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Title:

CLINICAL FACTORS ASSOCIATED WITH MONOZYGOTIC TWINNING AFTER SINGLE FROZEN EMBRYO TRANSFER

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Objective:

Multiple pregnancies from IVF have decreased dramatically over the last few decades, largely due to increased utilization of single embryo transfer (SET). However, multiple pregnancy is still possible from monozygotic splitting of a single embryo. Prior literature has focused on the incidence and predictors of monozygotic twinning (MZT) in fresh cycles with multiple embryo transfers, but these studies do not reflect changing trends in IVF practice, such as the increase in preimplantation genetic testing for aneuploidy (PGT-A) and freeze only cycles.1-8 The current study aimed to identify clinical factors that are significantly associated with monozygotic splitting after SET.

Design:

Retrospective case-control study

Material and Methods:

This single center, case control study included all clinical pregnancies that occurred after

SET with IVF from October 2002 to March 2020. IVF cycles that had more than one embryo transferred were excluded. Cycles that resulted in MZT were compared to those that resulted in singleton gestations. Patient age, anti-Müllerian hormone (AMH) level, basal antral follicle count (BAFC), body mass index (BMI), stimulation protocol, use of donor oocyte, type of transfer (fresh vs. frozen), cumulative gonadotropin (GND) dose, estradiol (E2) and progesterone (P4) level at time of surge, day of embryo development, number of oocytes retrieved, fertilization method, use of PGT-A, number of exposures to trophectoderm biopsy and vitrification-thawing, embryo sex, and modified Gardner morphology grading at time of cryopreservation were noted. Student's t-tests, chi-squared/Fisher's exact tests, and multivariate logistic regression were used for the analysis.

Results:

A total of 6,609 pregnancies after SET were identified, with 3.1% (n=205) of those resulting in MZT pregnancies. Patient age and BAFC did not differ significantly between MZT and singleton pregnancies (Table 1). Ovarian stimulation protocol (p=0.55), cumulative GND dose (3379.9 ± 1344.5 vs. 3372.8 ± 1297.5, p=0.94), estradiol (2426.6 ± 1220.3 vs. 2446.4 ± 1210.2, p=0.83) and progesterone (0.9 ± 0.5 vs. 0.9 ± 0.5, p=0.81) levels at surge, and number of oocytes retrieved (16.5 ± 10.3 vs. 17.2 ± 9.8, p=0.33) were similar in IVF cycles resulting in MZT and singleton gestations. Both groups had similar rates of utilizing donor oocytes (12.7 vs. 12.7%, p=0.98), frozen transfer (77.1 vs. 80.5%, p=0.22), ICSI (89.3 vs. 86.2%, p=0.06), and PGT-A with assisted hatching (AH) on day 3 (66.8 vs. 66.3%, p=0.88). There was no significant association between monozygotic splitting and day of embryo development (p=0.92), repeat trophectoderm biopsy (p=0.54), or repeat vitrification-warming (p=0.97). Embryos with expansion grade 3 (OR 2.37 95% CI 1.07-5.26, p=0.03) and grade 5 (OR 1.97, 95% CI 1.28-3.04, p<0.01) were more likely to split than fully hatched embryos. Embryos with TE grade A were also more likely to result in MZT gestations than those with TE grade B (OR 1.83, 95% CI 1.12-2.97, p=0.02) and grade C (OR 3.11, 95% CI 1.39-6.94, p<0.01), but no significant association was demonstrated with ICM grade (p=0.16). MZT pregnancies had a higher percentage of female embryos than singleton pregnancies (OR 1.73, 95% CI 1.09-2.72, p=0.02). The association between MZT pregnancies and TE grade and embryo sex remained statistically significant in the multivariate logistic regression, which controlled for relevant confounders.

Conclusion:

Monozygotic splitting confers significant risks to both the mother and neonate. The ability to predict the likelihood of twin gestation after SET using patient and cycle characteristics would allow providers to better counsel patients regarding the risk of SET and to select an embryo for transfer that would optimize obstetric outcomes. The current study showed female embryos with favorable TE grade are more likely to split. Future studies examining whether epigenetic factors related to embryo sex and morphology increase the likelihood of splitting would further clarify this relationship.

Support

None.

Table 1

Comparison of baseline demographics and cycle characteristics among MZT and singleton pregnancies following frozen SET

	MZT pregnancy (n=205)	Singleton pregnancy (n=6404)	p value
Patient age (years)	37.0 ± 5.1	36.6 ± 5.1	0.21
Oocyte age (years)	34.5 ± 5.0	34.1 ± 4.8	0.21
AMH (ng/mL)	3.4 ± 3.0	3.8 ± 4.3	0.36
BAFC	13.2 ± 8.1	13.4 ± 8.0	0.75
BMI (kg/m^2)	24.0 ± 4.9	24.1 ± 4.5	0.85
Ovarian stimulation protocol GnRH antagonist GnRH agonist downregulation Microflare OCP/Lupron Synthetic Other	 144 (75.4%) 2 (1.0%) 16 (8.4%) 4 (2.1%) 17 (8.9% 8 (4.2%)	 4546 (76.0%) 142 (2.4%) 414 (6.9%) 129 (2.2%) 596 (10.0%) 155 (2.6%)	0.55
Cumulative gonadotropin dose (IU)	3379.9 ± 1344.5	3372.8 ± 1297.5	0.94
Donor oocyte	26 (12.7%)	816 (12.7%)	0.98
Type of IVF cycle Fresh Frozen	 47 (22.9%) 158 (77.1%)	 1246 (19.5%) 5158 (80.5%)	0.22
Estradiol at surge (pg/mL)	2426.6 ± 1220.3	2446.4 ± 1210.2	0.83
Progesterone at surge (ng/mL)	0.9 ± 0.5	0.9 ± 0.5	0.81
Eggs retrieved	16.5 ± 10.3	17.2 ± 9.8	0.33
Fertilization method Conventional ICSI Split	 18 (9.1%) 176 (89.3%) 3 (1.5%)	 813 (13.3%) 5282 (86.2%) 36 (0.6%)	0.06

PGT-A with AH	137 (66.8%)	4246 (66.3%)	0.88
Day of embryo development at cryopreservation 3 5 6 7	 6 (2.9%) 128 (62.4%) 69 (33.7%) 2 (1.0%)	 168 (2.6%) 3961 (61.9%) 2216 (34.6) 59 (0.9%)	0.92
Number of trophectoderm biopsies 1 2 3	 139 (98.6%) 1 (0.7%) 1 (0.7%)	 4259 (98.0%) 67 (1.5%) 21 (0.5%)	0.54
Number of times vitrified and warmed 1 2	 156 (98.7%) 2 (1.3%)	 5901(98.7%) 67 (1.3%)	0.97
Expansion grade 1 2 3 4 5 6	 0 (0.0%) 1 (0.5%) 8 (4.0%) 92 (46.2%) 67 (33.7%) 31 (15.6%)	 14 (0.2%) 26 (0.4%) 141 (2.3%) 3321 (53.4%) 1419 (22.8%) 1296 (20.8%)	<0.01
ICM grade A B C D	 141 (71.6%) 45 (22.8%) 11 (5.6%) 0 (0.0%)	 4624 (75.9%) 1287 (21.1%) 179 (2.9%) 4 (0.1%)	0.16
Trophectoderm grade A B C D	 107 (54.3%) 73 (37.1%) 17 (8.6%) 0 (0.0%)	 2695 (44.2%) 2418 (39.7%) 966 (15.9%) 13 (0.2%)	<0.01
Embryo sex Female Male	 90 (63.8%) 51 (36.2%)	 2267 (52.2%) 2080 (47.8%)	<0.01

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