

biomarkers and explain the molecular mechanisms underlying the altered spermatogenesis. The objective of the study is to evaluate the blood plasma miR-20a-5p expression in infertile patients with non-obstructive azoospermia (NOA) compared to healthy normozoospermic men.

**Materials and methods:** From January 2018 to December 2019, 24 infertile couples were prospectively enrolled. All the patients were included into two groups: Group 1 infertile men with NOA, Group 2 healthy normozoospermic men belonging to couples with female infertility tubal factor who achieved pregnancy using IVF or ICSI. The expression of circulating miR-20a-5p was assessed by RT qPCR in plasma samples. A relative quantification strategy was adopted using the  $2^{-\Delta\Delta Cq}$  method to calculate the target miR-20a-5p expression with respect to miR-16-5p as endogenous control. Total cell-free RNA extracted from 0.5 ml plasma using the mirVana PARIS kit was submitted to RT-qPCR using TaqMan Advanced miRNA cDNA Synthesis Kit and TaqMan<sup>®</sup> Advanced miRNA Assays.

**Results:** Group 1 included 14 patients, Group 2 10 men. Mean male age was  $35.6 \pm 4.2$  years. Considering the Group 1, mean FSH value was  $19.4 \pm 7.8$  IU/l, LH  $8.5 \pm 3.4$  IU/l, TT  $12.5 \pm 3.9$  nmol/l, TSH  $2.0 \pm 1.1$  mIU/l, PRL  $10.5 \pm 3.2$  ng/ml. Mean right and left testicular volume (TV) was  $8.9 \pm 5.2$  ml and  $8.2 \pm 4.5$  ml, respectively. Group 2 showed hormonal levels and TV in the normal range. All NOA underwent testicular sperm extraction. Successful sperm retrieval (SR) with cryopreservation was found in 8/14 patients (overall SR rate: 57.1%). Mean sperm concentration was  $0.001 \pm 0.0001 \times 10^6$ /ml, motility  $0.2 \pm 0.6\%$ , biosystem straws collected  $3.2 \pm 2.0$ . Mean miR-20a-5p value was  $0.25 \pm 0.20$  and  $0.06 \pm 0.02$  in the Group 1 and Group 2, respectively. Thus, the relative expression of miR-20a-5p was significantly higher in patients affected by NOA than in healthy normozoospermic control subjects ( $p = 0.026$ ).

**Conclusions:** Blood plasma miR-20a-5p could represent a potential non-invasive diagnostic biomarker in infertile patients with non-obstructive azoospermia. A possible correlation of this marker with testicular histopathological findings could allow the clinician to correctly counsel the azoospermic patients in performing surgery for fertility purpose.

### SC11

Experience of oxygen-ozonotherapy in the management of erectile dysfunction in diabetic patients poor responder to 5-phosphodiesterase inhibitors. Preliminary results and follow-up

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**Introduction:** Erectile dysfunction (DE) has higher incidence in diabetic patients. Microvascular damages, persistent oxydant activity of free radicals (ROS), endothelial dysfunction are at the basis of frequent poor outcome of 5-phosphodiesterase (5-PDE) inhibitors for DE in this particular category of patients. Endothelial nitric oxide synthase expression increased significantly with ozone therapy in some animal models. In this study we have evaluated the effects of systemic oxygen-ozonotherapy (OOT), defined autohaemotherapy (AHMT) as possible supplementary therapy in non responders to 5PDE inhibitors.

**Materials and methods:** The rationale of OOT is in hormetic properties, i.e. paradoxical antioxidant effects obtained at low concentration of oxygen-ozone combination, aiming the reduction of all free oxydative molecules, especially nitroxic and oxygen radicals. We have selected 13 patients aged 50–70 years affected by type 2 diabetes in association with ED. All patients are diabetic in good glycemic control. All patients, non responders to 5PDE inhibitors, have been submitted to 10 weekly applications of autohaemotherapy (AHTM): from every patient we have taken venous sampling of 200 ml of blood enriched with an ozone gas mixture, administered by a specific medical device with a

concentration between 30 and 70 mcg/ml then immediately reinfused. During AHTM cycle and 2 month follow up all patients have taken daily tadalafil 5 mg. All patients have been evaluated with IIEF15 before and after 2 months follow up. All patients and their partners have been also evaluated by psychosexual counseling with weekly sitting, at the beginning for the selection, during all phases of AHTM therapy and during 2 month follow-up.

**Results:** All patients improve quality and quantity of erections with a rising response to 5PDE inhibitors during the 2 month follow up. Psychosexual couple counseling also show qualitative improvement of sexual relations. AHTM has been well tolerated to all patients. No side effect has been observed during the entire cycle of therapy and the 2 month follow up. All patients refer a personal daily improvement of glycemic control without any variation of therapy or lifestyle.

**Conclusions:** OOT could be beneficial in reducing the negative effects of diabetes on erectile dysfunction as a result of enhanced enzymatic activity in endothelial factors and reducing the effect of ROS. Preliminary results need more studies with a wider number of patients. Endothelial nitric oxide synthase expression could increase significantly with OOT with more beneficial systemic effects, with minimal contraindications and no side effects. OOT could rise the quality and quantity of erections in diabetic patients, improving the outcome in the use of 5PDE inhibitors and in glycemic control. For diabetic patients, especially for poor responders to 5PDE inhibitors a multidisciplinary approach and psychosexual counseling demonstrate good synergy to improve sexual relations and quality of life.

### SC12

Mid-Term effects of high-altitude on sexual hormonal parameters during a Himalayan expedition

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**Introduction:** The aim of this study is to assess the mid-term effects of altitude hypoxia on sexual hormonal parameters, comparing Italian trackers with native Nepalese porters who took part in a Himalayan expedition.

**Materials and methods:** Participants completed a combined circuit of 300 Km distance in 19 days with over 16000 meters of difference in altitude and average daily walk of 6 hours involving a demanding route with ascent and descent in the Himalayas, Nepal. The analyses were performed on two groups of participants: 6 Italians and 6 Nepalese. The effect of high-altitude on Hypothalamus-hypophysis-gonads axis (FSH, LH, Testosterone, Progesterone) was assessed for both groups. Samples were collected the day before the expedition beginning and the day after it was completed. The Italians had an additional sample after 10 days (follow-up sample).

Shapiro-Wilk test, Q-Q plots, Levene's test for equality of variances, Repeated Measures - ANOVA. were adopted. Significance ( $p$  value), effect size (partial  $\eta^2$ ) were reported.

**Results:** Nepalese participants had LH values higher than Italians at Pre expedition evaluation ( $4.260 \pm 2.416$  mUI/ml vs  $2.728 \pm 1.004$  mUI/ml, respectively), that was increased at Post expedition in both groups ( $4.598 \pm 1.605$  mUI/ml vs  $3.262 \pm 1.605$  mUI/ml). Among Nepalese, we found a more important reduction in FSH concentration from Pre and Post expedition ( $5.840 \pm 2.001$  mUI/ml to  $5.054 \pm 2.215$  mUI/ml) respect to Italians (from  $5.106 \pm 1.483$  mUI/ml at Pre to  $4.958 \pm 0.999$  mUI/ml at Post and down  $5.070 \pm 0.961$  at Follow up). Testosterone concentration decreased from Pre to Post expedition ( $p = 0.109$ ,  $\eta^2 p = 0.260$ ,  $\omega^2 p = 0.164$ ) also showed by post-hoc analyses, with Nepalese participants having a greater reduction (from  $4.557 \pm 0.823$  to  $3.397 \pm 1.304$  ng/ml) than Italians (from  $4.864 \pm 1.675$  to  $4.354 \pm 0.948$  ng/ml). At Follow up, Italians increased the baseline values ( $6.570 \pm 1.376$  ng/ml), as revealed by the Pre vs Post vs Follow Up