ORAL ABSTRACTS

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THE EFFECT OF ORCHIECTOMY ON FUNCTIONAL ASPECTS OF SPERM AND OXIDATIVE STRESS OF SEMINAL PLASMA FROM PATIENTS WITH TESTICULAR TUMORS

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Introduction: Testicular tumors, although rare, are the most frequent tumors in young men of reproductive age. Treatment is usually achieved by orchiectomy, followed or not by adjuvant therapy, with survival rates of 95% after five years. Because adjuvant therapy is often gonadotoxic, there is a concern with reproductive life. Currently, sperm banking is the only option that guarantees future fertility in these men. However, it remains to be answered if the best sample to cryopreserve is the pre- or the post-orchiectomy sample. Therefore, we wished to verify semen quality, sperm functional traits, and semen oxidative stress, before and after orchiectomy and suggest the better time to perform sperm banking in patients with testicular tumors.
Methods: This prospective study was carried out including 24 patients with testicular germ cell tumors, who provided one semen sample before orchiectomy of the affected testis and one at least 15 days after the surgery. Following semen analysis, an aliquot was used for analysis of (i) sperm DNA fragmentation (alkaline Comet assay, sperm were classified as high DNA integrity [Class I] to high DNA fragmentation [Class IV]); (ii) acrosome integrity (PNA-FITC); and (iii) mitochondrial activity (colorimetric staining of the midpiece, classified as Grade I [all mitochondria active] to IV [all mitochondria inactive]. Seminal plasma MDA levels were measured as markers of oxidative stress. Pre- and Post-orchiectomy samples were compared using a paired Student's T test (normal distributions) or a paired Wilcoxon test, when appropriate ($p<0.05$).
Results: Results are presented in table 1. A significant decrease in DNA fragmentation (p=0.019) and oxidative stress (p=0.014), and an increase in mitochondrial activity (p= 0.048) was observed after orchiectomy.
Conclusion: Based on our findings, the better time for sperm banking in patients with testicular tumors is after orchiectomy. Although cryopreservation of pre-orchiectomy samples remains an important conduct to preserve reproductive potential, post-orchiectomy samples should be preferred for assisted reproductive treatments.

Table 1. Value of seminal analysis and DNA fragmentation (Comet), mitochondrial activity (DAB), acrosome integrity (PNA – FITC) and lipid peroxidation (TBARS). Value presented as mean; standard deviation.

	Pre - orchiectomy	Post - orchiectomy	p
Volume (mL)	3.0; 1.24	3.2; 2.46	0.620*
Progressive (%)	51.9; 12.03	50.7; 10.16	0.819
Non-progressive (%)	42.4; 12.76	44.8; 9.79	0.646
Concentration (x 10^6/mL)	26.7; 24.93	24.2; 27.12	0.227*
Morphology (%)	4.4; 2.50	4.4; 2.10	0.714
Comet I (%)	7.0; 11.00	6.5; 7.00	0.36
Comet II (%)	62.5; 12.00	72.0; 18.00	0.019*
Comet III (%)	22.5; 8.00	14.5; 9.00	0.028*
Comet IV (%)	5.0; 8.00	3.0; 5.00	0.172
DAB I (%)	1.0; 3.00	1.0; 3.00	0.612
DAB II (%)	65.5; 29.00	65.0; 17.00	0.075
DAB III (%)	34.2; 15.32	28.1; 7.71	0.048*
DAB IV (%)	4.5; 7.00	4.0; 4.00	0.700
Intact acrosomes (%)	80.9; 8.66	80.4; 8.39	0.521
MDA (ng/ml)	9.5; 4.38	7.8; 2.79	0.014*

* - Significant difference
* - Nonparametric Wilcoxon test. Value present as median; interquartile range.

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CONSISTENT DIFFERENCES AMONG MEN IN CAPACITATION TIMING COULD PERSONALIZE IUI/ART

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Introduction: To become fertilization competent, sperm must first capacitate. This process depends on changes in membrane lipids, including removal of sterols and redistribution of the ganglioside GM1. Here, we examine the timing of capacitation among and within different individuals. Reliable differences in capacitation timing could be used to personalize IUI/ART protocols.
Methods: Semen samples from consenting men were liquefied, washed and aliquots incubated under non-capacitating (NC) or capacitating (CAP) conditions. Samples from fertile men (pregnant partner or recent father) and samples from men seeking fertility exams were incubated for three hours, fixed and analyzed immediately (day0) or after overnight incubation in fix (day1). In another trial, NC and CAP samples were incubated for three hours or 24 hours prior to fixation. Capacitation was assessed using localization of GM1 (Cap-ScoreTM).
Results: 102 samples from 36 fertile men were evaluated at day0 and day1. An increase in Cap-Score was observed in 81% (83/102) of CAP samples, with 44% (45/102) increasing more than 1 SD (7%). In 17 men seeking fertility treatment, 29% (5/17) increased 7% or more. To determine whether this change was physiological or an artifact of being in fix overnight, semen samples from nine fertile men were analyzed at day0, day1 and after 24 hours of incubation in medium and then fixed. All NC samples were equivalent (19±2, 23±2 and 20±1%) and were different from the CAP samples (28±1, 34±2 and 31±2%). Day1 CAP was significantly greater than Day0 CAP (p=0.03), but Cap-Scores for samples incubated overnight in fix or in capacitating media were the same (p=0.33). Consistent with prior literature, these data show that membrane changes involved in capacitation still occur in certain fixatives. These data suggest that sperm achieve capacitation at different times in different ejaculates. To see if this was reproducible for an individual, 91 samples from 25 fertile men were classified as either early or late capacitators (day1-day0>7). The average concordance of change within donors was 76%, showing that capacitation timing was highly consistent within men.
Conclusion: These data show that capacitation timing differed consistently among men. Determining capacitation rates could eventually lead to personalized management of infertility, including optimal times for IUI relative to ovulation and capacitation times prior to co-incubation of sperm and oocytes for IVF.
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