

for gamete donation. After liquefaction, we washed the sperm samples with PBS and performed TUNEL assay in different aliquots. This assay consists in measuring the existing breaks in DNA chain incorporating molecules marked with a fluorochrome. For the statistical analysis we used the SPSSv20.0, using the t-Student test.

**Main results and the role of chance:** We did not observe significant differences in DFI by adding a small volume of ethanol to the fresh sample required for Cinnamtannin-B1 resuspension. After 4 h incubation, we obtained a significant increase of DFI in the sperm sample without antioxidant (10.27 vs 16.47;  $p < 0.05$ ) suggesting that sperm DNA is damaged after incubation at 37°C. However, when we add Cinnamtannin-B1 to the semen sample at different concentrations, we observed a significant decrease in the DFI when we compared the samples incubated with 10 µM ( $9.47 \pm 1.39$ ) and 100 µM Cinnamtannin-B1 ( $9.73 \pm 1.36$ ) versus control ( $16.47 \pm 2.82$ ) ( $p < 0.05$ ). This results suggest that Cinnamtannin-B1 supplementation prevents the increase of DNA damage. In addition, this results also showed that antioxidant concentrations higher than 10 µM do not confer a greater protective effect.

**Limitations, reasons for caution:** We believe that sample size may be a limitation in this study as well as the use of normozoospermic samples. Further studies should be carried out to verify Cinnamtannin B-1 protective effect in samples with low seminal quality or high fresh sperm DNA fragmentation.

**Wider implications of the findings:** An interesting topic could be evaluating if addition of Cinnamtannin-B1 in sperm crioprotectors could be useful in sperm cryopreservation where spermatozoa are submitted to oxidative stress. Given the beneficial effect of Cinnamtannin B-1 *in vitro*, its use could also be considered as a feeding supplement especially in infertile men.

**Trial registration number:** not applicable.

**P-064 Reduced ejaculatory abstinence period may improve embryo quality for those with high sperm DNA fragmentation**

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**Study question:** Does reduced ejaculatory abstinence period improve embryo quality in ICSI cycles for those with high sperm DNA fragmentation (SDF) and repeated ART failures?

**Summary answer:** it seems that reduced ejaculatory abstinence period enhance overall embryo quality without compromising other semen parameters when compared to standard abstinence period

**What is known already:** Accumulating evidence suggests examination of the SDF particularly for those with repeated miscarriages or ART failures through ART. Despite controversies regarding the optimal cut-off point and methodology, several approaches have been suggested to overcome SDF related drawbacks such as oral intake of antioxidants, use of testicular spermatozoa obtained with either testicular sperm aspiration (TESA) or testicular sperm extraction (TESE) or sperm selection techniques. Yet, none of these interventions, alone or combined, have been unequivocally proven to be of clinical value to overcome the potential detrimental effect of high SDF on ART outcomes.

**Study design, size, duration:** This is an observational study conducted between 2015 and 2016 in a single center including 24 couples with >2 previous ART cycles. All couples were offered to undergo a new ICSI cycle using spermatozoa obtained by reduced abstinence period (1 hour following the first ejaculation on the day of oocyte retrieval) due to high SDF (>20%). Total of 23 cycles (SHORT) were compared with previous 49 standard cycles of couples (STAND), being their own controls.

**Participants/materials, setting, methods:** The average frequency of intercourse for the men before producing their first semen specimen for study was between once and twice weekly. Exclusion criteria for men were; azoospermia cases, failure to follow the ejaculatory abstinence protocol, current smoking, presence of varicocele, consumption of anti-oxidants or genital infection within 6 months. All female subjects had normal uterine cavity, hormone levels and karyotype. Poor responders, advanced age (>40 years) and cases with other medical disorders were excluded.

**Main results and the role of chance:** Total of 73 cycles were evaluated. Only 2 cycles were cancelled due to poor ovarian response and were not included. Blastocysts with ICM, A or B and trophectoderm grade A (i.e. AA or BA) were considered to be top quality. Significantly lower sperm count was detected in SHORT group, while significantly higher numbers of top quality blastocyst were detected. Accordingly, 25% implantation and 24% live birth rate was detected in SHORT group despite previous unsuccessful cycles.

**Table 1 Demographics.**

woman age (yrs)	33.5
infertility duration (yrs)	8.4
No of prev. cycles (N)	2.9
mean AMH (ng/ml)	2.5
mean AFC (N)	12

**Table 2 Semen parameters.**

	STAND (49)	SHORT (24)	
volume (ml)	2.8	1.3	<b>0.00</b>
total count	70.2	27.1	<b>0.00</b>
concent. (/ml)	29.8	21.9	NS
mean normal morphology %	4.7	5	NS
motility A+B %	48	53	NS
SDF %	33	-	NA

**Table 3 Embryology outcomes.**

	STAND (49)	SHORT (24)	
No of MII	8	8.5	NS
fertilization%	67	75	0.07
total No of embryos on day 3	4.7	6.3	<b>0.03</b>
No of good quality cleavage embryos	1.3	4	<b>0.00</b>
No of top quality blastocysts	0.6	3	<b>0.00</b>
No of transferred embryos	1.8	1.9	NS

**Limitations, reasons for caution:** This study is not a randomized controlled one and the main limitation is the lack of SDF data of SHORT group. Moreover, total antioxidant capacity and reactive oxygen species (ROS) levels are also not available.

**Wider implications of the findings:** Sperms obtained by reduced ejaculatory abstinence has been shown to reveal low SDF, ROS levels and higher antioxidant capacity. This has been validated even for normozoospermic cases. Therefore, performing ICSI with those spermatozoa seems to be effective in terms of top quality embryos particularly for those with repeated ART failure.

**Trial registration number:** NA.