To the Editor: Embryo development at the early cleavage stage is a complex and well-orchestrated biological process. During in vitro fertilization-embryo transfer (IVF-ET), embryos are routinely stratified according to morphological criteria and transferred on the morning of day 3 after fertilization. This stage of embryonic development is mainly regulated by maternal factors because the embryonic genome has not yet been fully activated.[1] Day 3 is a key period for embryos to accomplish embryonic genome activation (EGA). Embryos with higher developmental potential initiate EGA for subsequent development and implantation, while non-viable embryos undergo arrest.[2] However, it is difficult to precisely select embryos with EGA. Thus, implantation rates remain at 20% to 30% in IVF-ET.[3] During IVF-ET treatment, we found that more than half of day 3 embryos continued developing during an extended culture (EC) of 7 to 8 h combined with conventional competence based on an increased blastomere number and implantation, while non-viable embryos undergo arrest. We analyzed all oocyte retrieval cycles of women under 38 years with normal ovarian reserves and ≥2 available embryos on the morning of day 3 post-insemination.

In the EC group, embryos in one oocyte retrieval cycle were graded at 08:00 to 09:00 and continuously cultured for 7 to 8 h until 16:00 on day 3. We assessed developmental competence based on an increased blastomere number during the EC of 7 to 8 h combined with conventional morphological criteria. Continually developed embryos were preferred for transfer. In the control group, embryos were evaluated once at 08:00 to 09:00 on the morning of day 3, and then good-quality embryos were transferred before the others.

Ovarian stimulation and embryo treatments are described in Supplementary Material 1, http://links.lww.com/CM9/A242.

Clinical pregnancy was confirmed when a gestational sac with the presence of a fetal heartbeat was detected by transvaginal ultrasound examination 5 weeks after ET. Clinical outcomes were obtained via telephone follow-up with patients. Patients with live births were followed for 7 days after delivery. Perinatal and neonatal outcomes were evaluated. Deliveries included all live newborns and stillbirths after 28 weeks of gestation. Perinatal and neonatal mortality included stillbirths after 28 weeks of gestation and mortality within 1 week of a live birth.

Here, 963 oocyte retrieval cycles met inclusion criteria; 457 and 506 cycles in the EC and control groups, respectively. No significant differences were observed in patients’ characteristics and embryo development between the two groups [Supplementary Table 1, http://links.lww.com/CM9/A242].

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After the EC of 7 to 8 h on day 3, 57.63% of embryos continually developed, which were defined as ones with sustainable developmental potential (SDP). Clinical pregnancy outcomes of fresh ET cycles and frozen-thawed ET cycles were better in the EC group than in the control group, such as higher clinical pregnancy rates and implantation rates, and lower miscarriage rates [Supplementary Tables 2 and 3, http://links.lww.com/CM9/A242]. However, no differences were found in neonatal or obstetrical outcomes between the two groups.

There were 674 and 880 ET cycles in the EC and control groups, respectively. Total clinical pregnancy and total live birth rates in the EC group were significantly higher than the control group (55.04% vs. 48.98%, \( P = 0.018 \), and 46.29% vs. 37.95%, \( P < 0.001 \), respectively). The total implantation rate was higher in the EC group compared with the control group (34.48% vs. 29.67%, \( P = 0.003 \)). Similarly, the rates of live newborn infants per ET cycle and per transferred embryo were all significantly higher in the EC group compared with the control group (59.35% vs. 48.64%, \( P < 0.001 \), and 28.61% vs. 22.75%, \( P < 0.001 \), respectively). However, no significant difference was found in the cumulative live birth rates (68.27% vs. 66.01%, \( P = 0.369 \) [Table 1].

A similar number of oocyte retrieval cycles were needed for each group to achieve 100 live births or 100 live newborn infants. However, the number of ET cycles and the number of embryos transferred were significantly decreased in the EC group compared with the control group [Supplementary Figure 1A and 1B, http://links.lww.com/CM9/A242]. Kaplan-Meier survival analysis showed a similar cumulative probability of achieving one live birth in each group, but a shorter time for one live birth in the EC group compared with the control group [Supplementary Figure 1C, http://links.lww.com/CM9/A242].

In the vast majority of human embryos, EGA occurred by day 3, regardless of quality assessment or cell number.\(^4\) Thus, day 3 post-fertilization is a critical period for human embryos to initiate EGA. Consistent with previous studies, most available embryos (80.47%) in our study developed to the 4 to 8-cell stage on the morning of day 3. The EC of 7 to 8 h provided a short time for viable embryos to trigger EGA, and some embryos with SDP (57.63%) continued developing. Live birth rates were significantly improved in the EC group compared with the control group.

Sustainable developmental competence is one of the key indicators for viable embryos in quality assessment. Routinely, embryos that do not cleave during the preceding 24 h are graded as development arrest and generally not suggested to transfer.\(^1\) Here, embryos with SDP possessed a higher developmental potential than others. However, this strategy only increased the exposure duration of 7 to 8 h, which was much less than the blastocyst culture of

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Table 1: Clinical outcomes of patients after the extended 7–8 h culture of day 3 embryos.

| Outcomes                             | EC group   | Control group | \( P \)  |
|--------------------------------------|------------|---------------|---------|
| Oocyte retrieval cycle               | 457        | 506           |         |
| ET cycle                             |            |               |         |
| Fresh ET cycle                       | 262        | 317           |         |
| FET cycle                            | 412        | 563           |         |
| Total                                | 674        | 880           |         |
| Clinical pregnancy                   |            |               |         |
| Fresh ET cycle                       | 132 (50.38)| 146 (46.06)   | 0.300   |
| ET cycle                             | 239 (58.01)| 285 (50.62)   | 0.022   |
| Total                                | 371 (55.04)| 431 (48.98)   | 0.018   |
| Implantation                         |            |               |         |
| Fresh ET cycle                       | 177 (33.65)| 192 (29.49)   | 0.126   |
| FET cycle                            | 305 (34.98)| 366 (29.76)   | 0.011   |
| Total                                | 482 (34.48)| 558 (29.67)   | 0.003   |
| Miscarriage                          |            |               |         |
| Fresh ET cycle                       | 12 (9.09)  | 24 (16.44)    | 0.072   |
| FET cycle                            | 33 (13.81) | 59 (20.70)    | 0.039   |
| Total                                | 45 (12.13) | 83 (19.26)    | 0.006   |
| Live birth                           |            |               |         |
| Fresh ET cycle                       | 113 (43.13)| 116 (36.59)   | 0.109   |
| FET cycle                            | 199 (48.30)| 218 (38.72)   | 0.003   |
| Total                                | 312 (46.29)| 334 (37.95)   | <0.001  |
| Live newborn infant                  | 400        | 428           |         |
| Live newborn infant per ET cycle     | 400 (59.35)| 428 (48.64)   | <0.001  |
| Live newborn infant per transferred embryo* | 400 (28.61)| 428 (22.75)   | <0.001  |
| Cumulative live birth (% oocyte retrieval cycles) | 312 (68.27) | 334 (66.01)   | 0.369   |

Values are shown as \( n, n \) (%). \( \star \)transferred embryos, \( n=1398 \) in the EC group and \( n=1881 \) in the control group. EC: Extended culture; ET: Embryo transfer; FET: Frozen-thawed embryo transfer.

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1. \(^1\) Emory A, et al. J Assist Reprod Genet. 2019;36:397-400.
2. \(^2\) Zini L, et al. Fertil Steril. 2005;83:1141-8.
3. \(^3\) Bosco G, et al. Hum Reprod. 2009;24:1090-4.
4. \(^4\) Iams JD, et al. N Engl J Med. 1996;335:1505-11.
48 to 72 h. There was only 3.35% of embryos that were not eligible for transfer and discarded in the EC group, which was significantly lower than the blastocyst formation rate (approximately 50%). Furthermore, embryos without an increased blastomere in the EC group also exhibited some developmental potential. Thus, this strategy did not lead to a decline in cumulative live birth rates, but shortened the time for one live birth for most patients, and reduced the psychological burden and financial costs.

In conclusion, the current EC of day 3 embryos might provide a viable strategy to further improve the live birth rate during IVF-ET treatment.

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Conflicts of interest
None.

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