger follicle sizes (>15mm). In the multivariable analyses, smaller follicle sizes at trigger (>10 to ≤13mm, >13 to ≤15mm, >15mm to ≤17mm) were not significantly associated with a lower rate of cumulus-oocyte-complex (COC), metaphase II oocytes (MIIs), or blastulation compared to the >17mm group. In sensitivity analyses including the first cycle of each couple, the maturity rate among those with a COC retrieval was highest in follicle sizes >15 to ≤17mm (92.3%) and >13 to ≤15mm (91.7%), followed by >10 to ≤13mm (85.7%) and lowest in the >17mm group (58.8%). Five euploid blastocysts developed from 48 fertilized MIIs during the study period with follicle sizes at trigger 12mm (3), 14 mm (1), and 16mm (1). Four were transferred resulting in two live births, both developing from follicles with a size at trigger of 12mm.

Conclusion: The ideal follicle size for triggering oocyte maturation may be smaller in women with severely diminished ovarian reserve managed on a modified natural cycle compared to conventional cut-offs. The risk of OPU cancellation was higher in women triggered above 15 mm, and the yield of mature oocytes was not adversely affected in women triggered at >13 to ≤15mm compared to >15mm

to ≤17mm. Waiting for follicles to reach sizes above 17mm may be detrimental to achieving optimal outcomes.

Introduction

The administration of the final oocyte maturation medication prior to oocyte pick-up (OPU), often referred to as the "trigger", is a decisive step in Assisted Reproductive Technology (ART) treatments and is critical to achieving oocyte competency. The trigger mimics the mid-cycle LH surge through LH-like exposure by administration of hCG or by inducing a LH surge through administration of GnRH-agonist, leading to the resumption of the oocyte maturation and the acquisition of fertilization competence[1,2].

Before the availability of pituitary suppression protocols, the trigger was administered around a follicle size of 17mm to prevent spontaneous ovulation[3]. The ESHRE guideline on ovarian stimulation[4] recommends a lead follicle size between 16-22mm as the contemporary standard for trigger. This recommendation is based on the systematic review and meta-analysis of Chen et al [5], however, all included studies[6–12] were conducted in a normal responder population. Therefore, the ESHRE recommended follicle size at trigger might not apply to all patient populations, particularly patients with a severely diminished ovarian reserve.

Treatment of patients with a severely diminished ovarian reserve is challenging and often resorts to natural cycle (NC) / modified NC / mild stimulation approaches[13]. Clinically, ovarian aging with the loss of oocytes is reflected through shortened menstrual cycles, which is entirely attributed to a shorter follicular phase. The very early follicular phase of these cycles is characterized by accelerated follicle growth, early estradiol (E2) rise, and LH surge, resulting in earlier ovulation at smaller follicle sizes[14,15]. Triggering at the "optimal" follicle size in this patient population becomes a dilemma: follicles "too small" might yield immature (GV / MI oocytes) and follicles "too big" post-mature oocytes, both not competent for fertilization[16]. Moreover, waiting for bigger follicle size increases the risk of premature ovulation[17,18].

The current study aims to investigate whether triggering at smaller follicle size can maintain the reproductive potential of the cycle while decreasing risk of premature ovulation in women with

severely diminished ovarian reserve. Oocyte yield, oocyte maturity, and oocyte competence in modified natural IVF cycles with a solitary follicular growth and varying follicle sizes at the time of trigger, were compared.

Methods

Data from autologous ovarian stimulation cycles were extracted from the clinical data system from September 2017 to May 2023. Women who had at least one unsuccessful cycle of conventional ovarian stimulation with a high dosage of gonadotropins prior to the current cycle (cancelled due to no response after a high dosage stimulation (450 IU of gonadotropins) for a minimum of 7 days) and who underwent a modified natural cycle were included. If the patient was not amenorrhoeic or menopausal (amenorrhea > 12 months), there were no requirements for starting monitoring for spontaneous follicle growth. Those with only one dominant follicular (>10mm) visible on the day of trigger and who received their final maturation trigger were included. Since there was only one dominant follicle present, adopting this approach allowed the unambiguous identification of the retrieved oocyte to the source follicle with its documented follicle size at the trigger time. An ultrasound scan was done on the oocyte pick up (OPU) day prior to admission of the woman to the operation theatre to exclude premature ovulation. In the case of premature ovulation, diagnosed by the absence of the previously documented follicle, the OPU procedure was cancelled. The included cohort had one of three possible outcomes: OPU procedure with retrieval of a single cumulus-oocyte complex (COC), OPU without retrieval of any COC, and OPU cancellation due to premature ovulation after the trigger shot administration and before the OPU.

Ovarian stimulation protocol and OPU

Patients were monitored from day 2 / 3 of the cycle onwards. Ovarian stimulations were performed as modified natural IVF cycle treatments. In contrast to a standard ovarian stimulation in a GnRH-antagonist protocol with a stimulation start in the early follicular phase, administration of gonadotropins (either rFSH (recombinant Follicle-stimulating-hormone) or human-Menopausal-Gonadotropin (HMG)) together with pituitary suppression (GnRH-antagonist) was only started after a spontaneous follicle development with at least one follicle above 10mm was seen, independent of the cycle day. Follicles were measured through vaginal ultrasound and the follicle size was calculated

independently of the follicle shape as the mean of the two greatest diameters, measurements taken perpendicular to one another. Clinical experience and critical analysis of the outcomes in this patient group led to a change in the clinical management of this specific patient population over time with a shift towards triggering final oocyte maturation at smaller follicle sizes. However, as the approach to trigger at smaller follicle sizes evolved from the experience from daily clinical practice, no exact threshold of follicle size for triggering was set and the trigger decision was taken according to physicians' discretion. Final oocyte maturation was achieved by administration of either 10.000 IU of hCG or dual trigger (hCG and GnRH-analogue), at the physicians' discretion. Oocyte retrieval was performed 34 hours after administration of the trigger to reduce the risk of premature ovulation before the planned procedure. Patients had an ultrasonographic assessment prior to admission to the operation theatre and OPU was cancelled in case of premature ovulation. As per routine clinical practice for poor responders [19], follicles were aspirated and flushed three times under ultrasound guidance and the cumulus oocyte complex was identified.

Oocyte insemination, embryo culture, and biopsy

Oocytes were either inseminated by conventional IVF or ICSI, depending on the semen quality and the embryologists' judgement of the oocyte. Normal fertilization was assessed 17–20h after insemination or injection. Embryo development was monitored up to 7 days. Only expanded blastocysts of sufficient quality (BL3CC or higher, according to Gardner criteria [20]) were subjected to trophectoderm (TE) biopsy on day 5, day 6, or day 7 of embryo development and preimplantation genetic testing for aneuploidies (PGT-A) by Next Generation Sequencing, using PicoPlex technology (Rubicon Genomics, Inc; USA [21,22]. PGT-A was performed according to clinical standard procedure. Embryos were cryopreserved after biopsy.

Mature oocytes were vitrified for later use in patients who desired oocyte accumulation or in cases when the husband or partner was unable to deliver a semen sample due to any reason.

Statistical analysis

Continuous variables were presented as mean and standard deviation or median and quartiles depending on distribution characteristics. Categorical variables were presented as count and percentage unless otherwise specified in the tables. Analyses were performed with mixed-effects logistic regression models. Women's age, ovarian reserve parameters, body-mass index (BMI) and trigger type were potential confounders and were included in the multivariable analyses. Follicle size was modelled by creating four groups (>10 to ≤13mm, >13 to ≤15mm, >15 to ≤17mm, >17mm). In some instances where superiority of one group over the other was suspected (i.e., premature ovulation analysis), follicle size was split into two groups (≤15mm and >15) to increase the power of the analysis. Analyses were also performed using follicle size at trigger on continuous scale and restricted cubic splines to obtain the predicted probabilities of certain outcomes. Models were adjusted for trigger type, BMI, Age and AMH levels. The knot count was based on model fit that was assessed with Akaike Information Criterion. Those who had premature ovulation were treated as no COC or MII retrieval in analyses with those target outcomes. A sensitivity analysis analyzing the first cycle of patient was also undertaken. All analyses were conducted with R for Statistical Computing Software 4.2.0 (R Foundation, Vienna, Austria).

Ethical approval

The retrospective study protocol was reviewed and approved by the local Research Ethics Committee of ART Fertility Clinics (REFA099).

Results

A total of 160 cycles from 110 patients were included in the analysis. In the cohort, 32 patients contributed with more than one cycle to the analysis while 78 patients had only one cycle analyzed. Among those with repeat cycles, the median cycle count was 2 (IQR: 2-3) while the maximum cycle count per patient was 5. Patients' characteristics (age, body mass index (BMI), anti-Mullerian-Hormone (AMH), Antral Follicle Count (AFC) and follicle size on day of trigger (DoT) as well as the medication used for final oocyte maturation and grouped follicles sizes (>10-≤13mm, >13-≤15mm, >15-≤17mm and >17mm) on the DoT are summarized in Table 1, stratified according to patients who underwent an OPU procedure (n = 153) or OPU cancelation due to premature ovulation (n = 7). No significant differences in the patients' characteristics were seen between patients who had OPU and in whom OPU was cancelled due to premature ovulation. However, a notable difference between trigger types were observed between the groups (hCG only trigger 51.6% vs. 85.7%, OPU and no OPU group, respectively, p=0.168). Premature ovulation after administration of the trigger medication occurred in 2.6% and 0.0% of those with smaller follicle sizes at trigger (>10 to ≤13mm and >13 to ≤15mm groups, respectively) and increased to 8.3% and 10.3% in the larger follicle size at trigger groups of >15mm to ≤17mm and >17mm groups, respectively. Patients who received their trigger shot at smaller follicle sizes (≤15mm) had significantly lower premature ovulation and thus higher OPU rates (98.3% vs. 94.0%, adjusted OR: 8.55, 95% CI: 1.30 – 172.2, P=0.048) compared to those who received it at larger follicle sizes (>15mm). The predicted probabilities of OPU procedure according to follicle size at trigger on a continuous scale is available in Figure 1. There was a mild drop in OPU rates after 15mm follicle size at trigger. Due to apparent differences in trigger type, we investigated the interaction of trigger type with follicle size and their association with OPU cancelation. Most of the premature ovulations after the trigger show happened in hCG only trigger group (n=6) but the type of trigger did not appreciably affect the slope of trigger size, OPU probability (Figure 2).

COC and mature oocyte retrieval

COC retrieval rates were 72.4%, 61.1%, 73.7% and 73.7% for >17mm, >15 to ≤17mm, >13 to ≤15mm, >10 to ≤13mm groups, respectively (Table 2, supplementary Figure S1). In the multivariable analysis, smaller follicle size at trigger groups were not significantly associated with a lower rate of COC retrieval compared to >17mm group (Table 3). The predicted probabilities of COC retrieval according to follicle size at trigger on a continuous scale is available in Figure 1. The probabilities were mostly flat across the follicle size range. Retrieval of any MII oocyte analysis showed the rate of 48.3%, 55.6%, 63.2% and 55.3% for >17mm, >15 to ≤17mm, >13 to ≤15mm, >10 to ≤13mm groups, respectively (Table 2). Again, smaller follicle size at trigger groups were not significantly associated with a lower rate MII retrieval in the multivariable analysis (Table 3).

MII recovery rate among COCs and fertilization of MII oocytes

The odds of recovering an MII oocyte among patients who had a COC retrieved was not significantly different between follicle size at trigger groups with rates standing at 66.7%, 90.9%, 85.7% and 75.0% for >17mm, >15 to ≤17mm, >13 to ≤15mm, >10 to ≤13mm groups, respectively (Table 2). Among those who had an MII oocyte, fertilization odds were again without significant difference between follicle size at trigger groups with rates standing at 66.7%, 64.7%, 64.5% and 81.2% for >17mm, >15 to ≤17mm, >13 to ≤15mm, >10 to ≤13mm groups, respectively (Table 2). The predicted probabilities of MII retrieval among those who had at least one COC according to follicle size at trigger on a continuous scale is available in Figure 1. The probabilities peaked around >13 to ≤17mm range with no observable difference between >13 to ≤15mm and >15 to ≤17mm. However, maturity rates of retrieved COCs dropped in both tails for follicle size at trigger (>10 to ≤13mm and >17mm).

Blastulation odds and transfer outcomes

In cycles with a fertilized oocyte, odds of blastulation of sufficient quality was not significantly different between the groups and rates were 50%, 40%, 52.6% and 61.5% for >17mm, >15 to ≤17mm, >13 to ≤15mm, >10 to ≤13mm groups, respectively (Table 2). From 24 embryos which underwent genetic

testing, 70.84% (17) of the embryos developed from oocytes from follicles ≤ 15 mm (5 out of 12 mm follicle size, 2 out of 13 mm, 5 out of 14 mm and 5 out of 15 mm, respectively). Five embryos were euploid, 1 from a 16 mm sized follicle, 1 from 14 mm and 3 from 12 mm follicles. Four patients underwent a frozen ET, resulting in 2 live births, 1 early miscarriage and 1 patient did not conceive. Both live births resulted from oocytes which were retrieved from follicle sizes of 12 mm each. The source oocytes of the embryo, resulting in a miscarriage, was aspirated from a follicle of 16 mm and of the embryo which did not lead to a pregnancy from a follicle of 14 mm. One embryo remains cryopreserved.

Sensitivity analysis for first cycles

We have conducted a sensitivity analysis including the first cycle of every patient (n=110). While OPU cancelation was higher in follicle sizes above 15mm compared to 15mm and below (10.4% vs 1.6%, 5/43 vs 1/61), the difference did not reach statistical significance in the sensitivity analysis (OR: 7.09, 95% CI: 1.09 – 138.5, P=0.078). Retrieval rates for any COC was similar between the follicle size groups (68.0%, 56.5%, 68.6% and 77.8%, P=0.53,0.63 and 0.26, >17mm vs. >15 to ≤17mm, >13 to ≤15mm, and >10 to ≤13mm, respectively) (Supplementary Table S1). Retrieval rate of mature oocytes were higher in those who were triggered at smaller follicle sizes compared to >17mm. (40.0%, 52.2%, 62.9% and 66.7%, P=0.31, 0.033 and 0.025, >17mm vs. >15 to ≤17mm, >13 to ≤15mm, and >10 to ≤13mm, respectively). The maturity rate among those with a COC retrieval was highest in follicle sizes >15 to ≤17mm (92.3%) and >13 to ≤15mm (91.7), followed by >10 to ≤13mm (85.7%) and lowest in the >17mm group (58.8%). In adjusted analyses, those who were triggered at >13 to ≤15mm had significant higher maturity rates compared to those triggered above 17mm (adjusted OR: 12.6, 95% CI: 2.01-129.0, P=0.014) (Supplementary Table S1).

Discussion

The evidence gathered from our retrospective study suggests that when final oocyte maturation is administered at a follicle size ≤ 15mm in women with severely diminished ovarian reserve, they have a lower probability of premature ovulation and OPU cancellation. The likelihood of retrieving an oocyte seems similar for follicles larger or smaller than 15mm. The optimal maturity rates were observed between the range of 13 and 17 and maturity rates dropped in both ends of the follicle size range. Similar blastulation rates were also observed across the range. Live births resulted from oocytes retrieved out of follicles as small as 12mm. These results suggest that the optimal trigger strategy in women with severely diminished ovarian reserve deserves further investigation.

The ESHRE recommended follicle size at trigger of 16 to 22mm is based on studies [6–9,11,12], which did not compare their outcomes based on different follicle sizes at the time of final oocyte maturation, but according to "early" and "late" trigger by solely postponing trigger administration. Postponement of trigger implies further follicle growth and subsequently rising systemic hormonal levels[23], a factor known to negatively impact pregnancy rates in fresh embryo transfer (ET) cycles [24]. The studies are conflicted regarding the impact of delaying the trigger on the maturity and fertilization rates: whereas Clark et al[6], Tan et al[8] and Kolibianakis et al[9] did not see a significant difference in the fertilization rate, Kyrou et al[25] and Morley et al[12] described a lower maturity rate in the early trigger group. In cycles of normal responders, in which follicle sizes were measured at the OPU itself (trigger criteria: at least two follicles ≥ 18mm), a decrease in the odds of oocyte maturity and fertilization was seen with decreasing follicle sizes. Yet, mature oocytes, usable for fertilization, and good quality embryos, can be derived from smaller follicle oocytes, hence at a lesser frequency than from larger follicles[26].

Albeit offering a guideline for the question "when to trigger", the ESHRE[4] recommended "trigger follicle size" should be critically appraised for women with severely diminished ovarian reserve. Physiologically, the process of ovarian aging is characterized by a shortened follicular phase and cycle length[27]. Follicle growth and ovulation pattern differ between women of advanced reproductive age and younger women, with "older" patients having a significantly bigger mean diameter of the largest

follicle compared to the younger group in the follicular phase[15]. The follicle growth in the older women seems to slow down towards the midcycle and they tend to have a smaller lead follicle at ovulation (approximately 15.2mm)[15], compared to younger women.

Following the pathophysiologic changes in the aging ovary it seems only natural to mimic physiology by adapting the trigger time to a "smaller than usual" follicle size in these patients. In a pilot study, the group of Wu et al[28] triggered women (aged 41 to 43 years), when the lead follicle reached 16mm (early group) and compared the outcomes to same aged patients, triggered at a lead follicle size of 19-21mm (late group). The early group had less atretic and more immature oocytes, but still an increase in good quality embryos per cycle (all results statistically significant), compared to the late trigger group. Furthermore, a trend for an improved outcome for the early group was seen for clinical pregnancy per ET and implantation rate. This benefit of an "early trigger" is further supported by our findings. Our data suggest that in women of advanced reproductive age and diminished ovarian reserve oocytes are likely to mature earlier, presenting an age-related change which is clinically reflected in accelerated follicle growth and shortened cycles.

Besides the severely diminished ovarian reserve, patients in the present study had an advanced maternal age (mean age of 42 years) and a higher risk for aneuploid embryos[29]. Five cycles (3.12%) yielded a euploid embryo, four embryos were transferred in frozen ET cycles, resulting in three pregnancies and two (1.25%) live births. Undoubtedly, the low likelihood of a live birth could prompt ethical and financial concerns about the relevance of treating these patients with autologous oocytes. However, women/couples usually may still want to use their own oocytes rather than relying on oocyte donation, despite the poor prognosis. In countries, in which gamete donation is banned by socio-cultural or religious norms[30] or prohibited by law, this treatment option might present the "last straw".

The limitations of the study are the observational design, the small sample size, and the highly individualized stimulation strategies in this patient population. Most of the "insignificant" results reported in this study can be attributed to the small sample size, especially in analyses involving non-

vitrified embryos, but the point estimates do not suggest an impaired outcome with smaller follicles. It is also likely that the point estimates are biased due to model overfitting. However, the results should be interpreted as hypothesis-generating and not as definitive or unbiased associations. If replicated by other groups, we believe that the data presented generate sufficient equipoise to merit an interventional trial of early trigger in poor responders, which would be the only way to demonstrate the efficacy of such an intervention. Strengths are the strict inclusion criteria by including only patients with solitary follicle growth with measurements performed on the day of final oocyte maturation, so that the retrieved oocyte could unambiguously be assigned to the punctured follicle and its size on the day of trigger.

Conclusion

The current data suggest that women with a severely diminished ovarian reserve might benefit from triggering at a smaller follicle size as the risk of losing an oocyte following premature ovulation is reduced and mature oocytes with unrestricted oocyte competency and the potential to lead to a live birth can be obtained out of follicles as small as 12mm at the time of final oocyte maturation. Due to the small sample size, caused by the strict inclusion criteria, further studies evaluating the “optimal” follicle size at trigger in women affected by a severely diminished ovarian reserve are warranted to confirm the approach of an “early trigger”.

Data availability statement: The data underlying this article are available in the article and in its online supplementary material.

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Figure legends

Figure 1: Probabilities oocyte pick-up, cumulus-oocyte-complex retrieval and retrieved cumulus-oocyte-complex being meta-phase II according to regression analyses. Analyses are adjusted for age, AMH, BMI, AFC, and trigger type.

Figure 2: Probability of oocyte pick-up according to the follicle size at time of the trigger.

Tables

Table 1. Overall population characteristics, stratified whether oocyte pick up (OPU) procedure was performed or not (OPU vs No OPU)

Variables	OPU (n = 153)	No OPU* (n = 7)	P
Age (years)	43.0 (41.0 to 44.0)	43.0 (40.0 to 45.0)	0.98
BMI (kg/m2)	30.4 (28.0 to 32.9)	27.1 (24.3 to 31.0)	0.10
AMH (ng/ml)	0.11 (0.04 to 0.21)	0.07 (0.02 to 0.20)	0.43
AFC, number	1.0 (1.0 to 2.0)	1.00 (1.0 to 2.0)	0.93
Trigger type, count (%)			0.16
• **Dual trigger**	74 (48.4)	1 (14.3)	
• **hCG trigger**	79 (51.6)	6 (85.7)	
Follicle size, count (%)			0.082
• **>17mm**	26 (17.0)	3 (42.9)	
• **>15 to ≤17mm**	33 (21.6)	3 (42.9)	
• **>13 to ≤15mm**	57 (37.3)	0 (0.0)	
• **>10 to ≤13mm**	37 (24.2)	1 (14.3)	
Follicle size, count (%)			0.037
• **≤15mm**	94 (61.4)	1 (14.3)	
• **>15mm**	59 (38.6)	6 (85.7)	

*Premature ovulation and OPU cancellation after trigger shot has been given

AFC: Antral Follicle Count; AMH: anti-Mullerian hormone; BMI: Body Mass Index; hCG: human Chorion Gonadotropin; OPU: oocyte pick-up

Table 2. Crude stimulation outcomes in follicle size at trigger groups.

Outcome	>17mm	>15 to ≤17 mm	>13 to ≤15 mm	>10 to ≤13 mm	P¶
Any COC retrieved, %*	21/29 (72.4%)	22/36 (61.1%)	42/57 (73.7%)	28/38 (73.7%)	0.55
Any MII retrieved, % *	14/29 (48.3%)	20/36 (55.6%)	36/57 (63.2%)	21/38 (55.3%)	0.45
MII/COC ratio, % †	14/21 (66.7%)	20/22 (90.9%)	36/42 (85.7%)	21/28 (75%)	0.65
Fertilization rate, % ‡	4/6 (66.7%)	11/17 (64.7%)	20/31 (64.5%)	13/16 (81.2%)	0.40
Blastulation rate, %•	2/4 (50%)	4/11 (36.4%)	10/20 (50.0%)	8/13 (61.5%)	0.38

* All population

† Among those who had at least one COC retrieved

‡ Among those not vitrified

• Among those fertilized

¶ Cochrane-Armitage test for trend

COC: cumulus-oocyte complex; MII: metaphase-II

Table 3. Retrieving any COC and MII in the whole cohort, mature oocyte retrieval in patients with COC retrieval and fertilized MII in patients with MII retrieval and no vitrification

	Levels	NO	YES	OR, 95%CI, p-value (univariable)	Adjusted OR, 95%CI, p-value (multivariable)
	OUTCOME: ANY COC RETRIEVED				
Age (years)	Mean (SD)	41.3 (4.5)	42.2 (3.8)	1.06 (0.97-1.15, p=0.20)	1.04 (0.96-1.14, p=0.32)
BMI (kg/m2)	Mean (SD)	27.6 (5.0)	27.8 (5.0)	1.01 (0.94-1.08, p=0.83)	1.03 (0.96-1.11, p=0.40)
AMH (ng/ml)	Mean (SD)	0.2 (0.2)	0.1 (0.2)	0.44 (0.07-3.05, p=0.39)	0.60 (0.08-4.30, p=0.61)
AFC (no)	Mean (SD)	2.0 (1.4)	1.6 (1.0)	0.77 (0.57-1.03, p=0.077)	0.77 (0.56-1.05, p=0.098)
Trigger type no (%)	Dual trigger	24 (32.0)	51 (68.0)	-	-
	hCG	23 (27.1)	62 (72.9)	1.27 (0.64-2.52, p=0.49)	1.36 (0.64-2.91, p=0.42)
Follicle size no (%)	>17mm	8 (27.6)	21 (72.4)	-	-
	>15 to ≤17mm	14 (38.9)	22 (61.1)	0.60 (0.20-1.69, p=0.34)	0.60 (0.20-1.81, p=0.36)
	>13 to ≤15mm	15 (26.3)	42 (73.7)	1.07 (0.38-2.87, p=0.90)	1.15 (0.40-3.31, p=0.78)
	>10 to ≤13mm	10 (26.3)	28 (73.7)	1.07 (0.35-3.17, p=0.90)	1.15 (0.35-3.81, p=0.82)
	OUTCOME: ANY MII RETRIEVED				
Age (years)	Mean (SD)	41.6 (4.1)	42.2 (4.0)	1.03 (0.96-1.12, p=0.39)	1.03 (0.95-1.13, p=0.46)
BMI (kg/m2)	Mean (SD)	27.7 (4.7)	27.7 (5.1)	1.00 (0.94-1.07, p=0.99)	1.02 (0.94-1.09, p=0.68)
AMH (ng/ml)	Mean (SD)	0.2 (0.2)	0.1 (0.2)	0.49 (0.08-2.97, p=0.43)	0.71 (0.10-5.15, p=0.73)
AFC (no)	Mean (SD)	1.9 (1.3)	1.6 (1.0)	0.81 (0.60-1.07, p=0.15)	0.81 (0.59-1.11, p=0.19)
Trigger type no (%)	Dual trigger	34 (45.3)	41 (54.7)	-	-
	hCG	35 (41.2)	50 (58.8)	1.18 (0.63-2.22, p=0.59)	1.29 (0.61-2.70, p=0.50)
Follicle size no (%)	>17mm	15 (51.7)	14 (48.3)	-	-
	>15 to ≤17mm	16 (44.4)	20 (55.6)	1.34 (0.50-3.61, p=0.56)	1.41 (0.48-4.18, p=0.53)
	>13 to ≤15mm	21 (36.8)	36 (63.2)	1.84 (0.74-4.60, p=0.18)	2.09 (0.74-5.85, p=0.16)

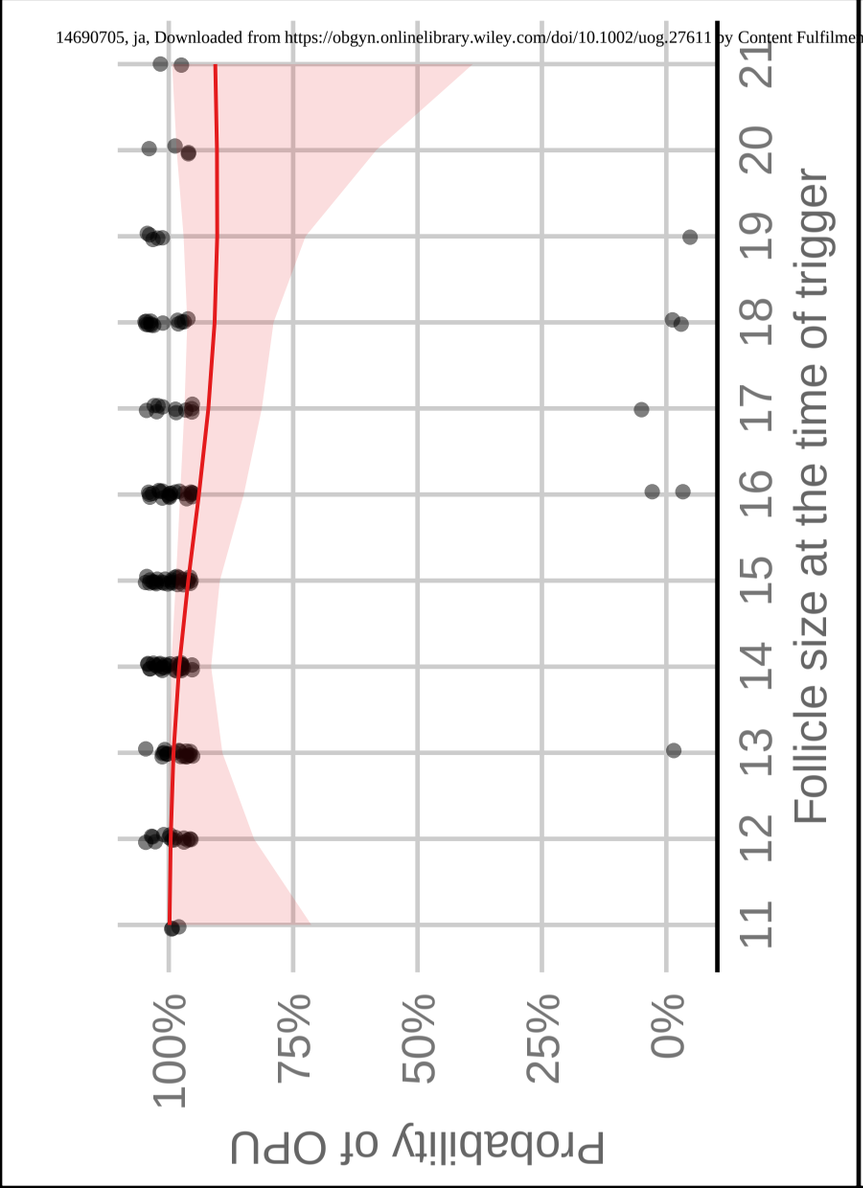
	>10 to ≤13mm	17 (44.7)	21 (55.3)	1.32 (0.50-3.52, p=0.57)	1.43 (0.46-4.40, p=0.53)
	OUTCOME: MII IN PATIENTS WITH A COC				
Age (years)	Mean (SD)	42.3 (3.0)	42.2 (4.0)	0.99 (0.86-1.12, p=0.86)	1.00 (0.70-1.41, p=0.98)
BMI (kg/m2)	Mean (SD)	28.0 (4.2)	27.7 (5.1)	0.99 (0.90-1.09, p=0.82)	1.01 (0.77-1.33, p=0.93)
AMH (ng/ml)	Mean (SD)	0.1 (0.2)	0.1 (0.2)	0.77 (0.07-14.46, p=0.84)	1.43 (0.00-540.73, p=0.90)
AFC (no)	Mean (SD)	1.7 (1.1)	1.6 (1.0)	0.96 (0.62-1.59, p=0.85)	0.89 (0.32-2.49, p=0.82)
Trigger type no (%)	Dual trigger	10 (19.6)	41 (80.4)	-	-
	hCG	12 (19.4)	50 (80.6)	1.02 (0.39-2.59, p=0.97)	1.08 (0.10-12.09, p=0.94)
Follicle size no (%)	>17mm	7 (33.3)	14 (66.7)	-	-
	>15 to ≤17mm	2 (9.1)	20 (90.9)	5.00 (1.03-37.07, p=0.066)	11.10 (0.29-420.80, p=0.19)
	>13 to ≤15mm	6 (14.3)	36 (85.7)	3.00 (0.86-10.90, p=0.086)	8.33 (0.46-151.99, p=0.15)
	>10 to ≤13mm	7 (25.0)	21 (75.0)	1.50 (0.43-5.32, p=0.52)	1.73 (0.08-35.82, p=0.72)
	OUTCOME: FERTILIZED MII				
Age (years)	Mean (SD)	43.4 (3.5)	41.5 (4.5)	0.88 (0.75-1.01, p=0.092)	0.89 (0.76-1.04, p=0.13)
BMI (kg/m2)	Mean (SD)	28.5 (6.2)	28.0 (4.6)	0.98 (0.88-1.08, p=0.69)	0.93 (0.83-1.05, p=0.23)
AMH (ng/ml)	Mean (SD)	0.2 (0.3)	0.1 (0.2)	0.33 (0.02-4.43, p=0.38)	0.18 (0.01-3.64, p=0.26)
AFC (no)	Mean (SD)	1.4 (0.7)	1.8 (1.1)	1.60 (0.90-3.41, p=0.16)	1.71 (0.90-3.26, p=0.10)
Trigger type no (%)	Dual trigger	12 (33.3)	24 (66.7)	-	-
	hCG	10 (29.4)	24 (70.6)	1.20 (0.44-3.35, p=0.72)	1.47 (0.47-4.58, p=0.504)
Follicle size no (%)	>17mm	2 (33.3)	4 (66.7)	-	-
	>15 to ≤17mm	6 (35.3)	11 (64.7)	0.92 (0.10-6.30, p=0.93)	0.78 (0.08-7.70, p=0.83)
	>13 to ≤15mm	11 (35.5)	20 (64.5)	0.91 (0.11-5.48, p=0.92)	0.69 (0.08-6.32, p=0.74)
	>10 to ≤13mm	3 (18.8)	13 (81.2)	2.17 (0.23-18.48, p=0.47)	1.71 (0.14-21.33, p=0.67)

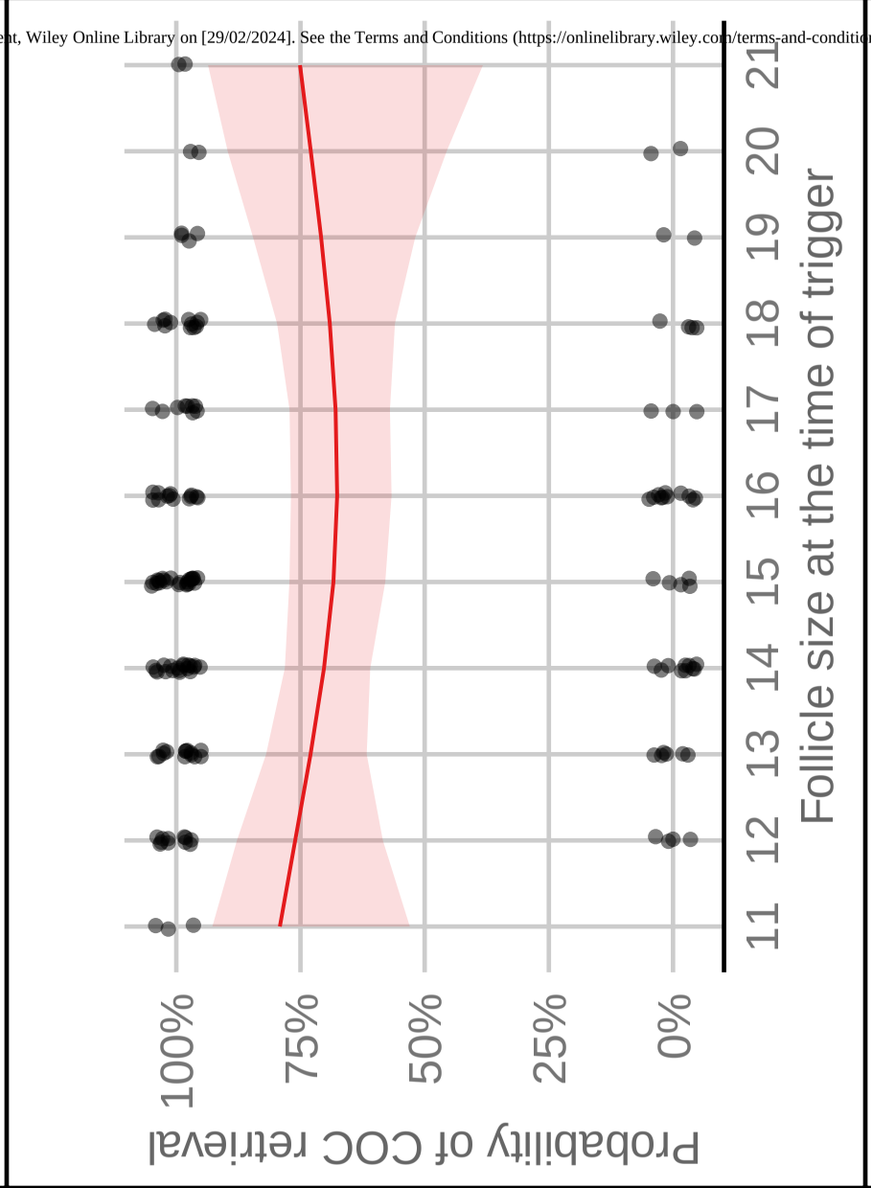
AFC: Antral Follicle Count; AMH: Anti-Muellerian-Hormone; BMI: Body Mass Index; CI: Confidence Interval; hCG: human Chorion Gonadotropin; SD: Standard Deviation; COC: cumulus-oocyte complex; MII: metaphase-II; OR: Odds Ratio.

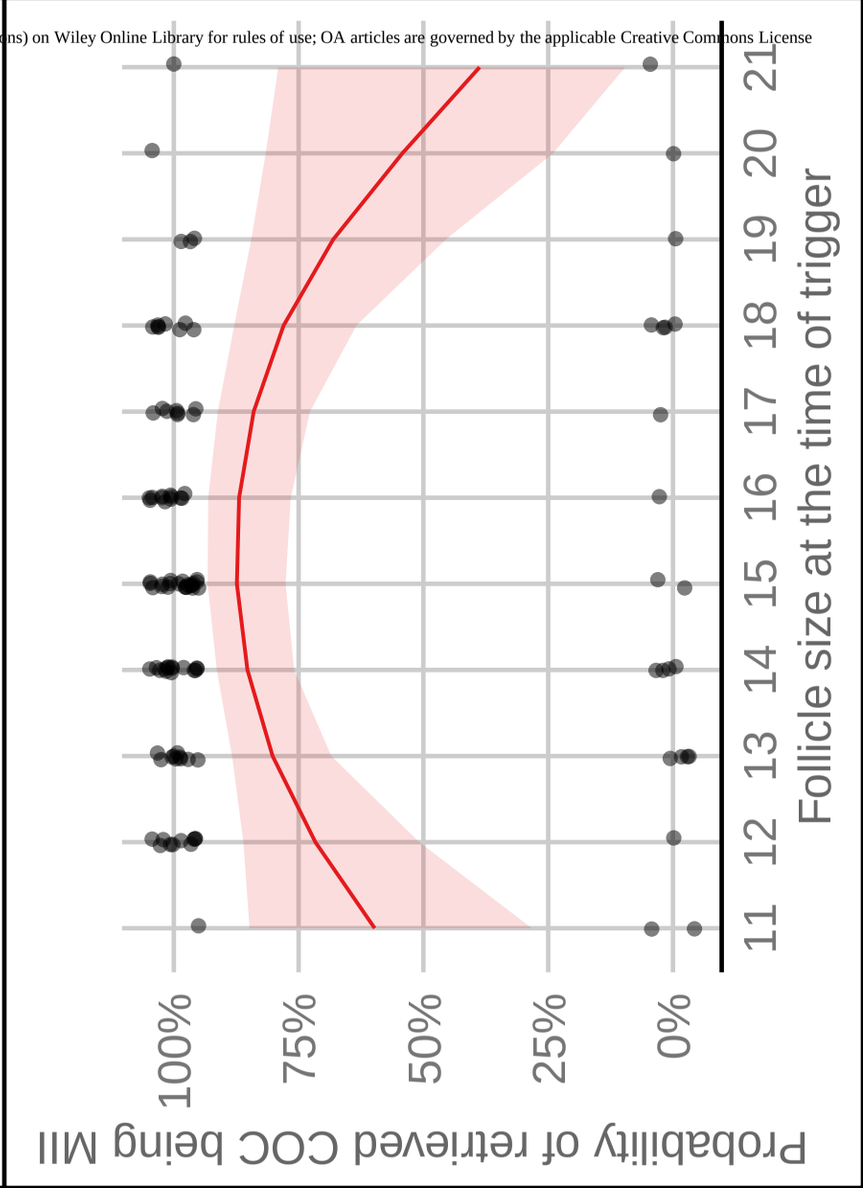
Table 4. Development of blastocyst of sufficient quality (BL3CC or higher) for a biopsy

	Levels	NO	YES	OR, 95%CI, p-value (univariable)	OR, 95%CI, p-value (multivariable)
	OUTCOME: BLASTULATION OF SUFFICIENT QUALITY				
Age (years)	Mean (SD)	42.7 (4.0)	40.1 (4.7)	0.86 (0.73-0.99, p=0.058)	0.84 (0.69-1.02, p=0.078)
BMI (kg/m2)	Mean (SD)	28.1 (4.0)	28.8 (4.7)	1.04 (0.90-1.20, p=0.60)	0.97 (0.79-1.20, p=0.78)
AMH (ng/ml)	Mean (SD)	0.1 (0.1)	0.2 (0.2)	5.62 (0.12-524.17, p=0.39)	55.90 (0.18-17183.27, p=0.16)
AFC (no)	Mean (SD)	1.7 (1.4)	1.8 (1.0)	1.09 (0.64-1.92, p=0.75)	0.71 (0.30-1.66, p=0.43)
Trigger type no (%)	Dual trigger	13 (54.2)	11 (45.8)	-	-
	hCG	9 (40.9)	13 (59.1)	1.71 (0.53-5.63, p=0.37)	4.24 (0.69-25.89, p=0.11)
Follicle size no (%)	>17mm	2 (50.0)	2 (50.0)	-	-
	>15 to ≤17mm	7 (63.6)	4 (36.4)	0.57 (0.05-6.34, p=0.63)	1.16 (0.09-15.06, p=0.91)
	>13 to ≤15mm	10 (50.0)	10 (50.0)	1.00 (0.10-9.73, p=0.99)	1.97 (0.17-22.93, p=0.58)
	>10 to ≤13mm	5 (38.5)	8 (61.5)	1.60 (0.15-17.28, p=0.68)	4.22 (0.26-69.81, p=0.31)

AFC: Antral Follicle Count; AMH: anti-Mullerian-Hormone; BMI: Body Mass Index; CI: Confidence Interval; hCG: human Chorion Gonadotropin; SD: Standard Deviation; COC: cumulus-oocyte complex; MII: metaphase-II; OR: Odds Ratio.







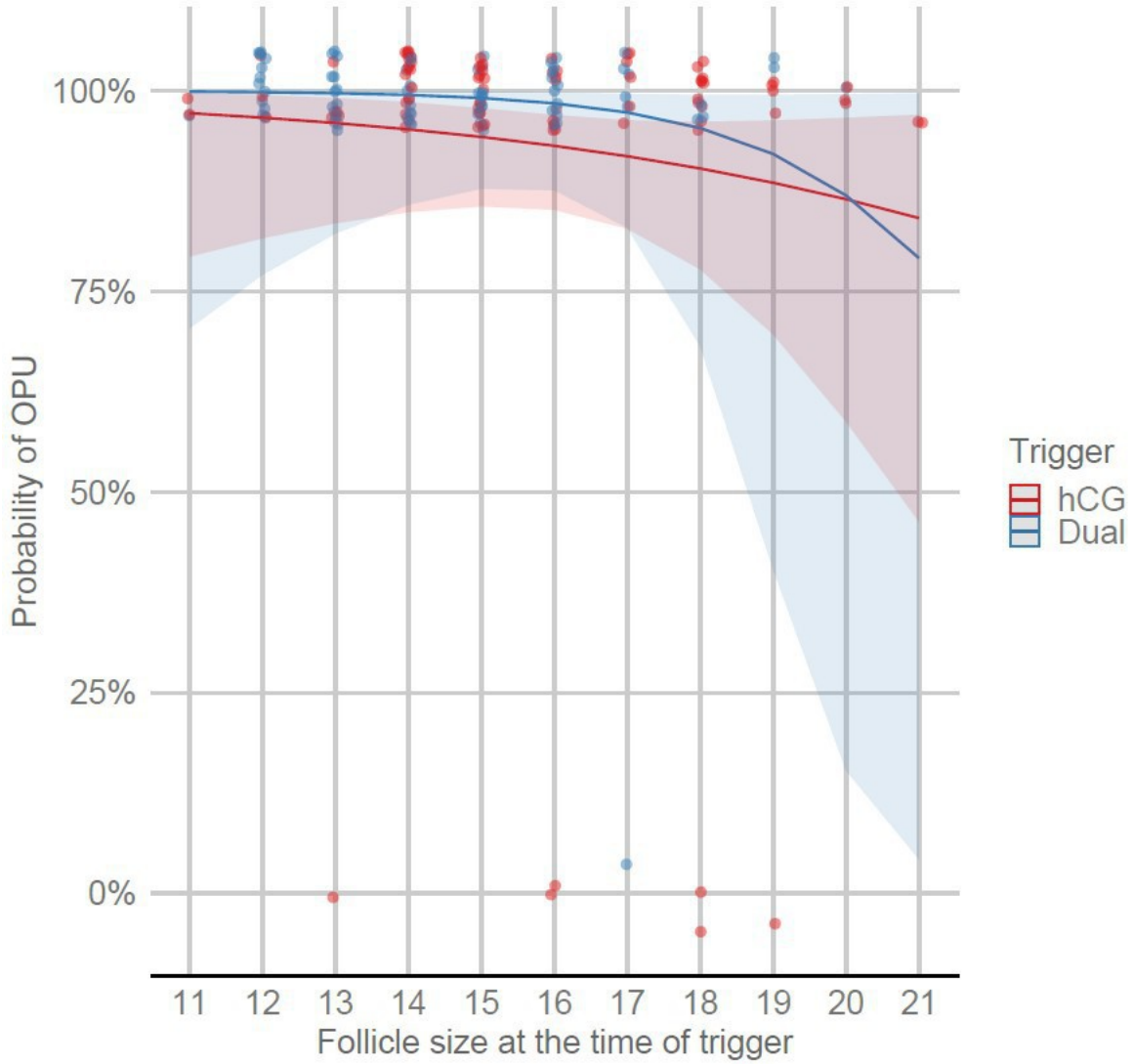


Figure 2.jpg