Neonatal and maternal outcomes after fresh blastocyst and cleavage stage embryo transfer in their first assisted reproductive technology

Mingze Du MD, Xingling Wang MD, Junwei Zhang MD, Zihua Liu MD, Jing Liu MD, Yichun Guan MD, Manman Liu MD, Wenxia Liu MD, Jiaheng Li MD

Du M, Wang X, Zhang J, et al. Neonatal and maternal outcomes after fresh blastocyst and cleavage stage embryo transfer in their first assisted reproductive technology. J Reprod Biol Endocrinol. 2017;1(2):19-22.

BACKGROUND: Blastocyst culture became a significant technology now. But it provided not only advantages but also disadvantages. Although prolonged *in vitro* exposure may cause damage to the embryo, but it would choose better embryo to transfer. Our purpose was to probe neonatal and maternal outcomes after blastocyst transfer versus cleavage stage embryo transfer.

METHODS: This is a retrospective cohort study from a single center (1477 singleton birth).

RESULTS: There was a high risk of preterm birth after blastocyst transfer versus cleavage stage embryo transfer (9.9% vs. 5.7%, p=0.02). But we did not find any difference in low birth weight, small for gestational age, large for gestational age, very preterm birth, very low birth weight, antepartum haemorrhage, placental abruption, placenta previa, post-partum haemorrhage and premature rupture of membranes, birth defects, perinatal mortality, APGAR score <7 at 5 min and gestational diabetes. Binary logistic regression indicated that body mass index is an important factor in small for gestational age (COR 0.86, 95% CI 0.80-0.92; AOR 0.86, 95% CI 0.80-0.92, P=0.00) and large for gestational age (COR 1.17, 95% CI 1.11-1.22; AOR 1.15, 95% CI 1.10-1.21, P=0.00). And the type of infertility, years of unwanted childlessness and parity also influenced large for gestational age.

CONCLUSION: Neonatal and maternal outcomes after cleavage stage embryo transfer were similar with blastocyst transfer except preterm birth. And body mass index played an important role in large for gestational age. Types of ectopic pregnancies so to preserve the woman's potential fertility.

KeyWords: Blastocyst; Cleavage stage embryo; Neonatal outcomes; Maternal outcomes

Abbreviations: LBW Low birth weight; LGA Large for gestational age; SGA Small for gestational age; VLBW Very low birth weight; ART Assisted reproductive technology; ICSI Intracytoplasmic sperm injection; IVF *In vitro* fertilization; PA Placental abruption; PP Placenta previa; PPH Post-partum hemorrhage; PROM Premature rupture of membranes; OR Odds ratio; CI Confidence interval

Recently, assisted reproductive technology (ART) was applied to more and more people (1). According to a report by the International Committee for Monitoring Assisted Reproductive Technologies World, the percentage of babies born from ART was estimated to increase by an average of 9.1 percent per year between 2008 and 2010 (2). In America, a total of 169,568 ART resulted in 56,028 live-birth deliveries and 68,782 infants born in 2014 (3).

With the increment of ART ratio, the neonatal and maternal outcomes were paid more and more attention. For instance, which one has a preferable neonatal and maternal outcome, blastocyst embryo transfer or cleavage stage embryo transfer? Numerous professors probed into this area at one time. Some studies pointed out that there was no significant difference in neonatal and maternal outcomes in different duration of *in vitro* culture (4-7). But other studies considered that there was an increased risk of preterm birth after blastocyst embryo transfer (8-10). And a recent meta-analysis found that the blastocyst embryo transfer was associated with risks of neonatal death, preterm birth, very preterm birth and large for gestational age (11). The closest meta-analysis indicated that the risk of preterm birth and very preterm birth decreased with cleavage stage embryo transfer in fresh cycle. However, there is no difference in frozen cycle (12).

The primary outcome was aim to investigate the neonatal and maternal outcomes for example preterm birth, low birth weight, small for gestational age, large for gestational age in different duration of *in vitro* culture. And we also compared very preterm birth, very low birth weight, sex ratio, antepartum haemorrhage, placental abruption, placenta previa, postpartum haemorrhage and premature rupture of membranes, birth defects, perinatal mortality, APGAR score <7 at 5min and gestational diabetes between two groups.

METHODS

Study design

This was a single-center retrospective study. All of data was provided by The Reproduction Center, The Third Affiliated Hospital of Zhengzhou University between January 2013 and August 2016. We collected data in our database. Women who underwent their first IVF/ICSI embryo transfer cycle were included. And we excluded patients who aged more than 35 years old to avoid age factor. If patients had intrauterine lesions and uterine malformation, we also eliminated them. We also only chose fresh embryo transfer to eliminate the biases. Data only included if the infants born after twenty weeks of gestation. Stillbirth also excluded in this study. Patients who underwent preimplantation genetic diagnosis (PGD)/preimplantation genetic screening (PGS) were excluded. Likewise, patients with donor oocytes were also excluded. We excluded it too by transvaginal ultrasound performed 30 days after transplantation to avoid vanishing twins (13). In total, 1477 singletons born after fresh embryo transfer.

Patients with GnRH agonist to ovulate, when 1-3 follicular diameter ≥ 20 mm or 60% dominated follicle ≥ 18 mm and serum luteinizing hormone (LH), estrogen (E2), progesterone (P) level were appropriate, 250 ug of Recombinant Human Chorionic Gonadotropin (Merck, Darmstadt, Germany) were injected. After trigger 36-38 h, oocyte retrieval underwent under ultrasonography. According to the stage of embryo, transplant surgery underwent after 3, 5 or 6 days after ovulation. Luteal support added the time after oocyte retrieval. We had two choices for patients. One was 60 mg im qd Progesterone Injection (Xianju, Zhejiang, China) and 20 mg po bid Dydrogesterone Tablets (Abbott, Illinois, America), the other was 90 mg Intravaginal administration qd Progesterone Sustained-release Vaginal Gel (Merck, Darmstadt, Germany) and 20 mg po bid Dydrogesterone Tablets (Abbott, Illinois, America). The luteal support continued to 45 days after transplantation.

Study and outcome factors

The original outcomes were preterm birth (PTB, <37 weeks), very preterm birth (VPTB, <32 weeks), low birth weight (LBW, <2500 g), very low birth

Reproduction Center, The Third Affiliated Hospital of Zhengzhou University, China

Correspondence: Xingling Wang, Reproduction Center, The Third Affiliated Hospital of Zhengzhou University, Kangfuqian Road, Zhengzhou, 450052, Henan, China. e-mail wangxl1616@126.com

Received: October 30, 2017, Accepted: October 31, 2017, Published: November 15, 2017

TABLE 1

Patient characteristic in blastocyst or cleavage stage group

Characteristics	Blastocyst (n=191)	Cleavage stage (n=1286)	P
Maternal age			
≤ 30	150	931	0.07[a]
>30	41	355	
Male age	29.13 ± 5.07	29.59 ± 4.52	0.19[c]
Method of treatment			
IVF	155	907	0.02 [a]
ICSI	36	379	
The type of infertility			
Primary infertility	113	795	0.48 [a]
Secondary infertility	78	491	
Years of unwanted childlessness			
≤ 2	94	534	
3-Feb	38	255	0.083 [a]
>3	59	497	
Parity			
0	172	1171	0.652 [a]
≥ 1	19	115	
Body mass index			
<18.5	16	86	
18.5-24.99	143	959	0.593 [a]
≥ 25	32	241	
Reason for infertility			
Male factor	50	423	
Tubal factor	109	630	0.096 [a]
Others	32	233	
Number of retrieved oocytes	17.90 ± 5.79	10.63 ± 4.56	0.00 [b]
Delivery mode			
Caesarean section	116	861	0.09 [a]
Spontaneous delivery	75	425	
Endometrial thickness	11.19 ± 2.19	10.97 ± 2.25	0.20 [c]
Gestational weeks	38.55 ± 1.73	38.76 ± 1.50	0.21[b]
Fetal gender			
Male	116	652	0.01 [a]
Female	75	634	
Transplanting time			
Weekday	141	917	0.47 [a]
Weekend	50	369	

TABLE 2

Neonatal outcome in blastocyst or cleavage stage group

Characteristics	Blastocyst	Cleavage stage	P
Z score	0.33	0.25	0.44[b]
Preterm birth	19 (9.9%)	72 (5.7%)	0.02[a]
Very preterm birth	1 (0.5%)	7 (0.6%)	0.97 [a]
LBW	5 (2.6%)	51 (4.0%)	0.36 [a]
VLBW	0 (0%)	5 (3.9%)	1.00 [a]
SGA	10 (5.2%)	115 (8.9%)	0.08 [a]
LGA	29 (15.2%)	173 (13.5%)	0.71 [a]
Birth defects	2 (1.0%)	6 (0.5%)	0.28 [a]
Perinatal mortality	2 (1.0%)	5 (0.4%)	0.23 [a]
APGAR score <7 at 5 min	3 (1.5%)	18 (1.4%)	0.75 [a]

[a]*Chi-square test*

[b]*Mann-Whitney test*

LBW Low birth weight; LGA Large for gestational age; SGA Small for gestational age; VLBW Very low birth weight

TABLE 3

Maternal outcome in blastocyst or cleavage stage group

Characteristics	Blastocyst	Cleavage stage	P
Preeclampsia	7 (3.7%)	50 (3.9%)	1.00[a]
PA	1 (0.5%)	7 (0.5%)	1.00[a]
PP	3 (1.6%	16 (1.3%)	0.73[a]
PPH	10 (5.2%)	45 (3.5%)	0.31[a]
Gestational diabetes	2 (1.0%)	18 (1.4%)	1.00[a]
PROM	7 (3.7%)	27 (2.1%)	0.18[a]

[a]*Chi-square test*

PA Placental abruption; PP Placenta previa; PPH Postpartum hemorrhage; PROM Premature rupture of membranes

weight (VLBW, <1500 g). Then, large for gestational age (LGA, >90th percentile) and small for gestational age (SGA, <10th percentile) were evaluated by percentile charts for Chinese newborns (14). Calculating standard deviation score (also known as Z-score) for IVF/ICSI infants by the equation $Z=(x-\mu)$: σ (15). x was the infant's birth weight, μ, was the mean birth weight at the same gestational age and gender in reference group. And sex ratio, antepartum haemorrhage, placental abruption, placenta previa, postpartum haemorrhage and premature rupture of membranes, birth defects, perinatal mortality, APGAR score <7 at 5 min and gestational diabetes were also discussed.

Statistical analysis

We compared the neonatal and maternal outcomes of IVF/ICSI pregnancy after blastocyst embryo transfer vs. cleavage stage embryo transfer by SPSS (Statistical package for the social sciences) software 22.0. For categorical variables, we used chi-square tests. If more than 20% cells have expected count less than 5, we used Fisher's exact test. For continuous variable, we used Student's t-test or Mann-Whitney test. And we also used binary logistic regression to analysis PTB, LBW, LGA and SGA. According to papers, these factors were analysis in binary logistic regression. Maternal age (categorical: ≤ 30 years, >30 years), Male age (continuous variable), Method of treatment

TABLE 4

Unadjusted and adjusted[a] risk for outcomes in singleton birth after IVF/ICSI

	COR (95% CI)	AOR (95% CI)	P	COR (95%CI)	AOR (95% CI)	P
Outcome	**Preterm birth**			**Low birth weight**		
Maternal age	0.97 (0.91-1.03)	0.91 (0.82-1.00)	0.06	1.02 (0.94-1.10)	0.96 (0.85-1.09)	0.56
Blastocyst/cleaved embryo	1.86 (1.10-3.17)	2.19 (1.15-4.18)	0.02	0.65 (0.26-1.65)	0.64 (0.23-1.82)	0.4
The type of infertility	0.86 (0.55-1.34)	0.81 (0.48-1.36)	0.42	1.30 (0.76-2.23)	1.52 (0.81-2.86)	0.19
Male/female	0.66 (0.43-1.02)	0.70 (0.45-1.09)	0.11	0.94 (0.55-1.60)	0.96 (0.56-1.65)	0.89
Years of unwanted childlessness	1.06 (0.97-1.15)	1.09 (0.99-1.20)	0.1	1.11 (1.01-1.22)	1.16 (1.03-1.30)	0.01
Parity	0.69 (0.30-1.62)	0.87 (0.34-2.23)	0.77	0.98 (0.39-2.50)	0.92 (0.33-2.60)	0.88
Delivery mode	1.55 (0.96-2.52)	1.46 (0.89-2.41)	0.14	1.29 (0.72-2.33)	1.27 (0.70-2.33)	0.44
Body mass index	1.04 (0.97-1.11)	1.04 (0.97-1.11)	0.32	0.96 (0.88-1.05)	0.94 (0.86-1.04)	0.21
Outcome	**Large for gestational age**			**Small for gestational age**		
Maternal age	1.04 (1.00-1.09)	1.03 (0.96-1.10)	0.41	0.98 (0.93-1.04)	0.98 (0.90-1.06)	0.62
Blastocyst/cleaved embryo	1.15 (0.75-1.76)	1.45 (0.87-2.42)	0.16	0.56 (0.29-1.09)	0.57 (0.27-1.19)	0.14
The type of infertility	1.03 (0.76-1.40)	0.59 (0.40-0.89)	0.01	0.89 (0.61-1.30)	1.00 (0.64-1.57)	1
Male/female	0.96 (0.71-1.29)	1.05 (0.77-1.43)	0.8	1.37 (0.95-1.98)	1.35 (0.93-1.96)	0.12
Years of unwanted childlessness	0.98 (0.92-1.04)	0.92 (0.85-0.99)	0.02	0.99 (0.92-1.08)	1.03 (0.94-1.12)	0.59
Parity	1.78 (1.14-2.77)	1.89 (1.09-3.28)	0.02	0.57 (0.26-1.25)	0.67 (0.28-1.57)	0.35
Delivery mode	3.08 (2.08-4.57)	2.79 (1.86-4.19)	0	0.81 (0.55-1.18)	0.88 (0.60-1.30)	0.52
Body mass index	1.17 (1.11-1.22)	1.15 (1.10-1.21)	0	0.86 (0.80-0.92)	0.86 (0.80-0.92)	0

[a]adjusted for maternal age, male age, method of treatment, type of infertility, years of unwanted childlessness, parity, body mass index, reason for infertility, number of retrieved oocytes, delivery mode, endometrial thickness, stage of embryos transferred and transplanting time

AOR Adjusted odds ratios; COR Crude odds ratios

(categorical: IVF, ICSI), type of infertility (categorical: primary infertility, secondary infertility), Years of unwanted childlessness (continuous variable), Parity (categorical: 0, ≥ 1), Body mass index (continuous variable), Reason for infertility (categorical: Male factor, Tubal factor, Others), Number of retrieved oocytes (continuous variable), Delivery mode (caesarean section, spontaneous delivery), Endometrial thickness (continuous variable), Stage of embryos transferred (categorical: cleavage stage embryos or blastocyst) and transplanting time (categorical: weekday, weekend). We did not analysis smoke for the reason that only 2.4% women smoked in China (16).

RESULTS

In the aggregate, 1477 live deliveries after blastocyst transfer (191, 12.9%) or cleavage stage transfer (1286, 87.1%) between January 2013 and August 2016.

In Table 1, a high risk of sex ratio imbalance to male was discovered (p=0.01). And in Table 2, we found that there was a high risk of preterm birth after blastocyst (9.9% vs. 5.7%, p=0.02) transfer. However, in Z score, very preterm birth, low birth weight, large for gestational age, small for gestational age, very low birth weight, birth defects, perinatal mortality and Apgar score <7 at 5 min, there was no difference after cleavage stage embryo transfer. Table 3 revealed that blastocyst group and cleavage stage group had semblable maternal outcome for instance preeclampsia, placental abruption, placenta previa, postpartum haemorrhage, premature rupture of membranes and gestational diabetes.

For the sake of probing the factors exhaustively which influenced preterm birth, low birth weight, large for gestational age and small for gestational age, binary logistic regression was used. In term of preterm birth, blastocyst transfer had an adverse outcome compared with cleavage stage embryo transfer (COR 1.86, 95% CI 1.10-3.17; AOR 2.19, 95% CI 1.15-4.18, P=0.02). To low birth weight, patients who had long years of unwanted childlessness would had high risk (COR 1.11, 95% CI 1.01-1.22; AOR 1.16, 95% CI 1.03-1.30, P=0.01). As for small for gestational age infants, we found that thin patients were more likely to born infants like that (COR 0.86, 95% CI 0.80-0.92; AOR 0.86, 95% CI 0.80-0.92, P=0.00). In the end, five factors and large for gestational age infants was relevant. Secondary fertility (COR 1.03, 95% CI 0.76-1.40; AOR 0.59, 95% CI 0.40-0.89, P=0.01), short duration of infertility (COR 0.98, 95% CI 0.92-1.04; AOR 0.92, 95% CI 0.85-0.99, P=0.02), women who had 0 parity (COR 1.78, 95% CI 1.14-2.77; AOR 1.89, 95% CI 1.09-3.28, P=0.02) and thin patients (COR 1.17, 95% CI 1.11-1.22; AOR 1.15, 95% CI 1.10-1.21, P=0.00) had a low risk of delivering a large for gestational age infant. And large for gestational age infants would have a large ratio of caesarean section (COR 3.08, 95% CI 2.08-4.57; AOR 2.79, 95% CI 1.86-4.19, P=0.00) Table 4.

DISCUSSION

In our study, we only found that there was an increased risk of preterm birth after blastocyst transfer. After adjusting potential confounding factors, we also discovered high risk of preterm birth with blastocyst transfer. Some studies also demonstrated this opinion. A recent meta-analysis revealed that there was a high risk of preterm birth especially in fresh embryo transfer (12). Kalra et al. compared 14743 singleton born after blastocyst and 32351 singleton after cleavage stage embryo transfer (9) and revealed that cleavage stage embryo transfer had a decreased risk of preterm birth (AOR: 1.39, 95% CI 1.29-1.50, P=0.00). And other studies also concluded a coincident result (8,11,17-20). In other neonatal and maternal outcomes, we did not find any difference in two groups especially. Although many researches also pointed that duration of *in vitro* culture do not influence birth weight (4,5,12,20,21), one study found that there was a low risk of low birth weight after blastocyst transfer (22). Moreover, Zhu et al. indicated that the duration of *in vitro* culture make a difference in birth weight (16). As for very preterm birth, some studies found that outcome was similar (4,5,7,20,23). However, a recent meta-analysis indicated that blastocyst had been worse (11). In our study, we did not found any advantage in very preterm birth after cleavage stage transfer. Almost all studies pointed that there was no difference between two groups in very low birth weight and we were no exception (4,8,23).

For large for gestational age, we did not find a risk after blastocyst. Furthermore, a binary logistic regression analysis indicated that large for gestational age was associated with the type of infertility, years of unwanted childlessness, parity, delivery mode and body mass index. Some studies also proved this opinion. Makinen et al found that body mass index, parity and culture had a significant effect on large for gestational age (15). Similarly, a low body mass index mother had a high risk of small for gestation age in our research. A large number of studies have revealed that the body mass index is related to gestational age (15,24-26). The exact reason for this was not clearly. Some scholar pointed that it may be related with lifestyle, maternal metabolism, insulin resistance, glucose homeostasis, fat oxidation and amino acid synthesis (27-29). And parity also made an influence on gestational age. Some meta-analysis also indicated that (30,31). Ng et al. found that previous pregnancy (AOR=2.03, 95% CI 1.08-3.81), caesarean section (AOR=1.98, 95% CI 1.10-3.55) and married mothers (AOR=1.85, 95% CI 1.00-3.42) had a high risk of large for gestational age babies (26).

In a recent meta-analysis, a higher male-female ratio after blastocyst transfer compared with cleavage-stage embryo transfer (OR:1.29, 95% CI 1.10-1.51) was found (32). Some professors also demonstrated that (33,34). Dumoulin et al. showed that higher mean log cell number of trophectoderm cells in ICSI male embryos compared with female in blastocyst (35). It might interfere with the process of imprinted X-inactivation. The other professor thought that a decrease in trophectoderm cells in female would cause female mortality in long duration of *in vitro* culture (36,37). So it made an influence on sex ratio.

We found that many articles had discussed this method. But in our study,

we optimized the inclusion criteria and exclusion criteria to get a better result. We only included the first cycle of patient to avoid confounding factors and repetition. And we excluded the intrauterine lesions and uterine malformation for instance bicornuate uterus, unicornuate uterus, septate uterus, endometrial tuberculosis and so on. Even so, we had some limitations. The sample in blastocyst transfer was small compared with cleavage stage transfer. And this study was a retrospective cohort study from a single center. Part of diagnoses was made in primary hospital, so it may cause an error.

CONCLUSION

In summary, we found that there was a high risk of preterm birth after blastocyst transfer. And the body mass index was an important factor in gestation age. The type of infertility, years of unwanted childlessness, parity, delivery mode and body mass index were related to large for gestational age. Finally, a higher male-female ratio after blastocyst transfer compared with cleavage-stage embryo transfer was showed.

FUNDING

This study was funded by Henan Province Health Department.

REFERENCES

1. Kushnir VA, Barad DH, Albertini DF, et al. Systematic review of worldwide trends in assisted reproductive technology 2004-2013. Reprod Biol Endocrinol. 2017;15:6.
2. Dyer S, Chambers GM, de Mouzon J, et al. International Committee for Monitoring Assisted Reproductive Technologies World Report: Assisted reproductive technology 2008, 2009 and 2010. Hum Reprod. 2016;31:1588-609.
3. Sunderam S, Kissin DM, Crawford SB, et al. Assisted reproductive technology surveillance - United States, 2014. MMWR Surveill Summ. 2017;66:1-24.
4. De Vos A, Janssens R, Van de Velde H, et al. The type of culture medium and the duration of *in vitro* culture do not influence birth weight of ART singletons. Hum Reprod. 2015;30:20-7.
5. Fernando D, Halliday JL, Breheny S, et al. Outcomes of singleton births after blastocyst versus non-blastocyst transfer in assisted reproductive technology. Fertil Steril. 2012;97:579-84.
6. Chambers GM, Chughtai AA, Farquhar CM, et al. Risk of preterm birth after blastocyst embryo transfer: A large population study using contemporary registry data from Australia and New Zealand. Fertil Steril. 2015;104:997-1003.
7. Oron G, Sokal-Arnon T, Son WY, et al. Extended embryo culture is not associated with increased adverse obstetric or perinatal outcome. Am J Obstet Gynecol. 2014;211(2):165.e1-7.
8. Dar S, Librach CL, Gunby J, et al. Increased risk of preterm birth in singleton pregnancies after blastocyst versus Day 3 embryo transfer: Canadian ART Register (CARTR) analysis. Hum Reprod. 2013;28:924-28.
9. Kalra SK, Ratcliffe SJ, Barnhart KT, et al. Extended embryo culture and an increased risk of preterm delivery. Obstet Gynecol. 2012;120:69-75.
10. Kallen B, Finnstrom O, Lindam A, et al. Blastocyst versus cleavage stage transfer in *in vitro* fertilization: differences in neonatal outcome? Fertil Steril. 2010;94:1680-83.
11. Martins WP, Nastri CO, Rienzi L, et al. Obstetrical and perinatal outcomes following blastocyst transfer compared to cleavage transfer: A systematic review and meta-analysis. Hum Reprod. 2016;31:2561-9.
12. Wang X, Du M, Guan Y, et al. Comparative neonatal outcomes in singleton births from blastocyst transfers or cleavage-stage embryo transfers: A systematic review and meta-analysis. Reprod Biol Endocrinol. 2017;15:36.
13. Sazonova A, Kallen K, Thurin-Kjellberg A, et al. Factors affecting obstetric outcome of singletons born after IVF. Hum Reprod. 2011;26:2878-86.
14. Yuan Y, Pang RY, Pan Y, et al. Percentile curves for gestational age specific birth weight of newborns. Chin J Child Healthc. 2011.
15. Makinen S, Soderstrom-Anttila V, Vainio J, et al. Does long *in vitro* culture promote large for gestational age babies? Hum Reprod. 2013;28:828-34.
16. Zhu J, Lin S, Li M, et al. Effect of *in vitro* culture period on birth weight of singleton newborns. Hum Reprod. 2014, 29:448-454.
17. Maheshwari A, Kalampokas T, Davidson J, et al. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of blastocyst-stage versus cleavage-stage embryos generated through *in vitro* fertilization treatment: a systematic review and meta-analysis. Fertil Steril. 2013;100(6):1615-21.e1-10.
18. Dar S, Lazer T, Shah PS, et al. Neonatal outcomes among singleton births after blastocyst versus cleavage stage embryo transfer: A systematic review and meta-analysis. Hum Reprod Update. 2014;20:439-48.
19. Maheshwari A, Hamilton M, Bhattacharya S. Should we be promoting embryo transfer at blastocyst stage? Reprod Biomed Online. 2016;32:142-6.
20. Maxwell SM, Melzer-Ross K, McCulloh DH, et al. A comparison of pregnancy outcomes between day 3 and day 5/6 embryo transfers: Does day of embryo transfer really make a difference? J Assist Reprod Genet. 2015;32:249-54.
21. Oron G, Nayot D, Son WY, et al. Obstetric and perinatal outcome from single cleavage transfer and single blastocyst transfer: a matched case-control study. Gynecol Endocrinol. 2015;31:469-72.
22. Ginstrom Ernstad E, Bergh C, Khatibi A, et al. Neonatal and maternal outcome after blastocyst transfer: a population-based registry study. Am J Obstet Gynecol. 2016;214(3):378.e1-378.e310.
23. Ishihara O, Araki R, Kuwahara A, et al. Impact of frozen-thawed single-blastocyst transfer on maternal and neonatal outcome: An analysis of 277,042 single-embryo transfer cycles from 2008 to 2010 in Japan. Fertil Steril. 2014;101:128-33.
24. Castro LC, Avina RL. Maternal obesity and pregnancy outcomes. Curr Opin Obstet Gynecol. 2002;14:601-6.
25. Surkan PJ, Hsieh CC, Johansson AL, et al. Reasons for increasing trends in large for gestational age births. Obstet Gynecol. 2004;104:720-726.
26. Ng SK, Olog A, Spinks AB, et al. Risk factors and obstetric complications of large for gestational age births with adjustments for community effects: results from a new cohort study. BMC Public Health. 2010;10:460.
27. Alderman BW, Zhao H, Holt VL, et al. Maternal physical activity in pregnancy and infant size for gestational age. Ann Epidemiol. 1998;8:513-9.
28. Metzger BE, Lowe LP, Dyer AR, et al. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med. 2008;358:1991-2002.
29. Nelson SM, Matthews P, Poston L. Maternal metabolism and obesity: modifiable determinants of pregnancy outcome. Hum Reprod Update. 2010;16:255-75.
30. Shah PS. Parity and low birth weight and preterm birth: A systematic review and meta-analyses. Acta Obstet Gynecol Scand. 2010;89:862-75.
31. Kozuki N, Lee AC, Silveira MF, et al. The associations of parity and maternal age with small-for-gestational-age, preterm and neonatal and infant mortality: A meta-analysis. BMC Public Health. 2013;13(Suppl 3):S2.
32. Chang HJ, Lee JR, Jee BC, et al. Impact of blastocyst transfer on offspring sex ratio and the monozygotic twinning rate: a systematic review and meta-analysis. Fertil Steril. 2009;91:2381-90.
33. Bu Z, Chen ZJ, Huang G, et al. Live birth sex ratio after *in vitro* fertilization and embryo transfer in China - An analysis of 121,247 babies from 18 centers. PLoS ONE. 2014;9(11):e113522.
34. Chen M, Du J, Zhao J, et al. The sex ratio of singleton and twin delivery offspring in assisted reproductive technology in China. Sci Rep. 2017;7:7754.
35. Dumoulin JC, Derhaag JG, Bras M, et al. Growth rate of human preimplantation embryos is sex dependent after ICSI but not after IVF. Hum Reprod. 2005;20:484-91.
36. Tarin JJ, Garcia-Perez MA, Hermenegildo C, et al. Changes in sex ratio from fertilization to birth in assisted-reproductive-treatment cycles. Reprod Biol Endocrinol. 2014;12:56.
37. Frazier LM, Grainger DA, Schieve LA, et al. Follicle-stimulating hormone and estradiol levels independently predict the success of assisted reproductive technology treatment. Fertil Steril. 2004;82:834-40.