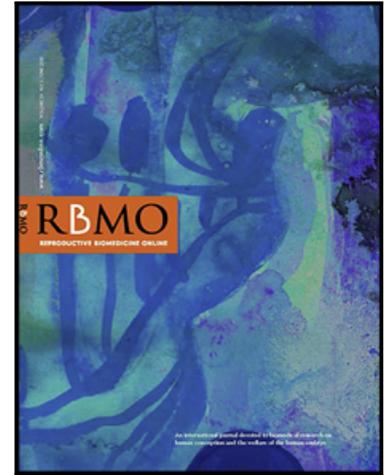# Journal Pre-proof

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Oocyte maturity, oocyte fertilization and cleavage stage embryo morphology are better in natural compared to high dose gonadotropin stimulated IVF cycles

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Declaration of interest: none.

Abstract

Research question

Does high dose gonadotropin stimulation have an effect on oocyte and early-stage embryo development?

Design

Retrospective study including 616 natural cycle IVF (NC-IVF) and 167 conventional IVF (cIVF) cycles. In total, 2110 oocytes were retrieved and analyzed in fresh cycles. In NC-IVF, only human chorionic gonadotropin to trigger ovulation was applied. In cIVF, antagonist protocols with daily 150-300IU of human menopausal gonadotropins were performed. The effect of gonadotropins on oocyte and early-stage embryo development was analyzed. Primary outcomes were the occurrence of mature (metaphase II) oocytes, of zygotes as well as of embryos with good morphology at the cleavage stage 2 days after oocyte retrieval.

Results

The mature oocyte ratio (number of mature oocytes/number of retrieved oocytes) was higher in NC-IVF compared to cIVF cycles (89% vs. 82%, adjusted (a) OR 1.79, p=0.001) as was the zygote ratio per retrieved oocytes (70% vs. 58%, aOR 1.76, p=0.001) and the zygote ratio per mature oocytes (79% vs. 71%, aOR 1.62, p=0.001). The percentage of zygotes, which developed into a cleavage stage embryo, was not different. For the transferred embryos, the probabilities of having a good embryo morphology with 4 blastomeres and a fragmentation of <10% (score 0) in cleavage stage embryos were found to be higher in NC-IVF (proportional aOR for 4 blastomeres 2.00, p<0.001; for a fragmentation score 0 aOR 1.87, p=0.003).

Conclusions

Oocyte maturity, oocyte fertilization as well as cleavage stage embryo morphology are affected by high dose gonadotropin stimulation in fresh IVF cycles.

Keywords: oocyte, fertilization, cleavage stage embryo, embryo morphology, natural cycle IVF.

Introduction

Gonadotropins have revolutionized in vitro fertilization (IVF) as they increase the number of follicles, retrievable oocytes and therefore the IVF success rate. However the majority of oocytes collected after ovarian stimulation are unable to develop into viable embryos because many of them are morphologically, cytogenetically or metabolically abnormal as described by Inge *et al.*, 2005; Dayal *et al.*, 2006; Patrizio *et al.*, 2007a; Patrizio and Silber, 2017 and reviewed by Patrizio *et al.*, 2007b.

A clinical study assessed the real biological efficiency of IVF calculating the live birth rate in relation to the number of oocytes retrieved and revealed that approximately only 5% of fresh oocytes lead to a live born child (Patrizio and Sakkas, 2009). With that said, the use of gonadotropins to obtain maximum numbers of oocytes is under debate (Fauser *et al.*, 1999; Edwards, 2007; Alper and Fauser, 2017). This raises the question, whether high dose exogenous gonadotropins may have a negative effect on oocyte quality, defined as the potential and ability to undergo meiotic maturation and fertilization, to achieve embryonic development and clinical pregnancy (Palmerini *et al.*, 2022).

Silber *et al.*, 2017 evaluated the intrinsic natural fertility of 14'185 natural cycle IVF oocytes and confirmed that the intrinsic fertility is greater in natural cycles than reported in cycles with gonadotropin hyperstimulation. In line with this, transferred cleavage stage embryos, generated in natural cycle IVF (NC-IVF), have a higher potential to generate a live birth

compared to embryos generated by gonadotropin stimulated IVF (aOR 1.85; 95%CI: 1.16-2.95) (Mitter *et al.*, 2021).

These differences might be due to changes of the physiology and endocrinology of follicles induced by exogenous gonadotropins as shown by Kollmann *et al.*, 2017 and Von Wolff *et al.*, 2014, von Wolff *et al.*, 2022.

However, studies on the effects of gonadotropin stimulation on oocyte and embryos quality are limited in humans. In mice and farm animals several studies have been performed. These studies revealed impaired oocyte and embryo quality induced by gonadotropin stimulation (Ertzeid and Storeng, 1992; Van Der Auwera and D'Hooghe, 2001; Lee *et al.*, 2017; Di Nisio *et al.*, 2018; Uysal *et al.*, 2018; Karl *et al.*, 2021). In line with this, a study in mice has shown that embryos from gonadotropin stimulated mice donors transferred to control recipients have a lower implantation rate when compared to embryos of unstimulated mice (Ertzeid and Storeng, 2001).

The reasons for the negative effects of gonadotropin stimulation might be manifold. Di Nisio *et al.*, 2018 have shown in mice that gonadotropin stimulation impairs the oocyte spindle and Uysal *et al.*, 2018 that the epigenetic mechanism of DNA methylation is altered. Furthermore, Lee *et al.*, 2017 revealed an increased mitochondrial deformity in mice oocytes after gonadotropin stimulation, characterized by the formation of vacuolated mitochondria.

In humans so far only one *in vivo* study directly analyzed the effect of gonadotropin stimulation on embryo quality (Ziebe *et al.*, 2004). Even though this study very elegantly performed an intra-individual comparison between embryos generated by gonadotropin stimulated IVF and embryos generated by natural thawing cycles of the same patients, the study was only performed in long agonist protocols and only focused on embryo morphology. We therefore aimed to evaluate the impact of gonadotropin stimulation in

antagonist protocols. Furthermore, we aimed not only to analyze the cleavage stage embryos as done by Ziebe *et al.*, 2004, but also focused on oocyte maturity and oocyte fertilization rate.

We compared oocytes and embryos generated from 616 cycles of natural cycle IVF (NC-IVF) with 167 cycles generated in conventional gonadotropin stimulated IVF (cIVF).

Materials and methods

Study population and participants

A retrospective, observational single centre study was performed between 2015 and 2019 at the University Women's Hospital of Bern. Polyfollicular cIVF cycles were only analyzed until September 2017 when embryo selection was introduced in Switzerland. Before September 2017, only zygote selection was possible which allowed comparison of NC-IVF and cIVF treatments. All laboratory technologies and media were the same in all IVF cycles analyzed. Women 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. Furthermore, social and medical freezing-cycles, thawing cycles, cIVF cycles with poor response (3 oocytes), cycles with more than two embryos transferred and cycles without oocyte retrieval were also excluded. Patients with polycystic ovary syndrome were not considered for the study because in most cases they present an irregular cycle, which does not allow to perform NC-IVF (Palomba *et al.*, 2017; Palomba, 2021).

After a detailed explanation of the IVF therapies modality including the differences between cIVF and NC-IVF, the women, in which both therapy modalities came into question, decided themselves which therapy they preferred to try. A switch to the other therapy modality was always possible after the completion of the cycle.

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.

IVF treatments

NC-IVF cycles were monitored using transvaginal ultrasound measurements of follicular diameter and endometrial thickness, together with determination of serum luteinizing hormone (LH) and estradiol (E2) levels by electrochemiluminescence analysis. When the diameter of the single follicle reached around 18 mm and the E2 concentration was expected to be 700-800 pmol/L, 5000 IU of human chorionic gonadotropin (hCG) (Choriomon®, IBSA Institute Biochimique SA, Lugano, Switzerland) was administered and patients were scheduled 36 hours later for oocyte retrieval. Oocyte pick-up was performed without anaesthesia using 19G single lumen needles and each single follicle was flushed five times (Kohl Schwartz et al., 2020). Embryos were transferred on day 2 or 3 as cleavage stage embryos. All mature oocytes were fertilized by intracytoplasmic sperm injection. Luteal phase support was applied using vaginal micronized progesterone (Utrogestan®, Vifor Pharma SA, Villars-sur-Glâne, Switzerland) in patients with a short luteal phase (< 12 days). Conventional gonadotropin stimulated IVF (cIVF) was performed by antagonist protocol using ovarian stimulation with human menopausal gonadotropins (Menotropin, 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 LH

surge. Ovulation was induced with recombinant hCG (Ovitrelle®, Merck AG, Switzerland) 36 hours before oocyte pick-up. Oocyte pick-up was performed in the operating theatre and all mature oocytes were fertilized by intracytoplasmic sperm injection (ICSI). In accordance with the Swiss law, most zygotes had to be frozen at the zygote stage and typically only 1 or 2 zygotes were cultured for two days to the cleavage stage. Embryo transfer was performed in analogy to NC-IVF two or three days after oocyte retrieval. Luteal phase support was applied using vaginal micronized progesterone (Utrogestan®, Vifor Pharma SA, Villars-sur-Glâne, Switzerland).

Outcome definition

Primary outcomes were the occurrence of mature (metaphase II) oocytes, zygotes, and the embryo with an ideal morphology at the cleavage stage 2 days after oocyte retrieval. Secondary outcomes included pregnancy and live birth.

Embryo morphology was determined based on the number of blastomeres, the percentage of fragmentation (10% = score 0; 11-20% = 1; 21-30% = 2; >30% = 3) and blastomere symmetry (equal = score 1, different = 2). Embryo morphology was assessed 44 ± 1 hour after ICSI. Embryos with 4 blastomeres, < 10% fragmentation and equal symmetry of blastomeres were assumed ideal (Giorgetti et al., 1995; Terriou et al., 2001). Pregnancy was defined as ultrasound detection of amniotic sac. Implantation and live birth ratios were defined as amniotic sacs per transferred embryo and birth of living child per amniotic sacs, respectively.

Statistical Analysis

Patients and cycles characteristics are presented in table 1, stratified by treatment as number and percentage for each categorical variable. For continuous variables, median and interquartile ranges, as well as the minimum and maximum observed values were reported. For the outcomes of interest we calculated the following statistics overall and within each treatment group:

- mature oocyte ratio (number of mature oocytes/number of retrieved oocytes),

- zygote ratio per retrieved oocyte (number of zygotes/number of retrieved oocytes),

- zygote ratio per mature oocyte (number of zygotes/number of mature oocytes = fertilization rate),

- implantation ratio (number of pregnancies/number of transferred embryos = implantation rate),

- live birth ratio (number of live births/number of clinical pregnancies).

Cleavage stage morphology of transferred embryos was evaluated by calculating the proportion of embryos with low fragmentation score (score 1), a high symmetry score (score 1) and an ideal number of blastomeres (n = 4).

The effect of treatment (NC-IVF vs. cIVF) on all the outcomes listed above was assessed independently using crude and adjusted Generalized Estimating Equation (i.e. GEE) models with an exchangeable data correlation matrix. When the number of events was n >40, models were adjusted for the confounding effects of age, number of previous embryo transfers (3 categories: [0], [1-2] and [3-6]) and causes of infertility. To account for the structure of our dataset (i.e., measures can be taken on different cycles from the same patient), we used a robust variance estimator and included a clustering effect of the patient into the GEE models. Finally, we assessed the robustness of our results by conducting a

sensitivity analysis for which we considered only the first reported cycle of each patient (supplement 1, 2).

Statistical analysis was conducted in R Version 4.2.1 (2022-06-23).

Results

The patient`s and cycle`s characteristics are shown in table 1.

In total 419 women, 22-42 years of age with regular menstrual cycles (25-35 days) and normal basal follicle-stimulating hormone (FSH) concentration (< 10 IU/L) undergoing NC-IVF and/or cIVF were included in the study. Of these 419 women, 279 (67%) underwent only NC-IVF, 129 (31%) only cIVF, and 11 (2.6%) both IVF treatment modalities (table 1). Our data set hence includes 783 cycles, 167 cIVF cycles (from 140 women, median number of cycles per patient = 1) and 616 NC-IVF cycles (from 290 women, median number of cycles per patient = 2) (table 1). To allow comparison of data only NC-IVF cycles performed between 2015 to 2019 and cIVF cycles performed between 2015 to 2017 were compared as embryo selection was prohibited in Switzerland until 2017. Accordingly, all cycles were performed without embryo selection.

For NC-IVF, the percentage of cycles with at least 1 retrieved oocyte was 80%. For cIVF, 1615 oocytes were retrieved (100% of the cycles). The median number of retrieved oocytes per cIVF cycle was 10.

Median female age at the time when the cycles were performed was 36.0 [33, 38] years for NC-IVF and 34.0 [31, 37] for cIVF. Overall infertility factors were male factors (n=206, 48%), female factors (n=72, 17%), female and male factors (n=66, 15%) and idiopathic infertility (n=83, 19%). Most common female factors were tubal pathologies and endometriosis rASRM I.

The main outcomes of the study are shown in tables 2 and 3 and figure 1.

The mature oocytes ratio was higher in the NC-IVF group than in the cIVF group (89% vs 82%, adjusted (a) OR 1.79, p=0.001) (table 2). In addition, the zygotes ratio per retrieved oocyte was also higher in NC-IVF (70% vs. 58%, aOR 1.76, p<0.001) (figure 1) as was the zygote ratio per mature oocytes (fertilization rate) (79% vs. 71%, aOR 1.62, p=0.001, table 2).

The percentage of zygotes which developed in a cleavage stage embryo was the same in both IVF treatment groups (97% for NC-IVF and 97% for cIVF, p=0.56, table 2).

Regarding the embryo morphology on day 2, the odds of having transferred embryos with an ideal number of blastomeres and a fragmentation score 0 were both found to be significantly higher in NC-IVF treatment (aOR 2.0, p<0.001 for 4 blastomeres; aOR 1.87, p=0.003 for fragmentation score 0). No significant difference was found for the symmetry of blastomeres (table 3).

The implantation ratio and live birth ratio per detected amniotic sacs were found to be similar in NC-IVF and cIVF cycles (clinical pregnancy: 15% vs. 19%, p=0.327; live birth: 76% vs. 68%, p 0.438).

For all outcomes listed above, similar results were found when considering only the first reported cycle of each patient (supplement 1, 2).

Discussion

The main objective of this study was to analyze the impact of gonadotropin stimulation on the maturity of oocytes, the fertilization of the oocytes and the morphology of cleavage stage embryos using the models of (natural) NC-IVF and (gonadotropin stimulated) cIVF.

The major finding of this study is the higher ratio of fertilized oocytes in NC-IVF compared to cIVF. Furthermore, it was found that the proportion of mature oocytes per retrieved oocytes

is higher in NC-IVF and that gonadotropin stimulation has a moderate but significant impact on embryo morphology.

The strength of the study is the high number of included cycles, the mono-centre design with the same laboratory techniques applied throughout the study period, the same embryologists categorizing the embryos to minimize the inter-observer variability of embryo morphology determination (Paternot *et al.*, 2011) and the legal situation in Switzerland, which prohibited embryo selection and thereby gave us the unique chance to compare NC-IVF and cIVF treatment cycles. Furthermore, all embryo transfers were performed fresh and 2-3 days after oocyte retrieval, which allowed us to compare the pregnancy and live birth ratio.

The weakness of the study is its retrospective design and the inclusion of several treatment cycles per patient as well that some patients performed both kind of IVF, even though this was considered in the statistical analysis by using a robust variance estimator that took care of arbitrary correlation among observations within a patient and by performing a sensitivity analysis considering only the first reported cycles of each patient.

Although the study is retrospective, we included in the analysis only patients with causes of infertility that should not have a direct impact on oocyte or embryo quality, mainly excluding all cases of endometriosis rASRM   II and all cases with testicular sperm extraction. NC-IVF was chosen as a model for natural cycles. Although, due to the administration of exogenous hCG, the cycles were not completely natural as hCG administration might have some functional impact on oocytes such as increasing the risk of spindle misalignment and chromosomal mis-segregation(Hodges *et al.*, 2002).

Our study compared for the first time not only oocyte and embryo parameters, but also analyzed the pregnancy and live birth ratios as embryo selection was not performed.

However, in cIVF a maximum of 2 zygote were cultivated and the surplus zygotes were cryopreserved by default, which might have led to some bias in pregnancy and live birth ratio, favouring cIVF.

We did not find a difference in pregnancy and live birth ratios. The reasons might be the low power due to the limited number of embryos and the rather low pregnancy ratio after the transfer of day 2/3 embryos. Furthermore, other confounders such as functional differences of the endometrium due to high blood concentrations of serum E2 or the use of GnRH antagonists as in cIVF might have had an impact on the success rates.

Furthermore, we have to be aware that although good embryo morphology has been shown to be a predictor of pregnancy in gonadotropin stimulated IVF (Giorgetti *et al.*, 1995; Terriou *et al.*, 2001), morphology in cleavage stage embryos does not correlate with the chromosomal status of the embryos (Majumdar *et al.*, 2017) which itself is a very relevant predictor of implantation. Due to these limitations of cleavage stage embryo morphology, we defined pregnancy and live birth ratios only as secondary study outcomes.

Interestingly, Mitter et al. 2021 also compared NC-IVF and cIVF cycles from the same IVF centre. In contrast to our study, they found a slightly higher pregnancy (aOR 1.87; 95% CI 1.21-2.91) and live birth ratio (aOR 1.85; 95%CI: 1.16-2.95) in NC-IVF after adjustment for maternal age, parity, primary or secondary infertility and indication for IVF. The reason for the difference might be the inclusion of a larger number of cycles, inclusion of only cycles with an embryo transfer, a different time period of the analysis and the use of registry data from the Swiss IVF registry.

Ziebe *et al.*, 2004 had also compared the morphology of embryos generated by NC-IVF and cIVF. They performed an intra-individual comparison, but analyzed fewer cycles (125 NC-IVF and 177 cIVF cycles) and included only long agonist protocols. The number of embryos with

4 cells was 59% in NC-IVF vs. 53% in cIVF and the number of embryos with < 10% fragmentation was 69% vs. 61%. The numbers were not statistically different, even though the morphology parameters appeared to be slightly better in NC-IVF. It remains unclear if the morphology of the embryos in the study by Ziebe *et al.*, 2004 would have reached significance if the number of cycles included in the study had been higher.

Our study also addressed the maturity of oocytes retrieved in NC-IVF vs. cIVF. As expected, the percentage of mature (metaphase II) oocytes was higher in NC-IVF. Even though this finding has not been described before, it was not unexpected as in cIVF large and medium sized follicles are aspirated whereas in NC-IVF only large follicles are aspirated.

We considered the maturity ratio because in general the number of immature oocytes is considered a marker of poor oocyte quality (Lee *et al.*, 2011; Astbury *et al.*, 2020). Astbury *et al.* compared patients in whom germinal vesicle staged immature oocytes where retrieved with patients without retrieval of immature oocytes. The presence of immature oocytes correlated significantly with lower implantation rate (11.8% vs. 30.2%, p = 0.02) and live birth rate (1.9% vs. 5.7%, p = 0.02). The authors concluded that the presence of immature oocytes reflects poor oocyte quality.

The most striking difference between NC-IVF and cIVF is the higher fertilization rate of mature oocytes, which has also not been published before. This result clinically supports the hypothesis that oocyte competence is negatively affected by high dose gonadotropin stimulation as demonstrated many studies on animals (Ertzeid and Storeng, 1992; Van Der Auwera and D'Hooghe, 2001; Lee *et al.*, 2017; Di Nisio *et al.*, 2018; Uysal *et al.*, 2018; Karl *et al.*, 2021). In fact, they show that gonadotropin stimulation impairs oocyte spindle (Di Nisio *et al.*, 2018), has an impact on epigenetic mechanism of DNA methylation (Uysal *et al.*, 2018) and may affect mitochondrial function (Lee *et al.*, 2017).

The process of follicle recruitment requires precise regulation and selection of follicles, involving several oocyte-granulosa cells bidirectional paracrine and junctional signalling, which is essential for the acquisition of oocyte competence for maturation and fertilization (Eppig, 2001). Furthermore, the nuclear and cytoplasmic maturity of the oocyte that accompanies follicular development plays a crucial role in facilitating fertilization and the early stages of embryonic development (Albertini *et al.*, 2003). The resumption of the first meiotic division is initiated by the preovulatory surge of LH by an indirect action mediated by cumulus cells. In case of cIVF the natural sequence of events including the source and changing of the hormone concentration is altered with the constantly high dose of gonadotropins.

Differences in follicular physiology can also be found at the endocrine and molecular level in follicular fluid. Follicular fluid is different in cIVF regarding its immune cell profile affecting heterogeneously many cytokines and leukocytes as well as lymphocytes (Kollmann *et al.*, 2017). The concentrations of LH, androgens and E2 (von Wolff *et al.*, 2014; von Wolff *et al.*, 2022) and the concentration of anti-Mullerian hormone (von Wolff et al., 2014), which is a marker for the potential of the embryo to implant (Ciepiela *et al.*, 2019) are significantly reduced in cIVF follicular fluid. Furthermore, gonadotropin stimulation has some effect on follicular fluid signalling proteins (Bersinger *et al.*, 2021) and disrupts the quantitative association of follicular fluid proteins with cumulus cell proteins and RNA (von Wolff *et al.*, 2022).

All these studies provide evidence that exogenous high dose gonadotropin stimulation does indeed affect oocyte function.

This raises the question if our study results also have clinical implications. Our findings might be relevant in poor and especially very poor responders. In very poor responders with a very

low ovarian reserve, gonadotropin stimulation does not increases oocyte yield, nor overall live birth rate. Gonadotropin stimulation might even decrease the implantation rate as shown by De Marco *et al.*, 2021 for advanced-age poor responders.

In conclusion,

our results contribute to the concept that high dose exogenous gonadotropins have an effect on oocyte and embryo quality. However, if this effect also has an impact on pregnancy and live birth rate remains to be evaluated.

Appendices

Table 1: Patients and cycles baseline characteristics in natural cycle IVF (NC-IVF) and conventional IVF (cIVF).

Table 2: Oocyte and embryo development in NC-IVF and conventional IVF (cIVF).

Table 3: Embryo morphology of cleavage stage embryos (day 2 after oocyte retrieval) in NC-IVF and conventional IVF (cIVF).

Figure 1: Mature (metaphase II) oocytes and zygotes (fertilized oocytes), both per retrieved oocytes in NC-IVF and conventional IVF (cIVF) (** = p <0.01; *** = p <0.001).

Supplement 1: Oocyte and embryo development in NC-IVF and conventional IVF (cIVF). Sensitivity analysis according to table 2 but with the inclusion of only the first reported NC-IVF or cIVF cycle of each patient.

Supplement 2: Embryo morphology of cleavage stage embryos (day 2 after oocyte retrieval) in NC-IVF and conventional IVF (cIVF). Sensitivity analysis according to table 3 but with the inclusion of only the first reported NC-IVF and cIVF cycle of each patient.

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Table 1: Patients and cycles baseline characteristics in natural cycle IVF (NC-IVF) and conventional IVF (cIVF) and overall.

	NC-IVF	cIVF	Overall[2]
Number of patients, n	290	140	419
Number of cycles, n	616	167	783
Number of patients who underwent more than 1 IVF cycle, n (%)	152 (52%)	23 (16%)	178 (42%)
Therapies performed by the patients, n (%)			
NC-IVF			279 (67%)
cIVF			129 (31%)
NC-IVF and cIVF			11 (2.6%)
Number of cycles per patient, n			
Median [IQR]	2 [1,3]	1 [1,1]	1 [1,2]
Range [min, max]	2 [1, 10]	1 [1, 4]	1 [1,10]
Number of retrieved oocytes, n total	495	1615	2110
Number of retrieved oocytes per patient, n			
Median [IQR]	1 [1,2]	10 [7,15]	2 [1,7]
Range [min, max]	1 [0, 7]	10 [4, 31]	2 [0,31]
Number of cycles with at least 1 retrieved oocyte, n (%)	495 (80%)	167 (100%)	662 (85%)
Female age at aspiration, years[1]			
Median [IQR]	36 [33, 38.85]	34 [31, 37]	36 [32, 38]
Range [min, max]	36 [22, 42]	34 [24, 42]	36 [22, 42]
Causes of infertility, n (%)			
Male factor	140 (48%)	68 (49%)	201 (48%)
Female factor	46 (16%)	26 (19%)	72 (17%)
Male and female	38 (13%)	29 (21%)	65 (16%)
Idiopathic	66 (23%)	17 (12%)	81 (19%)
Number of previous embryo transfers without pregnancy, n (%)[1]			
0	208 (72%)	106 (76%)	307 (73%)
1-2	68 (23%)	28 (20%)	94 (22%)
3-6	14 (4.8%)	6 (4.3%)	18 (4.3%)
Number of cycles with embryo transfer/cycles, n (%)	331/616 (54%)	139/167 (83%)	470/783 (60%)
Number of single embryo transfer/cycles with embryo transfer, n (%)	331/331 (100%)	26/139 (18.7%)	357/470 (76%)
Number of double embryo transfer/cycle with embryo transfer, n (%)	0/331 (0%)	113/139 (81.3%)	113/470 (24%)

[1]Values calculated from the mean value of each patient.
[2]As some patients underwent both treatments; the overall population is not a sum of the NC-IVF and cIVF populations.

Table 2: Oocyte and embryo development in NC-IVF and conventional IVF (cIVF)

	NC-IVF	cIVF	crude			adjusted		
			OR[a]	CI	p-Value	OR[a]	CI	p-Value
Retrieved oocytes	495	1615	-	-	-	-	-	-
Mature oocyte ratio	**441/495 (89%)**	**1326/1615 (82%)**	**1.79**	**[1.28, 2.49]**	**0.001**	**1.79**	**[1.26, 2.53]**	**0.001**
Zygote ratio per mature oocytes (Fertilization rate)	**348/441 (79%)**	**941/1326 (71%)**	**1.49**	**[1.13, 1.97]**	**0.004**	**1.62**	**[1.21, 2.18]**	**0.001**
Cleavage stage embryo ratio	339/348 (97%)	258/267[b] (97%)	1.31	[0.52, 3.29]	0.561	n.a.	n.a.	n.a
Embryo transfer ratio (day 2 transfer)	331/339 (98%)	252/258 (98%)	0.94	[0.29, 3.12]	0.925	n.a.	n.a.	n.a.

[a]OR >1 favor NC-IVF.
[b]72% of zygotes were frozen according to the Swiss law.

Table 3: Embryo morphology of cleavage stage embryos (day 2 after oocyte retrieval) in NC-IVF and conventional IVF (cIVF)

	NC-IVF	cIVF	crude			adjusted		
			OR[a]	CI	p-Value	OR[a]	CI	p-Value
Number of blastomeres			**1.93 (4 vs. <4 or >4)**	**[1.35, 2.75]**	**<0.001**	**2.00 (4 vs. <4 or >4)**	**[1.37, 2.90]**	**<0.001**
4	171 (52%)	90 (36%)						
<4 or >4	159 (48%)	162 (64%)						
Missing	1	0						
Fragmentation score			**1.92 (0 vs. 1-3)**	**[1.29, 2.85]**	**0.001**	**1.87 (0 vs. 1-3)**	**[1.24, 2.80]**	**0.003**
0	219 (67%)	129 (51%)						
1-3	110 (33%)	122 (49%)						
Missing	1	2						
Symmetry score			1.08 (1 vs. 2)	[0.75, 1.57]	0.677	1.09 (1 vs. 2)	[0.74, 1.60]	0.676
1	197 (60%)	144 (57%)						
2	133 (40%)	108 (43%)						
Missing	1	0						

[a]proportional OR. OR >1 favors NC-IVF.

Vitae

Isotta Magaton was born in St. Gallen, Switzerland and studied Medicine in Basel, Switzerland. She has been training in gynaecological endocrinology and reproductive medicine since 2019 at the University Women`s Hospital, Inselspital Bern, Switzerland. Her main interest is natural and minimal stimulation IVF.

Key Message

Oocyte maturity, oocyte fertilization and cleavage stage embryo are affected by high dose gonadotropin stimulation in fresh IVF cycles. However, if this effect also has an impact on pregnancy and live birth rate remains to be evaluated.

Figure 1