# Supplementary Information

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## List of participating members

The following persons participated in data collection and site monitoring: Yangyang Xu, Yuan Xu, Jinfeng Yuan and Shuzhen Tao at the Center for Clinical Reproductive Medicine, the First Affiliated Hospital of Nanjing Medical University; Xinyang Zhao and Tianxiao Liang at the Center of Reproductive Medicine, Department of Obstetrics and Gynecology, Shengjing Hospital of China Medical University; Zhen Li, Jing Liu, Xiaona Yu, Wenxia Liu, Meng Zhang, and Wenying Wang at the Reproductive Medicine Center, The Third Affiliated Hospital of Zhengzhou University; Yan Hong, and Bing Xu at the Center for Reproductive Medicine, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai Key Laboratory for Assisted Reproduction and Reproductive Genetics, Shanghai; Mei Yin at the Department of Reproductive Medicine Center, Maternal and Child Health Hospital in Guang Xi; Xin Wu, Yanling Niu, Ting Wang, Xiaoting Han, Junxiu Li, Jinmei Gao, Lingxia Ha, Mengling Song, Lili Ma and Liguo Pei at Reproductive Medicine Center, General Hospital of Ningxia Medical University; Yiqun Luo and Mingzhu Cao at the Center for Reproductive Medicine, Key Laboratory for Major Obstetric Diseases of Guangdong Province, The Third Affiliated Hospital of Guangzhou Medical University; All the medical staffs at the Department of Obstetrics and Gynecology, the First Affiliated Hospital of Anhui Medical University; Qingxia Meng and Huimin Li at the Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou Municipal Hospital; Shaodi Zhang, Yuanhui Chen and Xiaohang Xu at the Reproductive Medical Center, Henan Provincial People's Hospital; Taixiang Wu at Resman, a public management platform for medical research in China.

## Supplementary Tables

### Supplementary Table 1. Definition of secondary outcomes

| Term | Definition |
|---|---|
| Biochemical pregnancy | Biochemical pregnancy was defined as detection of serum β -HCG≥25 IU/L 14 days after embryo transfer. |
| Clinical pregnancy | Clinical pregnancy was defined as the presence of intrauterine gestation sacs at 30-35 days after embryo transfer. |
| Implantation | Implantation was defined as the number of gestational sacs per embryo transferred. |
| Ongoing pregnancy | Ongoing pregnancy was defined as a viable fetus with heartbeat at 12 weeks' gestation. |
| Ovarian hyperstimulation syndrome (OHSS) | OHSS was defined according to the Golan criteria. Moderate OHSS was diagnosed by presence of ascites on ultrasonography in addition to abdominal distension and discomfort with or without nausea, vomiting and/or diarrhea. Severe OHSS was diagnosed when there was clinical evidence of ascites and/or hydrothorax or breathing difficulties with or without hemoconcentration, coagulation abnormalities and diminished renal function. |
| Pregnancy loss | Pregnancy loss was defined as pregnancies that result in a spontaneous abortion or therapeutic abortion at any point throughout pregnancy. |
| Ectopic pregnancy | Ectopic pregnancy was one in which the embryo implanted at any site other than the endometrial lining of the uterus cavity. |
| Gestational diabetes mellitus (GDM) | GDM was defined as carbohydrate intolerance of variable severity with onset or first recognition during pregnancy as determined from the diagnosis in the obstetrical medical record. |
| Pre-eclampsia | Pre-eclampsia was defined as the development of gestational hypertension with proteinuria (≥300 mg/24-hour urine collection or 30 mg/dL in single urine sample) of new onset after 20 weeks of gestation. |
| Gestational hypertension | Gestational hypertension was defined as development of blood pressure greater than 140/90 mmHg after pregnancy without proteinuria or other signs of preeclampsia. |
| Premature rupture of membrane (PROM) | PROM was defined as rupture of the amniotic membranes before the onset of labor. |
| Preterm premature rupture of membrane (PPROM) | PPROM was defined as PROM occurs before 37 weeks of pregnancy |
| Preterm birth | Delivery of a fetus at less than 37 and more than 24 weeks' gestational age. |
| Very preterm birth | Delivery of a fetus at less than 32 and more than 24 weeks' gestational age. |
| Placenta previa | Placenta previa was used to describe a placenta that is implanted over or very near the internal cervical os. |
| Placental abruption | Placental abruption was defined as the early separation of a placenta from the lining of the uterus before completion of the second stage of labor, typically reserved for pregnancies over 20 weeks of gestation. |
| Congenital anomalies | Congenital anomalies were defined as structural or functional anomalies that occur during intrauterine life, including minor and major anomalies. |

| | |
|---|---|
| Postpartum hemorrhage | Postpartum hemorrhage was defined as blood loss ≥500 mL after vaginal birth or ≥1000 mL after cesarean delivery after completion of the third stage of labor. |
| Puerperal infection | Any bacterial infection of the genital tract after delivery and during puerperium. |
| Stillbirth | Stillbirth was defined as the death or loss of a baby before or during delivery at 20 weeks of pregnancy and later. |
| Neonatal death | Neonatal death was defined as deaths among live births during the first 28 completed days of life. |
| Miscarriage | Miscarriage refers to spontaneous abortion. It defines as the spontaneous pregnancy loss before the $24^{th}$ week of pregnancy. |
| Abortion | Abortion refers to induce/artificial abortion. It defines as the removal of pregnancy tissue, products of conception or the fetus and placenta (afterbirth) from the uterus. |
| Threatened miscarriage/abortion | Vaginal bleeding that occurs in the first 24 weeks of pregnancy. The cervical os is closed on physical exam. The patient may also experience abdominal cramping and pain. |
| Threatened preterm labor | Threatened preterm labor is defined as the progression of cervical dilatation and ripening caused by regular uterine contractions occurring at gestational age of 24+0 to 36+6. |
| Neonatal respiratory distress syndrome | Respiratory insufficiency with hypoxemia and compensatory tachypnea for newborns |
| Neonatal jaundice | Neonatal jaundice was a yellowing of skin and other tissues of a newborn infant |
| Neonatal infection | Neonatal infection was defined as a variety of infections in neonates caused by bacteria, fungi, virus, etc. as determined from the diagnosis in the neonatal medical record. |
| Small for gestational age | A birth weight below the 10th percentile for their gestational age. |
| Large for gestational age | A birth weight above the 90th percentile for their gestational age. |
| Macrosomia | Macrosomia was defined as birth weight over 4000 grams (8 pounds, 13 ounces), regardless of gestational age. |
| Low birth weight infant | Low birth weight was defined as birth weight less than 2500 grams (5 pounds, 8 ounces), regardless of gestational age. |
| Very low birth weight infant | Very low birth weight was defined as birth weight less than 1500 grams (3 pounds, 5 ounces), regardless of gestational age. |

## Supplementary Table 2: Live birth, pregnancy, and pregnancy loss after the first embryo transfer

| Outcomes, No. (%) | Blastocyst-stage embryo transfer group (n=486) | Cleavage-stage embryo transfer group (n=491) | Absolute difference (95% CI) | Relative risk (95% CI) * | *P* value † |
|---|---|---|---|---|---|
| **Live birth** † | 256 (52.7) | 200 (40.7) | 11.9 (5.7 to 18.2) | 1.29 (1.13 to 1.48) | 0.00018 |
| Singleton live birth | 250 (51.4) | 198 (40.3) | 11.1 (4.9 to 17.3) | 1.28 (1.11 to 1.46) | 0.00049 |
| Twin live birth | 6 (1.2) | 2 (0.4) | 0.8 (-0.3 to 2.0) | 3.03 (0.61 to 14.94) | 0.18 |
| **Pregnancy** | | | | | |
| Implantation per embryo ‡ | 293/489 (59.9) | 235/497 (47.3) | 12.6 (6.5 to 18.8) | 1.27 (1.13 to 1.43) | 6.97e-05 |
| Biochemical pregnancy § | 322 (66.3) | 258 (52.5) | 13.7 (7.6 to 19.8) | 1.26 (1.13 to 1.40) | 1.29e-05 |
| Clinical pregnancy ¶ | 296 (60.9) | 239 (48.7) | 12.2 (6.0 to 18.4) | 1.25 (1.11 to 1.40) | 0.00012 |
| Ongoing pregnancy ** | 267 (54.9) | 203 (41.3) | 13.6 (7.4 to 19.8) | 1.33 (1.16 to 1.52) | 2.12e-05 |
| **Pregnancy loss** | | | | | |
| Biochemical pregnancy loss | 26/322 (8.1) | 19/258 (7.4) | 0.7 (-3.7 to 5.1) | 1.10 (0.62 to 1.94) | 0.75 |
| Clinical pregnancy loss | 33/296 (11.1) | 32/239 (13.4) | -2.2 (-7.9 to 3.4) | 0.83 (0.53 to 1.31) | 0.43 |
| First trimester | 25/296 (8.4) | 30/239 (12.6) | -4.1 (-9.4 to 1.2) | 0.67 (0.41 to 1.11) | 0.12 |
| Second trimester | 8/296 (2.7) | 2/239 (0.8) | 1.9 (-0.3 to 4.0) | 3.23 (0.69 to 15.07) | 0.20 |

* Relative Risk are for the blastocyst-stage group versus cleavage-stage group. † *P*=2.22e-12 for noninferiority (with margin = -0.1, α = 0.025, one-sided). All *P* values are for superiority, two-sided. No adjustments were made for multiple comparison. Chi-square test was used. ‡ Implantation rate: number of gestational sacs divided by number of embryos that were transferred. § Biochemical pregnancy: serum human chorionic gonadotropin ≥25 IU/L 14 days after embryo transfer. ¶ Clinical pregnancy: detection of intrauterine gestation sacs at 30-35 days after embryo transfer. ** Ongoing pregnancy: detection of a viable fetus with heartbeat at 12 weeks' gestation.

## Supplementary Table 3: Live birth, pregnancy, and pregnancy loss after the second embryo transfer

| Outcomes, No. (%) | Blastocyst-stage embryo transfer group (n=189) | Cleavage-stage embryo transfer group (n=261) | Absolute difference (95 CI%) | Relative risk (95% CI) * | *P* value † |
|---|---|---|---|---|---|
| **Live birth** † | 89 (47.1) | 85 (32.6) | 14.5 (5.4 to 23.6) | 1.45 (1.15 to 1.82) | 0.002 |
| Singleton live birth | 87 (46.0) | 84 (32.2) | 13.8 (4.8 to 22.9) | 1.43 (1.13 to 1.81) | 0.003 |
| Twin live birth | 2 (1.1) | 1 (0.4) | 0.7 (-1.0 to 2.3) | 2.76 (0.25 to 30.24) | 0.57 |
| **Pregnancy** | | | | | |
| Implantation per embryo ‡ | 114/196 (58.2) | 101/274 (36.9) | 21.3 (12.3 to 30.3) | 1.58 (1.30 to 1.92) | 4.86e-06 |
| Biochemical pregnancy § | 123 (65.1) | 119 (45.6) | 19.5 (10.4 to 28.6) | 1.43 (1.21 to 1.69) | 4.28e-05 |
| Clinical pregnancy ¶ | 113 (59.8) | 102 (39.1) | 20.7 (11.5 to 29.9) | 1.53 (1.26 to 1.85) | 1.42e-05 |
| Ongoing pregnancy ** | 94/189 (49.7) | 87 (33.3) | 16.4 (7.3 to 25.5) | 1.49 (1.19 to 1.87) | 0.00046 |
| **Pregnancy loss** | | | | | |
| Biochemical pregnancy loss | 10/123 (8.1) | 17/119 (14.3) | -6.2 (-14.1 to 1.8) | 0.57 (0.27 to 1.19) | 0.15 |
| Clinical pregnancy loss | 21/113 (18.6) | 13/102 (12.7) | 5.8 (-3.8 to 15.5) | 1.46 (0.77 to 2.76) | 0.22 |
| First trimester | 17/113 (15.0) | 11/102 (10.8) | 4.3 (-4.7 to 13.2) | 1.40 (0.69 to 2.84) | 0.42 |
| Second trimester | 4/113 (3.5) | 2/102 (2.0) | 1.6 (-2.8 to 5.9) | 1.81 (0.34 to 9.65) | 0.69 |

* Relative Risk are for the blastocyst-stage group versus cleavage-stage group. † *P*=6.57e-08 for noninferiority (with margin = -0.1, α = 0.025, one-sided). All *P* values are for superiority, two-sided. No adjustments were made for multiple comparison. Chi-square test or Fisher's Exact Test was used. ‡ Implantation rate: number of gestational sacs divided by number of embryos that were transferred. § Biochemical pregnancy: serum human chorionic gonadotropin ≥25 IU/L 14 days after embryo transfer. ¶ Clinical pregnancy: detection of intrauterine gestation sacs at 30-35 days after embryo transfer. ** Ongoing pregnancy: detection of a viable fetus with heartbeat at 12 weeks' gestation.

## Supplementary Table 4: Live birth, pregnancy, and pregnancy loss after the third embryo transfer

| Outcomes, No. (%) | Blastocyst-stage embryo transfer group (n=52) | Cleavage-stage embryo transfer group (n=123) | Absolute difference (95% CI) | Relative risk (95% CI) * | *P* value† |
|---|---|---|---|---|---|
| **Live birth** † | 21 (40.4) | 36 (29.3) | 11.1 (-4.5 to 26.7) | 1.38 (0.90 to 2.12) | 0.15 |
| Singleton live birth | 20 (38.5) | 35 (28.5) | 10.0 (-5.4 to 25.4) | 1.35 (0.87 to 2.11) | 0.19 |
| Twin live birth | 1 (1.9) | 1 (0.8) | 1.1 (-2.9 to 5.2) | 2.37 (0.15 to 37.10) | 0.51 |
| **Pregnancy** | | | | | |
| Implantation per embryo ‡ | 27/60 (45.0) | 51/137 (37.2) | 7.8 (-7.2 to 22.7) | 1.21 (0.85 to 1.72) | 0.30 |
| Biochemical pregnancy § | 26 (50.0) | 56 (45.5) | 4.5 (-11.7 to 20.7) | 1.10 (0.79 to 1.53) | 0.59 |
| Clinical pregnancy ¶ | 24 (46.2) | 51 (41.5) | 4.7 (-11.4 to 20.8) | 1.11 (0.78 to 1.60) | 0.57 |
| Ongoing pregnancy ** | 21 (40.4) | 39 (31.7) | 8.7 (-7.0 to 24.3) | 1.27 (0.84 to 1.94) | 0.27 |
| **Pregnancy loss** | | | | | |
| Biochemical pregnancy loss | 2/26 (7.7) | 5/56 (8.9) | -1.2 (-13.9 to 11.4) | 0.86 (0.18 to 4.15) | 0.85 |
| Clinical pregnancy loss | 3/24 (12.5) | 10/51 (19.6) | -7.1 (-24.2 to 10.0) | 0.64 (0.19 to 2.11) | 0.53 |
| First trimester | 3/24 (12.5) | 9/51 (17.6) | -5.1 (-22.0 to 11.7) | 0.71 (0.21 to 2.38) | 0.74 |
| Second trimester | 0/24 (0.0) | 1/51 (2.0) | -2.0 (-5.8 to 1.8) | NA | 1.00 |

* Relative Risk are for the blastocyst-stage group versus cleavage-stage group. † *P*=0.004 for noninferiority (with margin = -0.1, α = 0.025, one-sided). All *P* values are for superiority, two-sided. No adjustments were made for multiple comparison. Chi-square test or Fisher exact test was used. ‡ Implantation rate: number of gestational sacs divided by number of embryos that were transferred. § Biochemical pregnancy: serum human chorionic gonadotropin ≥25 IU/L 14 days after embryo transfer. ¶ Clinical pregnancy: detection of intrauterine gestation sacs at 30-35 days after embryo transfer. ** Ongoing pregnancy: detection of a viable fetus with heartbeat at 12 weeks' gestation.

## Supplementary Table 5: Cumulative live births and pregnancy outcomes (per-protocol analysis)

| Outcomes, No. (%) | Blastocyst-stage embryo transfer (n=456) | Cleavage-stage embryo transfer (n=425) | Absolute difference (95% CI) * | Relative risk (95% CI) | *P* value † |
|---|---|---|---|---|---|
| **Primary outcome** | | | | | |
| Cumulative live births ‡ | 350 (76.8) | 291 (68.5) | 8.3 (2.4 to 14.2) | 1.12 (1.03 to 1.22) | 0.006 |
| Singleton live births | 344 (75.4) | 289 (68.0) | 7.4 (1.5 to 13.4) | 1.11 (1.02 to 1.21) | 0.014 |
| All twin live births | 6 (1.3) | 2 (0.5) | 0.8 (-0.4 to 2.1) | 2.80 (0.57 to 13.78) | 0.33 |
| Monozygotic twin live births | 6 (1.3) | 2 (0.5) | 0.8 (-0.4 to 2.1) | 2.80 (0.57 to 13.78) | 0.33 |
| **Secondary outcomes** | | | | | |
| Cumulative biochemical pregnancies § | 396 (86.8) | 348 (81.9) | 5.0 (0.2 to 9.8) | 1.06 (1.00 to 1.12) | 0.04 |
| Cumulative clinical pregnancies ¶ | 384 (84.2) | 332 (78.1) | 6.1 (0.9 to 11.3) | 1.08 (1.01 to 1.15) | 0.02 |
| Cumulative ongoing pregnancies ** | 361 (79.2) | 296 (69.6) | 9.5 (3.8 to 15.3) | 1.14 (1.05 to 1.23) | 0.001 |
| Cumulative pregnancy loss | | | | | |
| Biochemical pregnancy loss | 32 (7.0) | 29 (6.8) | 0.2 (-3.2 to 3.5) | 1.03 (0.63 to 1.67) | 0.91 |
| Clinical pregnancy loss (Miscarriage) | 45 (9.9) | 45 (10.6) | -1.8 (-6.7 to 3.1) | 0.86 (0.59 to 1.27) | 0.46 |
| Miscarriage <12 weeks gestation | 34 (7.5) | 41 (9.6) | -3.5 (-8.0 to 1.0) | 0.72 (0.47 to 1.10) | 0.13 |
| Miscarriage 12-24 weeks gestation | 11 (2.4) | 4 (0.9) | 1.7 (-0.4 to 3.7) | 2.38 (0.76 to 7.40) | 0.12 |
| Ectopic pregnancy | 4 (0.9) | 8 (1.9) | -1.3 (-3.1 to 0.6) | 0.44 (0.13 to 1.45) | 0.16 |
| Live birth after first embryo transfer | 248/450 (55.1) | 190/425 (44.7) | 10.4 (3.8 to 17.0) | 1.23 (1.08 to 1.41) | 0.002 |
| Live birth after second embryo transfer | 83/167 (49.7) | 74/211 (35.1) | 14.6 (4.7 to 24.6) | 1.42 (1.12 to 1.80) | 0.004 |
| Live birth after third embryo transfer | 19/43 (44.2) | 27/95 (28.4) | 15.8 (-1.6 to 33.2) | 1.55 (0.98 to 2.47) | 0.07 |
| Median time to livebirth since randomization, d †† | 340 (329−349) ‡‡ | 352 (340−375) ‡‡ | -15.2(-15.9 to -14.6) | 1.22 (1.04 to 1.42) § § | 0.01 ¶¶ |
| Number of unused frozen embryos | 4.2 ± 3.4 | 6.1 ± 4.0 | -1.9 (-2.4, -1.4) | | 5.18e-14 |
| Number of unused frozen embryos in women with a live birth | 4.7 ± 3.4 | 6.5 ± 3.8 | -1.8 (-2.3, -1.2) | | 1.17e-09 |
| Number of unused frozen embryos in women without a live birth | 2.4 ± 2.7 | 5.3 ± 4.3 | -2.8 (-3.7, -1.9) | | 1.22e-08 |
| Number of women without a frozen embryo | 61 (13.4) | 13 (3.1) | 10.3 (6.8 to 13.8) | 4.37 (2.44 to 7.84) | 3.44e-08 |
| Number of women without a frozen embryo without a livebirth | 38 (8.3) | 12 (2.8) | 5.5 (2.5 to 8.5) | 2.95 (1.56 to 5.57) | 0.00041 |
| Number of women without a frozen embryo with a livebirth | 23 (5.0) | 1 (0.2) | 4.8 (2.7 to 6.9) | 21.44 (2.91 to 158.04) | 1.18e-05 |

* Absolute differences in percentages are indicated in percentage points, and absolute differences in other values are indicated in units of that value.

† All *P* values are for superiority, two-sided. No adjustments were made for multiple comparison. Two sample *t*-test was used for continuous data; Chi-square test was used for categorical data.

‡ Cumulative live births were calculated from up to the first 3 embryo transfers in 1 year after randomization (with a 3-months extension for those affected by Covid-19) from one oocyte retrieval cycle. Live birth was defined as delivery of any neonate ≥24 weeks gestation that had a heartbeat and was breathing. *P*=5.40e-10 for noninferiority.

§ Biochemical pregnancy was defined as serum human chorionic gonadotropin ≥25 IU/L 14 days after embryo transfer.

¶ Clinical pregnancy was defined as detection of intrauterine gestation sacs at 30-35 days after embryo transfer.

** Ongoing pregnancy was defined as detection of a viable fetus with heartbeat at 12 weeks' gestation.

†† The length of time from randomization to 50% of the participants who achieved a livebirth.

‡‡ 95% confidence interval for median time to live birth.

§ § Hazard ratio (95% confidence interval). ¶¶ log-rank test.

## Supplementary Table 6: Cumulative obstetric and perinatal outcomes (per-protocol analysis)

| Outcomes, No. (%) | Blastocyst-stage embryo transfer (n=456) | Cleavage-stage embryo transfer (n=425) | Absolute difference (95% CI) * | Relative risk (95% CI) | *P* value |
|---|---|---|---|---|---|
| **Live births outcomes** | | | | | |
| Gestational age, mean (SD), weeks | 38.8 ±1.8 | 39.0±1.7 | -0.2 (-0.4 to 0.1) | | 0.28 |
| Birthweight, mean (SD), g | | | | | |
| Singleton | | | | | |
| No. of observations | 344 | 289 | | | |
| Mean weight | 3355.4 ± 507.1 | 3330.9 ± 524.6 | 24.4 (-56.3 to 105.1) | | 0.55 |
| Twin | | | | | |
| No. of observations | 12 | 4 | | | |
| Mean weight | 2257.5 ± 745.7 | 1837.5 ± 782.4 | 420.0 (-513.3 to 1353.3) | | 0.35 |
| Low birth weight † | 17 (3.7) | 14 (3.3) | 0.4 (-2.0 to 2.9) | 1.13 (0.56 to 2.27) | 0.73 |
| Very low birth weight ‡ | 5 (1.1) | 4 (0.9) | 0.2 (-1.2 to 1.5) | 1.17 (0.31 to 4.31) | 0.82 |
| Macrosomia § | 32 (7.0) | 30 (7.1) | -0.0 (-3.4 to 3.3) | 0.99 (0.62 to 1.61) | 0.98 |
| Small for gestational age ¶ | 22 (4.8) | 17 (4.0) | 0.8 (-1.9 to 3.5) | 1.21 (0.65 to 2.24) | 0.55 |
| Large for gestational age ** | 60 (13.2) | 46 (10.8) | 2.3 (-1.9 to 6.6) | 1.22 (0.85 to 1.74) | 0.29 |
| Sex ratio | | | | | |
| Male | 194/356 (54.5) | 147/293 (50.2) | 4.3 (-3.4 to 12.0) | 1.09 (0.94 to 1.26) | 0.27 |
| Female | 162/356 (45.5) | 146/293 (49.8) | -4.3 (-12.0 to 3.4) | 0.91 (0.78 to 1.07) | |
| Live birth without a complication | 121 (26.5) | 107 (25.2) | 1.4 (-4.4 to 7.1) | 1.05 (0.84 to 1.32) | 0.65 |
| **Maternal complications** | | | | | |
| Moderate or severe OHSS | 22 (4.8) | 14 (3.3) | 1.5 (-1.1 to 4.1) | 1.46 (0.76 to 2.82) | 0.25 |
| Gestational diabetes mellitus | 52 (11.4) | 47 (11.1) | 0.3 (-3.8 to 4.5) | 1.03 (0.71 to 1.50) | 0.87 |
| Preeclampsia or eclampsia | 4 (0.9) | 12 (2.8) | -1.9 (-3.7 to -0.2) | 0.31 (0.10 to 0.96) | 0.03 |
| Gestational hypertension | 18 (3.9) | 16 (3.8) | 0.2 (-2.4 to 2.7) | 1.05 (0.54 to 2.03) | 0.89 |
| Preterm premature rupture of membrane †† | 25 (5.5) | 6 (1.4) | 4.1 (1.7 to 6.4) | 3.88 (1.61 to 9.37) | 0.001 |
| Premature rupture of membrane | 49 (10.7) | 32 (7.5) | 3.2 (-0.6 to 7.0) | 1.43 (0.93 to 2.18) | 0.10 |
| Preterm birth ‡‡ | 30 (6.6) | 14 (3.3) | 3.3 (0.4 to 6.1) | 2.00 (1.07 to 3.71) | 0.03 |
| Spontaneous preterm birth § § | 23 (5.0) | 9 (2.1) | 2.9 (0.5 to 5.4) | 2.38 (1.11 to 5.09) | 0.02 |
| Iatrogenic preterm birth ¶¶ | 7 (1.5) | 5 (1.2) | 0.4 (-1.2 to 1.9) | 1.30 (0.42 to 4.08) | 0.65 |
| Very preterm birth (<32 Weeks) *** | 5 (1.1) | 4 (0.9) | 0.2 (-1.2 to 1.5) | 1.17 (0.31 to 4.31) | 0.82 |
| Placenta previa | 8 (1.8) | 2 (0.5) | 1.3 (-0.1 to 2.7) | 3.73 (0.80 to 17.46) | 0.14 |
| Placental abruption | 3 (0.7) | 2 (0.5) | 0.2 (-0.8 to 1.2) | 1.40 (0.23 to 8.33) | 0.71 |
| Placental accreta | 10 (2.2) | 5 (1.2) | 1.0 (-0.7 to 2.7) | 1.86 (0.64 to 5.41) | 0.24 |
| Other placental abnormality | 11 (2.4) | 7 (1.6) | 0.8 (-1.1 to 2.6) | 1.46 (0.57 to 3.74) | 0.42 |
| Postpartum hemorrhage | 8 (1.8) | 16 (3.8) | -2.0 (-4.2 to 0.2) | 0.47 (0.20 to 1.08) | 0.07 |
| **Neonatal complications** | | | | | |
| Therapeutic abortion or fetal reduction due to fetal congenital | 4 (0.9) | 3 (0.7) | 0.2 (-1.0 to 1.3) | 1.24 (0.28 to 5.52) | 0.77 |

| | | | | | |
|---|---|---|---|---|---|
| anomalies during 12 to 28 weeks of gestation ††† | | | | | |
| Stillbirth | 6 (1.3) | 3 (0.7) | 0.6 (-0.7 to 1.9) | 1.86 (0.47 to 7.41) | 0.57 |
| Neonatal hospitalization > 3 days | 55 (12.1) | 28 (6.6) | 5.5 (1.7 to 9.3) | 1.83 (1.18 to 2.83) | 0.006 |
| Neonatal jaundice | 114 (25.0) | 87 (20.5) | 4.5 (-1.0 to 10.1) | 1.22 (0.96 to 1.56) | 0.11 |
| Neonatal infection | 23 (5.0) | 10 (2.4) | 2.7 (0.2 to 5.2) | 2.14 (1.03 to 4.45) | 0.04 |
| Neonatal death among live newborns | 1 (0.2) | 2 (0.5) | -0.3 (-1.0 to 0.5) | 0.47 (0.04 to 5.12) | 0.95 |
| Congenital anomaly ††† | 15 (3.3) | 9 (2.1) | 1.2 (-1.0 to 3.3) | 1.55 (0.69 to 3.51) | 0.29 |

OHSS=ovarian hyperstimulation syndrome. Two-sided *P* values. No adjustments were made for multiple comparison.

Two sample *t*-test was used for continuous data; Chi-square test was used for categorical data.

* Absolute differences in percentages are indicated in percentage points, and absolute differences in other values are indicated in units of that value.

† Low birth weight was defined as a value of less than 2500g.

‡ Very low birth weight was defined as a value of less than 1500g.

§ Macrosomia was defined as a value of more than 4000g.

¶ Birthweight lower than 10th percentile.

** Birthweight higher than 90th percentile.

†† Preterm premature rupture of membrane in the table refers to premature rupture of membrane before 37 weeks gestational age.

‡‡ Preterm birth was defined as delivery at less than 37 weeks gestational age.

§ § Spontaneous preterm birth was defined as preterm delivery due to spontaneous labor resulting from preterm premature rupture of membranes, cervical factors and other reasons.

¶¶ Iatrogenic preterm birth was defined as a preterm birth resulting from a planned delivery due to maternal and/or fetal complications, including labor induction or cesarean section.

*** Very preterm birth was defined as delivery at less than 32 weeks gestational age.

††† Details of congenital anomaly were listed in supplementary Table 7.

## Supplementary Table 7. Details of congenital anomalies based on the intention-to-treat analysis

| Outcomes | Blastocyst-stage embryo transfer group (n=497) | Cleavage-stage embryo transfer group (n=495) | Absolute difference (95% CI) | *P* value |
|---|---|---|---|---|
| Total congenital anomalies, No. (%)* | 22/387 (5.7) | 14/336 (4.2) | 1.5 (-1.6 to 4.7) | 0.35 |
| Therapeutic abortion or fetal reduction due to fetal congenital anomalies during 12-28 weeks gestation, No. (%) | 6/416 (1.4)<br>1 with absence of radius of left forearm<br>1 due to abnormal development of left forearm<br>2 due to hydrocephalus<br>1 due to congenital cardiovascular anomalies (atrioventricular septal defect)<br>1 with toe deformity and thoracogastroschisis | 4/378 (1.1)<br>1 due to cervical lymphatic hygroma<br>1 due to talipes varus and hand deformity<br>1 due to meningocele<br>1 due to congenital cardiovascular anomalies (ventricular septal defect and aortic atresia) | 0.4 (-1.2 to 1.9) | 0.87 |
| Major congenital anomalies in live newborns, No. (%) | 11/381 (2.9)<br>2 with congenital choledochal cyst<br>1 with acardia and anencephaly<br>1 with laryngeal cartilage dysplasia<br>1 with polydactyly<br>4 with cardiovascular anomalies (atrial septal defect or patent ductus arteriosus)<br>1 with syndactyly with fusion of fourth and fifth toes<br>1 with pseudohermaphroditism | 9/332 (2.7)<br>1 with congenital absence of iris<br>1 with right inguinal hernia<br>1 with talipes varus<br>1 with congenital megacolon<br>2 with cardiovascular anomalies (atrial septal defect or atrial septal aneurysm)<br>1 with polydactyly and patent ductus arteriosus<br>1 with congenital torticollis<br>1 congenital intestinal malrotation with midgut torsion | 0.2 (-2.2 to 2.6) | 0.89 |
| Minor congenital anomalies in live newborns, No. (%) | 5/381 (1.3)<br>1 congenital malformation of external ear<br>4 patent foramen ovale | 1/332 (0.3)<br>1 patent foramen ovale | 1.0 (-0.3 to 2.3) | 0.29 |

* The denominator represents the number of live neonates plus the number of fetuses therapeutically terminated.

## Supplementary Table 8. Serious adverse events (all) and adverse events occurring in more than 2% of patients in either group (intention-to-treat)

| Outcomes, No. (%) | Blastocyst-stage embryo transfer group (n=497) | Cleavage-stage embryo transfer group (n=495) | Absolute difference (95% CI) | Relative risk (95% CI) | *P* value |
|---|---|---|---|---|---|
| **Maternal adverse events** | | | | | |
| **Before biochemical pregnancy** | | | | | |
| Moderate or severe OHSS * | 23/497 (4.6) | 17/495 (3.4) | 1.2 (-1.3 to 3.6) | 1.35 (0.73 to 2.49) | 0.34 |
| **After biochemical pregnancy** | | | | | |
| **First trimester** | | | | | |
| Ectopic pregnancy † | 7/430 (1.6) | 11/399 (2.8) | -1.1 (-3.1 to 0.9) | 0.59 (0.23 to 1.51) | 0.27 |
| Threatened abortion ‡ | 51/416 (12.3) | 39/378 (10.3) | 1.9 (-2.5 to 6.3) | 1.19 (0.80 to 1.76) | 0.39 |
| Hyperemesis ‡ | 3/416 (0.7) | 6/378 (1.6) | -0.9 (-2.4 to 0.6) | 0.45 (0.11 to 1.80) | 0.41 |
| **Second or third trimester** | | | | | |
| Gestational diabetes mellitus ‡ | 54/416 (13.0) | 55/378 (14.6) | -1.6 (-6.4 to 3.2) | 0.89 (0.63 to 1.26) | 0.52 |
| Preeclampsia or eclampsia ‡ | 5/416 (1.2) | 14/378 (3.7) | -2.5 (-4.7 to -0.3) | 0.32 (0.12 to 0.89) | 0.02 |
| Gestational hypertension ‡ | 20/416 (4.8) | 17/378 (4.5) | 0.3 (-2.6 to 3.2) | 1.07 (0.57 to 2.01) | 0.84 |
| Preterm premature rupture of membrane ‡ | 25/497 (5.0) | 8/495 (1.6) | 3.4 (1.2 to 5.6) | 3.11 (1.42 to 6.83) | 0.003 |
| Premature rupture of membrane ‡ | 51/416 (12.3) | 37/378 (9.8) | 2.5 (-1.9 to 6.8) | 1.25 (0.84 to 1.87) | 0.27 |
| Preterm delivery ‡ | 30/416 (7.2) | 18/378 (4.8) | 2.4 (-0.8 to 5.7) | 1.51 (0.86 to 2.67) | 0.15 |
| Very preterm delivery ‡ | 5/416 (1.2) | 4/378 (1.1) | 0.1 (-1.3 to 1.6) | 1.14 (0.31 to 4.20) | 0.85 |
| Placenta previa ‡ | 8/416 (1.9) | 2/378 (0.5) | 1.4 (-0.1 to 2.9) | 3.63 (0.78 to 17.01) | 0.15 |
| Placental abruption ‡ | 3/416 (0.7) | 2/378 (0.5) | 0.2 (-0.9 to 1.3) | 1.36 (0.23 to 8.11) | 0.73 |
| Placental accreta ‡ | 12/416 (2.9) | 6/378 (1.6) | 1.3 (-0.7 to 3.3) | 1.82 (0.69 to 4.79) | 0.22 |
| Other placental abnormality ‡ | 11/416 (2.6) | 8/378 (2.1) | 0.5 (-1.6 to 2.6) | 1.25 (0.51 to 3.07) | 0.63 |
| Cervical incompetence ‡ | 4/416 (1.0) | 6/378 (1.6) | -0.6 (-2.2 to 0.9) | 0.61 (0.17 to 2.13) | 0.64 |
| Anemia ‡ | 46/416 (11.1) | 44/378 (11.6) | -0.6 (-5.0 to 3.8) | 0.95 (0.64 to 1.40) | 0.80 |
| **After delivery** | | | | | |
| Postpartum hemorrhage § | 10/375 (2.7) | 17/331 (5.1) | -2.5 (-5.4 to 0.4) | 0.52 (0.24 to 1.12) | 0.09 |
| Puerperal infection § | 2/375 (0.5) | 0/331 (0.0) | 0.5 (-0.2 to 1.3) | .. | 0.50 |
| Postpartum anemia § | 12/375 (3.2) | 18/331 (5.4) | -2.2 (-5.3 to 0.8) | 0.59 (0.29 to 1.20) | 0.14 |
| **Neonatal adverse events** | | | | | |
| Therapeutic abortion or fetal reduction due to fetal congenital anomalies during 12 to 28 weeks of gestation ‡ | 6/416 (1.4) | 4/378 (1.1) | 0.4 (-1.2 to 1.9) | 1.36 (0.39 to 4.79) | 0.87 |
| Stillbirth ‡ | 8/416 (1.9) | 4/378 (1.1) | 0.9 (-0.8 to 2.5) | 1.82 (0.55 to 5.99) | 0.32 |
| Neonatal hospitalization > 3 days ¶ | 59/381 (15.5) | 32/332 (9.6) | 5.8 (1.0 to 10.7) | 1.61 (1.07 to 2.41) | 0.02 |
| Neonatal respiratory distress syndrome [e] | 7/381 (1.8) | 7/332 (2.1) | -0.3 (-2.3 to 1.8) | 0.87 (0.31 to 2.46) | 0.79 |
| Neonatal jaundice ¶ | 121/381 (31.8) | 101/332 (30.4) | 1.3 (-5.5 to 8.1) | 1.04 (0.84 to 1.30) | 0.70 |
| Neonatal infection ¶ | 24/381 (6.3) | 11/332 (3.3) | 3.0 (-0.1 to 6.1) | 1.90 (0.95 to 3.82) | 0.07 |
| Neonatal death among live newborns ¶ | 1/381 (0.3) | 2/332 (0.6) | -0.3 (-1.3 to 0.6) | 0.44 (0.04 to 4.78) | 0.90 |
| Congenital anomaly ¶ | 16/381 (4.2) | 10/332 (3.0) | 1.2 (-1.5 to 3.9) | 1.39 (0.64 to 3.03) | 0.40 |
| Intrauterine growth restriction ¶ | 1/381 (0.3) | 1/332 (0.3) | -0.0 (-0.8 to 0.7) | 0.87 (0.05 to 13.88) | 1.00 |
| Low birth weight ¶ | 21/381 (5.5) | 20/332 (6.0) | -0.5 (-3.9 to 2.9) | 0.91 (0.50 to 1.66) | 0.77 |
| Very low birth weight ¶ | 6/381 (1.6) | 5/332 (1.5) | 0.1 (-1.7 to 1.9) | 1.05 (0.32 to 3.40) | 0.94 |
| Macrosomia ¶ | 33/381 (8.7) | 36/332 (10.8) | -2.2 (-6.6 to 2.2) | 0.80 (0.51 to 1.25) | 0.33 |
| Small for gestational age ¶ | 23/381 (6.0) | 20/332 (6.0) | 0.0 (-3.5 to 3.5) | 1.00 (0.56 to 1.79) | 0.99 |
| Large for gestational age ¶ | 63/381 (16.5) | 54/332 (16.3) | 0.3 (-5.2 to 5.7) | 1.02 (0.73 to 1.42) | 0.92 |

OHSS=ovarian hyperstimulation syndrome. Two-sided *P* values. No adjustments were made for multiple comparison. Chi-square test or Fisher's Exact Test was used for categorical data.

* The denominator was number of women randomly assigned to each group. † The denominator was number of biochemical pregnancy. ‡ The denominator was number of clinical pregnancy. § The denominator was number of deliveries. ¶ The denominator was number of live newborns.

## Supplementary Table 9. Cumulative live births and pregnancy outcomes from all embryo transfers in 1 year and 3 months of randomization

| Outcomes, No. (%) | Blastocyst-stage embryo transfer (n=497) | Cleavage-stage embryo transfer (n=495) | Absolute difference (95% CI) * | Relative risk (95% CI) | *p* value† |
|---|---|---|---|---|---|
| **Primary outcome** | | | | | |
| Cumulative live births ‡ | 376 (75.7) | 341 (68.9) | 6.8 (1.2 to 12.3) | 1.10 (1.02 to 1.19) | 0.02 |
| Singleton live births | 366 (73.6) | 336 (67.9) | 5.8 (0.1 to 11.4) | 1.08 (1.00 to 1.18) | 0.046 |
| All twin live births | 10 (2.0) | 5 (1.0) | 1.0 (-0.5 to 2.5) | 1.99 (0.69 to 5.79) | 0.20 |
| Monozygotic twin live births | 7 (1.4) | 2 (0.4) | 1.0 (-0.2 to 2.2) | 3.49 (0.73 to 16.70) | 0.18 |
| **Secondary outcomes** | | | | | |
| Cumulative biochemical pregnancies § | 435 (87.5) | 412 (83.2) | 4.3 (-0.1 to 8.7) | 1.05 (1.00 to 1.11) | 0.06 |
| Cumulative clinical pregnancies ¶ | 422 (84.9) | 393 (79.4) | 5.5 (0.8 to 10.3) | 1.07 (1.01 to 1.13) | 0.02 |
| Cumulative ongoing pregnancies ** | 389 (78.3) | 349 (70.5) | 7.8 (2.4 to 13.2) | 1.11 (1.03 to 1.19) | 0.005 |
| Cumulative pregnancy loss | | | | | |
| Biochemical pregnancy loss | 37 (7.4) | 38 (7.7) | -0.2 (-3.5 to 3.1) | 0.97 (0.63 to 1.50) | 0.89 |
| Clinical pregnancy loss (Miscarriage) | 57 (11.5) | 58 (11.7) | -0.2 (-4.2 to 3.7) | 0.98 (0.69 to 1.38) | 0.90 |
| Miscarriage <12 weeks gestation | 46 (9.3) | 53 (10.7) | -1.5 (-5.2 to 2.3) | 0.86 (0.59 to 1.26) | 0.45 |
| Miscarriage 12-24 weeks gestation | 11 (2.2) | 5 (1.0) | 1.2 (-0.4 to 2.8) | 2.19 (0.77 to 6.26) | 0.13 |
| Ectopic pregnancy | 7 (1.4) | 12 (2.4) | -1.0 (-2.7 to 0.7) | 0.58 (0.23 to 1.46) | 0.24 |
| Median time to livebirth since randomization, d †† | 344 (334-353) ‡‡ | 373 (353-410) ‡‡ | -31.3(-32.1 to -30.4) | 1.23 (1.06 to 1.42) § § | 0.006 ¶¶ |
| Number of unused frozen embryos | 4.0 ± 3.4 | 5.7 ± 4.1 | -1.7 (-2.1 to -1.2) | | 2.21e-12 |
| Number of unused frozen embryos in women with a live birth | 4.7 ± 3.3 | 6.3 ± 4.0 | -1.7 (-2.2 to -1.1) | | 1.85e-09 |
| Number of unused frozen embryos in women without a live birth | 2.1 ± 2.6 | 4.4 ± 4.0 | -2.3 (-3.1 to -1.5) | | 1.04e-07 |
| Number of women without a frozen embryo | 76 (15.3) | 35 (7.1) | 8.2 (4.3 to 12.1) | 2.16 (1.48 to 3.16) | 4.01e-05 |
| Number of women without a frozen embryo without a livebirth | 49 (9.9) | 31 (6.3) | 3.6 (0.2 to 7.0) | 1.57 (1.02 to 2.43) | 0.04 |
| Number of women without a frozen embryo with a livebirth | 27 (5.4) | 4 (0.8) | 4.6 (2.5 to 6.8) | 6.72 (2.37 to 19.07) | 2.84e-05 |

OHSS=ovarian hyperstimulation syndrome.

* Absolute differences in percentages are indicated in percentage points, and absolute differences in other values are indicated in units of that value.

† All *P* values are for superiority, two-sided. No adjustments were made for multiple comparison. Two sample *t*-test was used for continuous data; Chi-square test was used for categorical data.

‡ Cumulative live births were calculated from up to the first 3 embryo transfers in 1 year after randomization (with a 3-months extension for those affected by Covid-19) from one oocyte retrieval cycle. Live birth was defined as delivery of any neonate ≥24 weeks gestation that had a heartbeat and was breathing. *P*=1.67e-09 for noninferiority.

§ Biochemical pregnancy was defined as serum human chorionic gonadotropin ≥25 IU/L 14 days after embryo transfer.

¶ Clinical pregnancy was defined as detection of intrauterine gestation sacs at 30-35 days after embryo transfer.

** Ongoing pregnancy was defined as detection of a viable fetus with heartbeat at 12 weeks' gestation.

†† The length of time from randomization to 50% of the participants who achieved a livebirth.

‡‡ 95% confidence interval for median time to live birth.

§ § Hazard ratio (95% confidence interval).

¶¶ log-rank test.

**Supplementary Table 10. Cumulative obstetric and perinatal outcomes from all embryo transfers in 1 year and 3 months of randomization**

| Outcomes, No. (%) | Blastocyst-stage embryo transfer group (n=497) | Cleavage-stage embryo transfer group (n=495) | Absolute difference (95% CI) * | Relative risk (95% CI) | *P* value |
|---|---|---|---|---|---|
| **Live births outcomes** | | | | | |
| Gestational age, mean (SD), weeks | 38.8 ±1.7 | 38.9 ±1.7 | -0.1 (-0.4 to 0.1) | | 0.39 |
| Birthweight, mean (SD), g | | | | | |
| Singleton | | | | | |
| No. of observations | 366 | 336 | | | |
| Mean weight | 3357.1 ±505.1 | 3333.2 ± 523.2 | 24.0 (-52.3 to 100.2) | | 0.54 |
| Twin | | | | | |
| No. of observations | 20 | 10 | | | |
| Mean weight | 2402.0 ± 621.0 | 2301.0 ± 708.3 | 101.0 (-457.4 to 659.4) | | 0.69 |
| Low birth weight † | 19 (3.8) | 18 (3.6) | 0.2 (-2.2 to 2.5) | 1.05 (0.56 to 1.98) | 0.88 |
| Very low birth weight ‡ | 5 (1.0) | 4 (0.8) | 0.2 (-1.0 to 1.4) | 1.24 (0.34 to 4.61) | 0.74 |
| Macrosomia § | 34 (6.8) | 37 (7.5) | -0.6 (-3.8 to 2.6) | 0.92 (0.58 to 1.43) | 0.70 |
| Small for gestational age ¶ | 23 (4.6) | 22 (4.4) | 0.2 (-2.4 to 2.8) | 1.04 (0.59 to 1.84) | 0.89 |
| Large for gestational age ** | 64 (12.9) | 56 (11.3) | 1.6 (-2.5 to 5.6) | 1.14 (0.81 to 1.59) | 0.45 |
| Sex ratio | | | | | |
| Male | 209/386 (54.1) | 173/346 (50.0) | 4.1 (-3.1 to 11.4) | 1.08 (0.94 to 1.25) | 0.26 |
| Female | 177/386 (45.9) | 173/346 (50.0) | -4.1 (-11.4 to 3.1) | 0.92 (0.79 to 1.07) | |
| Live birth without a complication | 131 (26.4) | 121 (24.4) | 1.9 (-3.5 to 7.3) | 1.08 (0.87 to 1.33) | 0.49 |
| **Maternal complications** | | | | | |
| Moderate or severe OHSS | 23 (4.6) | 17 (3.4) | 1.2 (-1.3 to 3.6) | 1.35 (0.73 to 2.49) | 0.34 |
| Gestational diabetes mellitus | 55 (11.1) | 58 (11.7) | -0.7 (-4.6 to 3.3) | 0.94 (0.67 to 1.34) | 0.75 |
| Preeclampsia or eclampsia | 5 (1.0) | 15 (3.0) | -2.0 (-3.8 to -0.3) | 0.33 (0.12 to 0.91) | 0.02 |
| Gestational hypertension | 20 (4.0) | 17 (3.4) | 0.6 (-1.8 to 2.9) | 1.17 (0.62 to 2.21) | 0.62 |
| Preterm premature rupture of membrane †† | 26 (5.2) | 8 (1.6) | 3.6 (1.4 to 5.9) | 3.24 (1.48 to 7.08) | 0.002 |
| Premature rupture of membrane | 52 (10.5) | 37 (7.5) | 3.0 (-0.6 to 6.5) | 1.40 (0.94 to 2.09) | 0.10 |
| Preterm birth ‡‡ | 31 (6.2) | 19 (3.8) | 2.4 (-0.3 to 5.1) | 1.63 (0.93 to 2.84) | 0.08 |
| Spontaneous preterm birth § § | 24 (4.8) | 10 (2.0) | 2.8 (0.6 to 5.1) | 2.39 (1.16 to 4.95) | 0.02 |
| Iatrogenic preterm birth ¶¶ | 7 (1.4) | 9 (1.8) | -0.4 (-2.0 to 1.2) | 0.77 (0.29 to 2.06) | 0.61 |
| Very preterm birth (<32 Weeks) *** | 5 (1.0) | 4 (0.8) | 0.2 (-1.0 to 1.4) | 1.24 (0.34 to 4.61) | 0.74 |
| Placenta previa | 8 (1.6) | 2 (0.4) | 1.2 (-0.0 to 2.4) | 3.98 (0.85 to 18.67) | 0.11 |
| Placental abruption | 3 (0.6) | 2 (0.4) | 0.2 (-0.7 to 1.1) | 1.49 (0.25 to 8.90) | 0.66 |
| Placental accreta | 12 (2.4) | 6 (1.2) | 1.2 (-0.5 to 2.9) | 1.99 (0.75 to 5.27) | 0.16 |
| Other placental abnormality | 11 (2.2) | 9 (1.8) | 0.4 (-1.4 to 2.1) | 1.22 (0.51 to 2.91) | 0.66 |
| Postpartum hemorrhage | 11 (2.2) | 18 (3.6) | -1.4 (-3.5 to 0.7) | 0.61 (0.29 to 1.28) | 0.18 |
| **Neonatal complications** | | | | | |
| Therapeutic abortion or fetal reduction due to fetal congenital | 6 (1.2) | 4 (0.8) | 0.4 (-0.8 to 1.6) | 1.49 (0.42 to 5.26) | 0.76 |

| | | | | | |
|---|---|---|---|---|---|
| anomalies during 12 to 28 weeks of gestation | | | | | |
| Stillbirth | 8 (1.6) | 4 (0.8) | 0.8 (-0.6 to 2.2) | 1.99 (0.60 to 6.57) | 0.25 |
| Neonatal hospitalization > 3 days | 58 (11.7) | 34 (6.9) | 4.8 (1.2 to 8.4) | 1.70 (1.13 to 2.55) | 0.009 |
| Neonatal jaundice | 121 (24.3) | 104 (21.0) | 3.3 (-1.9 to 8.5) | 1.16 (0.92 to 1.46) | 0.21 |
| Neonatal infection | 23 (4.6) | 13 (2.6) | 2.0 (-0.3 to 4.3) | 1.76 (0.90 to 3.44) | 0.09 |
| Neonatal death among live newborns | 1 (0.2) | 2 (0.4) | -0.2 (-0.9 to 0.5) | 0.50 (0.05 to 5.47) | 0.56 |
| Congenital anomaly | 17 (3.4) | 10 (2.0) | 1.4 (-0.6 to 3.4) | 1.69 (0.78 to 3.66) | 0.18 |

OHSS=ovarian hyperstimulation syndrome.

Two-sided *P* values. No adjustments were made for multiple comparison. Two sample *t*-test was used for continuous data; Chi-square test was used for categorical data.

* Absolute differences in percentages are indicated in percentage points, and absolute differences in other values are indicated in units of that value.

† Low birth weight was defined as a value of less than 2500g.

‡ Very low birth weight was defined as a value of less than 1500g.

§ Macrosomia was defined as a value of more than 4000g.

¶ Birthweight lower than 10th percentile.

** Birthweight higher than 90th percentile.

†† Preterm premature rupture of membrane in the table refers to premature rupture of membrane before 37 weeks gestational age.

‡‡ Preterm birth was defined as delivery at less than 37 weeks gestational age.

§ § Spontaneous preterm birth was defined as preterm delivery due to spontaneous labor resulting from preterm premature rupture of membranes, cervical factors and other reasons.

¶¶ Iatrogenic preterm birth was defined as a preterm birth resulting from a planned delivery due to maternal and/or fetal complications, including labor induction or cesarean section.

*** Very preterm birth was defined as delivery at less than 32 weeks gestational age.

**Supplementary Table 11. The association between type of embryo transfer cycle and two treatment groups in women with preeclampsia or eclampsia**

| | Blastocyst-stage Group (n=5) | Cleavage-stage Group (n=14) | p value* |
|---|---|---|---|
| Fresh cycle | 0/5(0.0%) | 6/14(42.9%) | 0.128 |
| Frozen cycle | 5/5(100.0%) | 8/14(57.1%) | |

*Fisher's Exact Test, two-sided.

**Supplementary Table 12. Logistic regression model to determine the adjusted treatment effect of blastocyst or cleavage stage transfer on preeclampsia.**

| | Unadjusted Odds Ratio (95% CI) | P value | Adjusted Odds Ratio (95% CI) | P value |
|---|---|---|---|---|
| Treatment arm, blastocyst transfer vs. cleavage-stage embryo transfer | 0.35 (0.12, 0.98) | 0.045 | 0.35 (0.12, 0.98) | 0.046 |
| Frozen vs. Fresh embryo transfer cycles | N.A. | N.A. | 0.80 (0.30, 2.14) | 0.657 |

N.A. indicates that the variable was not included the corresponding model. Two-sided *P* values. No adjustments were made for multiple comparison. Logistic regression model was used for analysis.

**Supplementary Table 13. Characteristics of embryo transfers between the two groups in the long-term follow-up cohort after the study period (1 year of randomization).**

| Characteristics | Blastocyst-stage embryo transfer group (n = 51) | Cleavage-stage embryo transfer group (n = 92) | *P* Value |
|---|---|---|---|
| No of embryos transferred, No. (%)[a] | | | |
| One embryo | 45/51 (88.2%) | 58/92 (63.0%) | 0.002 |
| Two embryos | 6/51 (11.8%) | 33/92 (35.9%) | |
| Three embryos | 0 (0.0%) | 1/92 (1.1%) | |
| Stage of embryo transferred, No. (%)[a] | | | |
| Blastocyst-stage embryo transfer | 51/51 (100.0%) | 38/92 (41.3%) | 1.31e-14 |
| Cleavage-stage embryo transfer | 0 (0.0%) | 54/92 (58.7%) | |

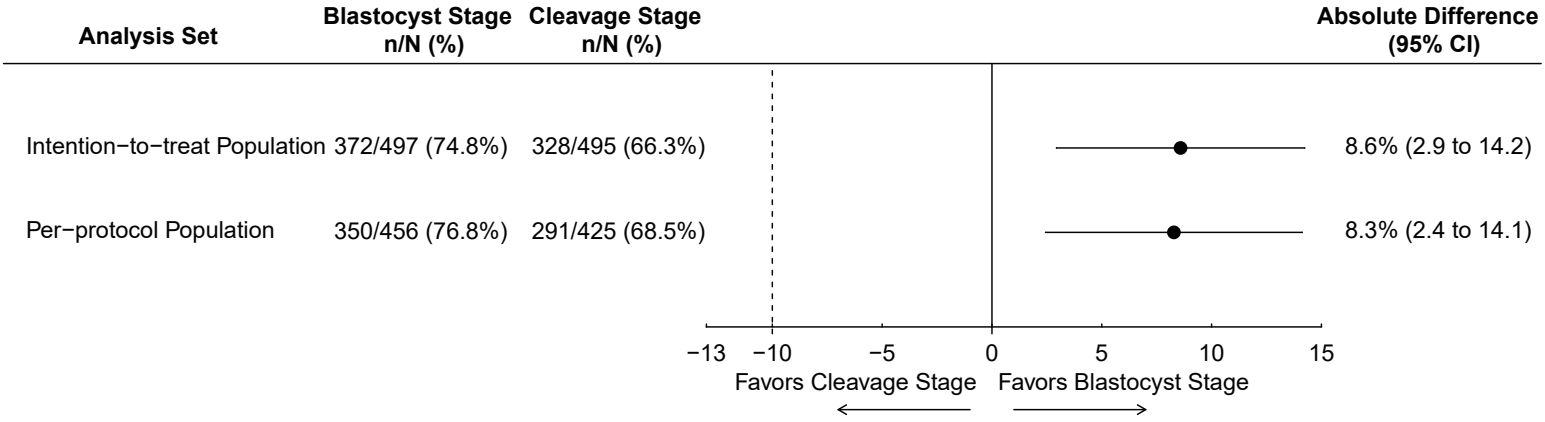
[a] Calculated based on the total number of embryo transfer cycles. Two-sided *P* values. No adjustments were made for multiple comparison. Fisher's Exact Test was used for analysis.

**Supplementary Table 14. Number and stage of embryos transferred leading to live births in the follow-up cohort after the study period (1 year of randomization).**

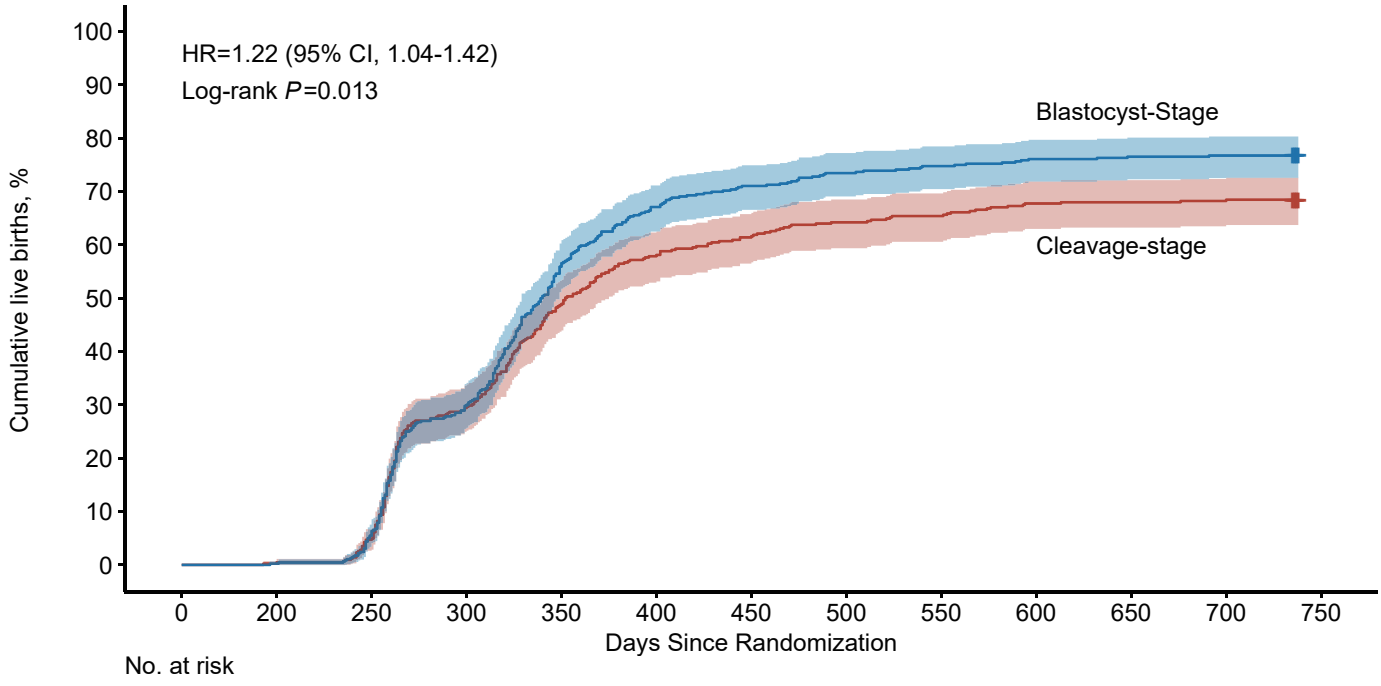
| Number and stage of embryos transferred No. (%) | Cumulative live births in blastocyst-stage group (n=26) | Cumulative live births in cleavage-stage group (n=43) |
|---|---|---|
| 1 cleavage-stage embryo | 0 (0.0) | 6 (14.0) |
| 1 blastocyst | 16 (61.5) | 14 (32.6) |
| 2 cleavage-stage embryos | 0 (0.0) | 10 (23.3) |
| 2 blastocysts | 5 (19.2) | 4 (9.3) |
| 1 cleavage-stage and 1 blastocyst-stage embryos | 0 (0.0) | 3 (7.0) |
| Natural conception | 5 (19.2) | 6 (14.0) |

**Supplementary Figure 1**
**Graphical summary and Kaplan-Meier curves for cumulative live births (per-protocol analysis)**

## A Principal analysis for the primary outcome




## B Kaplan-Meier curves for the primary outcome (per-protocol analysis)



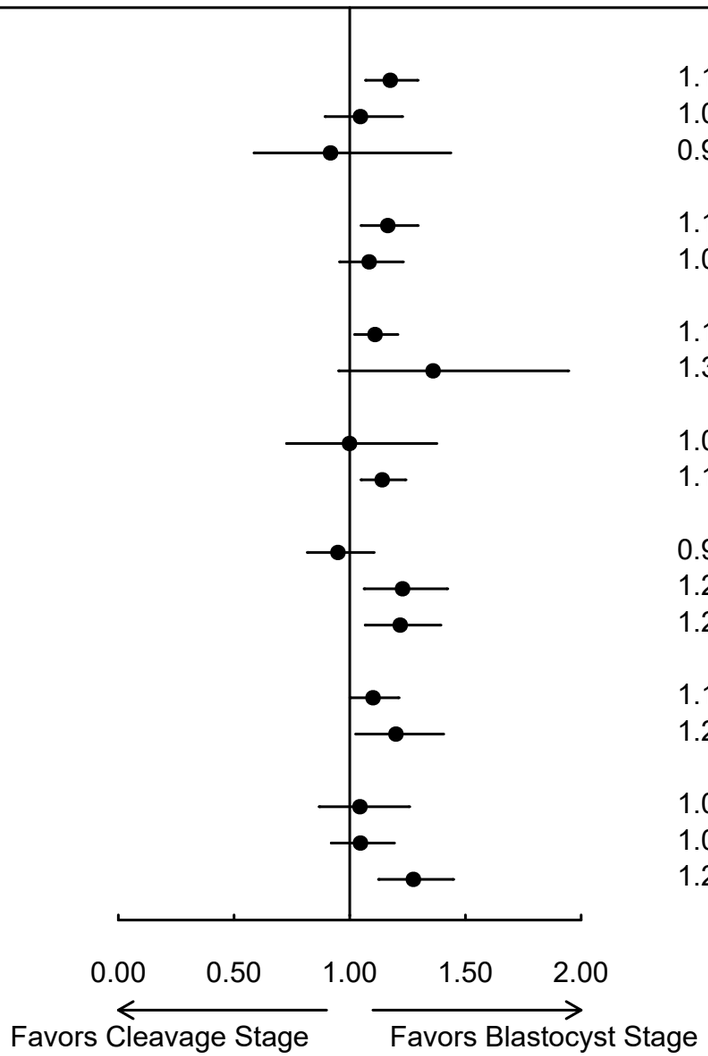

No. at risk

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cleavage-Stage | 425 | 424 | 405 | 300 | 218 | 179 | 164 | 152 | 147 | 137 | 136 | 135 | 0 |
| Blastocyst-Stage | 456 | 455 | 431 | 320 | 201 | 150 | 132 | 121 | 115 | 109 | 107 | 106 | 0 |

Note: HR, hazard ratio. Shaded areas indicate 95% confidence intervals (CI). Hazard ratio and the associated 95% CIs were estimated by using a Cox proportional hazards model. Source data are provided as a Source Data file.

## Supplementary Figure 2 Forest plot of post-hoc subgroup analyses for the primary outcome (intention-to-treat)

| | Blastocyst Stage * | Cleavage Stage * | Risk Ratio (95% CI) | $p_{interaction}$ |
|---|---|---|---|---|
| **Maternal age** | | | | 0.12 |
| ≤30 years | 80.2% (255/318) | 68.2% (202/296) | 1.18 (1.07 to 1.29) | |
| 30-35 years | 68.0% (102/150) | 65.0% (104/160) | 1.05 (0.89 to 1.23) | |
| >35 years | 51.7% (15/29) | 56.4% (22/39) | 0.92 (0.59 to 1.43) | |
| **Previous conception** | | | | 0.19 |
| 0 | 80.4% (205/255) | 69.0% (165/239) | 1.16 (1.05 to 1.29) | |
| ≥1 | 69.0% (167/242) | 63.7% (163/256) | 1.08 (0.96 to 1.23) | |
| **Previous IVF** | | | | 0.39 |
| No | 75.1% (350/466) | 67.7% (304/449) | 1.11 (1.02 to 1.20) | |
| Yes | 71.0% (22/31) | 52.2% (24/46) | 1.36 (0.95 to 1.94) | |
| **Ovarian reserve** | | | | 0.36 |
| AMH<1.2 ng/mL or AFC<5 | 65.8% (25/38) | 65.9% (27/41) | 1.00 (0.73 to 1.37) | |
| AMH≥1.2 ng/mL or AFC≥5 | 75.6% (347/459) | 66.3% (301/454) | 1.14 (1.05 to 1.24) | |
| **Estradiol** | | | | 0.01 |
| The lowest tertile (≤2932.69pg/mL) | 65.8% (104/158) | 69.4% (120/173) | 0.95 (0.82 to 1.10) | |
| The medium tertile (2932.69−4800.27pg/mL) | 79.3% (130/164) | 64.5% (91/141) | 1.23 (1.06 to 1.42) | |
| The highest tertile (>4800.27pg/mL) | 78.7% (137/174) | 64.6% (117/181) | 1.22 (1.07 to 1.39) | |
| **Progesterone** | | | | 0.37 |
| The lower stratum (<1.5ng/mL) | 74.1% (269/363) | 67.3% (237/352) | 1.10 (1.00 to 1.21) | |
| The higher stratum (≥1.5ng/mL) | 76.3% (100/131) | 63.6% (91/143) | 1.20 (1.03 to 1.40) | |
| **Ovarian response** | | | | 0.03 |
| Poor or Sub-optimal responders (≤9 oocytes) | 67.2% (78/116) | 64.4% (76/118) | 1.04 (0.87 to 1.26) | |
| Normal responders (10−15 oocytes) | 72.3% (141/195) | 69.1% (132/191) | 1.05 (0.92 to 1.19) | |
| Hyper-responders (>15 oocytes) | 82.3% (153/186) | 64.5% (120/186) | 1.28 (1.12 to 1.45) | |




*The denominators indicate the number of participants in each stratum for the two groups.

Note: AMH, anti-Müllerian hormone; AFC, antral follicle count. P for interaction is derived from Wald test using PROC LOGISTIC based on Wald Chi-square statistic.

This supplement contains the following items:

Original Protocol

# Cumulative live birth rates after cleavage-stage versus blastocyst-stage embryo transfer:

# A multicenter, prospective, randomized controlled trial

# (CLBR-CBSET)

Protocol Leader: Jiayin Liu, MD, PhD and Zi-Jiang Chen, MD, PhD

Data and Coordination Leader: Heping Zhang, PhD

Protocol version: Version 5.0

September 18, 2018

**CONTENTS**

## 1. Committee composition:

### 1.1 Protocol committee

**Table 1. Protocol committee**

| Name | Affiliation | Email address |
|---|---|---|
| Jiayin Liu | Clinical Center of Reproductive Medicine at the First Affiliated Hospital of Nanjing Medical University | jyliu_nj@126.com |
| Zi-Jiang Chen | Center for Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong University | chenzijiang@vip.163.com |

| | | |
|---|---|---|
| Richard S. Legro | Penn State University College of Medicine | rsl1@psu.edu |
| Heping Zhang | Yale University | heping.zhang@yale.edu |
| Xiang Ma | Clinical Center of Reproductive Medicine at the First Affiliated Hospital of Nanjing Medical University | sxmaxiang@126.com |
| Yuhua Shi | Center for Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong University | Shiyuhua2003@126.com |
| Yun Sun | Renji Hospital affiliated to Shanghai Jiaotong University School of Medicine | Syun163@163.com |

### 1.2 Steering Committee

**Table 2. Steering Committee**

| **Name** | **Affiliation** | **Email address** |
|---|---|---|
| **Chair** | | |
| Jiayin Liu | Clinical Center of Reproductive Medicine of the First Affiliated Hospital of Nanjing Medical University | jyliu_nj@126.com |
| **Co-investigators** | | |
| Zi-Jiang Chen | Center for Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong University | chenzijiang@vip.163.com |
| Richard S. Legro | Penn State University College of Medicine | rsl1@psu.edu |
| Heping Zhang | Yale University | heping.zhang@yale.edu |

### 1.3 Study sites and investigators

**Table 3. Study sites and investigators:**

| **No.** | **PIs** | **Study sites** | **Email** |
|---|---|---|---|
| 1 | Jiayin Liu<br>Xiang Ma | Clinical Center of Reproductive Medicine at the First Affiliated Hospital of Nanjing Medical University | jyliu_nj@126.com<br>sxmaxiang@126.com |
| 2 | Zi-Jiang Chen<br>Yuhua Shi | Center for Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong University | chenzijiang@vip.163.com<br>Shiyuhua2003@126.com |
| 3 | Yunxia Cao | The First Affiliated Hospital of Anhui Medical University | caoyunxia6@126.com |
| 4 | Yun Sun | Renji Hospital affiliated to Shanghai Jiaotong University School of Medicine | syun163@163.com |
| 5 | Bo Zhang | Guangxi Maternal and Child Health Hospital | cestbon269@sina.com |

| | | | |
|---|---|---|---|
| 6 | Jichun Tan | Shengjing Hospital of China Medical University | tjczjh@163.com |
| 7 | Jianqiao Liu | The Third Affiliated Hospital of Guangzhou Medical University | ljq88gz@163.com |
| 8 | Junli Zhao | General Hospital of Ningxia Medical University | zhaojunli6812@163.com |
| 9 | Hong Li | Suzhou Municipal Hospital | hongliszivf@163.com |
| 10 | Cuilian Zhang | Henan Provincial People's Hospital | luckyzcl@qq.com |
| 11 | Xingling Wang | The Third Affiliated Hospital of Zhengzhou University | wangxl208@126.com |

### 1.4 Data Coordination Committee

Prof. Heping Zhang from Yale University will lead the Data Coordination Committee with the help of personnel from the Department of Epidemiology and Biostatistics, School of Public Health, Nanjing Medical University, including registering the trial at ClinicalTrials.gov website (https://clinicaltrials.gov/).

### 1.5 Publication Committee

Members of the publication committee include Jiayin Liu, Zi-Jiang Chen, Xiang Ma, Yuhua Shi, Heping Zhang and Richard S. Legro.

## 2. Study synopsis

### 2.1 Objectives

The aim of this RCT is to compare differences in the efficacy and safety between cleavage-stage embryo transfer and blastocyst-stage embryo transfer in IVF/ICSI treatment cycle, taking into account of subsequent vitrified embryo transfers.

### 2.2 Patient Population

The patient population will include infertile patients between 20 and 40 years of age undergoing their first or second in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) cycle.

### 2.3 Study Design

This is a multi-center, prospective, randomized controlled clinical trial. A total of 980 infertile patients undergoing their first or second cycle of IVF or ICSI will be enrolled and randomized into two parallel treatment arms in a 1:1 ratio by site. Patients in group A will have a single cleavage-stage embryo transferred, and patients in group B will have a single blastocyst-stage embryo transferred. Each patient will be followed up until the first live birth is achieved or until all the embryos per oocyte retrieval are transferred (up to 5 single embryo transfers), including fresh and frozen-thawed embryo transfers. If a live birth is not achieved, single embryo transfers will be consecutively performed 5 times at most within 1.5 years follow-up duration. The follow-up period is 2.5 years from the day of randomization. Cumulative live birth rate (CLBR) will be analyzed according to appropriate statistical methods. All participants will receive a long GnRH-agonist, short GnRH-agonist or GnRH antagonist protocols. In frozen embryo transfer cycles, the endometrium will be prepared with natural ovulation cycles, minimal stimulation cycles or artificial cycles.

### 2.4 Treatment

All the participants will receive a long GnRH–agonist, short GnRH-agonist or GnRH antagonist protocol in combination with recombinant FSH (rFSH, Puregon). HCG will be administered for final oocyte maturation, and vaginal ultrasound-guided oocyte retrieval will be performed. Randomization will be performed on day 2/3 after oocyte retrieval, when at least 4 transferrable cleavage-stage embryos are achieved. Patients in group A will have a

single cleavage-stage embryos transferred. Patients in group B will have a single blastocyst-stage embryos transferred. If the previous embryo transfer does not result in a live birth, patients would go through cryopreserved cycles until a first live birth was achieved or all vitrified embryos were transferred (a maximum of 5 single embryo transfers) within the 2.5-year follow-up duration. Luteal phase support will be administered before embryo transfer. A pregnancy test will be performed 2 weeks after embryos transfer. If pregnancy is confirmed, luteal phase support will be continued until 10 weeks of gestation. The follow up will be continued until 6 weeks after delivery.

### 2.5 Primary outcome

The primary outcome is CLBR per patient until the first live birth from one initiating oocyte retrieval cycle.

### 2.6 Secondary outcomes

The secondary outcomes will include biochemical pregnancy rate, clinical pregnancy rate, implantation rate, cumulative clinical pregnancy rate, ongoing pregnancy rate, live birth rate, pregnancy loss rate, birth weight and sex ratio.

The safety parameters will include moderate or severe OHSS rate, ectopic pregnancy rate, multiple pregnancy rate, and incidence of obstetric and perinatal complications, such as gestational diabetes and hypertension, pre-eclampsia, placental previa, placental abruption, preterm delivery, neonatal hospitalization for >3 days, congenital anomalies and perinatal mortality.

### 2.7 Statistical Analysis

The primary analysis will use an intent-to-treat (ITT) approach to examine differences in CLBR with first live birth per oocyte retrieval in the two treatment arms. Categorical data will be represented as a frequency and a percentage; differences in these measures between treatment groups will be assessed by Chi-square analysis, with Fisher's Exact Test for expected frequencies less than 5. Continuous data will be expressed as mean ± SD, with student t test for testing differences between two groups. Inter-quantile ranges may be presented when non-normality of the continuous data is apparent.

A per protocol analysis and safety analysis will also be performed. The conservative and optimal estimate of the CLBR will be used to calculate the CLBR.

The Chi-square test will be used for the conservative CLBR between the two treatment groups. Optimal CLBR will be analyzed by using the Kaplan-Meier product-limit method, which censors data for subjects who do not return for treatment, and estimates the CLBR with 95% confidence intervals.

Furthermore, univariate/multivariate logistic will be performed to evaluate the influence of Age (≤35 or >35 years), AMH, number of oocytes retrieved, number of embryos, times of embryo transfer, duration to achieve pregnancy and BMI on pregnancy rate or CLBR. Any deviations from the previously described statistical plan will be described and justified in a protocol amendment. The results will be reported according to the CONSORT statement.

### 2.8 Anticipated Time to Completion

The study will be completed within the duration of 2.5 years after randomization. The anticipated time schedule is an 8 months enrollment period, a 18 months treatment period, and an 11 months pregnancy follow-up until 6 weeks after delivery. All embryos from one oocyte retrieval (up to 5 embryos) should be transferred within 1.5 years.

## 3. Backgrounds and Significance

More than 6 million infants are born by IVF/ICSI worldwide [1]. Embryo transfer is one of the most critical steps in IVF/ICSI. Traditionally, only cleavage-stage embryos on Day 3 were transferred. Over the past decades, the number of blastocyst-stage embryos transferred on Day 5 or 6 has been steadily increasing due to advances in IVF laboratories, such as embryo culture media. [2] It has been reported that in Australia, the proportion of blastocyst transfer cycles to cleavage transfer cycles has increased from less than 30% in 2004 to more than 60% in 2013[3,4]. National data from the United States and the United Kingdom show similar increases in the frequency of blastocyst transfers which represented approximately one third of IVF cycles in 2012. [5,6]

Extended culture to blastocyst stage exhibits several advantages over cleavage-stage embryo. It is well accepted that blastocyst-stage transfer mimics the conditions of physiological implantation, and as a result it may provide better embryo-endometrium synchronization and therefore higher chances of implantation [7]. Extended culture to the blastocyst stage also enables embryo self-selection, consequently the most viable embryos will survive and be selected for transfer. Evidence supports the notion that fresh blastocyst transfers result in significantly higher live birth rates and clinical pregnancy rates when compared with the fresh cleavage-stage transfers [8, 9]. However, the results are not conclusive when the transfer of subsequent frozen embryos is considered [8, 9].

The American Society for Reproductive Medicine and the fertility guidelines issued in 2013 by NICE have both expressed concern over the use of the blastocyst transfer [10, 11], because extending culture could yield fewer/no viable embryos available for freezing and subsequent thaw transfer, and further oocyte retrieval cycles will be required. Conversely, more ETs do not necessarily result in improved cumulative live birth, thus blastocyst transfer may relieve the burden of the additional transfers.

The results of studies comparing the outcomes between blastocyst- and cleavage-stage transfer are inconsistent. A Cochrane systematic review of 27 RCTs published in 2016 [8] reported that live birth rates (low-quality evidence) and clinical pregnancy rates (moderate-quality evidence) for fresh blastocyst transfer are higher compared with those of cleavage-stage transfer. The very low quality evidence shows no significant differences in the cumulative pregnancy rate between the two groups. Blastocyst transfer has a reduced embryo freezing rate. However, there was no difference between the groups in terms of multiple pregnancy and miscarriage rates. A well-designed retrospective study published in 2016[9] reported higher live birth rates in fresh single blastocyst transfer and similar cumulative live birth rate between the two groups. The study also proved that blastocyst stage transfers required a fewer number of embryos to obtain a live birth. However, two recent meta-analyses [12,13] comparing the outcomes of blastocyst and cleavage-stage transfer showed no significant differences in clinical pregnancy, live birth/ongoing pregnancy, cumulative live birth rate, as well as multiple pregnancy rate. One of the studies that included studies with vitrification reported a higher implantation rate and miscarriage rate after blastocyst transfer [12]. Both of the studies emphasized the low quality of included studies and the importance of performing a large-scale, well-designed RCT on this topic before robust conclusions can be drawn. Furthermore, two of the latest RCTs [14, 15] both failed to detect a significant difference in the efficacy of blastocyst transfer when compared to cleavage-stage transfer. However, both RCTs were conducted in single center with a limited number of treatment cycles.

Conventionally, IVF success has been calculated in terms of live birth rate per treatment attempt. As reproductive technology continues to develop, the number of FET cycles have been growing rapidly. Combined with an emphasis on reducing multiple pregnancies and increasing single embryo transfers, cumulative pregnancy rate per patients from one oocyte retrieval cycle reflect the true IVF success rate by considering all implantation attempts from both fresh and frozen embryos [16]. Vitrification technology has been proved to be the key for enhanced blastocyst cryopreservation, with a high survival rate of around 96%-98% [18]. Therefore, CLBR is able to be compared under more objective and fair conditions by using vitrification for both cleavage-stage embryos and blastocysts. To date, only five RCTs reported cumulative pregnancy rates after transferring both fresh and frozen embryos between blastocyst and cleavage-stage transfer [17]. Among these, four RCTs [19-22] in the early years based on slow-freezing reported that cleavage stage transfer resulted in a higher CLBR, while the current RCT [23] with vitrification showed higher cumulative ongoing pregnancy rates in blastocyst transfers, albeit in a small sample size. Recently another two retrospective cohort studies [9,24] comparing CLBRs of cleavage-stage and blastocyst embryos using vitrification methods for frozen embryos have been published. Both of the studies failed to show significant differences in CBLR between the two groups. However, they proved blastocysts required fewer embryo transfers and less time to achieve the first live birth, which seems to be a more effective and time-efficient method to obtain a live baby when compared with cleavage-stage embryo transfer. There is an absence of RCTs comparing CLBR of cleavage-stage and blastocyst embryos using vitrification methods for embryo cryopreservation.

The health of offspring born after blastocyst transfer has raised great concerns. Previous un-matched cohort studies [25, 26] reported extended culture resulted in adverse neonatal outcomes, such as a higher risk of preterm delivery, very preterm birth, large for gestational age (LGA) and perinatal mortality. However, most recent controlled studies did not confirm these conclusions [27-33]. In addition to extended culture effects, the influence of cryopreservation on these adverse outcomes should be considered. Slow-freezing techniques were reported to be related to an increased risk of LGA newborns [34]. In contrast, vitrification does not seem to be related to adverse neonatal outcomes for both cleavage-stage embryos and blastocysts [35-39]. A latest meta-analysis [40] compares the perinatal outcomes of singleton pregnancies from blastocysts with those from cleavage-stage transfer, stratified by fresh and frozen embryo-transfer

cycles. The results suggest cryopreservation of embryos can influence pregnancy outcome transfer in terms of preterm birth, very preterm birth, LGA, SGA and perinatal mortality. However, there is a lack of RCTs in this issue and the evidences available in the analysis are at a low level.

Well-designed RCTs are urgently needed to report the efficacy and safety of blastocyst transfers before a definitive conclusion can be drawn to guide the selection of the optimal transfer strategy. This is a randomized trial designed to determine the differences in pregnancy outcomes and newborn health between cleavage-stage embryo transfer and blastocyst-stage embryo transfer in IVF/ICSI treatment cycle, taking into account subsequent vitrified embryo transfers. The primary outcome is cumulative live birth rate (≥24 weeks gestation) per patient until the first live birth from one initiating oocytes retrieval cycle.

## 4. Objectives

This trial is designed to compare the efficacy and safety between cleavage-stage embryo transfer and blastocyst-stage embryo transfer in IVF/ICSI treatment cycle, taking into account subsequent vitrified embryo transfers.

The primary research hypothesis is that the CLBR of blastocyst-stage embryo transfers is non-inferior to that of cleavage-stage embryo transfers. A safety hypothesis will also be taken into consideration. We hypothesize both treatments have similar incidence of maternal and neonatal complications.

## 5. Study Population

The study population will include infertile patients between 20 and 40 years of age, undergoing their first or second IVF or ICSI cycle. Ovarian stimulation will be performed with long GnRH-agonist, short GnRH-agonist or GnRH antagonist with HCG trigger.

### 5.1 Inclusion criteria:

1. Women aged ≥20 and ≤40 years.

2. Women with the number of transferrable cleavage embryos ≥ 4;

3. Women undergoing their first or second cycle of IVF or ICSI.

### 5.2 Exclusion criteria

1. Women who have been diagnosed with uterine abnormalities (confirmed by three-dimensional ultrasonography or hysteroscopy), including malformed uterus (uterus unicornis, septate uterus or duplex uterus), submucous myoma, or intrauterine adhesion

2. Women who plan to undergo In Vitro Maturation (IVM);

3. Women who plan to undergo Preimplantation Genetic Diagnosis (PGD) /Preimplantation Genetic Screening (PGS).

4. Women who have hydrosalpinx visible on ultrasound.

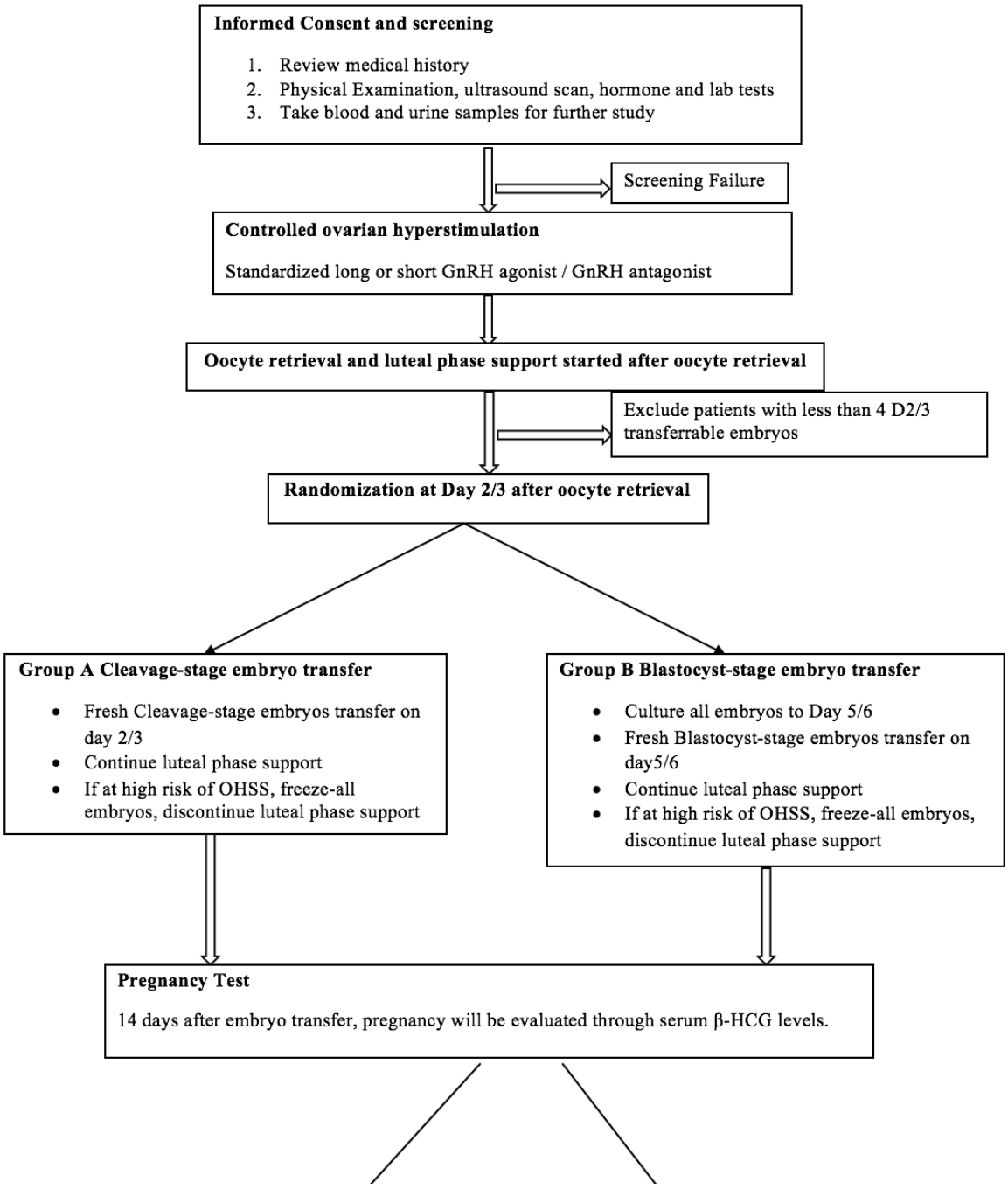
5. Women who have experienced recurrent spontaneous abortions, defined as 2 or more previous pregnancy losses.

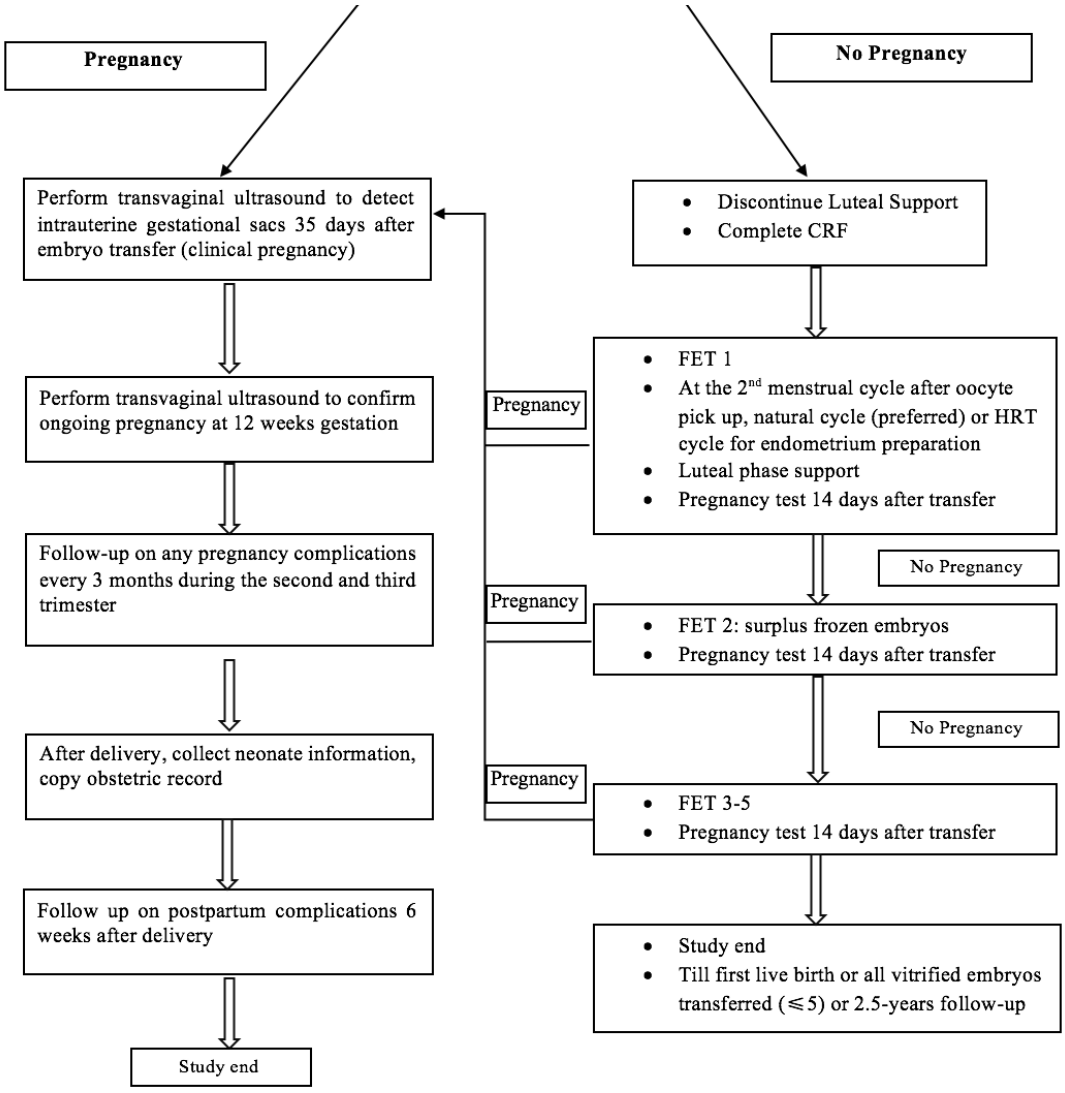
6. Women who have been developed a “freeze-all” treatment plan for purpose of subsequent surgery, such as salpingectomy due to hydrosalpinx after oocytes retrieval.

7. Women with contraindications to assisted reproductive technology and/or pregnancy, such as uncontrolled hypertension, symptomatic heart diseases, uncontrolled diabetes, undiagnosed liver disease or dysfunction (based on serum liver enzyme test results), undiagnosed renal disease or abnormal renal function, severe anemia, history of deep venous thrombosis, pulmonary embolus or cerebrovascular accident, history of or suspicious for cancer, undiagnosed vaginal bleeding.

## 6. Study procedures and visits

### 6.1 Study Procedure Flow Chart






## 6.2 Screening visit

Physicians will introduce this trial to potential participants who are interested in joining the trial, and refer the participants to investigators if the enrollment criteria are met. Participants who are willing to participate will receive an informed consent document explaining the trial in detail, especially the random assignment on day 2/3 after oocyte retrieval. Consent forms should be signed before any screening will be done. Screening visit will begin when patients finish all tests for preparation of IVF or ICSI. Participants will undergo a face-to-face screening visit to provide information on their demographic details, medication usage, previous history of diseases, menstruation, and infertility. This interview serves as further confirmation that the participant meets the inclusion criteria. The screening process will be recorded in the screening log, and the cause of screening failure will also be recorded. During this visit, the following procedures will be undertaken:

1) Obtain the signed informed consent
2) Perform a comprehensive history review consisting of demographic details, past medical history, associated medication status, menstrual history, infertility history and gynecological history;
3) Complete physical examinations including height, weight, BMI, circumference of the waist and hips, vital signs and pelvic exam; (If eligible pelvic exam has been performed within 6 months, just record them and no repeat will be required.)

4) A transvaginal ultrasound screen for uterus condition, ovary size, antral follicle count, and the thickness of the endometrium. If uterine abnormalities are suspected, a three-dimensional ultrasonography or hysteroscopy will be performed to confirm the diagnosis. (If eligible ultrasound has been performed within 6 months, just record them and no repeat will be required.)
5) Hormonal testing, including: serum follicle stimulating hormone (FSH), luteinizing hormone (LH), Total testosterone (TT), estradiol (E2), progesterone (P), prolactin (PRL), thyroid Stimulating Hormone (TSH), and anti-mullerian hormone (AMH) level. (If eligible results have been obtained within 6 months, just record them and no repeat will be required.)
6) Karyotypes tests of the couples. (If eligible karyotypes results have been performed previously, just record them and no repeat will be required.)
7) Safety lab tests including: CBC, hepatic and renal function tests, serum glucose and lipid level, coagulation analysis, TORCH screening, thyroid function tests, infectious disease screening, TCT, ECG. Insulin levels, the oral glucose tolerance test (OGTT), uric acid levels autoantibody levels and serum tumor markers are selective optional test based on patient's condition. (If eligible results have been obtained within 6 months, just record them and no repeat will be required.)
8) Complete the questionnaire for health survey (SF-36) and fertility quality of life (FertiQoL International);
9) Retrieval of blood sample for further studies. The volume of each sample is about 2-3ml, which will be numbered and stored in a -80°C refrigerator.

**6.3 Oocyte retrieval day visit**

During this visit, the following procedures will be completed:

1) Complete the form by recording parameters from COH procedure and oocyte pick-up procedure.
2) Exclude participants who fail to undergo oocyte retrieval procedure, obtain oocytes or complete sperm collection, have obvious hydrosalpinx, or plan to undergo "freeze all" policy.
3) Record adverse events and concomitant medication in the adverse event report form and concomitant medication record form.
4) Recommend retrieval of the granulosa cells, follicular fluid and blood samples.

**6.4 Day 2/3 after oocyte retrieval and fresh embryo transfer visit**

1) Record the parameters from In Vitro Fertilization procedure and embryo culture.
2) Exclude participants that fail to have ≥ 4 transferrable Day 2/3 embryos and have been developed a "freeze-all" treatment plan for purpose of subsequent surgery.
3) Randomize participants with ≥ 4 transferrable Day 2/3 embryos equally into two groups after oocyte retrieval. Group A will refer to the Day 2/3 embryo transfer group while group B will refer to the Day 5/6 embryo transfer group.
4) Record information regarding transferred embryos and progesterone support protocol if subjects in Group A undergo Day 2/3 embryo transfer in fresh cycle.
5) Record information regarding frozen embryos if subjects in Group A have embryos vitrified.
6) Inquire about and record adverse events and concomitant medication.

-If embryo transfer is not performed in fresh cycle and no embryos are vitrified, end of study visit should be performed, and cause of early termination will be recorded at the end of study visit form.

-If fresh embryo transfer is not conducted and all the embryos are vitrified, further FET visits should be performed.

**6.5 Day 5/6 after oocyte retrieval and fresh embryo transfer visit**

1) Participants in Group B will have all Day 2/3 embryos cultured to Day 5/6. Record information regarding blastocysts culture.
2) Record information regarding transferred embryos and progesterone support protocol if participants in Group B undergo embryo transfer in fresh cycle.
3) Record information regarding frozen embryos if participants in Group B have embryo vitrified.
4) Inquire about and record adverse events and concomitant medication in the specialized form for this visit.

-If no Day 5/6 embryos are obtained, end of study visit should be performed, and cause of early termination will be recorded in the end of study visit form.

-If embryo transfer is not performed in fresh cycle and no embryos are vitrified, end of study visit should be undertaken, and cause of early termination will be recorded in the end of study visit form.

-If fresh embryo transfer is not conducted and all the embryos are vitrified, further FET visit should be performed.

**6.6 Frozen embryo transfer (1-N) visit**

1) Record information regarding endometrium preparation, transferred embryos and progesterone support methods.
2) Record the number of surplus frozen embryos.
3) Inquire about and record adverse events and concomitant medication in the specialized forms for this visit.

-If patients with frozen embryos will not undergo FET procedure for any reason, end of study visit should be performed, and the cause will be recorded in the end of study visit form.

-If all thawed embryos are not transferrable, end of study visit will also be performed and recorded in the specialized form.

-If subjects do not obtain a first live birth, single frozen embryo transfers will be consecutively performed until a first live birth or 5 times at most within 1.5 years follow-up duration.

**6.7 Biochemical pregnancy test visit**

This visit will be performed on the 14th day after embryo transfer. Biochemical pregnancy is defined as a serum β – HCG level no less than 25 IU per liter. Fill out the specialized form for this visit.

-If the result of biochemical pregnancy is negative and embryos runs out for the next FET, an end of study visit will also be performed and recorded in the specialized form.

-If the result of biochemical pregnancy is negative but there are frozen embryos left, subsequent FET visits will be performed.

**6.8 Clinical pregnancy test visit**

The visit to test clinical pregnancy will take place on the 30-35th day after the embryo transfer. Clinical pregnancy is defined as the presence of an intrauterine gestation sac. If clinical pregnancy has been obtained, the follow-up schedule will be made. Investigators will check contact information with patients and require them to inform investigators of their delivery time.

A specialized form for this visit will be filled out. Concomitant medication and adverse events will be recorded as well.

-If the result of clinical pregnancy is negative and embryos run out for the next FET, an end of study visit and a summary of final pregnancy outcome will be performed and recorded in the specialized form.

-If the result of clinical pregnancy is negative but there are frozen embryos left, subsequent FET visits will be performed.

-If pregnancy is terminated prematurely, the cause will be recorded in the form for the outcome of the pregnancy.

**6.9 Follow-up visits**

The outcome of the pregnancy and any obstetrical or perinatal complications will be attained after review of obstetric medical records and neonatal medical records.

At 14 weeks gestation, the first-trimester pregnancy complications, including but not limited to OHSS, miscarriage, ectopic pregnancy or trophoblastic disease, will be collected from patients by telephone and by inspecting their medical records. The specialized form for the first pregnancy follow-up time point will be completed.

At 28 weeks gestation, the second-trimester pregnancy complications, including but not limited to abortion, gestational diabetes, pre-eclampsia/eclampsia, incompetent cervix, premature membrane rupture or placenta abruption will be evaluated by telephone call and by inspecting their medical records at the second pregnancy follow-up time point. The specialized form will be filled out.

At 37 weeks gestation, the third-trimester pregnancy complications including but not limited to preterm labor, placenta abruption, placenta accrete, placenta previa, pre-eclampsia/eclampsia, premature membrane rupture,

intrauterine growth retardation or amniotic fluid abnormality will be followed up by telephone call and by inspecting their medical records at the third pregnancy follow-up time point. The specialized form will be filled out.

At delivery, subjects are required to notify investigators of their delivery time. The delivery information, including gestational age, delivery mode, abnormality of placenta or delivery complications as well as infant information including birth weight and presence of birth defects will be followed up by telephone and by reviewing obstetric and neonatal medical records. The specialized form for the fourth follow-up time point will be completed. Recommend collecting the placenta and cord blood sample.

At 6 weeks after delivery, any complications of the mother (such as infection, postpartum depression, hemorrhage) or infant (such as neonatal respiratory distress syndrome, infection, jaundice, hospitalization, death) will be followed up by telephone and by reviewing obstetric and neonatal medical records. The specialized form for the fifth and final follow-up time point will be completed.

During the follow-up period, concomitant medication and adverse events must be recorded.

-If clinical pregnancy is achieved (premature pregnancy termination or live birth obtained), the specialized forms for pregnancy outcome after each ET and a summary of final pregnancy outcome (including times of ET, pregnancy details after each ET) after the end of all pregnancy follow-ups must be filled out.

-If a live birth is obtained, an end of study visit and a summary of final pregnancy outcome (including times of ET, pregnancy details after each ET) will also be performed and recorded in the specialized form.

-If live birth is not achieved during any pregnancy follow-up period and embryos run out for the next FET, an end of study visit will also be performed and recorded in the specialized form.

-If live birth is not achieved but there are frozen embryos left, subsequent FET visits will be performed until a maximum of 5 embryos transferred.

### 6.10 End of Study visit (by telephone)

The end of study visit will be performed if the following occurs:

1) The subjects achieve a first live birth and finish all pregnancy follow-ups (from either fresh ET or FET).
2) If subjects do not obtain a first live birth, all embryos per oocyte retrieval have been transferred (the transferable embryos < 5) or 5 consecutive single embryo transfers have been performed (the transferable embryos ≥ 5).
3) Subjects who complete the 2.5 year follow up (from the day of randomization).
4) No Day 5/6 embryo obtained
5) No transferrable embryos obtained after thawing
6) A live birth obtained by a natural pregnancy during the study
7) Subjects who are lost to follow up
8) Subjects who withdraw consent and/or refuse to continue participating.
9) Other reasons (for example, investigator's or subject's decision)

### 6.11 2.5 years follow up Visit (by telephone)

At the 2.5-year follow-up time point after randomization, subjects who do not reach the end of the study will be followed up by telephone to check and record their treatment or pregnancy status and outcomes.

## 7. Study Design

### 7.1 Type of design

This is a randomized controlled clinical trial comparing the cumulative live birth rate per oocyte retrieval between cleavage-stage embryo transfer and blastocyst-stage embryo transfer in 980 patients undergoing their first or second IVF/ICSI cycles across 11 reproductive centers in China. Eligible patients will be randomly divided into two treatment groups. Patients in the group A will have a single cleavage-stage embryo transferred per cycle, and patients in group B will have a single blastocyst-stage embryo transferred per cycle. All of the participants will receive a standardized long GnRH–agonist or short GnRH-agonist or GnRH antagonist ovarian stimulation protocol and standardized luteal phase support. Each patient will be followed up until the first live birth is achieved or all the embryos per oocyte retrieval are transferred (up to 5 single embryo transfers), including fresh and frozen-thawed embryo transfers. If a

live birth is not achieved, single embryo transfers will be consecutively performed 5 times at most within 1.5 years follow-up duration[9]. The follow-up period is 2.5 years from the day of randomization. Both "optimistic" and "conservative" cumulative live-birth rate will also be analyzed (see Section 9.2).

The blocked randomization method was used with the dynamic block size. The treatment ratio is 1:1 and the randomization was stratified by study sites. A data coordinate center (SAS Institute, Care, NC) in Yale University generated the randomization sequence with SAS (software version 9.2). This randomization sequence was input into an online central randomization system (http://www.medresman.org) kept blind from both investigators who enroll subjects and study coordinators. The randomization will occur on the day 2/3 after oocyte retrieval, when at least 4 embryos are achieved. Trained coordinators will draw one randomized number at a time using password-protected accounts. This study is designed as an open labeled one. Both investigators and subjects will be aware of the allocation after randomization.

### 7.2 Study intervention

#### 7.2.1 Controlled ovarian hyperstimulation:

GnRH-agonist or GnRH antagonist protocols will be employed for ovarian stimulation. Each site will use 1-2 fixed stimulation protocols to reduce potential bias.

- Long GnRH-agonist protocol
  1. Short-acting long GnRH-agonist protocol

GnRH agonist (Diphereline, Ipsen Pharma Biotech, France) will be administered with the dose of 0.05-0.1 mg daily on Day 21-25 of menstrual cycle for 14 -20 days. Once the downregulation criteria is met (LH≤5 IU/L, E2≤50 pg/ml, and endometrium thickness≤6mm), recombinant follicular stimulating hormone (rFSH, Puregon, MSD, Ravensburg, Germany) with dose of 75 to 225 IU per day will be initiated. The adjustment of gonadotropin dose is based on follicular development and serum hormone levels. Continual administration of GnRH agonist and gonadotropin last until the start of human chorionic gonadotropin (HCG) injection. HCG 4000-10000IU will be administered when at least 2 follicles are ≥ 18mm or 3 follicles are ≥ 17mm in mean diameter. Human menopausal gonadotropin (HMG) and recombinant human luteinizing hormone (r-hLH, Luveris, Merck Serono, Germany) could be combined or added if needed.

2. Long-acting long GnRH-agonist protocol

A quarter dose to a full dose (3.75mg) of GnRH agonist (Diphereline, Ipsen Pharma Biotech, France) will be administered on Day 1-2 of menstrual cycle or the luteal phase. After 14-28 days, recombinant follicular stimulating hormone (rFSH, Puregon, MSD, Ravensburg, Germany) with a dosage of 75 to 225 IU per day will be initiated, according to FSH and estradiol levels and follicle size. The gonadotropin dosage is adjusted based on follicular development and serum hormone levels. Continual administration of gonadotropin lasts until the start of human chorionic gonadotropin (HCG) injection. HCG 4000-10000IU will be administered when at least 2 follicles are ≥ 18mm or 3 follicles are ≥ 17mm in mean diameter. HMG and recombinant human luteinizing hormone (r-hLH, Luveris, Merck Serono, Germany) could be combined or added if needed.

- Short GnRH-agonist protocol

On day 2-3 of the menstrual cycle, GnRH agonist will be administered with a dosage of 0.05-0.1 mg daily, and recombinant follicular stimulating hormone (rFSH, Puregon, MSD, Ravensburg, Germany) with a dosage of 75 to 225 IU per day will be initiated. The adjustment of gonadotropin dose is based on follicular development and serum hormone levels. Continual administration of GnRH agonist and gonadotropin will last until the start of human chorionic gonadotropin (HCG) injection. HCG 4000-10000IU will be administered when at least 2 follicles are ≥ 18mm or 3 follicles are ≥ 17mm in mean diameter. HMG and recombinant human luteinizing hormone (r-hLH, Luveris, Merck Serono, Germany) could be combined or added if needed.

- GnRH antagonist protocol

Recombinant follicular stimulating hormone (rFSH, Puregon, MSD, Ravensburg, Germany) with dose of 75 to 225 IU per day will be administered on Day 2-3 of menstrual cycle for 5 days. The follicular development will be monitored by ultrasound and serum hormone levels. The dose of rFSH (Puregon) will be adjusted accordingly by investigators on each site. HMG and recombinant human luteinizing hormone (r-hLH, Luveris, Merck Serono, Germany) could be combined or added if needed. GnRH antagonist (Ganirelix, Orgalutran, MSD, Ravensburg,

Germany) 0.25mg will be given when at least one follicle is ≥12 mm in mean diameter until the trigger day (including the trigger day). HCG 4000-10000IU will be administered when at least 2 follicles are ≥ 18mm or 3 follicles are ≥ 17mm in mean diameter. If patients are at a high risk of OHSS, GnRHa or GnRHa + HCG could be used for trigger.

**7.2.2 Oocyte retrieval:**

Routine transvaginal ultrasound-guided follicle aspiration is to be performed 36 to 37 hours after HCG injection by experienced physicians.

**7.2.3 In-vitro fertilization and embryo culture:**

The oocytes will be inseminated by conventional IVF, intracytoplasmic sperm injection (ICSI) or early rescue ICSI according to the indications. On day 2/3 after oocyte retrieval, the quality of the embryos will be assessed by morphological criteria based mainly on the number and regularity of blastomeres as well as percentage fragmentation. Subjects with 4 or more transferrable embryos will be randomly assigned to undergo cleavage-stage or blastocyst-stage embryo transfer with the online central randomization system.

For subjects assigned to the cleavage-stage (Day 2/3) embryo transfer group, a single cleavage-stage embryo of the best quality will be transferred in a fresh cycle on Day 2/3 after oocyte retrieval. The surplus embryos, if any, will be vitrified for future FET if the fresh cycle does not result in a live birth. If a patient is at a high risk of OHSS, all embryos on Day 2/3 are allowed to be cryopreserved with vitrification for patient's safety. The FET cycle will be initiated on the second menstrual cycle after oocyte retrieval.

For subjects assigned to blastocyst-stage (Day 5/6) embryo transfer group, all embryos will be cultured to D5 or D6. A single blastocyst of the best quality will be transferred in a fresh cycle on D5 or D6 after oocyte retrieval (D5 embryo will be the prior choice). The surplus embryos, if any, will be vitrified for future FET in case the fresh cycle does not result in a live birth. If a patient is at a high risk of OHSS, all embryos on D5 or D6 can be cryopreserved with vitrification for patient's safety. The FET cycle will be initiated on the second menstrual cycle after oocyte retrieval.

**7.2.4 Embryo transfer and luteal phase support:**

The luteal phase support protocol for group A and B are the same. For patients who have fresh cleavage or blastocyst-stage embryos transferred, a single fresh embryo of top quality will be transferred Day 2/3 or D5/6 after oocyte retrieval. Luteal phase support with vaginal progesterone gel (Crinone, Merck Serono) 90mg per day and oral dydrogesterone 10mg twice daily will begin from the day of oocyte retrieval and continue until pregnancy determination 14 days after embryo transfer. If the patient is pregnant, vaginal progesterone gel together with dydrogesterone will continue to clinical pregnancy evaluation day (30-35th day after the embryo transfer) and oral dydrogesterone to 10 weeks gestation.

For those who have all embryos vitrified, luteal phase support will be stopped 7 days after randomization.

For patients who have frozen cleavage or blastocyst-stage embryos transferred, a single frozen embryo of top quality (Day 2/3 or Day 5/6) will be transferred. Endometrium preparation will be performed with natural cycle regimen, minimal stimulation cycle regimen or hormone replacement cycle regimen. The optimal choice for endometrium preparation is natural cycle ovulation or minimal stimulation that mimics a natural cycle. At the second cycle following oocyte retrieval, ovulation will be monitored using ultrasound. On ovulation day, luteal phase support will begin with 10mg oral dydrogesterone twice daily. Frozen-thawed cleavage-stage embryos and blastocysts will be transferred on day 2/3 or 5/6 after ovulation respectively. If the patient is pregnant, luteal phase support will continue until 10 weeks gestation, then gradually reduced.

For hormone replacement cycle regimen, the endometrium will also be prepared with oral E2 valerate (E2V) with a dose of 4-6mg daily initiated on day 1 to day 4 of menstrual cycle, vaginal progesterone gel (Crinone, Merck Serono) 90mg per day and oral dydrogesterone 10mg twice daily will be added when endometrial thickness reaches 7mm. Frozen-thawed cleavage-stage embryos and blastocysts will be transferred on day 4 or 5/6 after progesterone initiation respectively. If the patient is pregnant, E2V will be reduced gradually, vaginal progesterone gel will continue to clinical pregnancy evaluation day (30-35th day after the embryo transfer) and oral dydrogesterone to 10 weeks gestation.

**7.2.5 Pregnancy evaluation:**

Pregnancy will be determined 14 days after embryo transfer using serum β-HCG. If a biochemical pregnancy is achieved, a transvaginal ultrasound will be performed 30-35 days after embryo transfer to evaluate clinical pregnancy.

The ultrasound will record the number, size, location of gestational sacs and embryo buds as well as fetal heart beat and abnormal conditions. At 12 weeks of gestation, another ultrasound will be performed to confirm ongoing pregnancy. Moderate to severe OHSS will be recorded.

### 7.3 Study Endpoints

The primary endpoint will be cumulative live birth per patient from one initiated oocyte retrieval cycle. Live birth is defined as delivery of any neonate ≥24 weeks gestation with heart beat and breath.

Secondary efficacy endpoints will include biochemical pregnancy, clinical pregnancy, ongoing pregnancy, pregnancy loss rate and live birth. Biochemical pregnancy is defined as a serum β-HCG level of at least 25 IU/L 14 days after embryo transfer. Clinical pregnancy is defined by the presence of intrauterine gestation sacs at 30-35 days after embryo transfer. Ongoing pregnancy is defined as a viable pregnancy at 12 weeks gestation. Pregnancy loss is defined as a pregnancy that results in a spontaneous abortion or therapeutic abortion that occurred throughout pregnancy.

The safety endpoints will include moderate or severe OHSS rate, ectopic pregnancy, multiple pregnancy, and incidence of obstetric and perinatal complications, congenital anomalies and perinatal mortality.

### 7.4 Physical Exam

A standard pelvic exam will be performed to all patients by a study physician. Height and weight will be measured to a resolution of 0.1cm and 0.1kg, respectively on the screening visit. Patients are weighed in light clothing without shoes. After a 5-minutes rest, blood pressure will be measured in the patient's right arm in the sitting position. Repeated blood pressure measurements following acclimation to environment will be conducted if a blood pressure measurement is greater than or equal to 140/90 mmHg. Patients must have a normal Pap result prior to study entry.

### 7.5 Transvaginal Ultrasound Exams

A transvaginal ultrasound exam will be performed on days 1 to 5 of the menstrual cycle at the early follicular phase. The following parameters will be obtained, including: uterus dimensions (recorded by length × width × thickness), the thickness and type of the endometrium, uterine abnormalities, number and size of uterine myomas, ovarian size of both ovaries (recorded by length × width × thickness), the size of the largest ovarian follicle, and the count of antral follicles with 2 to 9 mm in diameter and ovarian morphology. To obtain ovarian size, the largest plane of the ovary is measured in two dimensions, followed by a third measurement by turning vaginal probe 90 degrees. Endometrial thickness is the largest anterior-posterior measurement of the endometrium in the sagittal plane.

### 7.6 Laboratory exam

All measurements for clinical evaluation (described in section 6.2) will be performed in the local lab of each study site. Hormone test for ovarian response monitoring and pregnancy test will be run in local lab as well.

## 8. Timeline and Recruitment Plan

The anticipated recruitment duration will be 8 months in 11 study sites. To enroll the 980 randomized subjects as planned, each site must have 80-100 participants randomized, which means that each site should contribute 10-12 randomized participants per month. The treatment period will be 18 months, during which subjects who do not obtain a live birth should perform single embryo transfers consecutively up to 5 times. Another 11 month period will be set for pregnancy follow-up until 6 weeks after delivery. In total, the follow-up period for each subject is 2.5 years from the day of randomization. In total, from initial recruitment to complete follow up, a total of 38 months will be required to complete the study (September 2018 to November 2021).

## 9. Statistical Analysis Plan

### 9.1 Sample size

According to the prospective study published in 2016, the CLBR were 52.6% and 52.5% for cleavage-stage and blastocyst-stage transfers respectively [9]. In present study, we plan to test the primary hypothesis that blastocyst-stage embryo transfers are non-inferior to cleavage-stage embryo transfers, assessed by CLBR. We assume that CLBR will be 52% in both cleavage-stage and blastocyst-stage embryo transfer groups.

For the sample size calculations, the significance level will be set at $\alpha = 0.025$ and the statistical power will be calculated as $1 - \beta = 0.80$. The ratio between groups will be 1:1. With a non-inferiority margin of 10%, it is estimated that a sample size of 392 subjects per treatment arm. With the addition of a dropout rate of 20%, the minimal sample size calculated is: 490 for group A, 490 for group B, 980 in total.

Sample size calculation

Significance level α: 0.025

1-β: 0.80

Cumulative Live birth rate in group A: 0.52

Cumulative Live birth rate in group B: 0.52

Delta (Δ) :10%

Ratio: 1:1

Minimum sample size: 392 for each group, 784 in total

In consideration of 20% drop-out rate: 490 for group A, 490 for group B, 980 in total.

### 9.2 Statistical analysis

The primary analysis will use an intent-to-treat (ITT) approach to examine differences in cumulative live birth rate with first live birth per oocyte retrieval in the two treatment arms. Intent-to-treat analyses will be performed in full analysis sample (FAS), which consists of all subjects who are randomly allocated to treatment, satisfy major entry criteria and are not lack of any data post randomization. Categorical data will be represented as a frequency and percentage; differences in these measures between treatment groups will be assessed by the Chi-square analysis, with a Fisher's Exact Test for expected frequencies less than 5. Continuous data will be expressed as mean ± SD, with student t test for testing differences between two groups. Inter-quantile ranges may be presented when non-normality of the continuous data is apparent.

A per protocol analyses will also be performed in per protocol sample (PPS) consisting of all subjects who were included in the FAS, complying with protocol and absent of major protocol deviations. Safety Analyses will be performed in the Safety population including all subjects who were randomly allocated to treatment and received embryo transfer at least one time.

There will be two ways to calculate the CLBR：

（Numerator is the No. of women obtain their first live birth, the Denominator is the No. of women who receive one oocyte retrieval）

- The conservative estimate of the cumulative live birth rate, which is based on the assumption that the women who do not return for a subsequent embryo transfer have no chance of a pregnancy resulting in a live birth.
- The optimal estimate of the cumulative live birth rate, which is based on the assumption that women who do not return for a subsequent embryo transfer would have the same chance of a pregnancy resulting in a live birth as those who do return for embryo transfer.

The Chi-square test will be used for conservative CLBR between the two treatment groups. Optimal CLBR will be analyzed by using the Kaplan-Meier product-limit method, which censors data for subjects who do not return for treatment, and estimates the CLBR with 95% confidence intervals.

Furthermore, univariate/multivariate logistic will be performed to evaluate the dependence of Age (≤35 or >35 years), AFC, AMH, number of oocytes retrieved, number of embryos, times of embryo transfer, duration to achieve pregnancy and BMI in relation to pregnancy rate or CLBR. Any deviations from the previously described statistical plan will be described and justified in a protocol amendment. The result will be reported according to the CONSORT statement. The interim analysis will not be performed in this study.

## 10. Adverse Events

### 10.1 Risks and Discomforts

Participating in this study will produce no risks beyond those standard-of-care noted in the IVF-ET procedure consent forms. Below is a table listing all procedures related to this trial and their discomforts and risk.

**Table 5 Risks and discomfort**

| Procedures and events | Discomforts and risks |
|---|---|
| Controlled ovarian hyperstimulation (COH) | Frequent subcutaneous injection, frequent venipuncture, frequent transvaginal ultrasound scan<br>Supra-physiologic estradiol may increase risk of cancer<br>Ovary torsion or ovary disruption |
| Ovarian hyperstimulation syndrome (OHSS) | Massive enlargement of ovaries, fluid in the abdominal cavity, bloating, nausea, vomiting<br>Severe cases may have fluid in thoracic cavity, breathing difficulties, oliguria even anuria, and may require hospitalization, medication or puncture drainage of fluid in abdomen or thorax. Very severe case may suffer from thrombosis, damage to liver or renal function, even death. |
| Oocyte retrieval | Anesthesia accident, pelvic organ injury, intra-abdominal hemorrhage, puncture site hemorrhage, infection<br>In serious case surgery or transfusion may be needed |
| ICSI | Microinjection may injure oocyte, pass unknown disease gene to next generation |
| Embryos transfer | Infection |
| Blastocyst culture | No transferrable blastocyst<br>Blastocyst formation rate is 60% |
| Embryo frozen and thaw | Embryos development stop, and the survival rate of thawed embryos is 95% |
| Standard venipuncture for blood work | Slight pain, blue mark at the site of puncture, infection or bleeding at the site |
| Transvaginal ultrasound | Abdominal or pelvic discomfort |
| Ectopic pregnancy | May require medicine or surgery treatment, in severe case pregnancy site rupture resulting intra-abdominal hemorrhage, even shock or death if treatment delayed |
| Multiple pregnancy | May require embryo reduction, increase risk of pregnancy complication and fetus abnormalities; Preterm delivery |
| Infertility treatment | Anxiety or emotional distress at various degree |

Patients are not expected to experience all of these complications. The treatment patients are randomly assigned to may have more complications or prove less effective than other available treatments.

To reduce the risk of OHSS, the initial gonadotropin dose will be individualized according to body weight, AMH level, pelvic surgery history ect. Gonadotropin dosage will be modified depending on your ovarian response. If high response occurs, the HCG trigger, embryo transfer, or even the cycle may be terminated to avoid OHSS. A single embryo will be transferred by qualified and experienced physicians to reduce the risk of multiple pregnancies. The ICSI procedure will be performed under indications. An investigator or a resident doctor will be on call 24 hours a day at each investigation site, if adverse events are present during the study.

Every possible effort will be taken to prevent injury resulting from the participation in the clinical trial. However, complications or injury could still arise during the study. If an adverse event occurs, medical treatment is available and will be provided at the usual charge. Neither financial compensation nor free medical treatment will be provided in the event of a research related injury.

**Table 4. Golan classification of ovarian hyperstimulation syndrome (OHSS) (1989)**

| | Size of ovaries | Grade | Symptoms |
|---|---|---|---|

| Classification | | | |
|---|---|---|---|
| Mild | 5~10cm | Grade 1 | Abdominal tension and discomfort |
| | | Grade 2 | Grade 1 signs plus nausea, vomiting, and/or diarrhea |
| Moderate | >10cm | Grade 3 | Grade 2 signs plus ultrasound evidence of ascites |
| Severe | >12cm | Grade 4 | Grade 3 signs plus clinical evidence of ascites and/or pleural effusion and dyspnea |
| | | Grade 5 | Grade 4 signs plus haemoconcentration increased blood viscosity, hypovolaemia, decreased renal perfusion, oliguria |

**10.2 Adverse Event Definitions**

An adverse event refers to any untoward or unfavorable medical occurrence associated with the subject's participation in the research, regardless if they are considered to be related to the study intervention. Adverse events can be any of the following:

- Physical signs or symptoms including side effects of medication
- Abnormal laboratory values
- Changes in vital signs, physical exam findings, or test results
- An increase in the frequency or intensity of a condition or illness that was present before participation in the study

A serious adverse event refers to any event that is temporally associated with the subject's participation in research that meets any of the following criteria:

- Death
- Life Threatening
- Severely or permanently disabling
- Requires in-patient hospitalization or prolongations of existing hospitalization
- Pregnancy loss after 20 weeks gestation
- Neonatal death up to 6 weeks after delivery
- Resulting in a congenital anomaly/birth defect
- Any event deemed serious by the PI onsite.

An unexpected adverse event is an adverse event not listed in the general investigation plan or protocol nor is it listed at the specificity or severity that has been previously observed and/or specified.

**10.3 Recording of Adverse Events**

All adverse events (serious or non-serious) and abnormal test results will be recorded in the patient's case report form, regardless of study group or suspected causal relationship to the study intervention(s). Report a diagnosis rather than a symptom. For all adverse events, sufficient information will be pursued and/or obtained to evaluate an adequate determination of the event's outcome and assess the casual relationship between the adverse event and the study interventions. Each event will be continuously followed up until resolved, stabilized, or up to 7 days after the last enrolled patient completes the follow-up.

Adverse events or abnormal test results deemed relevant to the study interventions will be followed up until the event or abnormal test result is resolved or stabilized at an acceptable level to the principal investigator.

**10.4 Causality and severity assessment**

The principal investigator will review documented adverse events and abnormal test results to determine:

- If abnormal test finding should be classified as an adverse event
- If the adverse event should be classified as a serious adverse event
- If the study intervention plausibly caused the adverse event

The conditions relating to the adverse event will be assessed to determine whether a relationship between the adverse event and the study exists. Record the causality of each event as "possibly related" or "not possibly related". The causality of the adverse event will be considered "possibly related" when causality is unknown. The maximum intensity of each adverse event will be evaluated and reported as one of the following:

1. Mild: events may or may not be volunteered by the patient. The patient is aware of and easily tolerates the event.
2. Moderate: the event poses sufficient discomfort to interfere with normal activities. A change in therapy may or may not be indicated.
3. Severe: side effects are almost always brought up by the patient. These events interfere with daily activities and usually require medical intervention.

### 10.5 Reporting of Serious Adverse Events and Unanticipated Problems

The PI onsite will report the SAE by completing a Serious Adverse Event Report Form within 24 hours of discovery and emailing the document in PDF form to the protocol PI. The site PI must determine and record on the form whether the SAE is anticipated or unanticipated and if it is related, possibly related, or unrelated to participation in the trial. The protocol PI must then determine if the SAE needs to be reported to the DCC. If the SAE is reported to the DCC, DCC staff will enter the SAE information in the central database and report to the DSMB. The DSMB will review the SAE upon receiving notification of the event via a closed-session email or conference call. The DSMB will send a report to the DCC within two weeks. If the SAE is life-threatening the report will be sent in one week. The DSMB report includes

- Statement indicating what information was reviewed by the DSMB
- The date of the review
- The DSMB's assessment of the reviewed information
- The DSMB's recommendation for the DCC, if any

The DCC will forward reportable events to protocol PI and all investigators. The protocol PI will evaluate the frequency and severity of the SAEs and determine if protocol modification and consent forms are required. Onsite PIs will the report the SAE to their IRB according to local IRB requirements.

Responsibilities of the PI at the site of the SAE:

1. Determine and record on the SAE form if adverse event is unanticipated or anticipated and possibly related or unrelated to participation in the research.
2. Report SAE by submitting the SAE form with the PI's signature in PDF format to the protocol leader via email:
   clbr_cbset_rct@163.com.
3. Reporting according to the following timeline:
   a. Unanticipated and related/possibly related SAE must be reported to the protocol PI within 1 business day of discovery.
   b. Anticipated and related/possibly related SAE must be reported to the protocol PI within 5 business days of discovery.
   c. Unrelated SAE (anticipated or unanticipated) must be reported to the protocol PI within 10 business days (no more than 3 weeks) of discovery.
   d. If the SAE is ongoing, the site PI will send follow-up reports to the protocol PI until the SAE is resolved.

## 11. Concomitant medication

If the following medications are used during this study, concomitant medication should be recorded on the specified form.

1) Anti-diabetic agents and anti-hypertension agent;
2) Treatment of endocrine or metabolic disorders such as hyperprolactinemia, hyperthyroidism or

hypothyroidism, insulin resistance, etc.

3) Folic acid supplement aimed at preventing neutral tubal defect;
4) For patients with abnormal bleeding/prolonged amenorrhea, progestin, micronized progesterone or dydrogesterone;
5) Medications which are not described in this protocol are used during the COH process and luteal support.
6) During the study period, some new diseases such as vaginitis are treated.
7) For patients with moderate or severe OHSS, clinical routine treatment, such as fluid infusion, albumin infusion, aspirin or preventive antibiotics will be used.
8) For patients with threaten abortion, an extra dose of progesterone will be approved for use. Concomitant medication will be recorded.
9) For patients with pregnancy complications, clinical standard care will be performed. Concomitant medication will be recorded.

## 12. Monitoring

### 12.1 Data and Safety Monitoring Board

An independent Data and Safety Monitoring Board (DSMB) will be established to review and interpret data generated from the study and review protocol revision prior to their implementation. The primary objectives of the DSMB are to ensure the safety of the subjects and preserve the integrity of the research data. The DSMB advises on research design issues, data quality and analysis, and research participant protections.

The DSMB will hold regular conference calls in English to review the protocol with respect to ethical and safety standards, monitor the safety of the trials, monitor the integrity of the data with respect to the original design of the study, and advise on study conduct. The DSMB will oversee progression of the trial, adjudicate adverse events, and decide on premature closure of the study. The conference calls will be coordinated by the DCC who will provide study updates via email prior to the calls.

The DSMB consists of five voting members who are impartial, independent of the investigators, and have no financial, scientific or other conflicts of interest with the study. The DSMB will consist of 3 members with relevant clinical expertise, 1 member with biostatistics expertise, and 1 human subject protection advocate.

**Table 7. DSMB Members**

| Name | Role on DSMB | Affiliation | High Level Responsibilities |
|---|---|---|---|
| Robert Rebar | Chair of DSMB | Western Michigan University Homer Stryker M.D. School of Medicine, USA | Chair the DSMB discussion and prepare written recommendations to IRB.<br>Ensure the safety of study subjects, the integrity of the research data.<br>Provide IRB with advice on the ethical and safe progression of studies conducted in the current project.<br>Advises on research design issues, data quality and analysis, and research participant protection for each prospective and on-going study. |
| TC LI | Voting member | Chinese University of Hong Kong | Ensure the safety of study subjects, the integrity of the research data.<br>Provide IRB with advice on the ethical and safety progression of current study. Advises on research design issues, data quality and analysis, and research participant protection for this prospective and on-going study. |
| Jun Zhang | Voting member | Xin Hua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, China | |
| Yan Liu | Voting member | Shanghai Changzheng Hospital，China | |
| Xiuqin Wang | Voting member | the First Affiliated Hospital of Nanjing Medical University，China | |

### 12.2 Ethics

Ethics approval has been sought from Ethics Committee at First Affiliated Hospital of Nanjing Medical University (2015-SR-018). All subjects will sign written informed consent forms before participating in the trial. The study will be conducted in compliance with the Declaration of Helsinki and Good Clinical Practice.

## 13. Data Handling and Record Keeping

Prof. Heping Zhang at Yale University will oversee the Data Collection and Management team made up of investigators from the Department of Epidemiology and Biostatistics, Nanjing Medical University.

### 13.1 Data entry and case report form

Investigators must record all requested data into the CRF and explain all the missing data. If a space on the CRF is not filled in for the reason that the procedure was not performed or the information was not collected, write "N/D". If the item is not applicable to the individual subject, write "N/A". If any entry error has been made, draw a single straight line through the incorrect entry and enter the correct data above it to correct the error. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS.

Case Report Forms (CRFs) will be made in WORD form and will be inputed into a Web-based data management system at http://www. medresman.org.cn/login.aspx. The Web data entry forms will be made in accordance with the WORD (paper) form.

### 13.2 Data security

To manage the data and monitor the process of the study, a web-based database, Clinical Trial Electronic Case Record Form (eCRF system) (http://www. medresman.org.cn/login.aspx), will be used to record the patients' data and results.

The database managers, Dr. Xiang Ma, Dr. Jing Wang, biostatistician Prof. Heping Zhang, and project leader Prof. Jiayin Liu, Prof. Zijiang Chen, are responsible for establishing the project space in the eCRF system and managing the users' accounts at each sub-center, establishing individual folders for each sub-center and assigning jurisdiction to the users.

The database managers have the highest authority to manage and monitor the data. The users of each sub-center will be allowed to enter their patients' information and study data into their individual folders. The database managers will make the final decision of what data will be disclosed to public. The private information of participants, including name, age, telephone number, will be strictly protected and never be disclosed.

The eCRF system service provider, Chinese Clinical Trial Registry, will be responsible for ensuring the safety of the database and the study data, maintaining the database, and providing technical support. However, the service provider does not have the right to revise data in the database. The contact person of the eCRF system is Prof. Taixiang Wu.

### 13.3 Data quality control

Data quality control will be managed at three different levels.

- The first level is the real-time logical checking built into the web-based data entry system. The research coordinators responsible for data entry at each participating site have the responsibility of ensuring accuracy of the data.
- The second level is the remote data monitor and validation which will be performed by the data manager and programmer at the DCC. Comprehensive data checks and regular manual checks will be conducted monthly by the data manager. Manual data checks within the database system will identify more complicated and less common errors. Errors will be fed back to the investigators at each site who will be required to check the data accuracy. The query will not be closed until the error has been corrected.
- The third level of quality control will be the site visits. During these visits, data in our database will be verified with the original study source or medical documents. Identified errors will be corrected. The site visits will ensure data quality and protection of patient's safety and privacy.

### 13.4 Audit

An audit will be implemented to ensure that only authorized additions, deletions, or alterations of information are inputted into the electronic record. This allows auditors the means to reconstruct significant details about study conduct

and collection of source data to verify the quality and integrity of the data. Computer generated and time-stamped audit trails will be implemented for tracking changes to electronic source documentation.

This is a multicenter clinical trial carried out within mainland China. Controls will be established to ensure the accuracy of the system's date and time. System documentation will explain time zone references as well as zone acronyms. Dates and times will include the year, month, day, hour, and minute provided by international standard-setting agencies. The ability to alter the date or time will be limited to authorized personnel, who will be notified if a system date or time discrepancy is detected.

In addition to internal safeguards built into the computerized system, external safeguards will be established. Study data will be stored in the servers housed at Clinical Center of Reproductive Medicine, First Affiliated Hospital of Nanjing Medical University, overseen by Prof. Heping Zhang. Records will be regularly backed up, and record logs will be kept to prevent a possible catastrophic loss and ensure the quality and integrity of the data.

## 14. Publication policy

### 14.1 Major Publications Authorship Order

The major manuscript is estimated to have up to 16 contributing authors. The authorship order for the participating sites will be based upon the recruitment of subjects, data accuracy and promptness of data reports. The rankings will start at position 6 and end at position 15. Data accuracy will be ranked by frequency of missing or false data entries at each participating site. If inquires show that data was accurately entered, they will not count against the data accuracy rate of the site. The site PI is responsible for documenting the contribution of the site's authors towards the study. Site investigators are encouraged to produce second hypotheses and write publications by sharing the data under the supervision of the publication committee.

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Final Protocol

# Cumulative live birth rates after cleavage-stage versus blastocyst-stage embryo transfer:
# A multicenter, prospective, randomized controlled trial
# (CLBR-CBSET)

Protocol Leader: Jiayin Liu, MD, PhD and Zi-Jiang Chen, MD, PhD
Data and Coordination Leader: Heping Zhang, PhD

Protocol version: Version 7.0
June 13, 2020

**CONTENTS**

## 1. Committee composition:

### 1.1 Protocol committee

**Table 1. Protocol committee**

| Name | Affiliation | Email address |
|---|---|---|
| Jiayin Liu | Clinical Center of Reproductive Medicine at the First Affiliated Hospital of Nanjing Medical University | jyliu_nj@126.com |
| Zi-Jiang Chen | Center for Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong University | chenzijiang@vip.163.com |

| | | |
|---|---|---|
| Richard S. Legro | Penn State University College of Medicine | rsl1@psu.edu |
| Heping Zhang | Yale University | heping.zhang@yale.edu |
| Xiang Ma | Clinical Center of Reproductive Medicine at the First Affiliated Hospital of Nanjing Medical University | sxmaxiang@126.com |
| Yuhua Shi | Center for Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong University | Shiyuhua2003@126.com |
| Yun Sun | Renji Hospital affiliated to Shanghai Jiaotong University School of Medicine | Syun163@163.com |

### 1.2 Steering Committee

**Table 2. Steering Committee**

| **Name** | **Affiliation** | **Email address** |
|---|---|---|
| **Chair** | | |
| Jiayin Liu | Clinical Center of Reproductive Medicine of the First Affiliated Hospital of Nanjing Medical University | jyliu_nj@126.com |
| **Co-investigators** | | |
| Zi-Jiang Chen | Center for Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong University | chenzijiang@vip.163.com |
| Richard S. Legro | Penn State University College of Medicine | rsl1@psu.edu |
| Heping Zhang | Yale University | heping.zhang@yale.edu |

### 1.3 Study sites and investigators

**Table 3. Study sites and investigators:**

| **No.** | **PIs** | **Study sites** | **Email** |
|---|---|---|---|
| 1 | Jiayin Liu<br>Xiang Ma | Clinical Center of Reproductive Medicine at the First Affiliated Hospital of Nanjing Medical University | jyliu_nj@126.com<br>sxmaxiang@126.com |
| 2 | Zi-Jiang Chen<br>Yuhua Shi | Center for Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong University | chenzijiang@vip.163.com<br>Shiyuhua2003@126.com |
| 3 | Yunxia Cao | The First Affiliated Hospital of Anhui Medical University | caoyunxia6@126.com |
| 4 | Yun Sun | Renji Hospital affiliated to Shanghai Jiaotong University School of Medicine | syun163@163.com |
| 5 | Bo Zhang | Guangxi Maternal and Child Health Hospital | cestbon269@sina.com |
| 6 | Jichun Tan | Shengjing Hospital of China Medical University | tjczjh@163.com |

| | | | |
|---|---|---|---|
| 7 | Jianqiao Liu | The Third Affiliated Hospital of Guangzhou Medical University | ljq88gz@163.com |
| 8 | Junli Zhao | General Hospital of Ningxia Medical University | zhaojunli6812@163.com |
| 9 | Hong Li | Suzhou Municipal Hospital | hongliszivf@163.com |
| 10 | Cuilian Zhang | Henan Provincial People's Hospital | luckyzcl@qq.com |
| 11 | Xingling Wang | The Third Affiliated Hospital of Zhengzhou University | wangxl208@126.com |

### 1.4 Data Coordination Committee

Prof. Heping Zhang from Yale University will lead the Data Coordination Committee with the help of personnel from the Department of Epidemiology and Biostatistics, School of Public Health, Nanjing Medical University, including registering the trial at ClinicalTrials.gov website (https://clinicaltrials.gov/).

### 1.5 Publication Committee

Members of the publication committee include Jiayin Liu, Zi-Jiang Chen, Xiang Ma, Yuhua Shi, Heping Zhang and Richard S. Legro.

## 2. Study synopsis

### 2.1 Objectives

The aim of this RCT is to compare differences in the efficacy and safety between cleavage-stage embryo transfer and blastocyst-stage embryo transfer in IVF/ICSI treatment cycle, taking into account of subsequent vitrified embryo transfers.

### 2.2 Patient Population

The patient population will include infertile patients between 20 and 40 years of age undergoing their first or second in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) cycle.

### 2.3 Study Design

This is a multi-center, prospective, randomized controlled clinical trial. A total of 980 infertile patients undergoing their first or second cycle of IVF or ICSI will be enrolled and randomized into two parallel treatment arms in a 1:1 ratio by site. Patients in group A will have a single cleavage-stage embryo transferred, and patients in group B will have a single blastocyst-stage embryo transferred. The outcomes of all the embryo transfers within 1 year after randomization will be followed up. Single embryo transfer (SET) is required for the first 3 embryo transfers within 1 year after randomization. For embryo transfers beyond the third within the 1 year, patients' treatment must follow their randomized allocation, and SET is no longer mandatory. Each patient will be observed until the study endpoint as defined in section 6.10. Cumulative live birth rate (CLBR) will be analyzed according to appropriate statistical methods. All participants will receive a long GnRH-agonist, short GnRH-agonist or GnRH antagonist protocols. In frozen embryo transfer cycles, the endometrium will be prepared with natural ovulation cycles, minimal stimulation cycles or artificial cycles. Due to the COVID-19 pandemic, the participants who were unable to undergo the embryo transfers in 1 year of randomization will have 3 months extension for frozen embryo transfers.

### 2.4 Treatment

All the participants will receive a long GnRH–agonist, short GnRH-agonist or GnRH antagonist protocol in combination with recombinant FSH (rFSH, Puregon). HCG will be administered for final oocyte maturation, and vaginal ultrasound-guided oocyte retrieval will be performed. Randomization will be performed on day 2/3 after oocyte retrieval, when at least 3 transferrable cleavage-stage embryos are achieved. Patients in group A will have a single cleavage-stage embryos transferred. Patients in group B will have a single blastocyst-stage embryos transferred. If the previous embryo transfer does not result in a pregnancy/live birth, patients would go through cryopreserved

cycles within 1 year after the randomization. Luteal phase support will be administered before embryo transfer. A pregnancy test will be performed 2 weeks after embryos transfer. If pregnancy is confirmed, luteal phase support will be continued until 10 weeks of gestation. The follow up will be continued until 6 weeks after delivery.

### 2.5 Primary outcome

The primary outcome is CLBR per patient until the first live birth from one oocyte retrieval cycle (Calculated using outcomes from the first three embryo transfers within 1 year after randomization. The study endpoint is defined in section 6.10).

### 2.6 Secondary outcomes

The secondary outcomes will include biochemical pregnancy rate, clinical pregnancy rate, implantation rate, cumulative clinical pregnancy rate, ongoing pregnancy rate, live birth rate, pregnancy loss rate, birth weight and sex ratio.

The safety parameters will include moderate or severe OHSS rate, ectopic pregnancy rate, multiple pregnancy rate, and incidence of obstetric and perinatal complications, such as gestational diabetes and hypertension, pre-eclampsia, placental previa, placental abruption, preterm delivery, neonatal hospitalization for >3 days, congenital anomalies and perinatal mortality.

### 2.7 Statistical Analysis

The primary analysis will use an intent-to-treat (ITT) approach to examine differences in CLBR with first live birth per oocyte retrieval in the two treatment arms, calculated using outcomes from the first three SETs within 1 year after randomization. If SET is not performed in the first three transfers, the study deviation should be reported, but the results will be calculated according to ITT principles. Categorical data will be represented as a frequency and a percentage; differences in these measures between treatment groups will be assessed by Chi-square analysis, with Fisher's Exact Test for expected frequencies less than 5. Continuous data will be expressed as mean ± SD, with student t test for testing differences between two groups. Inter-quantile ranges may be presented when non-normality of the continuous data is apparent.

A per protocol analysis and safety analysis will also be performed. The conservative and optimal estimate of the CLBR will be used to calculate the CLBR. The CLBR from the first 3 embryo transfers within 1 year of randomization will be calculated as the primary outcome. The CLBR from all the embryo transfers per oocyte retrieval within 1 year of randomization will be analyzed, but not as the primary outcome.

Due to the COVID-19 pandemic, the participants who were unable to undergo the embryo transfers in 1 year of randomization will have 3 months extension for frozen embryo transfers. The CLBR with and without the 3 months extension for frozen embryo transfers will be calculated separately. The CLBR with 3 months extension for frozen embryo transfers will be reported as the primary outcome.

The Chi-square test will be used for the conservative CLBR between the two treatment groups. Optimal CLBR will be analyzed by using the Kaplan-Meier product-limit method, which censors data for subjects who do not return for treatment, and estimates the CLBR with 95% confidence intervals.

Furthermore, univariate/multivariate logistic will be performed to evaluate the influence of Age (≤35 or >35 years), AMH, number of oocytes retrieved, number of embryos, times of embryo transfer, duration to achieve pregnancy and BMI on pregnancy rate or CLBR. Any deviations from the previously described statistical plan will be described and justified in a protocol amendment. The results will be reported according to the CONSORT statement.

### 2.8 Anticipated Time to Completion

The study will be completed within the duration of 2 years after randomization. The anticipated time schedule is an 8 months enrollment period, a 12 months treatment period, and an 11 months pregnancy follow-up until 6 weeks after delivery.

## 3. Backgrounds and Significance

More than 6 million infants are born by IVF/ICSI worldwide [1]. Embryo transfer is one of the most critical steps in IVF/ICSI. Traditionally, only cleavage-stage embryos on Day 3 were transferred. Over the past decades, the number of blastocyst-stage embryos transferred on Day 5 or 6 has been steadily increasing due to advances in IVF laboratories, such as embryo culture media. [2] It has been reported that in Australia, the proportion of blastocyst transfer cycles to

cleavage transfer cycles has increased from less than 30% in 2004 to more than 60% in 2013[3,4]. National data from the United States and the United Kingdom show similar increases in the frequency of blastocyst transfers which represented approximately one third of IVF cycles in 2012. [5,6]

Extended culture to blastocyst stage exhibits several advantages over cleavage-stage embryo. It is well accepted that blastocyst-stage transfer mimics the conditions of physiological implantation, and as a result it may provide better embryo-endometrium synchronization and therefore higher chances of implantation [7]. Extended culture to the blastocyst stage also enables embryo self-selection, consequently the most viable embryos will survive and be selected for transfer. Evidence supports the notion that fresh blastocyst transfers result in significantly higher live birth rates and clinical pregnancy rates when compared with the fresh cleavage-stage transfers [8, 9]. However, the results are not conclusive when the transfer of subsequent frozen embryos is considered [8, 9].

The American Society for Reproductive Medicine and the fertility guidelines issued in 2013 by NICE have both expressed concern over the use of the blastocyst transfer [10, 11], because extending culture could yield fewer/no viable embryos available for freezing and subsequent thaw transfer, and further oocyte retrieval cycles will be required. Conversely, more ETs do not necessarily result in improved cumulative live birth, thus blastocyst transfer may relieve the burden of the additional transfers.

The results of studies comparing the outcomes between blastocyst- and cleavage-stage transfer are inconsistent. A Cochrane systematic review of 27 RCTs published in 2016 [8] reported that live birth rates (low-quality evidence) and clinical pregnancy rates (moderate-quality evidence) for fresh blastocyst transfer are higher compared with those of cleavage-stage transfer. The very low quality evidence shows no significant differences in the cumulative pregnancy rate between the two groups. Blastocyst transfer has a reduced embryo freezing rate. However, there was no difference between the groups in terms of multiple pregnancy and miscarriage rates. A well-designed retrospective study published in 2016[9] reported higher live birth rates in fresh single blastocyst transfer and similar cumulative live birth rate between the two groups. The study also proved that blastocyst stage transfers required a fewer number of embryos to obtain a live birth. However, two recent meta-analyses [12,13] comparing the outcomes of blastocyst and cleavage-stage transfer showed no significant differences in clinical pregnancy, live birth/ongoing pregnancy, cumulative live birth rate, as well as multiple pregnancy rate. One of the studies that included studies with vitrification reported a higher implantation rate and miscarriage rate after blastocyst transfer [12]. Both of the studies emphasized the low quality of included studies and the importance of performing a large-scale, well-designed RCT on this topic before robust conclusions can be drawn. Furthermore, two of the latest RCTs [14, 15] both failed to detect a significant difference in the efficacy of blastocyst transfer when compared to cleavage-stage transfer. However, both RCTs were conducted in single center with a limited number of treatment cycles.

Conventionally, IVF success has been calculated in terms of live birth rate per treatment attempt. As reproductive technology continues to develop, the number of FET cycles have been growing rapidly. Combined with an emphasis on reducing multiple pregnancies and increasing single embryo transfers, cumulative pregnancy rate per patients from one oocyte retrieval cycle reflect the true IVF success rate by considering all implantation attempts from both fresh and frozen embryos [16]. Vitrification technology has been proved to be the key for enhanced blastocyst cryopreservation, with a high survival rate of around 96%-98% [18]. Therefore, CLBR is able to be compared under more objective and fair conditions by using vitrification for both cleavage-stage embryos and blastocysts. To date, only five RCTs reported cumulative pregnancy rates after transferring both fresh and frozen embryos between blastocyst and cleavage-stage transfer [17]. Among these, four RCTs [19-22] in the early years based on slow-freezing reported that cleavage stage transfer resulted in a higher CLBR, while the current RCT [23] with vitrification showed higher cumulative ongoing pregnancy rates in blastocyst transfers, albeit in a small sample size. Recently another two retrospective cohort studies [9,24] comparing CLBRs of cleavage-stage and blastocyst embryos using vitrification methods for frozen embryos have been published. Both of the studies failed to show significant differences in CBLR between the two groups. However, they proved blastocysts required fewer embryo transfers and less time to achieve the first live birth, which seems to be a more effective and time-efficient method to obtain a live baby when compared with cleavage-stage embryo transfer. There is an absence of RCTs comparing CLBR of cleavage-stage and blastocyst embryos using vitrification methods for embryo cryopreservation.

The health of offspring born after blastocyst transfer has raised great concerns. Previous un-matched cohort studies [25, 26] reported extended culture resulted in adverse neonatal outcomes, such as a higher risk of preterm delivery, very preterm birth, large for gestational age (LGA) and perinatal mortality. However, most recent controlled studies did not confirm these conclusions [27-33]. In addition to extended culture effects, the influence of cryopreservation on these adverse outcomes should be considered. Slow-freezing techniques were reported to be related to an increased risk of LGA newborns [34]. In contrast, vitrification does not seem to be related to adverse neonatal outcomes for both

cleavage-stage embryos and blastocysts [35-39]. A latest meta-analysis [40] compares the perinatal outcomes of singleton pregnancies from blastocysts with those from cleavage-stage transfer, stratified by fresh and frozen embryo-transfer cycles. The results suggest cryopreservation of embryos can influence pregnancy outcome transfer in terms of preterm birth, very preterm birth, LGA, SGA and perinatal mortality. However, there is a lack of RCTs in this issue and the evidences available in the analysis are at a low level.

Well-designed RCTs are urgently needed to report the efficacy and safety of blastocyst transfers before a definitive conclusion can be drawn to guide the selection of the optimal transfer strategy. This is a randomized trial designed to determine the differences in pregnancy outcomes and newborn health between cleavage-stage embryo transfer and blastocyst-stage embryo transfer in IVF/ICSI treatment cycle, taking into account subsequent vitrified embryo transfers. The primary outcome is cumulative live birth rate (≥24 weeks gestation) per patient until the first live birth from one initiating oocytes retrieval cycle.

## 4. Objectives

This trial is designed to compare the efficacy and safety between cleavage-stage embryo transfer and blastocyst-stage embryo transfer in IVF/ICSI treatment cycle, taking into account subsequent vitrified embryo transfers.

The primary research hypothesis is that the CLBR of blastocyst-stage embryo transfers is non-inferior to that of cleavage-stage embryo transfers. A safety hypothesis will also be taken into consideration. We hypothesize both treatments have similar incidence of maternal and neonatal complications.

## 5. Study Population

The study population will include infertile patients between 20 and 40 years of age, undergoing their first or second IVF or ICSI cycle. Ovarian stimulation will be performed with long GnRH-agonist, short GnRH-agonist or GnRH antagonist with HCG trigger.

### 5.1 Inclusion criteria:

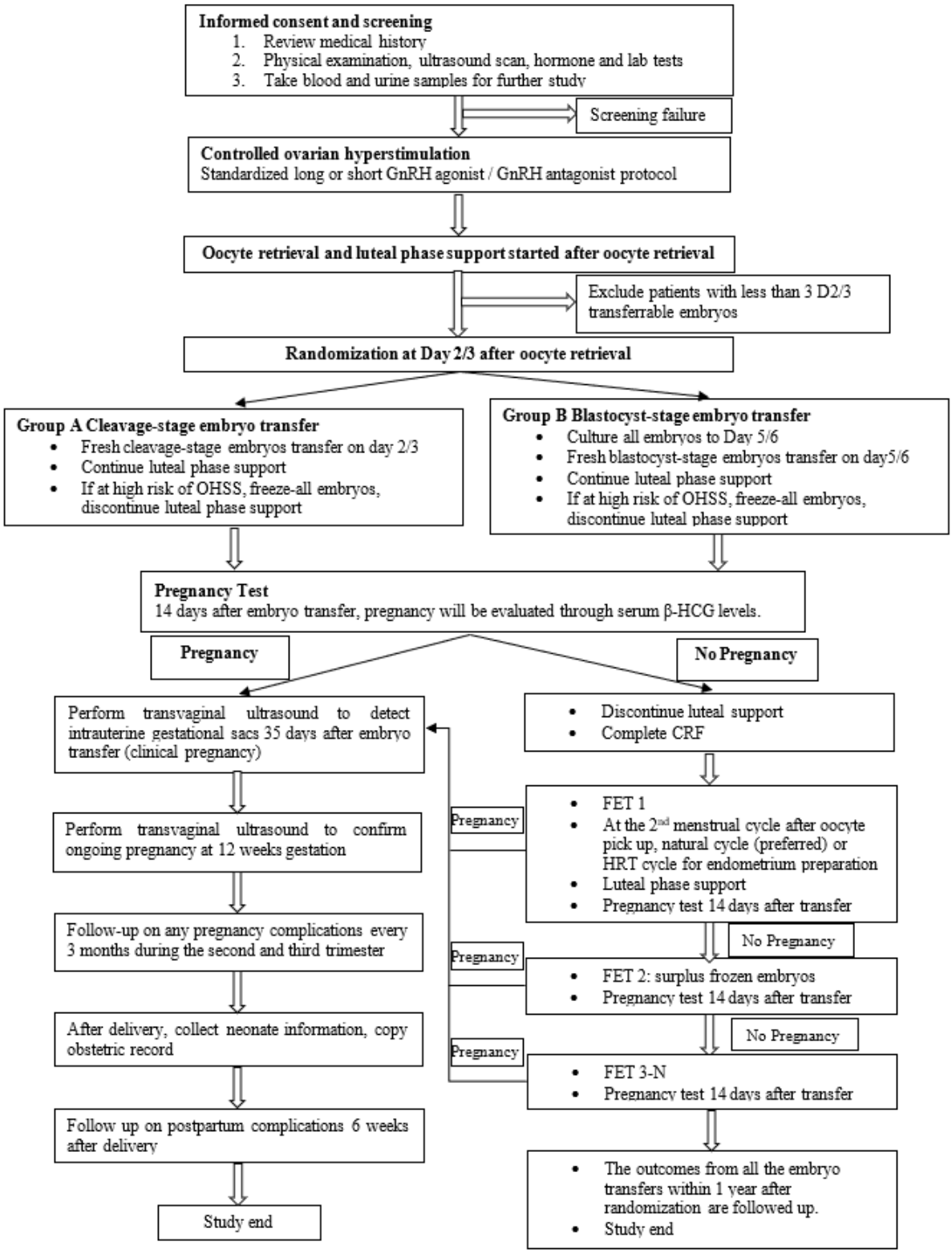
1. Women aged ≥20 and ≤40 years.
2. Women with the number of transferrable cleavage embryos ≥ 3;
3. Women undergoing their first or second cycle of IVF or ICSI.

### 5.2 Exclusion criteria

1. Women who have been diagnosed with uterine abnormalities (confirmed by three-dimensional ultrasonography or hysteroscopy), including malformed uterus (uterus unicornis, septate uterus or duplex uterus), submucous myoma, or intrauterine adhesion
2. Women who plan to undergo In Vitro Maturation (IVM);
3. Women who plan to undergo Preimplantation Genetic Diagnosis (PGD) /Preimplantation Genetic Screening (PGS).
4. Women who have hydrosalpinx visible on ultrasound.
5. Women who have experienced recurrent spontaneous abortions, defined as 2 or more previous pregnancy losses.
6. Women who have been developed a “freeze-all” treatment plan for purpose of subsequent surgery, such as salpingectomy due to hydrosalpinx after oocytes retrieval.
7. Women with contraindications to assisted reproductive technology and/or pregnancy, such as uncontrolled hypertension, symptomatic heart diseases, uncontrolled diabetes, undiagnosed liver disease or dysfunction (based on serum liver enzyme test results), undiagnosed renal disease or abnormal renal function, severe anemia, history of deep venous thrombosis, pulmonary embolus or cerebrovascular accident, history of or suspicious for cancer, undiagnosed vaginal bleeding.

## 6. Study procedures and visits

### 6.1 Study Procedure Flow Chart



### 6.2 Screening visit

Physicians will introduce this trial to potential participants who are interested in joining the trial, and refer the participants to investigators if the enrollment criteria are met. Participants who are willing to participate will receive an informed consent document explaining the trial in detail, especially the random assignment on day 2/3 after oocyte retrieval. Consent forms should be signed before any screening will be done. Screening visit will begin when patients finish all tests for preparation of IVF or ICSI. Participants will undergo a face-to-face screening visit to provide information on their demographic details, medication usage, previous history of diseases, menstruation, and infertility. This interview serves as further confirmation that the participant meets the inclusion criteria. The screening process will be recorded in the screening log, and the cause of screening failure will also be recorded. During this visit, the following procedures will be undertaken:

1) Obtain the signed informed consent
2) Perform a comprehensive history review consisting of demographic details, past medical history, associated medication status, menstrual history, infertility history and gynecological history;
3) Complete physical examinations including height, weight, BMI, circumference of the waist and hips, vital signs and pelvic exam; (If eligible pelvic exam has been performed within 6 months, just record them and no repeat will be required.)
4) A transvaginal ultrasound screen for uterus condition, ovary size, antral follicle count, and the thickness of the endometrium. If uterine abnormalities are suspected, a three-dimensional ultrasonography or hysteroscopy will be performed to confirm the diagnosis. (If eligible ultrasound has been performed within 6 months, just record them and no repeat will be required.)
5) Hormonal testing, including: serum follicle stimulating hormone (FSH), luteinizing hormone (LH), Total testosterone (TT), estradiol (E2), progesterone (P), prolactin (PRL), thyroid Stimulating Hormone (TSH), and anti-mullerian hormone (AMH) level. (If eligible results have been obtained within 6 months, just record them and no repeat will be required.)
6) Karyotypes tests of the couples. (If eligible karyotypes results have been performed previously, just record them and no repeat will be required.)
7) Safety lab tests including: CBC, hepatic and renal function tests, serum glucose and lipid level, coagulation analysis, TORCH screening, thyroid function tests, infectious disease screening, TCT, ECG. Insulin levels, the oral glucose tolerance test (OGTT), uric acid levels autoantibody levels and serum tumor markers are selective optional test based on patient's condition. (If eligible results have been obtained within 6 months, just record them and no repeat will be required.)
8) Complete the questionnaire for health survey (SF-36) and fertility quality of life (FertiQoL International);
9) Retrieval of blood sample for further studies. The volume of each sample is about 2-3ml, which will be numbered and stored in a -80°C refrigerator.

### 6.3 Oocyte retrieval day visit

During this visit, the following procedures will be completed:

1) Complete the form by recording parameters from COH procedure and oocyte pick-up procedure.
2) Exclude participants who fail to undergo oocyte retrieval procedure, obtain oocytes or complete sperm collection, have obvious hydrosalpinx, or plan to undergo "freeze all" policy.
3) Record adverse events and concomitant medication in the adverse event report form and concomitant medication record form.
4) Recommend retrieval of the granulosa cells, follicular fluid and blood samples.

### 6.4 Day 2/3 after oocyte retrieval and fresh embryo transfer visit

1) Record the parameters from In Vitro Fertilization procedure and embryo culture.
2) Exclude participants that fail to have ≥ 3 transferrable Day 2/3 embryos and have been developed a "freeze-all" treatment plan for purpose of subsequent surgery.
3) Randomize participants with ≥ 3 transferrable Day 2/3 embryos equally into two groups after oocyte retrieval. Group A will refer to the Day 2/3 embryo transfer group while group B will refer to the Day 5/6 embryo transfer group.
4) Record information regarding transferred embryos and progesterone support protocol if subjects in Group A undergo Day 2/3 embryo transfer in fresh cycle.
5) Record information regarding frozen embryos if subjects in Group A have embryos vitrified.

6) Inquire about and record adverse events and concomitant medication.

-If embryo transfer is not performed in fresh cycle and no embryos are vitrified, end of study visit should be performed, and cause of early termination will be recorded at the end of study visit form.

-If fresh embryo transfer is not conducted and all the embryos are vitrified, further FET visits should be performed.

**6.5 Day 5/6 after oocyte retrieval and fresh embryo transfer visit**

5) Participants in Group B will have all Day 2/3 embryos cultured to Day 5/6. Record information regarding blastocysts culture.
6) Record information regarding transferred embryos and progesterone support protocol if participants in Group B undergo embryo transfer in fresh cycle.
7) Record information regarding frozen embryos if participants in Group B have embryo vitrified.
8) Inquire about and record adverse events and concomitant medication in the specialized form for this visit.

-If no Day 5/6 embryos are obtained, end of study visit should be performed, and cause of early termination will be recorded in the end of study visit form.

-If embryo transfer is not performed in fresh cycle and no embryos are vitrified, end of study visit should be undertaken, and cause of early termination will be recorded in the end of study visit form.

-If fresh embryo transfer is not conducted and all the embryos are vitrified, further FET visit should be performed.

**6.6 Frozen embryo transfer (1-N) visit**

4) Record information regarding endometrium preparation, transferred embryos and progesterone support methods.
5) Record the number of surplus frozen embryos.
6) Inquire about and record adverse events and concomitant medication in the specialized forms for this visit.

-If patients with frozen embryos will not undergo FET procedure for any reason within 1 year after randomization, end of study visit should be performed, and the cause will be recorded in the end of study visit form.

-If all thawed embryos are not transferrable, end of study visit will also be performed and recorded in the specialized form.

-If subjects do not obtain a pregnancy/first live birth, the visits of FETs conducted within 1 year of randomization will be performed.

**6.7 Biochemical pregnancy test visit**

This visit will be performed on the 14th day after embryo transfer. Biochemical pregnancy is defined as a serum β – HCG level no less than 25 IU per liter. Fill out the specialized form for this visit.

-If the result of biochemical pregnancy is negative and embryos runs out for the next FET, an end of study visit will also be performed and recorded in the specialized form.

-If the result of biochemical pregnancy is negative but there are frozen embryos left, subsequent FET visits will be performed.

**6.8 Clinical pregnancy test visit**

The visit to test clinical pregnancy will take place on the 30-35th day after the embryo transfer. Clinical pregnancy is defined as the presence of an intrauterine gestation sac. If clinical pregnancy has been obtained, the follow-up schedule will be made. Investigators will check contact information with patients and require them to inform investigators of their delivery time.

A specialized form for this visit will be filled out. Concomitant medication and adverse events will be recorded as well.

-If the result of clinical pregnancy is negative and embryos run out for the next FET, an end of study visit and a summary of final pregnancy outcome will be performed and recorded in the specialized form.

-If the result of clinical pregnancy is negative but there are frozen embryos left, subsequent FET visits will be performed.

-If pregnancy is terminated prematurely, the cause will be recorded in the form for the outcome of the pregnancy.

**6.9 Follow-up visits**

The outcome of the pregnancy and any obstetrical or perinatal complications will be attained after review of obstetric medical records and neonatal medical records.

At 14 weeks gestation, the first-trimester pregnancy complications, including but not limited to OHSS, miscarriage, ectopic pregnancy or trophoblastic disease, will be collected from patients by telephone and by inspecting their medical records. The specialized form for the first pregnancy follow-up time point will be completed.

At 28 weeks gestation, the second-trimester pregnancy complications, including but not limited to abortion, gestational diabetes, pre-eclampsia/eclampsia, incompetent cervix, premature membrane rupture or placenta abruption will be evaluated by telephone call and by inspecting their medical records at the second pregnancy follow-up time point. The specialized form will be filled out.

At 37 weeks gestation, the third-trimester pregnancy complications including but not limited to preterm labor, placenta abruption, placenta accrete, placenta previa, pre-eclampsia/eclampsia, premature membrane rupture, intrauterine growth retardation or amniotic fluid abnormality will be followed up by telephone call and by inspecting their medical records at the third pregnancy follow-up time point. The specialized form will be filled out.

At delivery, subjects are required to notify investigators of their delivery time. The delivery information, including gestational age, delivery mode, abnormality of placenta or delivery complications as well as infant information including birth weight and presence of birth defects will be followed up by telephone and by reviewing obstetric and neonatal medical records. The specialized form for the fourth follow-up time point will be completed. Recommend collecting the placenta and cord blood sample.

At 6 weeks after delivery, any complications of the mother (such as infection, postpartum depression, hemorrhage) or infant (such as neonatal respiratory distress syndrome, infection, jaundice, hospitalization, death) will be followed up by telephone and by reviewing obstetric and neonatal medical records. The specialized form for the fifth and final follow-up time point will be completed.

During the follow-up period, concomitant medication and adverse events must be recorded.

-If clinical pregnancy is achieved (premature pregnancy termination or live birth obtained), the specialized forms for pregnancy outcome after each ET and a summary of final pregnancy outcome (including times of ET, pregnancy details after each ET) after the end of all pregnancy follow-ups must be filled out.

-If a live birth is obtained, an end of study visit and a summary of final pregnancy outcome (including times of ET, pregnancy details after each ET) will also be performed and recorded in the specialized form.

-If live birth is not achieved during any pregnancy follow-up period and embryos run out for the next FET, an end of study visit will also be performed and recorded in the specialized form.

-If live birth is not achieved but there are frozen embryos left, subsequent visits of FETs conducted within 1 year after randomization will be performed.

### 6.10 End of Study visit (by telephone)

The end of study visit will be conducted if the following occurs:

10) The subjects achieve their first live birth from the embryo transfers within 1 year after randomization.
11) An outcome from the last transfer within 1 year after randomization.
12) No embryo transfers within 1 year of randomization.
13) No Day 5/6 embryo obtained
14) No transferrable embryos obtained after thawing
15) A live birth obtained by a natural pregnancy during the study
16) Subjects who are lost to follow up
17) Subjects who withdraw consent and/or refuse to continue participating.
18) Other reasons (for example, investigator's or subject's decision)

### 6.11 2 years follow up Visit (by telephone)

At the 2 year follow-up time point after randomization, subjects who do not reach the end of the study will be followed up by telephone to check and record their treatment or pregnancy status and outcomes.

## 7. Study Design

### 7.1 Type of design

This is a randomized controlled clinical trial comparing the cumulative live birth rate per oocyte retrieval between cleavage-stage embryo transfer and blastocyst-stage embryo transfer in 980 patients undergoing their first or second IVF/ICSI cycles across 11 reproductive centers in China. Eligible patients will be randomly divided into two treatment groups. Patients in the group A will have a single cleavage-stage embryo transferred per cycle, and patients in group B will have a single blastocyst-stage embryo transferred per cycle. All of the participants will receive a standardized long GnRH–agonist or short GnRH-agonist or GnRH antagonist ovarian stimulation protocol and standardized luteal phase support. The outcomes from all the embryo transfers within 1 year after randomization will be followed up. If a pregnancy/live birth is not achieved, single embryo transfer is required for the first 3 embryo transfers within 1 year after randomization. For embryo transfers beyond the third within the 1 year, patient's treatment must follow their randomized allocation, and SET is no longer mandatory. The follow-up period is 2 years from the day of randomization. Both "optimistic" and "conservative" CLBR will also be analyzed (see Section 9.2). The CLBR from the first 3 single embryo transfers within 1 year after randomization will be calculated as the primary outcome. The CLBR of all the embryos transfers within 1 year after randomization will be analyzed as well, but not as the primary outcome. Due to the COVID-19 pandemic, the participants who were unable to undergo the embryo transfers in 1 year of randomization will have 3 months extension for frozen embryo transfers.

The blocked randomization method was used with the dynamic block size. The treatment ratio is 1:1 and the randomization was stratified by study sites. A data coordinate center (SAS Institute, Care, NC) in Yale University generated the randomization sequence with SAS (software version 9.2). This randomization sequence was input into an online central randomization system (http://www.medresman.org) kept blind from both investigators who enroll subjects and study coordinators. The randomization will occur on the day 2/3 after oocyte retrieval, when at least 4 embryos are achieved. Trained coordinators will draw one randomized number at a time using password-protected accounts. This study is designed as an open labeled one. Both investigators and subjects will be aware of the allocation after randomization.

### 7.2 Study intervention

#### 7.2.1 Controlled ovarian hyperstimulation:

GnRH-agonist or GnRH antagonist protocols will be employed for ovarian stimulation. Each site will use 1-2 fixed stimulation protocols to reduce potential bias.

- Long GnRH-agonist protocol

1. Short-acting long GnRH-agonist protocol

GnRH agonist (Diphereline, Ipsen Pharma Biotech, France) will be administered with the dose of 0.05-0.1 mg daily on Day 21-25 of menstrual cycle for 14 -20 days. Once the downregulation criteria is met (LH≤5 IU/L, E2≤50 pg/ml, and endometrium thickness≤6mm), recombinant follicular stimulating hormone (rFSH, Puregon, MSD, Ravensburg, Germany) with dose of 75 to 225 IU per day will be initiated. The adjustment of gonadotropin dose is based on follicular development and serum hormone levels. Continual administration of GnRH agonist and gonadotropin last until the start of human chorionic gonadotropin (HCG) injection. HCG 4000-10000IU will be administered when at least 2 follicles are ≥ 18mm or 3 follicles are ≥ 17mm in mean diameter. Human menopausal gonadotropin (HMG) and recombinant human luteinizing hormone (r-hLH, Luveris, Merck Serono, Germany) could be combined or added if needed.

2. Long-acting long GnRH-agonist protocol

A quarter dose to a full dose (3.75mg) of GnRH agonist (Diphereline, Ipsen Pharma Biotech, France) will be administered on Day 1-2 of menstrual cycle or the luteal phase. After 14-28 days, recombinant follicular stimulating hormone (rFSH, Puregon, MSD, Ravensburg, Germany) with a dosage of 75 to 225 IU per day will be initiated, according to FSH and estradiol levels and follicle size. The gonadotropin dosage is adjusted based on follicular development and serum hormone levels. Continual administration of gonadotropin lasts until the start of human chorionic gonadotropin (HCG) injection. HCG 4000-10000IU will be administered when at least 2 follicles are ≥ 18mm or 3 follicles are ≥ 17mm in mean diameter. HMG and recombinant human luteinizing hormone (r-hLH, Luveris, Merck Serono, Germany) could be combined or added if needed.

- Short GnRH-agonist protocol

On day 2-3 of the menstrual cycle, GnRH agonist will be administered with a dosage of 0.05-0.1 mg daily, and recombinant follicular stimulating hormone (rFSH, Puregon, MSD, Ravensburg, Germany) with a dosage of 75 to 225

IU per day will be initiated. The adjustment of gonadotropin dose is based on follicular development and serum hormone levels. Continual administration of GnRH agonist and gonadotropin will last until the start of human chorionic gonadotropin (HCG) injection. HCG 4000-10000IU will be administered when at least 2 follicles are ≥ 18mm or 3 follicles are ≥ 17mm in mean diameter. HMG and recombinant human luteinizing hormone (r-hLH, Luveris, Merck Serono, Germany) could be combined or added if needed.

- GnRH antagonist protocol

Recombinant follicular stimulating hormone (rFSH, Puregon, MSD, Ravensburg, Germany) with dose of 75 to 225 IU per day will be administered on Day 2-3 of menstrual cycle for 5 days. The follicular development will be monitored by ultrasound and serum hormone levels. The dose of rFSH (Puregon) will be adjusted accordingly by investigators on each site. HMG and recombinant human luteinizing hormone (r-hLH, Luveris, Merck Serono, Germany) could be combined or added if needed. GnRH antagonist (Ganirelix, Orgalutran, MSD, Ravensburg, Germany) 0.25mg will be given when at least one follicle is ≥12 mm in mean diameter until the trigger day (including the trigger day). HCG 4000-10000IU will be administered when at least 2 follicles are ≥ 18mm or 3 follicles are ≥ 17mm in mean diameter. If patients are at a high risk of OHSS, GnRHa or GnRHa + HCG could be used for trigger.

**7.2.2 Oocyte retrieval:**

Routine transvaginal ultrasound-guided follicle aspiration is to be performed 36 to 37 hours after HCG injection by experienced physicians.

**7.2.3 In-vitro fertilization and embryo culture:**

The oocytes will be inseminated by conventional IVF, intracytoplasmic sperm injection (ICSI) or early rescue ICSI according to the indications. On day 2/3 after oocyte retrieval, the quality of the embryos will be assessed by morphological criteria based mainly on the number and regularity of blastomeres as well as percentage fragmentation. Subjects with 3 or more transferrable embryos will be randomly assigned to undergo cleavage-stage or blastocyst-stage embryo transfer with the online central randomization system.

For subjects assigned to the cleavage-stage (Day 2/3) embryo transfer group, a single cleavage-stage embryo of the best quality will be transferred in a fresh cycle on Day 2/3 after oocyte retrieval. The surplus embryos, if any, will be vitrified for future FET if the fresh cycle does not result in a live birth. If a patient is at a high risk of OHSS, all embryos on Day 2/3 are allowed to be cryopreserved with vitrification for patient's safety. The FET cycle will be initiated on the second menstrual cycle after oocyte retrieval.

For subjects assigned to blastocyst-stage (Day 5/6) embryo transfer group, all embryos will be cultured to D5 or D6. A single blastocyst of the best quality will be transferred in a fresh cycle on D5 or D6 after oocyte retrieval (D5 embryo will be the prior choice). The surplus embryos, if any, will be vitrified for future FET in case the fresh cycle does not result in a live birth. If a patient is at a high risk of OHSS, all embryos on D5 or D6 can be cryopreserved with vitrification for patient's safety. The FET cycle will be initiated on the second menstrual cycle after oocyte retrieval.

**7.2.4 Embryo transfer and luteal phase support:**

The luteal phase support protocol for group A and B are the same. For patients who have fresh cleavage or blastocyst-stage embryos transferred, a single fresh embryo of top quality will be transferred Day 2/3 or D5/6 after oocyte retrieval. Luteal phase support with vaginal progesterone gel (Crinone, Merck Serono) 90mg per day and oral dydrogesterone 10mg twice daily will begin from the day of oocyte retrieval and continue until pregnancy determination 14 days after embryo transfer. If the patient is pregnant, vaginal progesterone gel together with dydrogesterone will continue to clinical pregnancy evaluation day (30-35th day after the embryo transfer) and oral dydrogesterone to 10 weeks gestation.

For those who have all embryos vitrified, luteal phase support will be stopped 7 days after randomization.

For patients who have frozen cleavage or blastocyst-stage embryos transferred, a single frozen embryo of top quality (Day 2/3 or Day 5/6) will be transferred. Endometrium preparation will be performed with natural cycle regimen, minimal stimulation cycle regimen or hormone replacement cycle regimen. The optimal choice for endometrium preparation is natural cycle ovulation or minimal stimulation that mimics a natural cycle. At the second cycle following oocyte retrieval, ovulation will be monitored using ultrasound. On ovulation day, luteal phase support will begin with 10mg oral dydrogesterone twice daily. Frozen-thawed cleavage-stage embryos and blastocysts will be transferred on day 2/3 or 5/6 after ovulation respectively. If the patient is pregnant, luteal phase support will continue until 10 weeks gestation, then gradually reduced.

For hormone replacement cycle regimen, the endometrium will also be prepared with oral E2 valerate (E2V) with a dose of 4-6mg daily initiated on day 1 to day 4 of menstrual cycle, vaginal progesterone gel (Crinone, Merck Serono) 90mg per day and oral dydrogesterone 10mg twice daily will be added when endometrial thickness reaches 7mm. Frozen-thawed cleavage-stage embryos and blastocysts will be transferred on day 4 or 5/6 after progesterone initiation respectively. If the patient is pregnant, E2V will be reduced gradually, vaginal progesterone gel will continue to clinical pregnancy evaluation day (30-35th day after the embryo transfer) and oral dydrogesterone to 10 weeks gestation.

### 7.2.5 Pregnancy evaluation:

Pregnancy will be determined 14 days after embryo transfer using serum β-HCG. If a biochemical pregnancy is achieved, a transvaginal ultrasound will be performed 30-35 days after embryo transfer to evaluate clinical pregnancy. The ultrasound will record the number, size, location of gestational sacs and embryo buds as well as fetal heart beat and abnormal conditions. At 12 weeks of gestation, another ultrasound will be performed to confirm ongoing pregnancy. Moderate to severe OHSS will be recorded.

## 7.3 Study Endpoints

The primary endpoint will be cumulative live birth per patient from one initiated oocyte retrieval cycle (Calculated using outcomes from the first three embryo transfers within 1 year of randomization). Live birth is defined as delivery of any neonate ≥24 weeks gestation with heart beat and breath.

Secondary efficacy endpoints will include biochemical pregnancy, clinical pregnancy, ongoing pregnancy, pregnancy loss rate and live birth. Biochemical pregnancy is defined as a serum β-HCG level of at least 25 IU/L 14 days after embryo transfer. Clinical pregnancy is defined by the presence of intrauterine gestation sacs at 30-35 days after embryo transfer. Ongoing pregnancy is defined as a viable pregnancy at 12 weeks gestation. Pregnancy loss is defined as a pregnancy that results in a spontaneous abortion or therapeutic abortion that occurred throughout pregnancy.

The safety endpoints will include moderate or severe OHSS rate, ectopic pregnancy, multiple pregnancy, and incidence of obstetric and perinatal complications, congenital anomalies and perinatal mortality.

## 7.4 Physical Exam

A standard pelvic exam will be performed to all patients by a study physician. Height and weight will be measured to a resolution of 0.1cm and 0.1kg, respectively on the screening visit. Patients are weighed in light clothing without shoes. After a 5-minutes rest, blood pressure will be measured in the patient's right arm in the sitting position. Repeated blood pressure measurements following acclimation to environment will be conducted if a blood pressure measurement is greater than or equal to 140/90 mmHg. Patients must have a normal Pap result prior to study entry.

## 7.5 Transvaginal Ultrasound Exams

A transvaginal ultrasound exam will be performed on days 1 to 5 of the menstrual cycle at the early follicular phase. The following parameters will be obtained, including: uterus dimensions (recorded by length × width × thickness), the thickness and type of the endometrium, uterine abnormalities, number and size of uterine myomas, ovarian size of both ovaries (recorded by length × width × thickness), the size of the largest ovarian follicle, and the count of antral follicles with 2 to 9 mm in diameter and ovarian morphology. To obtain ovarian size, the largest plane of the ovary is measured in two dimensions, followed by a third measurement by turning vaginal probe 90 degrees. Endometrial thickness is the largest anterior-posterior measurement of the endometrium in the sagittal plane.

## 7.6 Laboratory exam

All measurements for clinical evaluation (described in section 6.2) will be performed in the local lab of each study site. Hormone test for ovarian response monitoring and pregnancy test will be run in local lab as well.

# 8. Timeline and Recruitment Plan

The anticipated recruitment duration will be 8 months in 11 study sites. To enroll the 980 randomized subjects as planned, each site must have 80-100 participants randomized, which means that each site should contribute 10-12 randomized participants per month. The treatment period will be 12 months. Another 11 month period will be set for pregnancy follow-up until 6 weeks after delivery. In total, the follow-up period for each subject is 2 years from the day of randomization. In total, from initial recruitment to complete follow up, a total of 32 months will be required to complete the study (September 2018 to May 2021).

## 9. Statistical Analysis Plan

### 9.1 Sample size

According to the prospective study published in 2016, the CLBR were 52.6% and 52.5% for cleavage-stage and blastocyst-stage transfers respectively [9]. In the present study, we plan to test the primary hypothesis that blastocyst-stage embryo transfers are non-inferior to cleavage-stage embryo transfers, assessed by CLBR. We assume that CLBR will be 52% in both cleavage-stage and blastocyst-stage embryo transfer groups.

For the sample size calculations, the significance level will be set at $\alpha = 0.025$ and the statistical power will be calculated as $1 - \beta = 0.80$. The ratio between groups will be 1:1. With a non-inferiority margin of 10%, it is estimated that a sample size of 392 subjects per treatment arm. With the addition of a dropout rate of 20%, the minimal sample size calculated is: 490 for group A, 490 for group B, 980 in total.

Sample size calculation

Significance level α: 0.025

1-β: 0.80

Cumulative Live birth rate in group A: 0.52

Cumulative Live birth rate in group B: 0.52

Delta (Δ) :10%

Ratio: 1:1

Minimum sample size: 392 for each group, 784 in total

In consideration of 20% drop-out rate: 490 for group A, 490 for group B, 980 in total.

### 9.2 Statistical analysis

The primary analysis will use an intent-to-treat (ITT) approach to examine differences in cumulative live birth rate with first live birth per oocyte retrieval in the two treatment arms, calculated using outcomes from the first three embryo transfers within 1 year after randomization. ITT analyses will be performed on full analysis sets (FAS), which consist of all subjects who were randomly allocated into treatment groups. Subjects who don't satisfy major entry criteria and lack any data post randomization will be excluded. If SET is not performed in the first three transfers, the study deviation should be reported, but the results will be calculated according to ITT principles. Categorical data will be represented as a frequency and percentage; differences in these measures between treatment groups will be assessed by the Chi-square analysis, with a Fisher's Exact Test for expected frequencies less than 5. Continuous data will be expressed as mean ± SD, with student t test for testing differences between two groups. Inter-quantile ranges may be presented when non-normality of the continuous data is apparent.

A per protocol analyses will also be performed in per protocol sample (PPS) consisting of all subjects who were included in the FAS, complying with protocol and absent of major protocol deviations. Safety Analyses will be performed in the Safety population including all subjects who were randomly allocated to treatment and received embryo transfer at least one time.

The CLBR resulting from the first 3 single embryo transfers within 1 year after randomization will be calculated as the primary outcome. The CLBR from all the embryos transfers within 1 year after randomization will be analyzed as well, but not as the primary outcome.

There will be two ways to calculate the CLBR：

（Numerator is the No. of women obtain their first live birth, the Denominator is the No. of women who receive one oocyte retrieval）

- The conservative estimate of the cumulative live birth rate, which is based on the assumption that the women who do not return for a subsequent embryo transfer have no chance of a pregnancy resulting in a live birth.
- The optimal estimate of the cumulative live birth rate, which is based on the assumption that women who do not return for a subsequent embryo transfer would have the same chance of a pregnancy resulting in a live birth as those who do return for embryo transfer.

The Chi-square test will be used for conservative CLBR between the two treatment groups. Optimal CLBR will be analyzed by using the Kaplan-Meier product-limit method, which censors data for subjects who do not return for treatment, and estimates the CLBR with 95% confidence intervals.

Furthermore, univariate/multivariate logistic will be performed to evaluate the dependence of Age (≤35 or >35 years), AFC, AMH, number of oocytes retrieved, number of embryos, times of embryo transfer, duration to achieve pregnancy and BMI in relation to pregnancy rate or CLBR. Any deviations from the previously described statistical plan will be described and justified in a protocol amendment. The result will be reported according to the CONSORT statement. The interim analysis will not be performed in this study.

Due to the COVID-19 pandemic, the participants who were unable to undergo the embryo transfers in 1 year of randomization will have 3 months extension for frozen embryo transfers. The CLBR with and without the 3 months extension for frozen embryo transfers will be calculated separately. The CLBR with 3 months extension for frozen embryo transfers will be reported as the primary outcome.

## 10. Adverse Events

### 10.1 Risks and Discomforts

Participating in this study will produce no risks beyond those standard-of-care noted in the IVF-ET procedure consent forms. Below is a table listing all procedures related to this trial and their discomforts and risk.

**Table 5 Risks and discomfort**

| Procedures and events | Discomforts and risks |
|---|---|
| Controlled ovarian hyperstimulation (COH) | Frequent subcutaneous injection, frequent venipuncture, frequent transvaginal ultrasound scan<br>Supra-physiologic estradiol may increase risk of cancer<br>Ovary torsion or ovary disruption |
| Ovarian hyperstimulation syndrome (OHSS) | Massive enlargement of ovaries, fluid in the abdominal cavity, bloating, nausea, vomiting<br>Severe cases may have fluid in thoracic cavity, breathing difficulties, oliguria even anuria, and may require hospitalization, medication or puncture drainage of fluid in abdomen or thorax. Very severe case may suffer from thrombosis, damage to liver or renal function, even death. |
| Oocyte retrieval | Anesthesia accident, pelvic organ injury, intra-abdominal hemorrhage, puncture site hemorrhage, infection<br>In serious case surgery or transfusion may be needed |
| ICSI | Microinjection may injure oocyte, pass unknown disease gene to next generation |
| Embryos transfer | Infection |
| Blastocyst culture | No transferrable blastocyst<br>Blastocyst formation rate is 60% |
| Embryo frozen and thaw | Embryos development stop, and the survival rate of thawed embryos is 95% |
| Standard venipuncture for blood work | Slight pain, blue mark at the site of puncture, infection or bleeding at the site |
| Transvaginal ultrasound | Abdominal or pelvic discomfort |
| Ectopic pregnancy | May require medicine or surgery treatment, in severe case pregnancy site rupture resulting intra-abdominal hemorrhage, even shock or death if treatment delayed |
| Multiple pregnancy | May require embryo reduction, increase risk of pregnancy complication and fetus abnormalities; Preterm delivery |
| Infertility treatment | Anxiety or emotional distress at various degree |

Patients are not expected to experience all of these complications. The treatment patients are randomly assigned to may have more complications or prove less effective than other available treatments.

To reduce the risk of OHSS, the initial gonadotropin dose will be individualized according to body weight, AMH level, pelvic surgery history ect. Gonadotropin dosage will be modified depending on your ovarian response. If high response occurs, the HCG trigger, embryo transfer, or even the cycle may be terminated to avoid OHSS. A single embryo will be transferred by qualified and experienced physicians to reduce the risk of multiple pregnancies. The ICSI procedure will be performed under indications. An investigator or a resident doctor will be on call 24 hours a day at each investigation site, if adverse events are present during the study.

Every possible effort will be taken to prevent injury resulting from the participation in the clinical trial. However, complications or injury could still arise during the study. If an adverse event occurs, medical treatment is available and will be provided at the usual charge. Neither financial compensation nor free medical treatment will be provided in the event of a research related injury.

**Table 4. Golan classification of ovarian hyperstimulation syndrome (OHSS) (1989)**

| Classification | Size of ovaries | Grade | Symptoms |
|---|---|---|---|
| Mild | 5~10cm | Grade 1 | Abdominal tension and discomfort |
| | | Grade 2 | Grade 1 signs plus nausea, vomiting, and/or diarrhea |
| Moderate | >10cm | Grade 3 | Grade 2 signs plus ultrasound evidence of ascites |
| Severe | >12cm | Grade 4 | Grade 3 signs plus clinical evidence of ascites and/or pleural effusion and dyspnea |
| | | Grade 5 | Grade 4 signs plus haemoconcentration increased blood viscosity, hypovolaemia, decreased renal perfusion, oliguria |

### 10.2 Adverse Event Definitions

An adverse event refers to any untoward or unfavorable medical occurrence associated with the subject's participation in the research, regardless if they are considered to be related to the study intervention. Adverse events can be any of the following:

- Physical signs or symptoms including side effects of medication
- Abnormal laboratory values
- Changes in vital signs, physical exam findings, or test results
- An increase in the frequency or intensity of a condition or illness that was present before participation in the study

A serious adverse event refers to any event that is temporally associated with the subject's participation in research that meets any of the following criteria:

- Death
- Life Threatening
- Severely or permanently disabling
- Requires in-patient hospitalization or prolongations of existing hospitalization
- Pregnancy loss after 20 weeks gestation
- Neonatal death up to 6 weeks after delivery
- Resulting in a congenital anomaly/birth defect
- Any event deemed serious by the PI onsite.

An unexpected adverse event is an adverse event not listed in the general investigation plan or protocol nor is it listed at the specificity or severity that has been previously observed and/or specified.

### 10.3 Recording of Adverse Events

All adverse events (serious or non-serious) and abnormal test results will be recorded in the patient's case report form, regardless of study group or suspected causal relationship to the study intervention(s). Report a diagnosis rather than a symptom. For all adverse events, sufficient information will be pursued and/or obtained to evaluate an adequate determination of the event's outcome and assess the casual relationship between the adverse event and the study interventions. Each event will be continuously followed up until resolved, stabilized, or up to 7 days after the last enrolled patient completes the follow-up.

Adverse events or abnormal test results deemed relevant to the study interventions will be followed up until the event or abnormal test result is resolved or stabilized at an acceptable level to the principal investigator.

### 10.4 Causality and severity assessment

The principal investigator will review documented adverse events and abnormal test results to determine:

- If abnormal test finding should be classified as an adverse event
- If the adverse event should be classified as a serious adverse event
- If the study intervention plausibly caused the adverse event

The conditions relating to the adverse event will be assessed to determine whether a relationship between the adverse event and the study exists. Record the causality of each event as "possibly related" or "not possibly related". The causality of the adverse event will be considered "possibly related" when causality is unknown. The maximum intensity of each adverse event will be evaluated and reported as one of the following:

4. Mild: events may or may not be volunteered by the patient. The patient is aware of and easily tolerates the event.
5. Moderate: the event poses sufficient discomfort to interfere with normal activities. A change in therapy may or may not be indicated.
6. Severe: side effects are almost always brought up by the patient. These events interfere with daily activities and usually require medical intervention.

### 10.5 Reporting of Serious Adverse Events and Unanticipated Problems

The PI onsite will report the SAE by completing a Serious Adverse Event Report Form within 24 hours of discovery and emailing the document in PDF form to the protocol PI. The site PI must determine and record on the form whether the SAE is anticipated or unanticipated and if it is related, possibly related, or unrelated to participation in the trial. The protocol PI must then determine if the SAE needs to be reported to the DCC. If the SAE is reported to the DCC, DCC staff will enter the SAE information in the central database and report to the DSMB. The DSMB will review the SAE upon receiving notification of the event via a closed-session email or conference call. The DSMB will send a report to the DCC within two weeks. If the SAE is life-threatening the report will be sent in one week. The DSMB report includes

- Statement indicating what information was reviewed by the DSMB
- The date of the review
- The DSMB's assessment of the reviewed information
- The DSMB's recommendation for the DCC, if any

The DCC will forward reportable events to protocol PI and all investigators. The protocol PI will evaluate the frequency and severity of the SAEs and determine if protocol modification and consent forms are required. Onsite PIs will the report the SAE to their IRB according to local IRB requirements.

Responsibilities of the PI at the site of the SAE:

4. Determine and record on the SAE form if adverse event is unanticipated or anticipated and possibly related or unrelated to participation in the research.
5. Report SAE by submitting the SAE form with the PI's signature in PDF format to the protocol leader via email:
   clbr_cbset_rct@163.com.
6. Reporting according to the following timeline:

a. Unanticipated and related/possibly related SAE must be reported to the protocol PI within 1 business day of discovery.
b. Anticipated and related/possibly related SAE must be reported to the protocol PI within 5 business days of discovery.
c. Unrelated SAE (anticipated or unanticipated) must be reported to the protocol PI within 10 business days (no more than 3 weeks) of discovery.
d. If the SAE is ongoing, the site PI will send follow-up reports to the protocol PI until the SAE is resolved.

## 11. Concomitant medication

If the following medications are used during this study, concomitant medication should be recorded on the specified form.

1) Anti-diabetic agents and anti-hypertension agent;
2) Treatment of endocrine or metabolic disorders such as hyperprolactinemia, hyperthyroidism or hypothyroidism, insulin resistance, etc.
3) Folic acid supplement aimed at preventing neutral tubal defect;
4) For patients with abnormal bleeding/prolonged amenorrhea, progestin, micronized progesterone or dydrogesterone;
5) Medications which are not described in this protocol are used during the COH process and luteal support.
6) During the study period, some new diseases such as vaginitis are treated.
7) For patients with moderate or severe OHSS, clinical routine treatment, such as fluid infusion, albumin infusion, aspirin or preventive antibiotics will be used.
8) For patients with threaten abortion, an extra dose of progesterone will be approved for use. Concomitant medication will be recorded.
9) For patients with pregnancy complications, clinical standard care will be performed. Concomitant medication will be recorded.

## 12. Monitoring

### 12.1 Data and Safety Monitoring Board

An independent Data and Safety Monitoring Board (DSMB) will be established to review and interpret data generated from the study and review protocol revision prior to their implementation. The primary objectives of the DSMB are to ensure the safety of the subjects and preserve the integrity of the research data. The DSMB advises on research design issues, data quality and analysis, and research participant protections.

The DSMB will hold regular conference calls in English to review the protocol with respect to ethical and safety standards, monitor the safety of the trials, monitor the integrity of the data with respect to the original design of the study, and advise on study conduct. The DSMB will oversee progression of the trial, adjudicate adverse events, and decide on premature closure of the study. The conference calls will be coordinated by the DCC who will provide study updates via email prior to the calls.

The DSMB consists of five voting members who are impartial, independent of the investigators, and have no financial, scientific or other conflicts of interest with the study. The DSMB will consist of 3 members with relevant clinical expertise, 1 member with biostatistics expertise, and 1 human subject protection advocate.

**Table 7. DSMB Members**

| Name | Role on DSMB | Affiliation | High Level Responsibilities |
|---|---|---|---|
| Robert Rebar | Chair of DSMB | Western Michigan University Homer Stryker M.D. School of Medicine, USA | Chair the DSMB discussion and prepare written recommendations to IRB.<br>Ensure the safety of study subjects, the integrity of the research data. |

| | | | |
|---|---|---|---|
| | | | Provide IRB with advice on the ethical and safe progression of studies conducted in the current project.<br>Advises on research design issues, data quality and analysis, and research participant protection for each prospective and on-going study. |
| TC LI | Voting member | Chinese University of Hong Kong | Ensure the safety of study subjects, the integrity of the research data.<br>Provide IRB with advice on the ethical and safety progression of current study. Advises on research design issues, data quality and analysis, and research participant protection for this prospective and on-going study. |
| Jun Zhang | Voting member | Xin Hua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, China | |
| Yan Liu | Voting member | Shanghai Changzheng Hospital，China | |
| Xiuqin Wang | Voting member | the First Affiliated Hospital of Nanjing Medical University，China | |

### 12.2 Ethics

Ethics approval has been sought from Ethics Committee at First Affiliated Hospital of Nanjing Medical University (2015-SR-018). All subjects will sign written informed consent forms before participating in the trial. The study will be conducted in compliance with the Declaration of Helsinki and Good Clinical Practice.

## 13. Data Handling and Record Keeping

Prof. Heping Zhang at Yale University will oversee the Data Collection and Management team made up of investigators from the Department of Epidemiology and Biostatistics, Nanjing Medical University.

### 13.1 Data entry and case report form

Investigators must record all requested data into the CRF and explain all the missing data. If a space on the CRF is not filled in for the reason that the procedure was not performed or the information was not collected, write “N/D”. If the item is not applicable to the individual subject, write “N/A”. If any entry error has been made, draw a single straight line through the incorrect entry and enter the correct data above it to correct the error. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS.

Case Report Forms (CRFs) will be made in WORD form and will be inputed into a Web-based data management system at http://www.medresman.org.cn/login.aspx. The Web data entry forms will be made in accordance with the WORD (paper) form.

### 13.2 Data security

To manage the data and monitor the process of the study, a web-based database, Clinical Trial Electronic Case Record Form (eCRF system) (http://www.medresman.org.cn/login.aspx), will be used to record the patients’ data and results.

The database managers, Dr. Xiang Ma, Dr. Jing Wang, biostatistician Prof. Heping Zhang, and project leader Prof. Jiayin Liu, Prof. Zijiang Chen, are responsible for establishing the project space in the eCRF system and managing the users’ accounts at each sub-center, establishing individual folders for each sub-center and assigning jurisdiction to the users.

The database managers have the highest authority to manage and monitor the data. The users of each sub-center will be allowed to enter their patients’ information and study data into their individual folders. The database managers will make the final decision of what data will be disclosed to public. The private information of participants, including name, age, telephone number, will be strictly protected and never be disclosed.

The eCRF system service provider, Chinese Clinical Trial Registry, will be responsible for ensuring the safety of the database and the study data, maintaining the database, and providing technical support. However, the service provider does not have the right to revise data in the database. The contact person of the eCRF system is Prof. Taixiang Wu.

### 13.3 Data quality control

Data quality control will be managed at three different levels.

- The first level is the real-time logical checking built into the web-based data entry system. The research coordinators responsible for data entry at each participating site have the responsibility of ensuring accuracy of the data.
- The second level is the remote data monitor and validation which will be performed by the data manager and programmer at the DCC. Comprehensive data checks and regular manual checks will be conducted monthly by the data manager. Manual data checks within the database system will identify more complicated and less common errors. Errors will be fed back to the investigators at each site who will be required to check the data accuracy. The query will not be closed until the error has been corrected.
- The third level of quality control will be the site visits. During these visits, data in our database will be verified with the original study source or medical documents. Identified errors will be corrected. The site visits will ensure data quality and protection of patient's safety and privacy.

### 13.4 Audit

An audit will be implemented to ensure that only authorized additions, deletions, or alterations of information are inputted into the electronic record. This allows auditors the means to reconstruct significant details about study conduct and collection of source data to verify the quality and integrity of the data. Computer generated and time-stamped audit trails will be implemented for tracking changes to electronic source documentation.

This is a multicenter clinical trial carried out within mainland China. Controls will be established to ensure the accuracy of the system's date and time. System documentation will explain time zone references as well as zone acronyms. Dates and times will include the year, month, day, hour, and minute provided by international standard-setting agencies. The ability to alter the date or time will be limited to authorized personnel, who will be notified if a system date or time discrepancy is detected.

In addition to internal safeguards built into the computerized system, external safeguards will be established. Study data will be stored in the servers housed at Clinical Center of Reproductive Medicine, First Affiliated Hospital of Nanjing Medical University, overseen by Prof. Heping Zhang. Records will be regularly backed up, and record logs will be kept to prevent a possible catastrophic loss and ensure the quality and integrity of the data.

## 14. Publication policy

### 14.1 Major Publications Authorship Order

The major manuscript is estimated to have up to 16 contributing authors. The authorship order for the participating sites will be based upon the recruitment of subjects, data accuracy and promptness of data reports. The rankings will start at position 6 and end at position 15. Data accuracy will be ranked by frequency of missing or false data entries at each participating site. If inquires show that data was accurately entered, they will not count against the data accuracy rate of the site. The site PI is responsible for documenting the contribution of the site's authors towards the study. Site investigators are encouraged to produce second hypotheses and write publications by sharing the data under the supervision of the publication committee.

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# CLBR-CBSET Study Protocol: Summary of Changes

Before the trial began, we did very thorough work and made four major changes to the protocol based on discussions in the Steering Committee and the Data and Safety Monitoring Committee. Version 5 (18 September 2018) was the official protocol followed by our trial and officially approved by the IRB. During the course of the trial, the steering committee and protocol committee proposed amendments to the protocol twice based on the progress of the trial. All protocol amendments have been approved by the IRB before being conducted and were overseen by the DSMB. We summarized in detail the changes from the original to the final protocol, as well as the rationale and elaboration of the amendments.

1. The number of times and duration of single embryo transfers were revised. (Version 6, 16 December 2018)

The steering committee and protocol committee noted that single embryo transfer after 3 failed embryo transfer cycles was difficult to follow in clinical practice, and that approximately 80% of patients were expected to achieve a live birth in the first 3 embryo transfer cycles. Therefore, the protocol was amended from "single embryo transfers will be consecutively performed 5 times at most within 1.5 years follow-up duration" to "Single embryo transfer is required for the first 3 embryo transfers within 1 year after randomization. For embryo transfers beyond the third within the 1 year, patients' treatment must follow their randomized allocation, and SET is no longer mandatory". In accordance with this amendment, the primary outcome was restricted to the outcomes from the first three embryo transfers within 1 year after randomization. "The cumulative livebirth rate from all the embryo transfers within the study period will be analyzed as well, but not as the primary outcome." was added to the statistical plan.

2. The minimum number of transferrable cleavage-stage embryos required for randomization was reduced from four to three. (Version 6, 16 December 2018)

The steering committee and protocol committee noted that over 90% of patients with $\geq 3$ transferrable cleavage-stage embryos could obtain blastocysts, based on the current blastocyst formation rates. In addition, the high rate of screening failure rate under the original protocol inhibited the study progress. Therefore, the number of embryos required for randomization was modified from four to three.

3. The duration of frozen embryo transfers was extended by 3 months due to COVID-19. (Version 7, 13 June 2020)

Many participants were unable to undergo the embryo transfers in 1 year of randomization due to the COVID-19 pandemic. Therefore, the steering committee decided to extend the frozen embryo transfer period by 3 months for these participants.

**CLBR-CBSET Study Protocol: Summary of Changes**
From Version 5.0 to Version 6.0

Below are the details of the amendments.

| Item | Section(s) | Protocol Version 5.0 (September 18, 2018)<br>Before change | Protocol Version 6.0 (December 16, 2018)<br>After change | Rationale |
|---|---|---|---|---|
| Treatment of subjects and Follow-up period | 2.3 study design;<br>2.4 treatment;<br>2.8 anticipated time to completion<br>6.6 Frozen transfer visit<br>6.9 and 6.11 Follow-up visit, title in table of content<br>7.1 type of design<br>8 timeline the treatment period | Each patient will be followed up until the first live birth is achieved or until all the embryos per oocyte retrieval are transferred (**up to 5 single embryo transfers**), including fresh and frozen-thawed embryo transfers. If a live birth is not achieved, single embryo transfers will be consecutively performed 5 times at most **within 1.5 years** follow-up duration.<br>The follow-up period is 2.5 years from the day of randomization. | The outcomes of all the embryo transfers within 1 year after randomization will be followed up. **Single embryo transfer (SET) is required for the first 3 embryo transfers within 1 year after randomization**. For embryo transfers beyond the third within the 1 year, patients' treatment must follow their randomized allocation, and SET is no longer mandatory.<br>The follow-up period is 2 years from the day of randomization. | Due to content revisions |
| Study outcome | 2.5 primary outcome<br>7.3 study endpoint | The primary outcome is CLBR per patient until the first live birth from one initiating oocyte retrieval cycle. | CLBR per patient until the first live birth from one oocyte retrieval cycle **(Calculated using outcomes from the first three embryo transfers within 1 year after randomization).** | Due to content revisions |
| Inclusion criteria | 5.1 inclusion criteria<br>6.4 Day 2/3 after oocyte retrieval visit<br>7.2.3 In-vitro fertilization and embryo culture | number of transferrable cleavage embryo ⩾ **4** | number of transferrable cleavage embryo ⩾ **3** | Due to content revisions |
| Statistical analysis | 2.7 and 9.2 Statistical analysis | The primary analysis will use an intent-to-treat (ITT) approach to examine differences in CLBR with first live birth per oocyte retrieval in the two treatment arms. | The primary analysis will use an intent-to-treat (ITT) approach to examine differences in CLBR with first live birth per oocyte retrieval in the two treatment arms, **calculated using outcomes from the first three embryo transfers within 1 year after randomization.** | Addition,<br>Due to content revisions |

| | | | | |
|---|---|---|---|---|
| | | | **If SET is not performed in the first three transfers, the study deviation should be reported, but the results will be calculated according to ITT principles.**<br><br>**The CLBR from the first 3 embryo transfers within 1 year of randomization will be calculated as the primary outcome. The CLBR from all the embryo transfers per oocyte retrieval within 1 year of randomization will be analyzed, but not as the primary outcome.** | |
| End of study visit | 6.10 end of study visit | 1) The subjects achieve a first live birth and finish all pregnancy follow-ups (from either fresh ET or FET).<br>2) If subjects do not obtain a first live birth, all embryos per oocyte retrieval have been transferred (the transferable embryos < 5) or 5 consecutive single embryo transfers have been performed (the transferable embryos ≥ 5).<br>3) Subjects who complete the 2.5 year follow up (from the day of randomization). | 1) The subjects achieve their first live birth from the embryo transfers within 1 year after randomization.<br>2) An outcome from the last transfer within 1 year after randomization.<br>3) No embryo transfers within 1 year of randomization. | To be consistent with the treatment and follow-up period |

## CLBR-CBSET Study Protocol: Summary of Changes

From Version 6.0 to Version 7.0

| Item | Section(s) | Protocol Version 6.0 (December 16, 2018)<br>Before change | Protocol Version 7.0 (June 13, 2020)<br>After change | Rationale |
|---|---|---|---|---|
| Treatment of subjects and Follow-up period | 2.3 study design<br>7.1 type of study design | NA | Due to the COVID-19 pandemic, the participants who were unable to undergo the embryo transfers in 1 year of randomization will **have 3 months extension for frozen embryo transfers**. | Due to content revisions |
| Statistical analysis | 2.7 and 9.2 statistical analysis | NA | The CLBR with and without the 3 months extension for frozen embryo transfers will be calculated separately. The CLBR with 3 months extension for frozen embryo transfers will be reported as the primary outcome. | Addition,<br>Due to content revisions |