Extended culture of day 3 embryos improves live birth rate in *in vitro* fertilization-embryo transfer

Xiao-Peng Zhao[1,2], Ling Wang[2], Lu Ma[1], Di Zuo[1], Fei Chang[2], Li-Yuan Zhang[2], Jun-Long Jia[2], Shao-Kang Mo[2], Yuan-Kun Sang[2], Bin-Bin Ma[3], Fang Xu[1]

[1]Key Laboratory of Fertility Preservation and Maintenance, Ministry of Education, School of Basic Medical Sciences, Ningxia Medical University, Yinchuan, Ningxia 750004, China;
[2]Center for Reproductive Medicine, The 940th Hospital of Joint Logistics Support Force of Chinese People's Liberation Army, Lanzhou, Gansu 730050, China;
[3]Research Center for Regenerative Medicine, Shanghai East Hospital, School of Life Sciences and Technology, Tongji University, Shanghai 200120, China.

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 from 08:00–09:00 to 16:00, and live birth rates increased when these embryos were transferred. Then, this strategy was used to improve live birth rates during IVF-ET.

This study retrospectively analyzed the pregnancy outcomes of patients undergoing IVF-ET treatment at the Center for Reproductive Medicine of the 940th Hospital between January 2012 and December 2017. All procedures performed in studies strictly complied with the 1964 *Helsinki Declaration* and its later amendments or comparable ethical standards, as well as the relevant laws and regulations of China. The study was approved by the Ethics Committee of the 940th Hospital (No. 2019KYLL001). The authors certify that they obtained all appropriate patient consent forms.

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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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Correspondence to: Fang Xu, Key Laboratory of Fertility Preservation and Maintenance, Ministry of Education, School of Basic Medical Sciences, Ningxia Medical University, 1160 Shengli Street, Yinchuan, Ningxia 750004, China
E-Mail: xufang@nxmu.edu.cn

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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* (%). * 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.

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,[3,4] 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. We concluded that the majority of embryos with SDP were more likely to be those which had initiated EGA or had the potential to initiate EGA later. The EC of day 3 embryos may help to identify embryos with higher developmental potential for ET, and improve the live birth rate of ET cycles.

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.[5] 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

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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