Time-lapse imaging reveals differences in growth dynamics of embryos after in vitro maturation compared with conventional stimulation

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Objective: To study the impact of in vitro maturation (IVM) on embryonal development with the use of time-lapse imaging.
Design: Retrospective case-control study.
Setting: University hospital.
Patient(s): In total, 294 embryos were cultured after intracytoplasmic sperm injection treatment of three groups: patients with polycystic ovary syndrome (PCOS) and IVM (n = 105; group 1 [G1]), patients after conventional stimulation without PCOS (n = 115; G2) and with PCOS (n = 74; G3). In total, 171 embryos were finally analyzed (57 G1, 65 G2, and 49 G3).
Intervention(s): Data of 23 PCOS patients (30 IVM cycles) from January 2012 to July 2015 were matched according to age and number of oocytes to patients after conventional stimulation without PCOS (n = 30; 30 cycles) and with PCOS (n = 16; 19 cycles). Markers of embryo development were analyzed at different time points. Pregnancy rates (PRs) and live birth rates (LBRs) were recorded.
Main Outcome Measure(s): Morphokinetic differences in embryo development after IVM compared with conventional stimulation with or without PCOS.
Result(s): The rate of good-quality embryos was significantly lower in G1. Embryo development in G1 was significantly accelerated to the time of appearance of two pronuclei but slowed down by the time of reaching 6-cell stage and remained slower compared with embryos of G2 and G3. PRs as well as LBRs did not differ significantly among the study groups.
Conclusion(s): Although growth dynamics of embryos from G1 differ from G2 and G3 and the rate of good-quality embryos was lower in IVM embryos, PRs and LBRs did not differ significantly. (Fertil Steril® 2017;107:606–12. ©2016 by American Society for Reproductive Medicine.)
Key Words: Time-lapse imaging, in vitro maturation, morphokinetic parameters, clinical pregnancy rate

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In vitro maturation (IVM) is a well established reproductive technique, particularly for patients with polycystic ovary syndrome (PCOS) or at high risk of ovarian hyperstimulation syndrome (OHSS) (1), with a live birth rate (LBR) from 16.5% to 23.5% (2). However, the method is applied in only a few IVF clinics worldwide, because it requires a more intense workload and has lower success rates than conventional in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI). IVM is mainly applied in PCOS women at high risk of OHSS during hormonal stimulation for IVF/ICSI (3). Besides the OHSS risk, PCOS patients are known to have an abnormal follicle recruitment as well as disturbed oocyte maturation and development (4–6). Therefore, evaluation of embryo development after IVM seems to be of particular interest for PCOS patients, because IVM of oocytes before fertilization might lead to a different developmental competence of the resulting embryo, which could be reflected by developmental kinetics,

Received September 13, 2016; revised December 16, 2016; accepted December 21, 2016; published online January 27, 2017.
S.R. attended an IVM workshop sponsored by Origio, Berlin, Germany, and received grants from Merck Serono and MSD. J.E.D. has nothing to disclose. J.W. has nothing to disclose. M.M. CEO of a consultancy company (Ilabcomm), which received honorarium for consultancy from Vitrolife. B.T. received grants as lecturer from Merck Serono, MSD, Teva, and Ferring. T.S. received grants as lecturer from Bayer Health Care and Medesso/Qonsilus.
S.R., J.E.D., B.T., and T.S., should be considered similar in author order.
Supported by Deutsche Forschungsgemeinschaft (STR 387/4-1).
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Fertility and Sterility® Vol. 107, No. 3, March 2017 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2016.12.026

such as cell-cycle lengths and timing of morphokinetic events during the preimplantation phase.

Routine clinical time-lapse imaging (TLI) was established in 2008 and since then has emerged as a noninvasive method to analyze morphokinetic events underlying developmental processes at high temporal resolution for up to 5–6 days of embryo development (7–9). Several time intervals during TLI have been proposed as predictive factors for good-quality embryos (GQEs); e.g., timing of pronuclear (2PN) breakdown, cleavage times during the first three rounds of mitosis, and the time span between ICSI and 5-cell embryo formation (10–14). A prospective randomized double-blinded controlled study including 843 infertile couples showed a higher ongoing pregnancy rate (PR) of 51.4% in the TLI group with the use of predictive factors compared with 41.7% in the conventional incubation group with the use of standard embryo assessment (10).

There is evidence that embryos from PCOS women have a decelerated development from ICSI to 2PN breakdown, first cleavage, and cleavage to 3 and 4 cells compared with women without PCOS (4). This difference disappeared after the 7-cell stage (4). Most recently, Walls et al. published data concerning morphokinetic parameters of embryos derived from IVM-PCOS patients compared with PCOS patients with conventional ICSI. They were able to demonstrate that the embryos from the PCOS-IVM group showed a higher degree of multinucleation and a higher rate of embryo arrest (11).

To analyze different end points during embryo development in PCOS patients undergoing IVM or conventional ICSI, we analyzed morphokinetic events with the use of TLI and added a second control group consisting of non-PCOS ICSI patients. We hypothesized that both IVM and PCOS affect embryo development, which might be detected by monitoring cleavage kinetics. In addition, PRs and LBRs of the study population were recorded.

MATERIALS AND METHODS

Data Collection Study Population

The data of 23 IVM patients with clinically proven PCOS according to the Rotterdam criteria (12) and an additional male infertility factor due to abnormal spermiogram according to World Health Organization criteria (13) or a fertilization failure in a former IVF attempt, attending the Department of Gynecologic Endocrinology and Fertility Disorders, Ruprecht-Karls University Heidelberg from January 2012 to July 2015, were collected. IVM was offered to all PCOS patients, explaining the possible advantages (e.g., avoiding OHSS) and disadvantages (e.g., possible imprinting defects). In a process of shared decision making, patients had free choice to opt for an IVM or conventional ICSI cycle. Each PCOS patient undergoing an IVM treatment followed by ICSI (n = 30; group 1 [G1]) was matched according to age and number of oocytes by patients without PCOS and conventional hormonal stimulation followed by ICSI due to male factor infertility (n = 30; group 2 [G2]) who attended our department during the same time period. In addition, the data of a second control group were collected, including 16 patients and 19 cycles with PCOS and a male factor for infertility and conventional stimulation followed by ICSI (group 3 [G3]).

Hormonal Stimulation and Oocyte Pick-Up

IVM procedures were performed as described previously (1). Briefly, after 3 days of stimulation with the use of 125 IU FSH per day (Puregon; MSD), ovulation was induced with the use of 250 μg recombinant hCG (Ovitrelle; Merck Serono), and 36 hours later ovum pick-up was performed under ultrasound-guided control with the use of a 17-gauge needle and a reduced aspiration pressure of 100 mm Hg (Cook).

Oocytes that were mature on the day of ovum pick-up 36 hours after ovulation trigger were inseminated on the same day. Immature oocytes were initially collected in Medicult IVM System (82214010A; Origio) LAG medium and subsequently cultured for 24 $\pm$ 1 hours in Medicult IVM System IVM medium, supplemented with 0.075 IU/mL FSH (Gonal-F; Merck-Serono), 0.1 IU/mL hCG (Predalon; MSD), and 10% patient's serum according to the manufacturers' instructions.

In G2, 15 patients underwent a GnRH agonist protocol and 15 patients a GnRH antagonist protocol, whereas in G3, 9 patients underwent a GnRH agonist protocol in 11 cycles and 7 patients a GnRH antagonist protocol in 8 cycles.

IVF Laboratory Management: Fertilization, Embryo Culture, Embryo Grading, and Embryo Transfer

Insemination was performed by means of ICSI in Sydney IVF Gamete Buffer (K-SIGB-50; Cook Medical). Immediately after the ICSI procedure all inseminated oocytes were placed individually in an Embryoslide (Vitrolife) prefilled and -equilibrated with the use of Sydney IVF Cleavage Medium (K-SICM-20; Cook Medical) covered with paraffin oil (10100060A; Origio), and cultured in an Embryoscope time-lapse incubation system (Vitrolife) with an atmosphere of 5.0% O_2 and 6.4% CO_2. The concentration of CO_2 was set to adjust the pH of the culture medium within a range of 7.25–7.35. Media change was performed on the 3rd day of culture by replacing the spent medium with preequilibrated Sydney IVF Blastocyst Medium (K-SIBM-20; Cook Medical). After medium change, the Embryoslide was reinserted into the incubator and the culture continued until transfer or vitrification.

Grading of cleavage-stage embryos on days 2 and 3 was performed as follows (modified from Balaban et al. [14]): grade A = embryos with blastomeres of equal size and cytoplasmic fragmentation <10%; grade B = embryos with blastomeres of equal size and cytoplasmic fragmentation 10%–25%; grade C = embryos with blastomeres of equal or unequal size and cytoplasmic fragmentation 26%–50%; grade D = embryos with fragmentation >50%. Blastocysts were scored according to Gardner et al. (15).

Good quality embryos (GQEs) were defined as follows: on day 2, 2- to 4-cell, grade A/B; on day 3, 5- to 8-cell, grade A/B; on day 4, 9- to 16-cell and grade A/B, compacting or fully compacted morula; and on day 5, blastocyst grade $\geq$3BB.

In total, 294 embryos (105 G1, 115 G2, and 74 G3) were cultured. Presence of 2PN was checked 17 ± 1 hours after insemination (hpi) and a maximum of up to eight oocytes with 2PN were incubated up to 5 days according to the German Embryo Protection Act (Supplemental Table 1; available online at www.fertstert.org). Supernumerary PN-stage oocytes were cryopreserved by means of slow freezing (Sydney IVF Cryopreservation Kit, K-SICS-5000; Cook) or vitrification (Kitazato Vitrification Media, VT801, 91171). Supernumerary GQEs, which were not transferred, were cryopreserved by means of vitrification.

Embryo transfer was performed with the use of ultrasound guidance and a suitable embryo transfer catheter (Guardia Access K-JETS-7019 or Guardia Access ET K-JETS-7019-ET; Cook) on day 2–5. Patients had a serum pregnancy test 14 days after insemination of the oocytes. The test was considered to be positive when hCG was >10 mIU/mL. Clinical pregnancy was verified with the use of ultrasound (visibility of one or more gestational sacs; multiple gestational sacs were counted as one clinical pregnancy) 10 days after the positive pregnancy test (16).

Time-Lapse Imaging

Embryos after IVM treatment or conventional hormonal stimulation were cultured in a closed time-lapse imaging incubator (Embryoscope; Vitrolife). Images were recorded every 10 minutes in seven focal planes and a z-interval of 15 μm. The time point of insemination was defined as the mid-time of the ICSI treatment. Time-lapse imaging was started immediately after the ICSI treatment.

Morphokinetic variables were documented in hpi by means of the Embryoviewer station connected to the Embryoscope. Durations of morphokinetic events were provided in hours. Annotated developmental features included time until extrusion of polar body 2 (tPB2), appearance (tPNa) and fading (tPNf) of the two PNs, time to *n*-cell stage (t*n*; *n* = 2–9), time to morula stage (tM), time to start of blastulation (tSB), time to blastocyst (tB), and time to expanding blastocyst (tEB). Calculated morphokinetic features included duration of embryonic cell cycle (ECC) 1 (t2 − tPB2), ECC2 (t4 − t2), ECC3 (t8 − t4), and synchronicity of cell divisions s2 (t4 − t3) and s3 (t8 − t5). PNa was defined as the first frame with a detectable PN. PNf was defined as the first frame when PNs could no longer be visualized (17). Embryos were scored as morula if >90% of the embryo volume was compacted (not considering excluded blastomeres). SB was defined as the first frame with visible signs of cavitation. Blastocyst corresponds to blastocyst stage 2 according to Gardner et al. (15). Expanding blastocyst was defined as the first frame with a visible thinning of the zona pellucida (17). Data were calculated for transferred embryos and supernumerary embryos that were cryopreserved on the day of transfer (n = 171).

Statistics

Statistical analysis was performed with the use of IBM SPSS Statistics for Mac (version 22.0). We analyzed data with the use of a closed testing procedure (18). Continuous data were analyzed by means of the Kruskal-Wallis *H* and Mann-Whitney *U* test, all other data with the use of the chi-square test. *P* values <.05 were considered to be statistically significant. Data are presented as figures and tables when appropriate.

Ethics Approval

The study was approved by the local Ethics Committee of the Ruprecht-Karls University of Heidelberg.

RESULTS

Study Population

Patient characteristics are presented in Table 1 and differed significantly regarding antimüllerian hormone levels (*P*<.001) but not in age or body mass index (BMI). Only one PCOS patient in the IVM group suffered from hyperandrogenism. Regarding OHSS, two patients from G3 developed OHSS in the analyzed ICSI cycle, whereas in G1 and G2 no OHSS was diagnosed.

Time-Lapse Imaging

The number of oocytes retrieved after IVM or conventional stimulation and maturation, fertilization, and transfer rates are summarized in Supplemental Table 1 and Figure 1. A total of 294 2PN oocytes (105 G1, 115 G2, and 74 G3) were kept in culture and 167 2PN oocytes (33 G1, 71 G2, and 63 G3) were cryopreserved for future use.

In total, 46/105 (43.8%) IVM embryos (G1) were discarded owing to an abnormal development, as were 50/115 (43.5%) embryos from G2 and 25/74 (33.8%) embryos from G3. Criteria for discarding embryos included degeneration, developmental arrest for ≥24 hours and direct cleavage from 1- to 3-cell stage. The number of GQEs was significantly lower among IVM embryos compared with the control groups (G1: 21/105 (20.0%); G2: 40/115 (34.8%); G3: 36/74 (48.6%); *P*<.001; Supplemental Table 1). In G1, 57 embryos were transferred and two supernumerary GQEs were cryopreserved on day 2 (n = 1) or 5 (n = 1). Of the 57 transferred embryos, two were not cultured in the Embryoscope and therefore were not included in the analysis. In G2, 56 embryos were transferred and nine GQEs were cryopreserved on day 4 (n = 1) or 5 (n = 8). In G3, 39 embryos were transferred and 10 GQEs were cryopreserved on day 4 (n = 4) or 5 (n = 6; Fig. 1).

There was no difference in tPB2 among the groups (Fig. 2; Supplemental Table 2, available online at www.fertstert.org). tPNa was significantly different between G1 and G2 as well as between G1 and G3. Transferred IVM embryos reached tPNa faster than control ICSI embryos from non-PCOS as well as PCOS patients (5.55 hpi [G1], 6.61 hpi [G2], and 6.38 [G3]; *P*<.001; Supplemental Table 2; Fig. 2A). During the next developmental steps (tPNf, t2 to t5), no significant differences were present among the study subgroups.

However, embryos from IVM patients (G1) reached t6 significantly later than embryos from PCOS control embryos (G3), and this deceleration was still present at t7, t8, t9+, tM, tSB, tB, tEB, ECC2, ECC3, and s3 (Supplemental Table 2; Fig. 2). In addition, significant differences were present

TABLE 1

Characteristics of the study population, mean ± SD (range).

Group	Age (y)	BMI (kg/m^2)	AMH (ng/mL)
G1 (n = 23 patients)	32.09 ± 4.27 (22–41)	26.06 ± 6.82 (16.3–42.1)	7.91 ± 5.78 (2.19–23.0) (n = 22)[a]
G2 (n = 30 patients)	33.47 ± 4.16 (26–42)	23.46 ± 2.65 (19.5–30.1)	2.57 ± 2.39 (0.26–10.35) (n = 28)[a,b]
G3 (n = 16 patients)	34.69 ± 4.60 (24–40)	25.53 ± 4.83 (19.0–36.9)	7.98 ± 4.28 (3.06–15.00) (n = 12)[b]
P value	ns	ns	[a,b]< .001

Note: AMH = antimüllerian hormone; BMI = body mass index; G1 = in vitro maturation patients with PCOS; G2 = patients without PCOS; G3 = PCOS patients; n.s. = no significant differences.
[a,b]*P*< .001 between G1 and G2 and between G2 and G3.

Roesner. IVM and time-lapse imaging. Fertil Steril 2016.

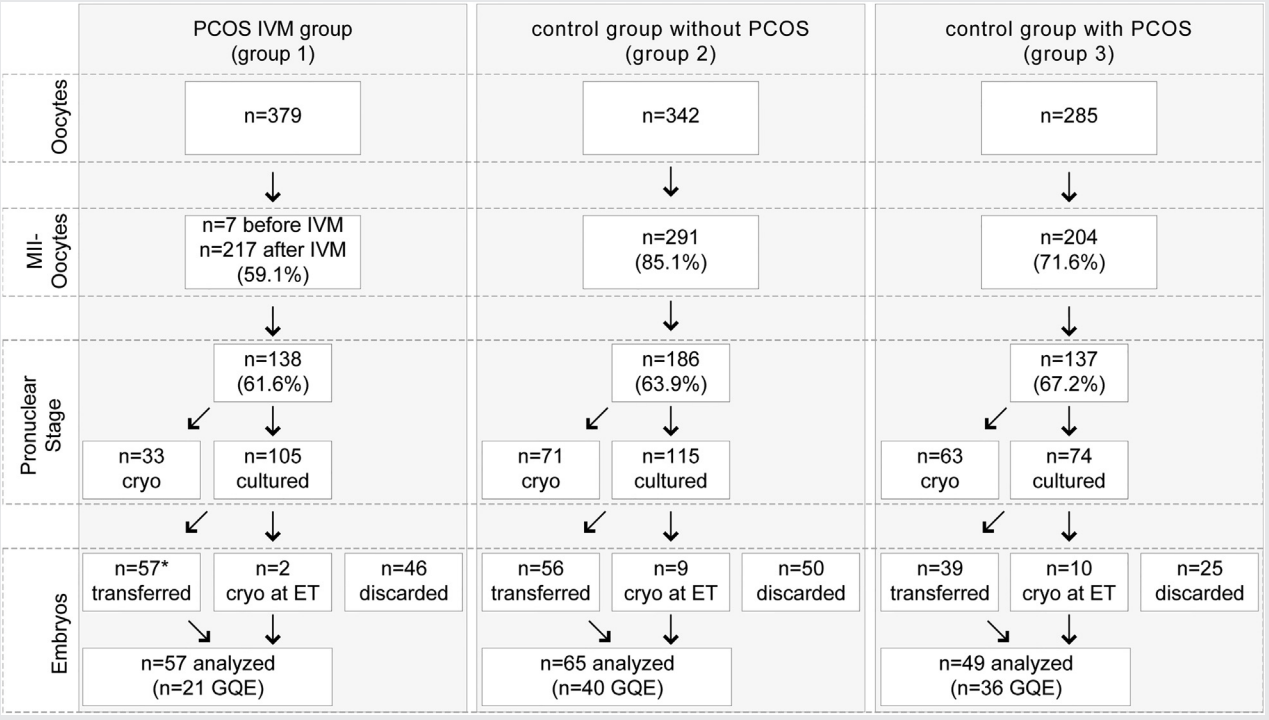
between G2 and G3 regarding embryo development from the t7 stage onward, including t8, tM, tSB, tB, tEB, and ECC3 (Supplemental Table 2; Fig. 2).

When comparing embryos from IVM patients with embryos from non-PCOS patients, significant differences beyond tPNa were present only at ECC3 (Supplemental Table 2; Fig. 2B). Overall, development of IVM embryos proceeded faster until tPNa, then slowed down and remained slow until blastocyst stage. Time to blastulation as well as time to expanded blastocyst was fastest in embryos from PCOS control patients (G3). Regarding embryonic cell cycles and synchronicity of cell cycle lengths, IVM embryos from PCOS patients (G1) had longer durations than the other study subgroups, although not all parameters were significantly different (Fig. 2B).

Pregnancy and Live Birth Rates

The PR in IVM cycles (G1) was lower than in both control groups (G1: 36.7% [11/30]; G2: 56.7% [17/30]; G3: 47.4% [9/19]), but this was not significant (*P*=.299; Supplemental Table 3, available online at www.fertstert.org). Regarding clinical PR, the highest success rates were present in the non-PCOS control group (G2: 56.7%), but again no significant difference was present. Accordingly, the LBR in IVM cycles was lower without reaching significance (G1: 20.0%

FIGURE 1



Study population and recruitment of the embryos selected for time-lapse imaging. cryo = cryopreserved; ET = embryo transfer; GQE = good-quality embryos; IVM = in vitro maturation; MII = metaphase II; PCOS = polycystic ovary syndrome; *Two embryos were not cultured in the Embryoscope.

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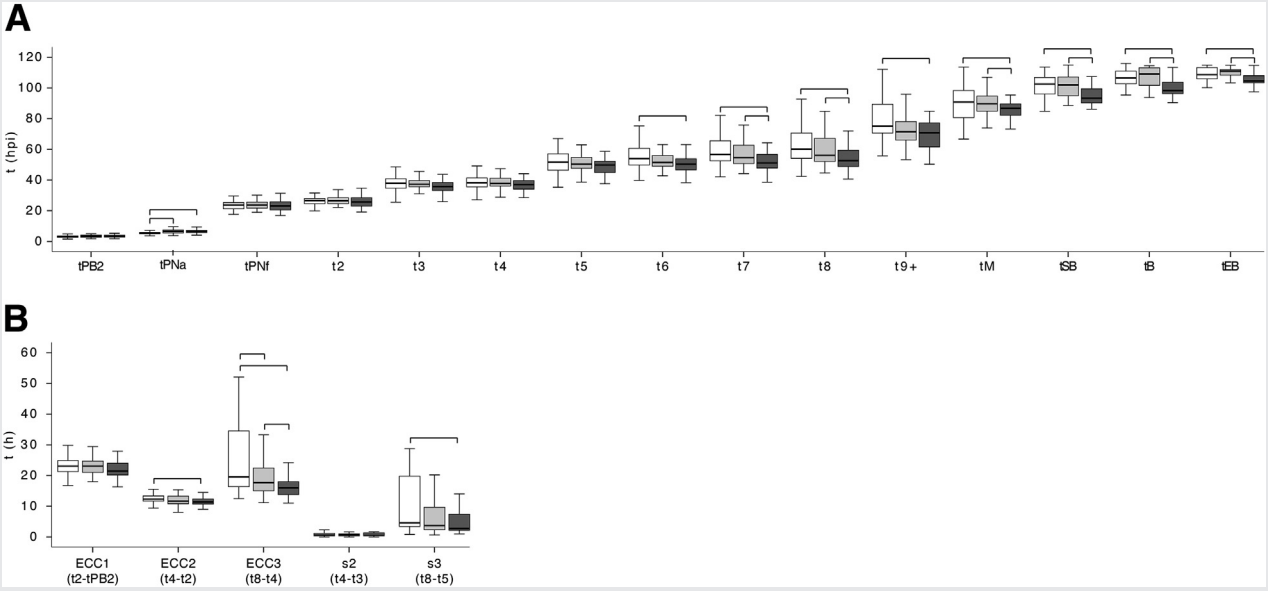
FIGURE 2



Differences in morphokinetics assessed by time-lapse imaging within the study population. (**A**) *Box plots* depicting morphokinetic times (in hours after insemination [hpi]). (**B**) *Box plots* depicting morphokinetic durations (in hours). *White*: in vitro maturation with polycystic ovary syndrome (PCOS); *light gray*: non-PCOS control; *dark gray*: PCOS control. Significant differences ($P<.05$) are indicated between the marked groups.

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[6/30]; G2: 40.0% [12/30 plus one ongoing pregnancy and three pregnancies with unknown outcome]; G3: 42.1% [8/19]; $P=.159$).

DISCUSSION

In this retrospective case-control study, TLI was applied to embryos of PCOS patients after IVM or standard ICSI procedure to compare them with embryos of non-PCOS ICSI patients. IVM patients had significantly fewer mature oocytes and a lower rate of GQEs than patients after conventional stimulation with and without PCOS. Our findings are in accordance with other data (19-21), because they also showed that IVM is associated with a lower rate of mature oocytes compared to conventional IVF or ICSI as well as a higher rate of embryo arrest compared with PCOS and non-PCOS patients with conventional ICSI. However, in our study, PRs and ongoing PRs were the same in all three groups.

Although oocytes from all three groups resumed and completed meiosis at a similar time, as indicated by tPB2, tPNa was significantly faster in oocytes derived from IVM cycles. This suggests that after activation of the oocyte, chromatin decondensation and/or pronuclear envelope formation might be accelerated in oocytes from IVM patients compared with oocytes after conventional stimulation of non-PCOS patients and PCOS patients. After PN formation, developmental rates between the groups were similar until t5. Development of embryos from IVM patients slowed down after t6. This might be linked to the finding that "human gene expression first occurs between the 4- and 8-cell stages of preimplantation development" (22). The time needed for IVM might therefore directly influence embryo development and competence (23). Although no difference in oocyte morphology between matured oocytes after IVM and metaphase II oocytes after conventional stimulation has been described (24), genes reflecting oocyte competence seem to be altered after in vitro maturation of human oocytes (3). TLI has been studied extensively to predict the developmental competence of embryos (7–9, 25–29). In the present study, embryos from IVM patients developed significantly more quickly to tPNa but slowed down until reaching t6 and then remained slower compared with conventional ICSI patients with or without PCOS.

Our study data are in contrast to those of Walls et al. (11), who did not see any difference in morphokinetic development between the study populations when comparing embryos progressing to the blastocyst stage; in particular, no accelerated or decelerated development was present in IVM embryos in PCOS compared with non-PCOS patients (11). Our IVM approach is slightly different from the protocol used by Walls et al. In contrast to their study, the FSH priming in our study was performed for only 3 days compared with 3–6 days (11, 19), and Walls et al. did not trigger ovulation with the use of hCG (11, 19). The rate of mature oocytes at the day of oocyte pick-up in our study was low (7/379), so effects of hCG priming on the overall maturation of oocytes are rather unlikely. Although the vast majority of retrieved oocytes were morphologically immature, hCG triggering might, at least in part, explain the differences in the morphokinetic parameters. In addition, Walls et al. used a different composition

of IVM medium, with higher concentrations of FSH and hCG: rFSH 0.1 IU/mL versus 0.075 IU/mL; hCG 0.5 IU/mL versus 0.1 IU/mL. This may also have led to differences in morphokinetic patterns compared with our results. Moreover, the selection of embryos included in the study of Walls et al. was different, because they only included embryos that developed to blastocysts. Because of national regulations (German Embryo Protection Act) it was possible to culture only a limited number of embryos in our study. Supernumerary oocytes were cryopreserved at the 2PN stage. Among the remaining cultured embryos, we included only those that were either transferred or cryopreserved. Among those were also embryos of poor quality that did not develop to blastocysts until day 5. These selection differences might have contributed to the conflicting results. Above all, inclusion criteria for patients were not the same: Walls et al. included only patients with age <37 years and BMI <35 kg/m^2. Therefore, results might have been influenced by distinctive metabolic disturbances in patients with BMI >35 kg/m^2 or age >37 years leading to a decrease of ovarian reserve.

In our study, embryos from patients with PCOS reached different time points during early embryo development significantly faster or slower compared with embryos from patients without PCOS. Wissing et al. published data indicating that embryos of hyperandrogenic PCOS patients developed slower than embryos from normoandrogenic PCOS patients or non-PCOS control subjects (4), whereas no significant difference was present in early embryo development analyzed with the use of TLI between normoandrogenic PCOS patients and control subjects. The differences in embryo kinetics diminished after the 7-cell stage (4). In our study population, only one IVM patient suffered from hyperandrogenism, so no subgroup analysis was performed according to differences in androgen concentration. In addition, the differences in embryo kinetics within our study subgroups persisted after the 7-cell stage.

Owing to our small study population no significant difference was present when PRs and LBRs were analyzed. The highest PR was achieved in the non-PCOS control group (56.7%). Because of the German Embryo Protection Act, PRs in Germany may differ from countries with less restrictive regulations. However, PRs in Germany published in the German IVF registry (30–32) vary from 28.7% to 30.3% (2012–2014) and are thus lower than the PRs achieved in our study population (30.0%–56.7%). Walls et al. reported CPRs of 43.8% in the IVM and 36.2% in the IVF group and LBRs of 18.8% (IVM) and 31.0% (IVF), which are in accordance with our own LBRs (20.0%–42.1%) (11).

A limitation of the present study is the lack of molecular and genetic data, because IVM may lead to abnormal follicle recruitment as well as disturbed oocyte maturation. It is also assumed that IVM may cause genetic disorders based on imprinting defects, e.g., Beckwith-Wiedemann or Angelman syndrome (33). So far, aneuploidy of IVM embryos has been addressed in several studies, but there is still insufficient data to demonstrate a higher aneuploidy rate in embryos after IVM (34, 35). Because of the German Embryo Protection Act, routine preimplantation genetic screening is not feasible for IVM patients. However, mature oocytes after IVM did not show abnormal methylation patterns (36), and recently we were able to show that no epigenetic abnormalities in the umbilical cord blood of children born after IVM treatment of the mother were present (37), rendering a major genetic impact on our TLI findings rather unlikely. Furthermore, we analyzed the development of children conceived with the use of IVM and found no differences in their development up to 2 years compared with children conceived after conventional stimulation for IVF and ICSI (paper submitted). In addition, other studies dealing with the outcome of children after IVM found no abnormalities in anthropometric or mental development data (38–43). Nevertheless, our data are preliminary and cannot serve as predictive markers for developmental competence of IVM embryos yet. Owing to the small sample size of the present study, further studies including a larger number of IVM patients are needed to confirm our results and establish potential morphokinetic time points.

In summary, our findings show that, despite fewer GQEs in the IVM group, PRs and LBRs were equal in all groups. IVM avoids the risks of OHSS, so IVM can be offered to all patients at high risk for OHSS, e.g., patients with PCOS or after OHSS in a former cycle with conventional stimulation (44). In our study we wanted to detect possible morphokinetic variables that may give us the ability to evaluate embryogenesis for choosing the best-quality embryo. To interpret the annotated parameters correctly, knowledge of the influence of patient-specific characteristics (e.g., PCOS) and different stimulation protocols (e.g., IVM, conventional stimulation for IVF or ICSI) on morphokinetics is needed. Our findings will help us to interpret the results obtained by TLI to increase PR and—important for patients—LBR.

CONCLUSION

TLI is a noninvasive method to analyze embryo potential and might give more insight in the early stages of embryo development after IVM compared with conventional stimulation, thus contributing to the safety of IVM for a select group of patients. Because of the small sample size of the present study, further studies with a larger population are needed to confirm our results.

Acknowledgments: The authors thank Christel Enders, Sabrina Hoffmann, and Julia Jauckus for technical assistance in the IVF lab; Riku Togawa for assistance in the selection of patient data; and Thomas Bruckner (Institute of Medical Biometry and Informatics, University of Heidelberg) for support in statistical analysis.

REFERENCES

1. Roesner S, von Wolff M, Eberhardt I, Beuter-Winkler P, Toth B, Strowitzki T. In vitro maturation: a five-year experience. Acta Obstet Gynecol Scand 2012; 91:22–7.
2. Das M, Son WY, Buckett W, Tulandi T, Holzer H. In-vitro maturation versus IVF with GnRH antagonist for women with polycystic ovary syndrome: treatment outcome and rates of ovarian hyperstimulation syndrome. Reprod Biomed Online 2014;29:545–51.
3. Guzman L, Ortega-Hrepich C, Polyzos NP, Anckaert E, Verheyen G, Coucke W, et al. A prediction model to select PCOS patients suitable for

IVM treatment based on anti-müllerian hormone and antral follicle count. Hum Reprod 2013;28:1261–6.
4. Wissing ML, Bjerge MR, Olesen AI, Hoest T, Mikkelsen AL. Impact of PCOS on early embryo cleavage kinetics. Reprod Biomed Online 2014;28:508–14.
5. Wood JR, Dumesic DA, Abbott DH, Strauss JF 3rd. Molecular abnormalities in oocytes from women with polycystic ovary syndrome revealed by microarray analysis. J Clin Endocrinol Metab 2007;92:705–13.
6. Teixeira Filho FL, Baracat EC, Lee TH, Suh CS, Matsui M, Chang RJ, et al. Aberrant expression of growth differentiation factor-9 in oocytes of women with polycystic ovary syndrome. J Clin Endocrinol Metab 2002;87:1337–44.
7. Pribenszky C, Matyas S, Kovacs P, Losonczi E, Zadori J, Vajta G. Pregnancy achieved by transfer of a single blastocyst selected by time-lapse monitoring. Reprod Biomed Online 2010;21:533–6.
8. Meseguer M, Herrero J, Tejera A, Hilligsoe KM, Ramsing NB, Remohi J. The use of morphokinetics as a predictor of embryo implantation. Hum Reprod 2011;26:2658–71.
9. Meseguer M, Rubio I, Cruz M, Basile N, Marcos J, Requena A. Embryo incubation and selection in a time-lapse monitoring system improves pregnancy outcome compared with a standard incubator: a retrospective cohort study. Fertil Steril 2012;98:1481–9.e10.
10. Rubio I, Galan A, Larreategui Z, Ayerdi F, Bellver J, Herrero J, et al. Clinical validation of embryo culture and selection by morphokinetic analysis: a randomized, controlled trial of the Embryoscope. Fertil Steril 2014;102:1287–94.e5.
11. Walls ML, Hunter T, Ryan JP, Keelan JA, Nathan E, Hart RJ. In vitro maturation as an alternative to standard in vitro fertilization for patients diagnosed with polycystic ovaries: a comparative analysis of fresh, frozen and cumulative cycle outcomes. Hum Reprod 2015;30:88–96.
12. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 2004;81:19–25.
13. Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HW, Behre HM, et al. World Health Organization reference values for human semen characteristics. Hum Reprod Update 2010;16:231–45.
14. Balaban B, Brison D, Calderón G, Catt J, Conaghan J, Cowan L, et al. Alpha Scientists in Reproductive Medicine, ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod 2011;26:1270–83.
15. Gardner DK, Schoolcraft WB. In vitro culture of human blastocysts. In: Jansen R, Mortimer D, editors. Toward reproductive certainty: fertility and genetics beyond. Carnforth, U.K: Parthenon; 1999:378–88.
16. Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K, et al. International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009. Fertil Steril 2009;92:1520–4.
17. Ciray HN, Campbell A, Agerholm IE, Aguilar J, Chamayou S, Esbert M, et al. Proposed guidelines on the nomenclature and annotation of dynamic human embryo monitoring by a time-lapse user group. Hum Reprod 2014;29:2650–60.
18. Marcus R, Peritz E, Gabriel KR. Closed testing procedures with special reference to ordered analysis of variance. Biometrika 1976;63:655–60.
19. Walls ML, Ryan JP, Keelan JA, Hart R. In vitro maturation is associated with increased early embryo arrest without impairing morphokinetic development of useable embryos progressing to blastocysts. Hum Reprod 2015;30:1842–9.
20. Child TJ, Phillips SJ, Abdul-Jalil AK, Gulekli B, Tan SL. A comparison of in vitro maturation and in vitro fertilization for women with polycystic ovaries. Obstet Gynecol 2002;100:665–70.
21. Gremeau AS, Andreadis N, Fatum M, Craig J, Turner K, McVeigh E, et al. In vitro maturation or in vitro fertilization for women with polycystic ovaries? A case-control study of 194 treatment cycles. Fertil Steril 2012;98:355–60.
22. Braude P, Bolton V, Moore S. Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. Nature 1988;332:459–61.
23. Son WY, Lee SY, Lim JH. Fertilization, cleavage and blastocyst development according to the maturation timing of oocytes in in vitro maturation cycles. Hum Reprod 2005;20:3204–7.
24. Coticchio G, Guglielmo MC, dal Canto M, Fadini R, Mignini Renzini M, de Ponti E, et al. Mechanistic foundations of the metaphase II spindle of human oocytes matured in vivo and in vitro. Hum Reprod 2013;28:3271–82.
25. Hashimoto S, Kato N, Saeki K, Morimoto Y. Selection of high-potential embryos by culture in poly(dimethylsiloxane) microwells and time-lapse imaging. Fertil Steril 2012;97:332–7.
26. Cruz M, Garrido N, Herrero J, Perez-Cano I, Munoz M, Meseguer M. Timing of cell division in human cleavage-stage embryos is linked with blastocyst formation and quality. Reprod Biomed Online 2012;25:371–81.
27. Wong CC, Loewke KE, Bossert NL, Behr B, De Jonge CJ, Baer TM, et al. Noninvasive imaging of human embryos before embryonic genome activation predicts development to the blastocyst stage. Nat Biotechnol 2010;28:1115–21.
28. dal Canto M, Coticchio G, Mignini Renzini M, de Ponti E, Novara PV, Brambillasca F, et al. Cleavage kinetics analysis of human embryos predicts development to blastocyst and implantation. Reprod Biomed Online 2012;25:474–80.
29. Campbell A, Fishel S, Bowman N, Duffy S, Sedler M, Hickman CF. Modelling a risk classification of aneuploidy in human embryos using noninvasive morphokinetics. Reprod Biomed Online 2013;26:477–85.
30. Deutsches IVF-Register. Deutsches IVF-Register—jahrbuch 2012. J Reprod Med Endocrinol 2012, Sonderheft 2:1–48.
31. Deutsches IVF-Register. Deutsches IVF-Register—jahrbuch 2013. J Reprod Med Endocrinol 2013;5–6:239–73.
32. Deutsches IVF-Register. Deutsches IVF-Register—jahrbuch 2014. J Reprod Med Endocrinol 2014;6:511–45.
33. Chen Z, Robbins KM, Wells KD, Rivera RM. Large offspring syndrome: a bovine model for the human loss-of-imprinting overgrowth syndrome Beckwith-Wiedemann. Epigenetics 2013;8:591–601.
34. Requena A, Bronet F, Guillen A, Agudo D, Bou C, Garcia-Velasco JA. The impact of in-vitro maturation of oocytes on aneuploidy rate. Reprod Biomed Online 2009;18:777–83.
35. Zhang XY, Ata B, Son WY, Buckett WM, Tan SL, Ao A. Chromosome abnormality rates in human embryos obtained from in-vitro maturation and IVF treatment cycles. Reprod Biomed Online 2010;21:552–9.
36. Kuhtz J, Romero S, de Vos M, Smitz J, Haaf T, Anckaert E. Human in vitro oocyte maturation is not associated with increased imprinting error rates at LIT1, SNRPN, PEG3 and GTL2. Hum Reprod 2014;29:1995–2005.
37. Pliushch G, Schneider E, Schneider T, El Hajj N, Rosner S, Strowitzki T, et al. In vitro maturation of oocytes is not associated with altered deoxyribonucleic acid methylation patterns in children from in vitro fertilization or intracytoplasmic sperm injection. Fertil Steril 2015;103:720–7.e1.
38. Buckett WM, Chian RC, Holzer H, Dean N, Usher R, Tan SL. Obstetric outcomes and congenital abnormalities after in vitro maturation, in vitro fertilization, and intracytoplasmic sperm injection. Obstetrics Gynecol 2007;110:885–91.
39. Cha KY, Chung HM, Lee DR, Kwon H, Chung MK, Park LS, et al. Obstetric outcome of patients with polycystic ovary syndrome treated by in vitro maturation and in vitro fertilization–embryo transfer. Fertil Steril 2005;83:1461–5.
40. Fadini R, Mignini Renzini M, Guarnieri T, Dal Canto M, De Ponti E, Sutcliffe A, et al. Comparison of the obstetric and perinatal outcomes of children conceived from in vitro or in vivo matured oocytes in in vitro maturation treatments with births from conventional ICSI cycles. Hum Reprod 2012;27:3601–8.
41. Foix–l'Helias L, Grynberg M, Ducot B, Frydman N, Kerbrat V, Bouyer J, et al. Growth development of French children born after in vitro maturation. PLoS One 2014;9:e89713.
42. Shu-Chi M, Jiann-Loung H, Yu-Hung L, Tseng-Chen S, Ming IL, Tsu-Fuh Y. Growth and development of children conceived by in-vitro maturation of human oocytes. Early Hum Dev 2006;82:677–82.
43. Söderstrom-Anttila V, Salokorpi T, Pihlaja M, Serenius-Sirve S, Suikkari AM. Obstetric and perinatal outcome and preliminary results of development of children born after in vitro maturation of oocytes. Hum Reprod 2006;21:1508–13.
44. Sauerbrun-Cutler MT, Vega M, Keltz M, McGovern PG. In vitro maturation and its role in clinical assisted reproductive technology. Obstet Gynecol Surv 2015;70:45–57.

SUPPLEMENTAL TABLE 1

Number of oocytes and embryos cultured in vitro.

Group	Oocytes, n	Maturation rate, n (%)	Fertilization rate, n (%)	Embryos cultured per cycle, mean ± SD (range)	Embryos transferred per cycle, mean ± SD (range)	GQEs, n (%)	Transfers, n (%)
G1 (n = 30 cycles)	379	224 (59.1)	138 (61.6)	3.50 ± 1.17 (1–6)	1.90 ± 0.71 (1–3)	21/105 (20.0)	57/105 (54.3)
G2 (n = 30 cycles)	342	291 (85.1)	186 (63.9)	3.83 ± 1.23 (1–8)	1.87 ± 0.35 (1–2)	40/115 (34.8)	56/115 (48.7)
G3 (n = 19 cycles)	285	204 (71.6)	137 (67.2)	3.89 ± 1.05 (2–5)	2.05 ± 0.23 (2–3)	36/74 (48.6)	39/74 (52.7)
P value		< .001	ns	ns	ns	< .001	

Note: G1 = in vitro maturation patients with PCOS; G2 = patients without PCOS; G3 = PCOS patients; GQEs = good quality embryos.

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SUPPLEMENTAL TABLE 2

Morphokinetics monitored by time-lapse imaging.

Parameter	Group	n	Mean ± SD (median, range)	*P* value
tPB2	G1	57	3.28 ± 1.16 (3.27, 1.01–8.92)	.245
	G2	58	3.69 ± 1.62 (3.44, 1.81–12.76)	
	G3	46	3.54 ± 0.93 (3.41, 1.81–6.06)	
tPNa	G1	57	5.66 ± 1.25 (5.55, 3.69–11.25)	< .001
	G2	60	6.93 ± 2.36 (6.61, 3.79–17.13)	
	G3	49	6.41 ± 1.19 (6.38, 4.10–9.41)	
tPNf	G1	56	23.72 ± 3.71 (23.70, 17.66–36.88)	.453
	G2	60	24.42 ± 3.65 (23.67, 18.96–36.11)	
	G3	46	23.46 ± 3.66 (23.19, 16.89–35.90)	
t2	G1	56	26.52 ± 3.79 (26.16, 19.99–39.21)	.337
	G2	65	27.85 ± 5.16 (26.51, 22.11–43.02)	
	G3	49	26.54 ± 4.87 (25.79, 19.22–41.23)	
t3	G1	54	37.13 ± 5.37 (37.91, 24.21–48.66)	.121
	G2	64	38.39 ± 5.35 (37.26, 28.17–53.70)	
	G3	47	36.03 ± 3.93 (35.76, 25.93–43.75)	
t4	G1	52	38.34 ± 4.94 (38.30, 25.58–51.70)	.364
	G2	63	39.43 ± 5.39 (37.79, 28.84–54.37)	
	G3	45	38.15 ± 6.14 (37.13, 28.59–64.93)	
t5	G1	46	52.14 ± 9.13 (51.68, 35.28–83.02)	.07
	G2	63	52.53 ± 8.16 (50.39, 38.68–73.45)	
	G3	45	48.79 ± 6.00 (49.77, 37.60–65.26)	
t6	G1	45	55.58 ± 9.54 (53.95, 39.76–83.86)	.028
	G2	62	54.04 ± 8.15 (51.47, 42.74–82.09)	
	G3	44	50.96 ± 7.30 (50.34, 38.27–70.61)	
t7	G1	43	59.57 ± 10.75 (56.63, 42.07–92.03)	.004
	G2	61	56.91 ± 8.88 (54.70, 44.24–83.09)	
	G3	43	52.77 ± 8.58 (51.11, 38.60–76.17)	
t8	G1	40	63.39 ± 13.08 (60.02, 42.40–92.70)	.005
	G2	61	59.52 ± 10.73 (56.04, 44.58–99.52)	
	G3	43	55.02 ± 9.34 (52.60, 40.65–80.62)	
t9+	G1	34	78.28 ± 13.14 (75.06, 55.74–112.05)	.024
	G2	55	73.28 ± 10.36 (71.37, 53.27–100.69)	
	G3	39	69.95 ± 11.53 (70.82, 50.27–111.78)	

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SUPPLEMENTAL TABLE 2

Continued.

Parameter	Group	n	Mean ± SD (median, range)	*P* value
tM	G1	34	90.34 ± 11.51 (90.87, 66.71–113.55)	.038
	G2	54	89.63 ± 9.17 (89.71, 63.61–106.92)	
	G3	32	85.52 ± 5.81 (86.72, 73.23–95.32)	
tSB	G1	29	101.36 ± 7.82 (102.51, 84.77–113.58)	.003
	G2	40	101.10 ± 7.47 (101.98, 88.55–114.88)	
	G3	20	94.84 ± 5.64 (93.37, 86.14–107.47)	
tB	G1	19	106.10 ± 5.68 (106.46, 95.32–115.94)	.002
	G2	34	106.86 ± 6.45 (109.00, 93.80–114.52)	
	G3	20	100.11 ± 6.67 (98.31, 90.38–113.31)	
tEB	G1	12	108.98 ± 4.47 (108.64, 100.17–114.84)	.022
	G2	17	109.45 ± 4.17 (110.86, 98.51–114.77)	
	G3	21	105.69 ± 5.36 (104.54, 96.19–118.29)	
ECC1	G1	56	23.27 ± 3.44 (16.75–33.96)	.203
	G2	58	23.29 ± 3.12 (17.98–32.24)	
	G3	46	22.90 ± 4.90 (16.33–38.18)	
ECC2	G1	52	12.11 ± 3.20 (1.00–18.41)	.023
	G2	63	11.90 ± 2.76 (1.83–23.53)	
	G3	45	11.97 ± 4.77 (1.68–35.01)	
ECC3	G1	40	24.87 ± 11.18 (12.50–52.07)	.001
	G2	61	20.12 ± 7.64 (11.18–49.58)	
	G3	43	17.61 ± 6.85 (3.67–41.01)	
s2	G1	52	1.33 ± 2.31 (0.00–10.84)	.995
	G2	63	1.09 ± 2.13 (0.00–13.00)	
	G3	45	2.15 ± 4.72 (0.00–24.34)	
s3	G1	40	10.91 ± 10.99 (0.83–45.01)	.034
	G2	61	7.25 ± 7.70 (0.67–31.75)	
	G3	43	6.50 ± 7.27 (1.00–29.08)	

Note: tPB2 = time to extrusion of polar body 2; tPNa = time to appearance of two pronuclei; tPNf = time to appearance of two pronuclei; *tn* = time to *n*-cell stage; tM = time to morula stage; tSB = time to start of blastulation; tB = time to blastocyst; tEB = time to expanding blastocyst; ECC = embryonic cell cycle; ECC1 = t2 − tPB2; ECC2 = t4 − t2; ECC3 = t8 − t4; s2 = synchronicity of cell divisions t4 − t3; s3 = synchronicity of cell divisions t8 − t5; other abbreviations as in Supplemental Table 1.

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SUPPLEMENTAL TABLE 3

Success rates in the study population.

Parameter and group	d2	d2/3 mix	d3	d3/4 mix	d4	d5	Total
Pregnancy rate							
G1	1/6	1/1	2/4	1/1	0/0	6/18	11 (36.7%)
G2	0/1		3/3		4/7	10/19	17 (56.7%)
G3	0/2		1/3		3/5	5/9	9 (47.4%)
P value							.299
Biochemical pregnancies							
G1	0/6	0/1	0/4	0/1	0/0	2/18	2 (6.7%)
G2	0/1		0/3		0/7	0/19	0 (0.0%)
G3	0/2		0/3		0/5	1/9	1 (5.3%)
P value							.373
Clinical pregnancies							
G1	1/6	1/1	2/4	1/1	0/0	4/18	9 (30.0%)
G2	0/1		3/3		4/7	10/19	17 (56.7%)
G3	0/2		1/3		3/5	4/9	8 (42.1%)
P value							.113
Miscarriage							
G1	1	0	1	0	0	1	3 (10.0%)
G2	0		0		0	1	1 (3.3%)
G3	0		0		0	0	0 (0.0%)
P value							.257
Live birth							
G1	0	1	1	1	0	3	6 (20%)
G2	0		2 + 1[a]		2 + 2[a]	8 + 1[a]	12 (40.0%)
G3	0		1		3	4	8 (42.1%)
P value							.159

Note: G1: n = 30 cycles; G2: n = 30 cycles; G3: n = 19 cycles; d = day; other abbreviations as in Supplemental Table 1.

[a] Ongoing pregnancy(ies) or pregnancy(ies) with unknown status.

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