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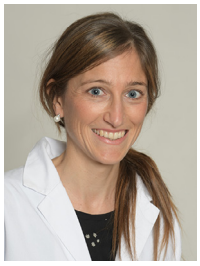
ARTICLE

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



BIOGRAPHY

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.

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KEY MESSAGE

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

ABSTRACT

Research question: Does high-dose gonadotrophin stimulation have an effect on oocyte and early-stage embryo development?

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

Results: The mature oocyte rate (number of mature oocytes/number of retrieved oocytes) was higher in NC-IVF than cIVF cycles (89% versus 82%, adjusted odds ratio [aOR] 1.79, $P = 0.001$), as was the zygote rate per oocyte retrieved (70% versus 58%, aOR 1.76, $P = 0.001$) and the zygote rate per mature oocyte (79% versus 71%, aOR 1.62, $P = 0.001$). The percentage of zygotes that developed into cleavage-stage embryos was no different. For the transferred embryos, the probability of having a good embryo morphology with four blastomeres and a fragmentation of <10% (score 0) in cleavage-stage embryos was found to be higher in NC-IVF (proportional aOR for four blastomeres 2.00, $P < 0.001$; aOR 1.87 for a fragmentation score of 0, $P = 0.003$).

Conclusions: Oocyte maturity, oocyte fertilization and morphology of the cleavage-stage embryo are affected by high-dose gonadotrophin stimulation in fresh IVF cycles.

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KEYWORDS

Cleavage-stage embryo
Embryo morphology
Fertilization
Natural cycle IVF
Oocyte

INTRODUCTION

Gonadotrophins have revolutionized IVF as they increase the number of follicles and retrievable oocytes, and therefore the IVF success rate. However, the majority of oocytes collected after ovarian stimulation cannot develop into viable embryos because many of them are morphologically, cytogenetically or metabolically abnormal (described by *Inge et al., 2005*; *Dayal et al., 2006*; *Patrizio et al., 2007a*; *Patrizio and Silber, 2017*; reviewed by *Patrizio et al., 2007b*).

A clinical study has assessed the real biological efficiency of IVF by 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*). That said, the use of gonadotrophins to obtain maximum numbers of oocytes is under debate (*Fauser et al., 1999*; *Edwards, 2007*; *Alper and Fauser, 2017*). This raises the question of whether high-dose exogenous gonadotrophins might have a negative effect on oocyte quality, defined as the potential and ability to undergo meiotic maturation and fertilization, and to achieve embryonic development and clinical pregnancy (*Palmerini et al., 2022*).

Silber and colleagues (*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 is reported in cycles with gonadotrophin stimulation. 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 with embryos generated by gonadotrophin-stimulated IVF (adjusted odds ratio [aOR] 1.85, 95% confidence interval [CI] 1.16–2.95) (*Mitter et al., 2021*). These differences might be due to changes in the physiology and endocrinology of follicles induced by exogenous gonadotrophins, as shown by Kollmann and co-workers (*Kollman et al., 2017*) and Von Wolff and colleagues (*Von Wolff et al., 2014*, *2022*).

However, studies on the effects of gonadotrophin stimulation on oocyte and embryo quality are limited in humans. Several studies have been performed in mice and farm animals. revealing impaired oocyte and embryo quality induced by gonadotrophin 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 gonadotrophin-stimulated donor mice transferred to control recipients had a lower implantation rate compared with embryos from unstimulated mice (*Ertzeid and Storeng, 2001*).

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

In humans, so far only one in-vivo study has directly analysed the effect of gonadotrophin stimulation on embryo quality (*Ziebe et al., 2004*). Although this study very elegantly performed an intra-individual comparison between embryos generated by gonadotrophin-stimulated IVF and embryos generated in natural cycles in the same patients, the study was performed only in long agonist protocols and focused only on embryo morphology. The objective of the current study was therefore to evaluate the impact of gonadotrophin stimulation in antagonist protocols. Furthermore, it aimed not only to analyse cleavage-stage embryos, as was done by Ziebe and colleagues (*Ziebe et al., 2004*), but also to focus on oocyte maturity and oocyte fertilization rate.

Oocytes and embryos generated from 616 cycles of NC-IVF were compared with those from 167 cycles generated in conventional (gonadotrophin-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 analysed only until September 2017, when embryo selection was introduced in Switzerland. Before September 2017, only zygote selection was possible, which allowed a comparison of NC-IVF and cIVF treatments. All the laboratory technologies and media were the same in all the IVF cycles analysed.

The following groups were excluded from the study: women with endometriosis stage II or more by the 1996 revised American Society of Reproductive Medicine (ASRM) classification of endometriosis as diagnosed by laparoscopy or clinical and ultrasound analysis; women with fibroids as diagnosed by ultrasonography; women with other uterine pathology (e.g. uterine polyps or adhesions); and cases where sperm collection had been by testicular sperm. Furthermore, social and medical freezing cycles, thawing cycles, cIVF cycles with a poor response (≤3 oocytes), cycles with more than two embryos transferred and cycles without oocyte retrieval were also excluded. Participants with polycystic ovary syndrome were not considered for the study because in most cases they present an irregular cycle, which does not allow NC-IVF to be performed (*Palomba et al., 2017*; *Palomba, 2021*).

After a detailed explanation of the IVF therapy modalities including the differences between cIVF and NC-IVF, the women themselves decided which therapy they preferred to try. A switch to the other therapy modality was always possible after 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 May 2020).

IVF treatments

NC-IVF cycles were monitored using transvaginal ultrasound measurements of follicular diameter and endometrial thickness, together with a determination of serum LH and oestradiol concentrations by electrochemiluminescence analysis. When the diameter of the single follicle reached around 18 mm and the oestradiol concentration was expected to be 700–800 pmol/l or more, 5000 IU of human chorionic gonadotrophin (HCG; Choriomon) was administered and the women were scheduled for oocyte retrieval to take place 36 h later. Oocyte retrieval was performed without anaesthesia using 19G single-lumen needles and each follicle was flushed five times (Kohl *Schwartz et al.,*

2020). All mature oocytes were fertilized by intracytoplasmic sperm injection (ICSI). Embryos were transferred on day 2 or 3 as cleavage-stage embryos. Luteal-phase support was applied using vaginal micronized progesterone (Utrogestan) in women with a short luteal phase (<12 days).

Conventional gonadotrophin-stimulated IVF (cIVF) was performed with an antagonist protocol using ovarian stimulation with human menopausal gonadotrophins (Menotropin or Merional) at a dosage of 150–300 IU and Ganirelix (Orgalutran) to inhibit the LH surge. Ovulation was induced with recombinant HCG (Ovitrelle) 36 h before oocyte retrieval. Oocyte retrieval was performed in the operating theatre and all mature oocytes were fertilized using ICSI. In accordance with Swiss law, most zygotes had to be frozen at the zygote stage and typically only one or two zygotes were cultured for 2 days to the cleavage stage. Embryo transfer was performed in analogy to NC-IVF 2 or 3 days after oocyte retrieval. Luteal-phase support was applied using vaginal micronized progesterone (Utrogestan).

Outcome definition

Primary outcomes were the occurrence of mature (metaphase II) oocytes, zygotes and embryos with an ideal morphology at the cleavage stage 2 days after oocyte retrieval. Secondary outcomes included implantation rate 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 h after ICSI. Embryos with four blastomeres, less than 10% fragmentation and equal symmetry of the blastomeres were assumed to be ideal (*Giorgetti et al., 1995*; *Terriou et al., 2001*). Clinical pregnancy was defined as the ultrasound detection of at least one amniotic sac. Implantation and live birth rates were defined as amniotic sacs per transferred embryo and the birth of a living child per clinical pregnancy, respectively.

Statistical analysis

Patient and cycles characteristics are presented in TABLE 1, stratified by treatment as the number and percentage for each categorical variable. For continuous variables, the medians and interquartile ranges, as well as minimum and maximum observed values, were reported.

For the outcomes of interest, the following statistics were calculated overall and within each treatment group: mature oocyte rate (number of mature oocytes/number of oocytes retrieved), zygote rate per retrieved oocyte (number of zygotes/number of oocytes retrieved), zygote (fertilization) rate per mature oocyte (number of zygotes/number of mature oocytes), implantation rate (number of amniotic sacs/number of transferred embryos) and live birth rate (number of live-born infants/number of clinical pregnancies).

The cleavage-stage morphology of transferred embryos was evaluated by calculating the proportion of embryos with a low fragmentation score (score 1), a high

TABLE 1 BASELINE CHARACTERISTICS OF THE PARTICIPANTS AND CYCLES IN NATURAL CYCLE IVF, CONVENTIONAL IVF AND OVERALL

Characteristics	NC-IVF	cIVF	Overall[a]
Number of patients	290	140	419
Number of cycles	616	167	783
Number of patients who underwent more than one IVF cycle, *n* (%)	152 (52)	23 (16)	178 (42)
Therapies undergone by the patients, *n* (%)			
NC-IVF	–	–	279 (67)
cIVF	–	–	129 (31)
NC-IVF and cIVF	–	–	11 (2.6)
Number of cycles per patient			
Median (IQR)	2 (1–3)	1 (1–1)	1 (1–2)
Range (min, max)	2 (1, 10)	1 (1, 4)	1 (1, 10)
Total number of retrieved oocytes	495	1615	2110
Number of retrieved oocytes per patient			
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[b]			
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* (%)			
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*/*N* (%)	331/616 (54)	139/167 (83)	470/783 (60)
Number of single-embryo transfers/cycles with embryo transfer, *n*/*N* (%)	331/331 (100)	26/139 (19)	357/470 (76)
Number of double-embryo transfers/cycles with embryo transfer, *n*/*N* (%)	0/331 (0)	113/139 (81)	113/470 (24)

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

symmetry score (score 1) and an ideal number of blastomeres ($n = 4$).

The effect of treatment (NC-IVF versus cIVF) on all the outcomes listed above was assessed independently using crude and adjusted generalized estimating equation (GEE) models with an exchangeable data correlation matrix. When the number of events was more than 40, the models were adjusted for the confounding effects of age, number of previous embryo transfers (three categories: 0, 1–2 and 3–6) and causes of infertility. To account for the structure of the dataset (i.e. measures could be taken during different cycles in the same participant), a robust variance estimator was used that included a clustering effect of the participant into the GEE models. Finally, the robustness of the results was assessed by conducting a sensitivity analysis for which only the first reported cycle for each woman was considered (Supplementary Tables 1 and 2)

Statistical analysis was conducted in R Version 4.2.1 (2022-06-23; The R Foundation, Austria).

RESULTS

The participant and cycle 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 FSH concentrations (<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). The dataset thus includes 783 cycles, 167 cIVF cycles (from 140 women, median number of cycles per participant = 1) and 616 NC-IVF cycles (from 290 women, median number of cycles per participant = 2) (TABLE 1). To allow a comparison of the data only NC-IVF cycles performed between 2015 and 2019 and cIVF cycles performed between 2015 and 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 one 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.

The median female age and interquartile range when the cycles were performed was 36.0 (33–38.85) years for NC-IVF and 34.0 (31–37) years for cIVF. Overall infertility factors were male factors ($n = 201$, 48%), female factors ($n = 72$, 17%), female and male factors ($n = 65$, 16%) and idiopathic infertility ($n = 81$, 19%). The most common female factors were tubal pathologies and endometriosis stage I using the revised ASRM criteria.

The main outcomes of the study are shown in TABLES 2 and 3 and FIGURE 1.

The mature oocyte rate was higher in the NC-IVF group than the cIVF group (89% versus 82%, aOR 1.79, $P = 0.001$; TABLE 2). In addition, the rate of zygotes per oocytes retrieved was also higher in NC-IVF (70% versus 58%, aOR 1.76, $P < 0.001$; FIGURE 1) as was the zygote rate per mature oocyte (fertilization rate) (79% versus 71%, aOR 1.62, $P = 0.001$; TABLE 2).

The percentage of zygotes that developed into cleavage-stage embryos was the same in both IVF treatment groups (97% for NC-IVF and 97% for cIVF, $P = 0.561$; TABLE 2).

Regarding embryo morphology on day 2, the odds of having transferred embryos with an ideal number of blastomeres and a fragmentation score of 0 were both found to be significantly higher in NC-IVF treatment (aOR 2.0, $P < 0.001$ for four blastomeres; aOR 1.87, $P = 0.003$ for a fragmentation score of 0). No significant difference was found for the symmetry of the blastomeres (TABLE 3). For all the outcomes listed in TABLES 2 and 3, similar results were found when considering only the first reported cycle for each participant (Supplementary Tables 1 and 2).

The implantation rate and live birth rate per detected amniotic sac were found to be similar in NC-IVF and cIVF cycles (implantation rate: 15% [51/331] versus 19% [47/252], $P = 0.327$, all singleton pregnancies; live birth: 76% versus 68% [39 versus 32], $P = 0.438$).

DISCUSSION

The main objective of this study was to analyse the impact of gonadotrophin 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 gonadotrophin-stimulated cIVF.

This study's major finding is the higher rate of fertilized oocytes in NC-IVF compared with cIVF. Furthermore, it was found that the proportion of mature oocytes per

TABLE 2 OOCYTE AND EMBRYO DEVELOPMENT IN NC-IVF AND CONVENTIONAL IVF

Parameters	NC-IVF	cIVF	Crude			Adjusted[a]		
			OR[b]	CI	*P*-value	OR[b]	CI	*P*-value
Retrieved oocytes	495	1615	–	–	–	–	–	–
Mature oocytes/ retrieved oocytes, *n/N* (%)	441/495 (89)	1326/1615 (82)	1.79	1.28– 2.49	0.001	1.79	1.26–2.53	0.001
Zygote/mature oocytes (Fertilization rate), *n/N* (%)	348/441 (79)	941/1326 (71)	1.49	1.13–1.97	0.004	1.62	1.21– 2.18	0.001
Cleavage stage embryos/zygotes, *n/N* (%)	339/348 (97)	258/267[c] (97)	1.31	0.52–3.29	0.561	n.a.	n.a.	n.a
Day 2 embryos transferred/cleavage-stage embryos, *n/N* (%)	331/339 (98)	252/258 (98)	0.94	0.29–3.12	0.925	n.a.	n.a.	n.a.

[a] Crude OR adjusted for age, number of previous embryo transfers (three categories: 0, 1–2 and 3–6) and causes of infertility

[b] OR >1 favours NC-IVF.

[c] 72% of zygotes were frozen, according to Swiss law.

CI, confidence interval; cIVF, conventional IVF; NC-IVF, natural cycle IVF; OR, odds ratio.

TABLE 3 EMBRYO MORPHOLOGY OF TRANSFERRED CLEAVAGE-STAGE EMBRYOS ON DAY 2 AFTER OOCYTE RETRIEVAL IN NC-IVF AND CONVENTIONAL IVF

Morphological characteristics	NC-IVF	cIVF	Crude			Adjusted[a]		
			OR[b]	CI	*P*-value	OR[b]	CI	*P*-value
Number of blastomeres			1.93 (4 versus <4 or >4)	1.35, 2.75	<0.001	2.00 (4 versus <4 or >4)	1.37, 2.9	<0.001
4, *n* (%)	171 (52)	90 (36)						
<4 or >4, *n* (%)	159 (48)	162 (64)						
Missing, *n* (%)	1	0						
Fragmentation score, *n* (%)			1.92 (0 versus 1-3)	1.29, 2.85	0.001	1.87 (0 versus 1-3)	1.24, 2.80	0.003
0, *n* (%)	219 (67)	129 (51)						
1–3, *n* (%)	110 (33)	122 (49)						
Missing, *n* (%)	2	1						
Symmetry score, *n* (%)			1.08 (1 versus 2)	0.75, 1.57	0.677	1.09 (1 versus 2)	0.74, 1.60	0.676
1	197 (60)	144 (57)						
2	133 (40)	108 (43)						
Missing	1	0						

[a] Crude odds ratios adjusted for age, number of previous embryo transfers (three categories: 0, 1–2 and 3–6) and causes of infertility.

[b] Proportional OR. OR >1 favours NC-IVF.

CI, confidence interval; cIVF, conventional IVF; NC-IVF, natural cycle IVF; OR, odds ratio.

oocytes retrieved was higher in NC-IVF and that gonadotrophin stimulation has a moderate but significant impact on embryo morphology.

The strength of the study is the high number of included cycles, the single-centre design with the same laboratory techniques applied throughout the study period, the same embryologists categorizing the embryos to minimize the interobserver variability in embryo morphology determination (*Paternot et al., 2011*) and the legal situation in Switzerland, which prohibited embryo selection and thereby gave the study the unique chance to compare NC-IVF and cIVF treatment cycles. Furthermore, all embryo transfers were fresh and were performed 2–3 days after oocyte retrieval, which allowed a comparison of the implantation rate and live birth rate resulting from the transfer of embryos that were all at the cleavage-stage.

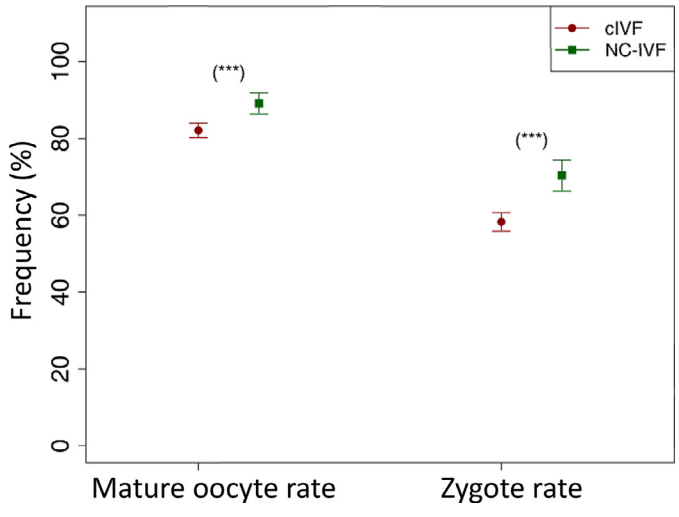

FIGURE 1 Mature (metaphase II) oocytes and zygotes (fertilized oocytes) per oocyte retrieved in natural cycle IVF (NC-IVF) and conventional IVF (cIVF) and their associated 95% confidence intervals. *** Crude $P < 0.001$. Adjustment for the analysis for age, number of previous embryo transfers (three categories: 0, 1–2 and 3–6) and causes of infertility obtained an adjusted odds ratio of 1.76 (CI 1.37–2.26, $P = 0.001$) for the number of zygotes per oocyte retrieved and of 1.79 (CI 1.26–2.53; $P < 0.001$) for the number of mature oocytes per oocyte retrieved.

The weaknesses of the study are its retrospective design and the inclusion of several treatment cycles per participant, as well as the fact that some women underwent both kinds of IVF, even though this was considered in the statistical analysis by using a robust variance estimator that took care of arbitrary correlations among observations within an individual, and by performing a sensitivity analysis considering only the first reported cycles for each patient.

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

This study, for the first time, not only compared oocyte and embryo parameters, but also analysed the pregnancy and live birth rates as embryo

selection was not performed. However, in cIVF a maximum of two zygotes were cultured and the surplus zygotes were by default cryopreserved, which might have led to some bias in pregnancy and live birth rates, favouring cIVF.

No difference was found in pregnancy and live birth rates. The reasons might be the low power due to the limited number of embryos and the rather low pregnancy rate after the transfer of day 2/3 embryos. Furthermore, other confounders such as functional differences in the endometrium due to high blood concentrations of serum oestradiol or the use of gonadotrophin-releasing hormone antagonists as in cIVF might have had an impact on the success rates.

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

Interestingly, Mitter and colleagues (*Mitter et al., 2021*) also compared NC-IVF and cIVF cycles from the same IVF centre. In contrast to the current study, they found slightly higher pregnancy (aOR 1.87, 95% CI 1.21–2.91) and live birth (aOR 1.85, 95% CI 1.16–2.95) rates in NC-IVF after adjusting 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, the inclusion of only cycles with an embryo transfer, a different time period for the analysis and the use of registry data from the Swiss IVF registry.

Ziebe and co-workers (*Ziebe et al., 2004*) also compared the morphology of embryos generated by NC-IVF and cIVF. They performed an intra-individual comparison but analysed fewer cycles (125 NC-IVF and 177 cIVF cycles) and included only long agonist protocols. The percentage of embryos with four or more cells was 59% in NC-IVF versus 53% in cIVF, and the percentage of embryos with less than 10% fragmentation was 69% versus 61%. The values were not statistically different, even though the morphological parameters appeared to be slightly better in NC-IVF. It remains unclear whether the morphology of the embryos in the study by Ziebe and co-workers (*Ziebe et al., 2004*) would have reached significance if the number of cycles included in the study had been higher.

The current study also addressed the maturity of oocytes retrieved in NC-IVF versus 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.

The mature oocyte rate was considered because in general a higher number of immature oocytes is considered a marker of poor oocyte quality (*Lee et al., 2011*; *Astbury et al., 2020*). Astbury and colleagues (*Astbury et al. 2020*) compared patients in whom germinal vesicle stage immature oocytes were retrieved with those in whom immature oocytes were not retrieved. The presence of immature oocytes showed a significant correlation with a lower implantation rate (11.8% versus 30.2%, $P = 0.02$) and live birth rate (1.9% versus 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 gonadotrophin stimulation, as demonstrated by 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 gonadotrophin stimulation impairs oocyte spindles (Di *Nisio et al., 2018*), has an impact on epigenetic mechanisms of DNA methylation (*Uysal et al., 2018*) and may affect mitochondrial function (*Lee et al., 2017*).

The process of follicle recruitment requires a precise regulation and selection of follicles, involving several bidirectional paracrine and junctional signalling mechanisms in oocyte–granulosa cells, which are 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 pre-ovulatory surge of LH via an indirect action mediated by the cumulus cells. In cIVF the natural sequence of events including the source and changes of the hormone concentrations is altered by the constantly high dose of gonadotrophins.

Differences in follicular physiology can also be found at the endocrine and molecular levels in follicular fluid. Follicular fluid is different in cIVF in terms of its immune cell profile, heterogeneously affecting many cytokines and leukocytes as well as lymphocytes (*Kollmann et al., 2017*). The concentrations of LH, androgens and oestradiol (*von Wolff et al., 2014*, *2022*), as well as the concentration of anti-Müllerian 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, gonadotrophin 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 gonadotrophin stimulation does indeed affect oocyte function.

This raises the question of whether the results of the current study also have clinical implications. The findings might be relevant in poor and especially very poor responders. In poor responders (according to the Bologna criteria; *Ferraretti et al., 2011*) with a very low ovarian reserve, gonadotrophin stimulation does not increase oocyte yield or overall live birth rate. Gonadotrophin stimulation might even decrease the implantation rate, as shown by De Marco and colleagues (*De Marco et al., 2021*) for advanced-age poor responders.

In conclusion, these results contribute to the concept that high-dose exogenous gonadotrophins have an effect on oocyte and embryo quality. However, whether this effect also has an impact on pregnancy and live birth rate remains to be evaluated.

DATA AVAILABILITY

Data will be made available on request.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.rbmo.2022.11.008.

REFERENCES

Albertini, DF, Sanfins, A, Combelles, CMH, 2003. Origins and manifestations of oocyte maturation competencies. Reprod. Biomed. Online 6, 410–415 Reproductive Healthcare Ltd.

Alper, MM, Fauser, BC, 2017. Ovarian stimulation protocols for IVF: is more better than less? Reprod. Biomed. Online 34, 345–353 Elsevier Ltd.

Astbury, P, Subramanian, GN, Greaney, J, Roling, C, Irving, J, Homer, HA, 2020. The Presence of Immature GV– Stage Oocytes during IVF/ICSI Is a Marker of Poor Oocyte Quality: A Pilot Study. Med. Sci. 8, 4.

Auwera I Van Der, D, Hooghe, T, 2001. Superovulation of female mice delays embryonic and fetal development. Hum. Reprod. 16, 1237–1243.

Bersinger, NA, Eisenhut, M, Stute, P, Wolff, M von, 2021. Gonadotropin Stimulation Has Only a Limited Effect on the Concentration of Follicular Fluid Signalling Proteins: An Antibody Array Analysis. Int. J. Reprod. Med. 2021, 1–7.

Ciepiela, P, Dulęba, AJ, Kario, A, Chełstowski, K, Branecka-Woźniak, D, Kurzawa, R, 2019. Oocyte matched follicular fluid anti-Müllerian hormone is an excellent predictor of live birth after fresh single embryo transfer. Hum. Reprod. 34, 2244–2253.

Dayal, MB, Kovalevsky, G, Patrizio, P, 2006. Rate of blastocyst development from excess embryos remaining in culture after day 3 embryo transfer. Int. J. Fertil. Womens Med. 51, 136–139.

Edwards, RG, 2007. IVF, IVM, natural cycle IVF, minimal stimulation IVF - Time for a rethink. Reprod. Biomed. Online 15, 106–119.

Eppig, JJ, 2001. Oocyte control of ovarian follicular development and function in mammals. Reproduction 122, 829–838.

Ertzeid, G, Storeng, R, 1992. Adverse effects of gonadotrophin treatment on pre- and postimplantation development in mice. J. Reprod. Fertil. 96, 649–655.

Ertzeid, G, Storeng, R, 2001. The impact of ovarian stimulation on implantation and fetal development in mice. Hum. Reprod. 16, 221–225.

Fauser, BCJM, Devroey, P, Yen, SSC, Gosden, R, Crowley, J, Baird, DT, Bouchard, P, 1999. Minimal ovarian stimulation for IVF: Appraisal of potential benefits and drawbacks. Hum. Reprod. 14, 2681–2686.

Ferraretti, AP, Marca, A La, Fauser, BCJM, Tarlatzis, B, Nargund, G, Gianaroli, L, 2011. ESHRE consensus on the definition of 'poor response to ovarian stimulation for in vitro fertilization: The Bologna criteria. Hum. Reprod. 26, 1616–1624.

Giorgetti, C, Terriou, P, Auquier, P, Hans, E, Spach, JL, Salzmann, J, Roulier, R, 1995. Implantation: Embryo score to predict implantation after in-vitro fertilization: Based on 957 single embryo transfers. Hum. Reprod. 10, 2427–2431.

Hodges, CA, Ilagan, A, Jennings, D, Keri, R, Nilson, J, Hunt, PA, 2002. Experimental evidence that changes in oocyte growth influence meiotic chromosome segregation. Hum. Reprod. 17, 1171–1180.

Inge, GB, Brinsden, PR, Elder, KT, 2005. Oocyte number per live birth in IVF: Wer Steptoe and Edwards less wasteful? Hum. Reprod. 20, 588–592.

Karl, KR, Jimenez-Krassel, F, Gibbings, E, Ireland, JLH, Clark, ZL, Tempelman, RJ, Latham, KE, Ireland, JJ, 2021. Negative impact of high doses of follicle-stimulating hormone during superovulation on the ovulatory follicle function in small ovarian reserve dairy heifers. Biol. Reprod. 104, 695–705.

Kollmann, Z, Schneider, S, Fux, M, Bersinger, NA, Wolff, M Von, 2017. Gonadotrophin stimulation in IVF alters the immune cell profile in follicular fluid and the cytokine concentrations in follicular fluid and serum. Hum. Reprod. 32, 820–831.

Lee, JE, Kim, SD, Jee, BC, Suh, CS, Kim, SH, 2011. Oocyte maturity in repeated ovarian stimulation. Clin. Exp. Reprod. Med. 38, 234–237.

Lee, M, Ahn, J Il, Lee, AR, Ko, DW, Yang, WS, Lee, G, Ahn, JY, Lim, JM, 2017. Adverse effect of superovulation treatment on maturation, function and ultrastructural integrity of murine oocytes. Mol. Cells 40, 558–566.

Majumdar, G, Majumdar, A, Verma, I, Upadhyaya, K, 2017. Relationship between Morphology, Euploidy and Implantation Potential of Cleavage and Blastocyst Stage Embryos. J. Hum. Reprod. Sci. 10, 49–57.

Marco, MP De, Montanari, G, Ruscito, I, Giallonardo, A, Ubaldi, FM, Rienzi, L, Costanzi, F, Caserta, D, Schimberni, M, Schimberni, M, 2021. Natural Cycle Results in Lower Implantation Failure than Ovarian Stimulation in Advanced-Age Poor Responders Undergoing IVF: Fertility Outcomes from 585 Patients. Reprod. Sci. 28, 1967–1973.

Mitter, V, Grädel, F, Koh Schwartz, AS, Vo Wolff, M, 2021. P–693 Gonadotropin stimulation reduces the implantation and live birth but not the miscarriage rate – a study based on the comparison of stimulated and unstimulated IVF. Hum. Reprod. 36.

Nisio, V Di, Rossi, G, Palmerini, MG, Macchiarelli, G, Tiboni, GM, Cecconi, S, 2018. Increased rounds of gonadotropin stimulation have side effects on mouse fallopian tubes and oocytes. Reproduction 155, 245–250.

Palmerini, MG, Antonouli, S, Macchiarelli, G, Cecconi, S, Bianchi, S, Khalili, MA, Nottola, SA, 2022. Ultrastructural Evaluation of the Human Oocyte at the Germinal Vesicle Stage during the Application of Assisted Reproductive Technologies. Cells 11, 1636 [Internet].

Palomba, S, 2021. Is fertility reduced in ovulatory women with polycystic ovary syndrome? An opinion paper. Hum. Reprod. 36, 2421–2428.

Palomba, S, Daolio, J, Sala GB, La, 2017. Oocyte Competence in Women with Polycystic Ovary Syndrome. Trends Endocrinol. Metab. 28, 186–198.

Paternot, G, Wetsels, AM, Thonon, F, Vansteenbrugge, A, Willemen, D, Devroe, J, Debrock, S, D'Hooghe, TM, Spiessens, C, 2011. Intra- and interobserver analysis in the morphological assessment of early stage embryos during an IVF procedure: A multicentre study. Reprod. Biol. Endocrinol. 9.

Patrizio, P, Bianchi, V, Lalioti, MD, Gerasimova, T, Sakkas, D, 2007a. High rate of biological loss in assisted reproduction: It is in the seed, not in the soil. Reprod. Biomed. Online 14, 92–95.

Patrizio, P, Fragouli, E, Bianchi, V, Borini, A, Wells, D, 2007b. Molecular methods for selection of the ideal oocyte. Reprod. Biomed. Online 15, 346–353.

Patrizio, P, Sakkas, D, 2009. From oocyte to baby: a clinical evaluation of the biological efficiency of in vitro fertilization. Fertil. Steril. 91, 1061–1066.

Patrizio, P, Silber, S, 2017. Improving IVF: is there a limit to our ability to manipulate human biology? J. Assist. Reprod. Genet. 34, 7–9.
Schwartz, ASK, Calzaferri, I, Roumet, M, Limacher, A, Fink, A, Wueest, A, Weidlinger, S, Mitter, VR, Leeners, B, Wolff, M von, 2020. Follicular flushing leads to higher oocyte yield in monofollicular IVF: a randomized controlled trial. Hum. Reprod. 35, 2253–2261 [Internet]Oxford University Press.
Silber, SJ, Kato, K, Aoyama, N, Yabuuchi, A, Skaletsky, H, Fan, Y, Shinohara, K, Yatabe, N, Kobayashi, T, 2017. Intrinsic fertility of human oocytes. Fertil. Steril. 107, 1232–1237.
Terriou, P, Sapin, C, Giorgetti, C, Hans, E, Spach, JL, Roulier, R, 2001. Embryo score is a better predictor of pregnancy than the number of transferred embryos or female age. Fertil. Steril. 75, 525–531.
Uysal, F, Ozturk, S, Akkoyunlu, G, 2018. Superovulation alters DNA methyltransferase protein expression in mouse oocytes and early embryos. J. Assist. Reprod. Genet. 35, 503–513.
Wolff, M von, Eisenhut, M, Stute, P, Bersinger, NA, 2022. Gonadotrophin stimulation reduces follicular fluid hormone concentrations and disrupts their quantitative association with cumulus cell mRNA. Reprod. Biomed. Online 44, 193–199.
Wolff, M Von, Kollmann, Z, Flück, CE, Stute, P, Marti, U, Weiss, B, Bersinger, NA, 2014. Gonadotrophin stimulation for in vitro fertilization significantly alters the hormone milieu in follicular fluid: A comparative study between natural cycle IVF and conventional IVF. Hum. Reprod. 29, 1049–1057.
Ziebe, S, Bangsbøll, S, Schmidt, KLT, Loft, A, Lindhard, A, Andersen, AN, 2004. Embryo quality in natural versus stimulated IVF cycles. Hum. Reprod. 19, 1457–1460 Oxford University Press.

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