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**CONCLUSIONS:** The trend to increased male: female live birth SSR coincides with the wider application of blastocyst transfer in this study. Both blastocyst developmental stage and blastocyst grade significantly predict male sex.

**IMPACT STATEMENT:** Understanding the potential reasons for sex-ratio imbalance following fertility treatment helps us develop strategies to balance SSR in the future. How to reach a natural sex ratio by balancing blastocyst and cleavage stage embryo transfer deserves future investigation.

**SUPPORT:** None.

**P-92** 6:30 AM Monday, October 24, 2022

**COMPARISON OF LABORATORY OUTCOMES FROM DIFFERENT SPERM SOURCES USING FRESH AND FROZEN DONOR EGGS IN A SINGLE HIGH VOLUME LABORATORY.**



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**OBJECTIVE:** Use of frozen donor eggs for ART has increased substantially. The convenience of using frozen donor eggs in advantageous and addresses limitations present with fresh donor egg cycles. However, compared to fresh donor cycles, the number of frozen eggs provided per cycle is low. Thus, high developmental competence of frozen eggs is critical to ensure success, especially with certain sperm sources. Fertilization and blastocyst development from fresh and frozen donor eggs following injection with varying sperm sources were compared.

**MATERIALS AND METHODS:** Data from ICSI cycles using fresh or frozen donor egg over a 21 month period were examined. Cycles using fresh ejaculated (Ejac) and testicular sperm (TEST) were analyzed separately. Rates of fertilization (fert) per mature egg as well as good quality blastocyst (GQB) development >3BB per 2PN were compared on days 5, 6 and 7. Data were analyzed using Fisher's Exact test, p<0.05.

**RESULTS:** In fresh donor eggs, fert rates differed by sperm source with TESE sperm yielding lower rates than Ejac. Using frozen eggs, no difference in fert was noted between sperm sources. Fert rates using just Ejac sperm or just TEST sperm did not differ between egg types. Using either fresh or frozen donor eggs, GQB rates were lower for TESE sperm than Ejac on all days. No differences were noted using just TEST sperm between fresh or frozen eggs. Using Ejac sperm, frozen donor eggs yielded lower GQB on all days. A higher increase in GQB from D6 to 7 was obtained using frozen eggs than fresh eggs. Overall, GQB was higher for fresh eggs on D5 (34.1% vs. 24.9%) D6 (60.4% vs. 51.1%) and D7 (61.6% vs 56.4%) compared to frozen eggs p <0.005.

n	Fresh Donor Egg		Frozen Donor Egg	
	1277	25	2532	26
Fert rate (%)	82.5 <sup>a</sup>	60.0 <sup>b</sup>	80.5	76.9
D5 GQB (%)	34.9 <sup>a1</sup>	4.0 <sup>b</sup>	23.9 <sup>a2</sup>	15.4 <sup>b</sup>
D5/6 GQB (%)	62.2 <sup>a1</sup>	36.0 <sup>b</sup>	48.7 <sup>a2</sup>	26.9 <sup>b</sup>
D5/6/7 GQB (%)	63.4 <sup>a1</sup>	36.0 <sup>b</sup>	54.3 <sup>a2</sup>	26.9 <sup>b</sup>

Different letter superscript indicates significant difference between sperm source within an egg type. Different numeric superscript indicates significant difference within a sperm type between egg sources.

**CONCLUSIONS:** Testicular sperm yielded lower blastocyst rates in both fresh and frozen donor eggs compared to ejaculated sperm. Frozen donor eggs yielded fewer high quality blastocysts than fresh eggs on all days examined and did not catch up by day 7, indicating that slower development was not the only issue. Frozen donor eggs may benefit from extended observations, checking development later on day 6 or on day 7 to ensure all blastocysts are identified.

**IMPACT STATEMENT:** Frozen donor eggs display reduced blastocyst formation compared to fresh donor eggs using different sperm sources and may benefit from extended culture.

**P-93** 6:30 AM Monday, October 24, 2022

**OPTIMAL INTERVENTION TO OBTAIN SPERMS WITH GOOD DNA QUALITY – ROLE OF MACS VS MICROFLUIDICS IN SPERM SORTING.**



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**OBJECTIVE:** In Individuals with raised Sperm DNA Fragmentation Index (DFI), which sperm selection technique, Magnetic Activated Cell Sorting (MACS) or Microfluidics Sperm Sorting (MF) helps obtain sperms with good DNA quality.

**MATERIALS AND METHODS:** This is an ongoing observational cohort study conducted at a private teaching hospital between November 2020 and October 2021. Men undergoing IVF cycles were randomly allocated to MACS (58) or MF (30) sperm sorting. Sperm DFI testing was performed on neat samples and post processed samples. Couples with one failed IVF, sperm count >5millions/ml, and raised Sperm DFI (>25%) were included in the study. Sperm DFI testing was performed by SCSA method. Based on randomization, MACS or MF was done as per the manufacturer's instruction. DFI assessment was done on neat and post intervention samples. The efficiency of the sperm processing method to reduce the DFI value post intervention was the primary outcome. We also evaluated the percentage of samples whose DFI values were <15%, 15-25%, and >25% respectively post intervention. MedCalc® Software Version 20.022 was used for statistical analysis.

**RESULTS:** MF group showed an average Pre- Interventional DFI Value of 24.75% & Post-Intervention DFI Value of 5.19%. The mean Rate of Change in DFI was observed to be 81.39% (P=0.008), with the highest frequency of 100% and a lower frequency of 6.06%.

MACS group showed an average Pre-Interventional DFI Value of 44.44% & Post-Interventional DFI Value of 21.56%. The mean Rate of Change in DFI was observed to be 54.47% (P=0.007), with the highest frequency of 100% and a lower frequency of 3.70%.

Post-Intervention DFI value Distribution:

**MICROFLUIDICS**

52 samples had DFI <15%

1 sample - 15% - 25%

5 samples - >25%

**MACS**

8 samples had DFI <15%

8 sample - 15% - 25%

14 samples - >25%

MF sperm sorting seems to be efficient in offering a better rate of change in DFI values. Irrespective of the DFI value of neat samples, a higher proportion of semen samples seem to have a DFI value of less than 15% in the MF sperm sorting group post intervention.

**CONCLUSIONS:** Microfluidics seems like a beneficial intervention to optimize sperm selection for Individuals with raised sperm DFI. MF sperm sorting seems to be efficient in offering a better rate of change in DFI values. Irrespective of the DFI value of neat samples, a higher proportion of semen samples seem to have a DFI value of less than 15% in the MF sperm sorting group post intervention

**IMPACT STATEMENT:** For individuals with a history of failed implantation and raised sperm DFI, MF sperm sorting seems to be a beneficial intervention to obtain sperm with good DNA quality.

**P-94** 6:30 AM Monday, October 24, 2022

**SPERM CONCENTRATION, SPERM MOTILITY AND TOTAL MOTILE SPERM COUNT ARE NOT AFFECTED BY CORONAVIRUS DISEASE 2019 (COVID-19) INFECTION.**



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**OBJECTIVE:** The purpose of this study is to compare the parameters of sperm analysis in a group of healthy sperm donors before and after Coronavirus Disease 2019 (COVID-19) infection.

TABLE 1.

	Mean	N	Std. Deviation	Std. Error Mean	P value
Pre-Covid Abstinence (Day)	4.5385	13	1.85362	.51410	0.607 <sup>b</sup>
Post-Covid Abstinence (Day)	4.2308	13	1.53590	.42598	
Pre-Covid Volume (mL)	3.9462	13	1.99065	.55211	0.889 <sup>b</sup>
Post-Covid Volume (mL)	3.9846	13	2.09239	.58032	
Pre-Covid Concentration (M/ml)	53.8689	13	37.86822	10.50275	0.313 <sup>a</sup>
Post-Covid Concentration (M/ml)	64.8634	13	43.96596	12.19396	
Pre-Covid A+B (%)	41.3077	13	21.70017	6.01854	0.852 <sup>a</sup>
Post-Covid A+B (%)	41.9231	13	20.35707	5.64604	
Pre-Covid TMS (M)	112.5763	13	94.04360	26.08300	0.570 <sup>a</sup>
Post-Covid TMS (M)	127.5823	13	96.70131	26.82012	

<sup>a</sup>paired samples t test

<sup>b</sup>Wiloxon's test

TMSC: Total Motile Sperm Count

**MATERIALS AND METHODS:** The study included semen analysis from eligible sperm donors aged 25-56 who had abstinence of 3-9 days and provided sperm before and after COVID-19 infection. Data were obtained from the patients who applied to our infertility clinic for semen analysis or in vitro fertilization (IVF) treatment. Ejaculate volume (mL), average concentration (M/mL), percent motility (percent), and total motile sperm count (M) were the primary outcomes. Data were compared and analyzed by paired samples t test and Wiloxon's test.

**RESULTS:** A total of 13 qualified sperm donors met inclusion criteria for this study. There was no significant difference in concentration, motility, or total motile sperm count in the patients' semen parameters before and after the infection ( $p > 0.05$ ) (Table 1).

**CONCLUSIONS:** COVID-19, a novel coronavirus disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has sparked a global pandemic that hit the world in 2020, offering a huge challenge to healthcare systems and affected populations (1). One of the known effects of SARS-CoV-2 infection is invasion or damage to the male reproductive system. To enter host cells, the virus uses the transmembrane serine protease 2 (TMPRSS2) and the angiotensin-converting enzyme 2 (ACE2) receptor (2). Furthermore, ACE2 is extensively expressed in testicular tissue, and SARS-CoV-2 has been found in semen (3). SARS-CoV-2 can also infect cells through the host cell receptor CD147 (basigin, BSG), a transmembrane glycoprotein crucial for the blood-testis barrier's integrity (BTB) (4). Sperm quality measures were not significantly different in qualified, otherwise healthy sperm donors prior to COVID infection and after recovery.

**IMPACT STATEMENT:** SARS-CoV-2 infection has no impact on sperm concentration, motility, or total motile sperm count in healthy, eligible donors.

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#### MONOZYGOTIC TWINS AFTER IVF: CAN TIME-LAPSE TECHNOLOGY PREDICT MONOZYGOTIC TWIN PREGNANCY?

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**OBJECTIVE:** The purpose of this study was to evaluate morphokinetic of embryos that resulted in monozygotic twin pregnancies (MZTP) after a single embryo transfer (SET), with matched time-lapse data of embryos that resulted in a singleton pregnancy (SP) after SET.

**MATERIALS AND METHODS:** For this retrospective matched case-control, we reviewed data from 2018 to 2021 and ten MZTP were identified. As the disparity of cases was too large to use full data, a proportion of 1:2 case-control was adopted. We used women age and quality of blastocyst transferred as matching factors, and two controls were selected for each case. All resulted pregnancies come from a SET. Morphokinetic data of transferred blastocysts were recorded. To explore the risk factors for MZT, we used a generalized estimated equation (GEE) model to adjust for potential confounders and to calculate the adjusted odds ratio. The statistical significance was established at  $P < 0.05$ . Additionally, all time-lapse videos from MZTP and SP groups were individually reviewed by an experienced embryologist.

**RESULTS:** A GEE model with gamma distribution verified possible differences in embryonic morphokinetics through measurements of development in 10 moments (time to: PN fade - tPNf; two-cells - t2; three-cells - t3; four-cells - t4; five-cells - t5; eight-cells - t8; nine-cells - t9; morula - tM; start of blastulation - tSB; blastocyst - tB), in relation to the type of pregnancy developed (SP or MZTP). We found no significant differences in embryonic morphokinetic measures over time (Wald: 0.010, df: 1,  $p = 0.919$ ) between the SP and MZTP groups, as well as in interaction between groups and morphokinetics (Wald: 9.148, df: 9,  $p = 0.424$ ). The mean time of each moment evaluated was not significantly different between SP and MZTP embryos ( $p > 0.05$ ). In the time-lapse videos reviewing, no sign of embryo splitting or doubled inner cell mass (ICM) were observed. It is also interesting to note, all blastocyst who become MZTP had inner cells mass classified as A or B in the morphological evaluation.

**CONCLUSIONS:** Embryo morphokinetics whose result in MZTP are similar to SP. There are no sign which indicates embryo splitting or doubled ICM until the fifth day of embryo development, evaluated by time-lapse videos.

**IMPACT STATEMENT:** Division occurring at the blastocyst stage (70-75%) is believed to account for mostly of MZTP. The division shortly after fertilization (20-30%) or in later stages of pregnancy are supposed to be minority (1-2%). Thus, time-lapse technology would allow us to observe embryo splitting in most blastocysts which become MZTP. More than a decade after the time-lapse imaging surge, there are rare report of ICM splitting in the literature. Our study corroborates with others on the absence of morphokinetic differential or visual signal of ICM splitting in blastocyst at fifth day of development that became MZTP. Thus, the theory about the timing of embryonic division generating MZTP appears not to be consistent with more recent assisted reproductive practices.

**SUPPORT:** No financial support was required.

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