reproduction techniques (ART) may increase the basal rate of DNA damage. Altogether, this may produce a reduction in the expected fertility rates. Unfortunately, both in freshly ejaculated and in cryopreserved samples, the mechanisms and processes involved in Sperm DNA Fragmentation (SDF) are not fully illuminated. In mammalian species it is known that SDF, far to be a static concept, an increase in the rate of Sperm DNA Fragmentation (rSDF: increase of SDF values through time before fertilization) is produced. Thus, the dynamic behaviour of SDF, more than the static view of this parameter, could be a key factor to understand the potential capacity of male to produce a normal pregnancy. Within the general tendency reported by other authors on different seminal characteristics after sperm capacitation, the most parsimonious hypothesis is that the values of SDF would be improved after capacitation. The aim of this investigation was to analyze the increase of SDF values over time in donors with proven fertility, using an experimental model which emulates the steps of sperm handling as used for ART, using fresh and frozen-thawed and capacitated samples. A dynamic approach of SDF was used to assess such hypothesis.

**Material & Methods:** SDF assessment was performed in fresh and frozen-thawed and capacitated samples (density gradient isolation) from the same individual after 0.5, 1.5, 4.5, 6, 24, 48 and 72 hours of incubation in a humidified atmosphere of 5% CO2 in air at 37°C. Study was performed using 15 male donors with proven fertility for a maximum of six births at the reproductive medicine center. SDF fragmentation was assessed under fluorescence microscopy and image analysis software using Halosperm, the commercial variant of the sperm chromatin dispersion methodology.

**Results:**
1. No significant differences in SDF were obtained when fresh and frozen-thawed and capacitated sperm at a basal time were compared.
2. The rSDF showed by both samples is different and capacitation selects for sperm subpopulations which exhibit a lower range of variance for SDF.
3. Gradi-ent-isolated human sperm capacitated after frozen-thawing select for sperm populations with a more stable rate of SDF than that observed in freshly ejaculated semen samples.
4. Capacitation does not select for the best levels for DNA damage in all individuals when compared with the values and rSDF in fresh semen samples.

**Conclusion:** Dynamic assessment of SDF offers additional and different information to that obtained from a static viewpoint. In the particular case of this experiment, it is concluded that cryopreservation of sperm samples does not affect the basal level of DNA damage observed in each individual when compared to those obtained in fresh samples. Interestingly, sperm capacitation after thawing renders individuals semen samples more predictable in their dynamic behaviour for an increasing rSDF than the rates observed in fresh semen samples. Sperm selection is not providing the best levels of SDF after capacitation for all individuals. Within the field of DNA damage, we are imperatively called to select the most efficient semen samples by minimizing the severity of DNA damage, for example, being scientifically conscientious of the timing for using the sperm samples in ART according to the dynamic behavior that each sperm sample presents. It is therefore recommended that chromatin changes through time might be used as an additional parameter for the assessment of sperm quality after capacitation or in every circumstance of semen handling for ART.

**O-219 Oral**

**Activity of maturation promoting factor, but not of microtubule-activated protein kinase, decreases over time in frozen-thawed human oocytes**

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**Introduction:** Following cryopreservation, the developmental ability of the human oocyte may be affected by diverse forms of cell damage, including perturbances in the level of regulatory proteins. In this study, in frozen-thawed human mature oocytes, we examined the biochemical activity of maturation promoting factor (MPF) and microtubule-activated protein kinase (MAPK), two key regulators of the meiotic and mitotic cell cycles that ensure meiotic arrest, normal spindle configuration and chromosome condensation at the metaphase II (MII) stage.

**Material & Methods:** Surplus human oocytes were donated by consenting IVF patients. Following cumulus cells removal, oocytes showing normal mor-...
MOPS-KOH, 12 mM MgCl2, 12 mM EGTA, 0.1 mM EDTA, 1 mM DTT, 2.3 mM NaVO4, 2 mM NaF, 0.8 mM PMSF, 15 µg/mL leupeptin, 30 µg/mL aprotinin, 0.1% (w/v) PVA, 1 mg/mL histone H1 (type III-S from calf thymus), myelin basic protein (1 mg/mL, MBP), 2.2 M protein kinase inhibitor peptide, and 2.5 MBq/mL γ-[32P]-ATP. The reaction was started after the addition of γ-[32P]-ATP and performed for 30 min at 37°C. Phosphorylations of substrates histone H1 and MBP were considered as signs of MPF and MAPK activity, respectively. Proteins were separated on 1D SDS-PAGE electrophoresis and radioactive bands were analyzed by gel autoradiography. The pixel intensity of a preselected set area was measured using Kodak Image Analysis Software 1D 3.6. The mean band intensity of fresh controls was assumed to correspond to 100 arbitrary units and the mean band intensities of the other groups were quantified comparatively to this value. Analysis of variance (ANOVA) was used to assess the significance of differences in MPF and MAPK activity among groups. P < 0.05 was considered significant.

Results: In frozen-thawed oocytes that where cultured for 1 hour after thawing, the relative intensity (RI) of the MPF bands was statistically different (98.2) from the fresh control. However, MPF band intensity was significantly reduced (RI = 73.3, P < 0.05) in samples cultured for 2 hours after freezing-thawing. Conversely, comparable intensities of the MAPK bands were observed in fresh or cryopreserved oocytes cultured for 1 or 2 hours (RI = 100, 96.8, and 98.7, respectively).

Conclusions: In human oocytes, slow-freezing appears to partly influence the activity of cell cycle regulatory proteins. After thawing, MAPK is unaffected over a period of two hours. During the same interval, MPF activity is initially maintained unaltered but undergoes a significant decrease thereafter. This may have significant implications for the use of frozen-thawed oocytes. In particular, considering the observed delayed reduction in MPF activity, it might be appropriate to limit to 1 hour the post-thaw period in which oocytes are cultured before sperm microinjection, thereby preventing possible losses in spindle and chromosome configuration, or premature exit from the MII arrest.

O-221 Oral Oocyte cryopreservation is an efficient alternative to embryo freezing

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Introduction: Italian law, introduced in 2004, imposes that no more than three oocytes can be fertilized and the same law prohibits embryo cryopreservation. Thawing and transfer of the embryos frozen prior to this law is still allowed. Oocytes cryopreservation is permitted, which currently is the only alternative to embryo freezing. Efficiency of oocyte cryopreservation has been significantly lower than embryo freezing, however modified protocols have resulted in improved outcomes. The aim of this study is to evaluate if the oocyte cryopreservation can serve as a viable alternative to embryo freezing in terms of clinical outcomes.

Materials and Methods: Study period was between March 2004 and December 2008. Patient inclusion criteria: 1) female age < 39 years (at the time of the oocyte collection) and 2) cryopreserved oocytes or embryos. Group A: transfer of frozen/thawed embryos and Group B: transfer of embryos obtained from cryopreserved oocytes. Excellent and good quality supernumerary embryos were frozen using slow freezing protocol with 1.5 M propanol (PROH) and 0.1 M sucrose. Good quality supernumerary oocytes were frozen using three different protocols: 1) slow freezing with 1.5 M PROH and 0.1 M sucrose; 2) vitrification on gold grids with 1.5 M and 5.5 M ethylene glycol (EG) and 1 M sucrose; 3) vitrification on cryoprotectants with 1.33 M (7.5%) and 2.66 M (15%) EG, 1.06 M (7.5%) and 2.12 (15%) dimethyl sulfoxide (DMSO) and 0.5 M sucrose. Slow-frozen embryos and oocytes were thawed using in 1.0, 0.5 and 0.0 M PROH and 0.1 M sucrose. Vitrified oocytes were warmed with sucrose 1 M, 0.5 M, 0.25 M, 0.125 M and 0 M (for gold grids) or 1 M, 0.5 M and 0.0 M (for cryoprotectants). Statistical analysis were performed using Fisher’s exact and Mann-Whitney tests at the level of P ≤ 0.05.

Results: Groups A and B include 207 and 125 thawing/warming cycles, respectively. Mean female age ± SE at the day of the freezing was not statistically different between the two groups (33.1 ± 0.21 in Group A and 33.4 ± 0.29 in Group B). In Group A, 678 embryos were thawed and 454 of them survived (66.9%) whereas in Group B, 343 out of 849 thawed/warmed oocytes survived (40.4%, P ≤ 0.001). More patients had embryo-transfer in Group A (88.9%, N = 193) as compared to Group B (64.8%, N = 81, P ≤ 0.001). Embryos transferred were 449 in Group A and 176 in Group B. There were not significant differences in pregnancy rate per transfer (28.5% of patients (N = 55) in Group A and 29.6% (N = 24) in Group B), in implantation rate (8.0% of embryos (N = 449) in Group A and 11.9% (N = 176) in Group B) and in