serum levels of 17ß-estradiol and SHBG were found. Overweight and obese patients had a significantly higher percentage of spermatozoa with low MMP and 31.9 ± 9.3%, respectively) and a significantly lower percentage of alive spermatozoa (56.5 ± 6.9% and 54.1 ± 5.8%, respectively) compared to controls (low MMP = 2.8 ± 1.1%; alive = 77.8 ± 1.9%). The percentage of spermatozoa with PS externalization (10.7 ± 3.7% and 16.1 ± 3.4%, respectively), an early sign of apoptosis, or with abnormal chromatin compactness (20.0 ± 2.3% and 20.6 ± 3.1%, respectively) was significantly higher in overweight and obese patients compared to controls (PS externalization = 2.7 ± 0.5%; chromatin abnormality = 13.9 ± 0.7%). Lastly, the percentage of patients with fragmented DNA was higher in both overweight (4.1 ± 0.8%) and obese (5.6 ± 1.3%) patients compared to controls (2.3 ± 0.7%), but the difference reached the statistical significance only in the latter. Correlation analysis showed that the BMI correlated negatively with total sperm count, progressive motility, normal forms and percentage of viable spermatozoa and positively with spermatozoa with low MMP, PS externalization and DNA fragmentation, but not with chromatin compactness.

Discussion: These results showed that healthy overweight and obese men have worst conventional sperm parameters, sperm mitochondrial function and chromatin/DNA integrity. All these parameters, but chormatin abnormality, correlated significantly with BMI. The lack of andrological, systemic and/or endocrinological diseases suggest that the increased body weight has a negative impact on these parameters. Given their relevant role played on couple's fertility, we suggest to include a body weight losing program among the therapeutic strategies of male infertility.

P-025 Effects of density gradient centrifugation and swim up techniques on sperm DNA integrity

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Introduction: A wide variety of techniques for sperm preparation are currently available, the most commonly used are density gradient centrifugation and *swim up*. Although these techniques appear to be effective in selecting functional sperm for assisted reproductive technologies (ART), to date, there is no consensus about which is the best method for isolating functionally competent spermatozoa and neither about the effect of these semen processing techniques on sperm DNA integrity.

Sperm DNA damage has been linked with poor semen quality and reduced fertility. Since there is concern about the effects of DNA fragmentation in ART success and embryo quality, the evaluation of the DNA damage caused by some of the protocols of semen processing is of interest. In this study we aim to examine and compare the effect of density gradient centrifugation and *swim up* processing techniques on DNA integrity within populations of spermatozoa from normozoospermic and non normozoospermic subjects.

Material and Methods:

Sperm preparation

One hundred and forty nine human semen samples were used in this study. Samples were obtained by masturbation after 3-5 days of sexual abstinence. After liquefaction of semen, standard semen parameters were obtained according to WHO guidelines. All of the semen samples used had a minimum concentration of 5 million spermatozoa/ml and absence of leukocy-tospermia.

Swim up. Samples diluted in IVF plus (Vitrolife, Göteborg, Sweden) were centrifuged at 1400 rpm for 10 minutes, the sperm pellet resuspended in IVF plus and incubated for 1h at 37°C under an atmosphere of 5% CO_2 in air. After the incubation period, the entire supernatant was aspirated and analysed.

Density gradient centrifugation. Semen samples were placed on top of a 50%-90% Spermgrad (Vitrolife, Göteborg, Sweden)/IVF plus gradient, centrifuged for 20 minutes at 1200 rpm and the pellet resuspended in IVF plus. Subsequently, the *swim up* protocol described above was performed.

Sperm DNA fragmentation analysis

Sperm Chromatin Dispersion (SCD) test. DNA fragmentation index (DFI) and DNA degradation index (DDI) of the samples were determined by using the Halosperm® kit (Halotech DNA SL, Madrid, Spain).

2 tailed (2T) Comet assay. Single stranded (ss) and double stranded (ds) DFI of the samples were determined by using the 2T comet assay (Enciso et al. 2009).

Results: SCD test DNA damage analysis revealed that neat non normozoospermic semen samples presented a significantly (U Mann Whitney, $p < 0.05$) higher mean DFI and DDI than normozoospermic samples.

After semen processing with density gradient and *swim up* techniques, mean sperm DFI and DDI, assessed by the SCD test, were significantly reduced (U Mann Whitney, $p < 0.05$) in both *swim up*-treated and density gradient-treated spermatozoa in both groups of subjects. Similarly, the percentage of spermatozoa with ds DNA breaks assessed by the 2T comet assay, was also significantly reduced (U Mann Whitney, $p < 0.05$) in both *swim up*-treated and density gradient-treated spermatozoa in both groups of subjects. However, the percentage of spermatozoa with ss DNA breaks assessed by the 2T comet assay, was significantly (U Mann Whitney, $p < 0.05$) reduced only in density gradient-treated but not in *swim up*-treated spermatozoa in the groups of subjects analysed.

Conclusions: The semen processing techniques *swim up* and density gradient centrifugation recover spermatozoa with improved DNA quality. Both methods are efficient in eliminating highly DNA damaged and double stranded DNA damaged spermatozoa but the density gradient centrifugation method is more efficient than the *swim up* technique in eliminating single stranded DNA damaged spermatozoa.

Reference:

1 Enciso M, Sarasa J, Agarwal A, Fernández JL, Gosálvez J. Reproductive BioMedicine Online, Vol. 18 (5): 609-616. 2009.

P-026 Can prepubertal human testicular tissue be cryopreserved by vitrification?

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Introduction: Cryopreservation of testicular tissue from prepubertal boys with cancer has emerged as an ethically acceptable strategy to preserve their fertility, since sperm banking cannot be considered in these patients. Controlled slow-freezing (SF) with dimethylsulfoxide (DMSO) as a permeating cryoprotectant (CP) has proved to be a promising approach to preserve immature human testicular biopsies. Nevertheless, an efficient cryopreservation protocol has not yet been established. Vitrification (V) might constitute a better approach by avoiding ice crystal formation and subsequent freeze injuries. Using a long-term in vitro organotypic culture system, our aim was to evaluate the efficiency of V to preserve spermatogonial cell (SPGc) survival and seminiferous tubule (ST) integrity of prepubertal human testicular tissue, since these parameters are essential for initiation of spermatogenic processes.

Material and Methods: Testicular tissue was obtained from two patients (6 and 12 years of age) before starting gonadotoxic treatment. Controlled SF was performed with a programmable freezer using a freezing solution containing DMSO (0.7 mol/l) and sucrose (0.1 mol/l). Increased concentrations of CP and faster cooling rates were used for V. After dehydration in DMSO (2.8 mol/l) and ethylene glycol (2.8 mol/l) solution, samples were placed in open cryostraws and directly plunged into liquid nitrogen. Fresh tissue (FR), used for control purposes, and thawed and warmed biopsies were cultured for 10 days. ST integrity was evaluated by light microscopy on stained sections of tissue fixed in Bouin's solution. SPGc survival and proliferation were evaluated by immunohistochemistry using, respectively, MAGE-A4 and Ki67 antibodies on sections of tissue fixed in formalin.

Results: Similar ST morphology was observed in both cryopreserved tissues and FR controls after long-term organotypic culture. Histological characteristics of SPGc and Sertoli cells were preserved, as well as cell-cell cohesion and cell adhesion to the basement membrane in all three groups (FR, SF and V). Pyknotic nuclei were found in the ST of FR and cryopreserved cultured tissue retrieved from the oldest boy. These cells were more frequently seen after cryopreservation, but no difference was noted between the SF and V methods. Survival of SPGc evidenced by MAGE-A4-positive immunostaining was confirmed in all cryopreserved tissue and FR controls after long-term culture. The ability of SPGc to proliferate after cryopreservation and culture was proved in all three groups by positive Ki67 immunostaining.

Conclusion: Vitrification is a convenient method for cryopreservation of immature human testicular tissue, since the process appears to be faster and cheaper than SF. As this technique preserves ST integrity and allows survival and proliferation of human SPGc in long-term organotypic culture, V might be considered as an alternative to SF, and thus a promising strategy to preserve the reproductive capacity of young boys. The functional characteristics of cryopreserved SPGc should be further evaluated through xenotransplantation experiments to determine whether SF and V are equally efficient approaches.