from 1993 to 2009. All TC patients were evaluated within 1 month after unilateral orchidectomy. Sperm parameters (ejaculate volume, sperm concentration, total sperm count, forward motility) were evaluated according to WHO 1999 guideline.

Results: The mean age and sperm concentration was 27 years (range 16-42), 21.69 M/mL ( ± SD 20.5) in the TC group and 25 years (range 16-34), 31,35 M/mL ( $\pm$  SD 23.6) in the HD group. In the 104 TC patients 48 (46.1%) olygozoospermic and 13 (16.2%) azoospermic individuals were detected. Only 43 (41.3%) of them had more than 40 million total sperm count/ejaculate. The average forward motility was 26.7% (  $\pm$  SD 5.4). Histological data were available for 65 TC patients. The sperm concentration was reduced both in seminoma patients (N = 32, 22.64 M/ml  $\pm$  SD 15.5) and in non-seminoma (embryonal carcinoma, yolk sac tumors, choriocarcinoma) TC patients (n = 33, 22.3 M/ ml ± SD 16.5). Thirteen TC patients and six HD patients were azoospermic. Among the 45 HD patients 14 (31.2%) were olygozoospermic, 6 (13.2%) were azoospermic and only 25 (55.5%) normozoospermic. The average forward motility was 25,9% ( ± SD 5.56).

Conclusions: Spermatogenesis is defected in most testicular cancer patients, and Hodgkin disease also negatively influences the sperm parameters. On average, normal sperm count can be detected only in half of the oncologic patients apply for sperm cryopreservation. The decreased progressive motility suggests that with the reduced sperm concentration the sperm function is also affected, suggesting decreased fertilizing potential. Functional testing, chromatin assays and chromosomal analysis of samples would improve the assessment of the fertility prognosis of these patients before cryopreservation.

## P-073 Sperm apoptotic markers as related to clinical and hormonal characteristics, and fertility potential

B. Golob<sup>1</sup>, B. Zorn<sup>1</sup>, A. Ihan<sup>2</sup>, A. Kopitar<sup>2</sup>, M. Kolbezen<sup>1</sup> <sup>1</sup>University Medical Centre Ljubljana, Department of Obstetrics and Gynecology Reproductive Unit Andrology Centre, Ljubljana, Slovenia

<sup>2</sup>University of Ljubljana Faculty of Medicine, Institute of Microbiology and Immunology, Ljubljana, Slovenia

Introduction: The origin and significance of apoptosis markers changes in ejaculated sperm are debated.

Material and Methods: Semen and serum samples from men of infertile couples were evaluated. The study involved the determination of classical sperm characteristics in neat semen and sperm apoptotic markers i.e. changes in plasma membrane phospholipid asymmetry, mitochondrial membrane potential (MMP) and DNA integrity by SCSA in semen prepared by density gradient using flow cytometry. Serum FSH was measured by a solid-phase, two-site chemiluminescent immunometric assay. Couples were followed for 36 to 60 months. In the study we enrolled 143 couples and divided them according to the method of conception: in 76 the female partner could conceive naturally or with intrauterine insemination (IUI) and in 88 the female partner underwent IVF or ICSI; 41 (29%) couples were lost to follow-up. Statistical analysis was based on calculations of correlations (Spearman test), logistic regression, and comparison using Mann-Whitney test.

Results: Male age was significantly correlated to the number of non apoptotic cells (r = -0.244, p = 0.004) and to DNA denaturation (r = 0.206, p = 0.004) 0.014). Testicular volume was correlated to non apoptotic cells (r = 0.259, p = 0.002), MMP (r = 0.295, p < 0.001) and DNA denaturation (r = -0.168, p = 0.045). FSH was correlated to apoptotic cells (r = 0.227, p = 0.008) and MMP (r = -0.262, p = 0.002). A significant correlation was observed between apoptotic cells, cells with normal MMP, DNA denaturation and classical sperm characteristics. In men whose partners conceived naturally or after IUI sperm DNA denaturation was significantly lower in comparison with those who did not conceive  $(7.7\% \pm 2.2\% \text{ vs. } 19.1\% \pm 2.3\%, p = 0.024)$ . Women whose male partner had sperm DNA denaturation > 10% had four times lower chance to conceive naturally. In men undergoing IVF or ICSI, sperm apoptotic markers were not predictive of pregnancy.

Conclusions: Changes in plasma membrane phospholipid asymmetry, mitochondrial membrane potential (MMP) and DNA integrity are related to male age and important indicators of spermatogenesis, i.e. to testicular volume and serum FSH. Though apoptotic markers do not predict pregnancy in IVF and ICSI, they predict natural pregnancy. Therefore, sperm apoptotic markers provide a clinical indication for routine use in infertility evaluation.

## P-074 DNA fragmentation and semen alterations in elite triathletes

D. Vaamonde<sup>1</sup>, M.E. Da Silva-Grigoletto<sup>2</sup>, J.M. Garcia-Manso<sup>3</sup>, R. Vaamonde-Lemos<sup>1</sup>, S.C. Oehninger<sup>4</sup>

<sup>1</sup>University of Cordoba - Faculty of Medicine, Morphological Sciences, Cordoba, Spain

<sup>2</sup>Junta de Andalucia, Andalusian Center of Sports Medicine, Cordoba, Spain <sup>3</sup>University of Las Palmas Gran Canaria - School of Physical Activity and Sport Sciences, Physical Education Department, Las Palmas de Gran Canaria, Spain

<sup>4</sup>Jones Institute for Reproductive Medicine, Obstetrics and Gynecology, Eastern Virginia Medical School

Introduction: One of the sports modalities that has gained greater popularity in the last years is triathlon. Its practice demands high physical requirements from its participants, making the athletes undergo high training volumes and competitions of long duration in the modalities of cycling, swimming and running.

Among the different varieties of triathlon, the most extenuating is the Ironman, where athletes compete to swim 3800m, pedal for 180km, and run 42km; this means that high level athletes have to spend between 9 to 10 hours performing physical exercise in a continuous manner. It is easily understood that such competition imposes an elevated stress on the athlete's organism.

Previous studies have related this sports practice to semen alterations (Vaamonde et al., 2009), especially when training volume is high. The observations lead us to hypothesize that significant alterations in DNA may also be present. Therefore, the objective of the present study was to assess DNA fragmentation and semen parameters in high-level triathletes.

Material and Methods: Nine high-level triathletes voluntarily participated from the present study. Semen and training parameters were carefully analyzed. The subjects, aged  $27 \pm 3$  had been practicing triathlon for 5 years in average. They have a mean VO<sub>2</sub>max above 70 ml/kg/min. In brief, their mean annual training regime is as follows: 416km of swimming, 13000km of cycling, and 2600km of running. The mean values for the last week of training undertaken by the athletes composing the sample were: 4km of swimming, 220 km of cycling, and 14 km of running.

The triathletes were asked to keep the standard days of abstinence for proper semen assessment. Likewise, indications were also given regarding coffee, drugs, and alcohol consumption. In addition, and as a preventive measure, they considerably diminished training volume during the last week, and especially for two days before semen collection in order to avoid an acute effect on semen quality. On the day of semen collection, subjects completed a questionnaire ruling out any possible effect on the HPG axis for reasons other than sports practice.

Sample normality was assessed following standard WHO values for volume, concentration and total number, and velocity; morphology normalcy, on the contrary, was assessed following Kruger's strict criteria. For DNA fragmentation evaluation, we used the sperm chromatin dispersion (SCD) test.

Results: Mean values for volume, concentration, and velocity were among normal ranges. However, both morphology normalcy and DNA fragmentation showed abnormal values ( $5.3 \pm 2.7\%$ , and  $20.4 \pm 6.1\%$ , respectively). Additionally, we observed an excess of round cells above normal range in all samples but two

Conclusions: The altered value observed in the sperm fragmentation test supports the hypothesis that high loads of endurance training alter semen parameters and may interfere with the athlete's fertility potential. **References:** 

- Vaamonde D, Da Silva-Grigoletto ME, García-Manso JM, Vaamonde-Lemos R, Swanson RJ, Oehninger SC. Response of semen parameters to three training modalities. Fertil Steril. 2009 Dec;92(6):1941-6.

## P-075 The importance of carefully screening the ejaculate in non-obstructive azoospermic men

G. Walis<sup>1</sup>, D. Monahan<sup>1</sup>, Q.V. Neri<sup>1</sup>, E. Ermolovich<sup>1</sup>, Z. Rosenwaks<sup>1</sup>, G.D. Palermo<sup>1</sup>

<sup>1</sup>Weill Cornell Medical College, Ronald O. Perelman & Claudia Cohen Center for Reproductive Medicine, New York NY, U.S.A.

Introduction: At time of initial consultation for infertility, a semen analysis is a relevant component of the male work-up. When no spermatozoa are seen in a counting chamber, often a centrifugation of the specimen is required to confirm