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P-561 6:30 AM Wednesday, October 20, 2021

MELATONIN ATTENUATES TESTICULAR DYSFUNCTION AND SPERM DAMAGE INDUCED BY CHEMOTHERAPY REGIMEN BASED ON BLEOMYCIN, ETOPOSIDE, AND CISPLATIN (BEP): AN EXPERIMENTAL STUDY. Mojtaba Moradi, DVM Department of Clinical Sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran.

OBJECTIVE: To determine the protective effects of exogenous melatonin following exposure to Bleomycin, Etoposide, and Cisplatin (BEP) chemotherapy on sperm parameters and DNA integrity, testes nitro-oxidative status, as well as on histopathological, inflammatory, and apoptotic alternations in rats testes.

MATERIALS AND METHODS: Adult male Wistar rats were randomly segregated into six groups (n=10/group). Groups 1, 3, and 4 were intraperitoneally injected with the vehicle, 10 and 20 mg/kg of melatonin, respectively. Other groups received one cycle of 21 days of 0.5 therapeutically relevant dose levels of BEP (0.75 mg/kg Bleomycin, 7.5 mg/kg Etoposide and 1.5 mg/kg Cisplatin) with or without melatonin. Sperm parameters, including sperm count, motility, viability, morphology, and sperm chromatin integrity using aniline blue (AB), toluidine blue(TB), and Chromomycin A3 (CMA3) were analyzed. Besides, testosterone level, histopathology and stereology of testes, the levels of malondialdehyde (MDA), nitric oxide (NO), and total antioxidant capacity (TAC) in testes; the expression of apoptosis-related genes such as Bcl2, Bax, Caspase-3, p53, and TNF-α (Real-time PCR and Immunohistochemistry) were assessed.

RESULTS: According to our results, the quantitative analysis of the testes' stereological procedures, QRT-PCR examination, and immunohistochemical (IHC) staining revealed that melatonin reversed the BEP-induced compromised spermatogenesis (P<.05). In this regard, melatonin rectified BEP-induced disturbance on sperm count, motility, viability, morphology. Additionally, co-administration of 10 and 20 mg/kg of melatonin could restore BEP-induced alteration in sperm DNA methylation and DNA fragmentation as compared with the BEP group. Moreover, melatonin enhanced the antioxidant status of the testis by elevating TAC and ameliorating MDA and NO levels. More interestingly, QRT-PCR analysis revealed that melatonin therapy prevented BEP-induced apoptosis in the testis by attenuating apoptosis-associated genes such as Bcl-2, Bax, Caspase-3, and p53. (P<.01). In this continuum, the co-administration of 10 and 20 mg/kg of melatonin with the BEP regimen decreased significantly the population of p53 and TNF-α positive cells while augmented the expression of Bcl-2 protein in the spermatogenic cells line in comparison to the BEP group.

CONCLUSIONS: Together, BEP chemotherapy adversely affects testicular functions leading to compromise of sperm parameters and chromatin integrity. Although not completely, melatonin therapy contributes to preserving testes from BEP-evoked damages via preventing nitro-oxidative stress, inflammation, and apoptosis. Since our experimental design mostly resembles the chemotherapy protocol used in humans, these findings may have widespread clinical implications for minimizing chemotherapy-related male sub/infertility and shortening the fertility recovery time in patients receiving the BEP regimen.

IMPACT STATEMENT: BEP chemotherapy causes a high rate of male infertility and even long-term genotoxicity, which has emerged as a major public health concern.

SUPPORT: Kermanshah University of Medical Sciences, Iran and Razi University.

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THE USE OF FRESH COMPARED TO FROZEN EJACULATED SPERM HAS MINIMAL IMPACT ON FRESH EMBRYO TRANSFER CYCLE REPRODUCTIVE OUTCOMES. Nahid Punjani, MD MPH,[1] Pietro Bortoletto, MD,[2] Phillip A. Romanski, MD,[2] Caroline Kang, MD, PhD,[1] Steven Spandorfer, MD,[2] James A. Kashanian, MD[1] [1]Weill Cornell Medicine, New York, NY; [2]The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY.

OBJECTIVE: To compare the reproductive outcomes of utilizing fresh versus frozen ejaculated sperm in fresh embryo transfer (ET) cycles.

MATERIALS AND METHODS: Fresh ET cycles between 2013-2019 from a single institution were reviewed. All fresh, autologous, first time IVF cycles were included, and cycles using donor or surgically retrieved sperm were excluded. Maternal age was stratified as <35, ≥35 to <40 and ≥40 years. Sperm concentration was stratified as ≥15, <15 to ≥5, and <5 mil/ml. Outcomes included clinical intrauterine pregnancy (IUP), miscarriage and live birth. A multivariable logistic regression model was adjusted for developmental stage of embryo at time of transfer and paternal age.

RESULTS: A total of 6372 couples were included. Of these, 5957 (93.5%) utilized fresh ejaculated sperm and 415 (6.5%) frozen ejaculated sperm. The proportion using frozen sperm increased with lower sperm concentrations: 212 (4.0%) for ≥15 mil/ml, 54 (12.2%) for <15 to ≥5 mil/ml and 149 (24.4%) for <5 mil/ml. On multivariable logistic regression, fresh ejaculated sperm among those with maternal age >40 and sperm concentrations of <15 to ≥5 mil/ml, were associated with greater odds of a clinical IUP (see Table). No significant differences were observed for miscarriage or live birth.

CONCLUSIONS: For couples conceiving via fresh ET, the use of fresh versus frozen sperm has minimal impact on cycle outcome unless the male has an abnormal sperm concentration (<15 to ≥5mil/ml) and female is >40 years but no impact on live birth rates.

IMPACT STATEMENT: The use of fresh or frozen ejaculated sperm has minimal impacts on outcomes in fresh ET cycles stratifying for sperm concentration and maternal age.

Table – Cycle outcomes of fresh vs. frozen (referent) ejaculated sperm

	<35		≥35-40		>40	
Maternal Age	OR	95%CI	OR	95%CI	OR	95%CI
Concentration ≥15 (n=5318)						
Clinical IUP	1.08	0.64-1.80	1.11	0.67-1.82	0.74	0.45-1.22
Miscarriage	2.15	0.52-9.00	1.21	0.43-3.39	0.56	0.28-1.12
Live Birth	0.92	0.55-1.53	1.03	0.62-1.72	0.93	0.51-1.69
Concentration 15 to ≥5 (n=444)						
Clinical IUP	1.26	0.42-3.74	1.70	0.56-5.14	**13.30**	**1.41-125.46**
Miscarriage	0.56	0.11-2.89	-	-	-	-
Live Birth	1.55	0.52-4.65	1.23	0.40-3.72	5.99	0.72-50.09
Concentration <5 (n=610)						
Clinical IUP	0.80	0.50-1.28	0.91	0.56-1.47	0.68	0.41-1.13
Miscarriage	0.87	0.37-2.07	0.73	0.30-1.78	0.69	0.27-1.67
Live Birth	0.83	0.52-1.34	0.96	0.59-1.56	0.72	0.43-1.21

York Community Trust

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A DOUBLE-BLIND, PROSPECTIVE TEST DEMONSTRATES AN ASSOCIATION BETWEEN CAPACITATION STATUS AND PREGNANCY. Bryan Kloos, PhD,[1] Andrew J. Levi, MD,[1] Kristen Bender, MS,[1] G Charles Ostermeier, PhD[2] [1]Park Avenue Fertility, Trumbull, CT; [2]Androvia LifeSciences, Mountainside, NJ.

OBJECTIVE: Semen Analysis (SA) often fails to predict fertility, apart from extreme cases, highlighting the need for advanced sperm testing. Cap-Score™ is a validated test that uses changes in G_{M1} localization patterns to identify sperm that can and cannot capacitate. Since capacitation is requisite for fertilization, men must produce sufficient sperm with this ability for pregnancy generation. The purpose of this study was to use a double-blind prospective analysis to evaluate how predictive a previously defined Cap-Score reference range (Cardona, et al. 2017) was, of male fertility.

MATERIALS AND METHODS: Cap-Score and SA were performed (n=107) with clinical Intrauterine Insemination (IUI) outcomes available for 24 at the time of analysis. The chance of pregnancy outcome was predicted as either low (n=9) or normal (n=15), based solely on the previously defined reference range. IUI was done blinded to Cap-Score evaluation. Absolute and cumulative pregnancy rates were compared over 1-3 rounds of IUI using a Kaplan-Meier survival analysis. Semen analysis measures were

compared between pregnant (n=8) and not-pregnant (n=16) groups using weighted t-tests, with the weights assigned by the number of IUI rounds.

RESULTS: Men having low Cap-Scores showed reduced absolute and cumulative pregnancy outcomes (absolute: predicted low [0%] vs. predicted normal [53%; p=0.001]; cumulative predicted low vs normal: 0 vs 33, 0 vs 58, and 0 vs 58% for cycles 1, 2, and 3 [n=24, 11, and 4 rounds of IUI; p=0.025]). Only Cap-Score (35.4±1.7 vs 31.3±2.2; p=0.04) and motility (81.1±2.2 vs 73.9±3.5; p=0.02), differed between the pregnancy groups. No differences were detected between these groups in semen volume (p=0.47), sperm concentration (p=0.83), total motile sperm (p=0.84), or in male (p=0.07) and female age (p=0.06).

CONCLUSIONS: Cap-Score was associated with a man's chance of generating a pregnancy, substantiating previous work (Schinfeld, et al. 2018). While motility differed between the pregnancy groups, all men were above the 40% WHO cut-off. All pregnancies occurred within the first two rounds of IUI within the normal Cap-Score group. Quickly identifying men with reduced fertility, rather than after multiple failed IUI attempts, was modeled to improve outcome and save money (Babigumira, et al 2018). Several studies support the improvement of capacitation through lifestyle changes and(or) surgical intervention. Thus, depending on time, resource and treatment goals, multiple options are available for men with reduced Cap-Scores beyond intracytoplasmic sperm injection.

IMPACT STATEMENT: Accurately identifying male fertility is critical in the treatment of the couple seeking fertility assistance. The sooner patients are on the correct treatment path, the better their experience and expected outcome.

Reference

Cardona, et al. 2017. Mol Repro and Devel. 84(5):423-435.
Schinfeld, et al. 2018. Mol Repro and Devel. 285(8-9):654-664
Babigumira, et al. 2018. JARG 35:99-106

SUPPORT: Androvia LifeSciences provided Cap-Scores.

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ICSI OUTCOMES IN INFERTILITY MALE WITH MULTIPLE MORPHOLOGY ABNORMALITIES OF THE SPERM FLAGELLA CAUSED BY *DNAH1* MUTATION.

Ke Feng, MM., Haibin Guo, MD. Henan Provincial Peoples's hospital.

OBJECTIVE: To investigate the intracytoplasmic sperm injection (ICSI) outcomes in infertility male with multiple morphology abnormalities of the sperm flagella (MMAF) caused by *DNAH1* gene mutation.

MATERIALS AND METHODS: A total of 39 patients with multiple morphology abnormalities of the sperm flagella (MMAF) were analyzed retrospectively in the Reproductive Medicine Center of Henan Provincial People's Hospital from February 2018 to January 2020. 12 out of 39 patients were clearly caused by *DNAH1* gene mutation, including 10 patients with compound heterozygous mutation and 2 patients with homozygous mutation. The other 27 patients didn't have known genes causing MMAF. According to whether caused by *DNAH1* gene mutation or not, they were divided into *DNAH1* positive group and *DNAH1* negative group. 100 cases of male infertility patients with ICSI treatment during the same period were chose as control group. The differences in ART cycles and pregnancy outcomes were analyzed between study groups.

RESULTS: There were statistically significant differences in numbers of both eggs and M II eggs between the *DNAH1* positive group, the *DNAH1* negative group and the control group (17.08±5.32, 9.59±3.98, 10.44±6.33 , $P < 0.05$; 14.58±5.18, 6.78±3.38, 8.32±5.31 , $P < 0.05$). There was no statistically significant difference in implantation rate, clinical pregnancy rate, the embryo miscarriage rate and the live birth rate (68.42% VS 54.35% VS 58.54% , $P > 0.05$; 75% VS 66.67% VS 76%, $P > 0.05$; 16.67% VS 16.67% VS 21.05%, $P > 0.05$; 58.33% VS 55,56% VS 58%, $P > 0.05$). 9 out of 12 couples of male infertility caused by *DNAH1* mutation received a total of 9 cycles of egg extraction, forming 71 D3 embryos, 9 times of the first fresh or frozen embryo transplantation, and 10 biological offspring were obtained.

CONCLUSIONS: DNAH1 gene mutation do not reduce the ICSI outcome of MMAF patients, including implantation rate, clinical pregnancy rate, and live birth rate

IMPACT STATEMENT: ICSI is an effective technology for patients with MMAF caused by *DNAH1* gene mutation to obtain their biological offspring.

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OXIDATIVE STRESS TESTING AND ANTIOXIDANT TREATMENT OF MALE INFERTILITY – SURVEY OF CURRENT CLINICAL PRACTICES.

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OBJECTIVE: To determine the pattern of prescribing oxidative stress (OS) tests and antioxidants (AOX) treatments in the clinical management of male infertility.

MATERIALS AND METHODS: We created an internet survey as a data collection tool to investigate the use of OS testing, and AOX utilization by reproductive specialists involved in the care of male infertile patients. Also, scientific literature was analyzed to identify the top 100 most cited articles related to the topic, where we analyzed the percentage of studies investigating OS markers along with AOX treatment.

RESULTS: A total of 1,305 participants from 88 countries responded to the survey, with only 34.3% reporting OS testing in clinical practice (Table 1). Of these, 59% and 55.8% recommended OS testing for patients with idiopathic and unexplained infertility, respectively. Lifestyle related risk factors were recorded as the commonest indications for OS testing (i.e. smoking, alcohol consumption or drug abuse/misuse, 74.0%; advanced paternal age, 66.1%).

A total of 1,260 responded to the question on the AOX prescription, with 85.3% of participants recommending AOX as a therapeutic option (Table 1). Surprisingly, of those participants who did not use OS testing as a part of male infertility evaluation (n=857), a high percentage (79.2%) reported prescribing AOX treatment. These results agreed with the analysis of the scientific literature based on the citation rate, where OS testing and sperm DNA damage testing were carried out only in 51% and 23% of the clinical trials included (35 and 16 out of 69, respectively).

CONCLUSIONS: OS is a well-established cause of sperm dysfunction and it contributes to male infertility. However, its investigation is widely neglected in the clinical management of male infertility, where empirical AOX treatment is commonly practiced. The same trend is reported in scientific research, where a large number of publications investigate the effects of AOX treatment on male fertility outcomes without analyzing OS markers.

IMPACT STATEMENT: Our study highlighted a potential clinical concern as, without testing for OS, there exists the risk of not identifying the male population which may really benefit from AOX treatment.

SUPPORT: This research was supported by the American Center for Reproductive Medicine, Cleveland Clinic.

Table 1. Online survey responses of practitioners on oxidative stress testing, and antioxidant prescription.

	Continent					
Variable	Asia	South America	Europe	Africa	North America	Australia
OS testing (n=447)	148 (33.1%)	135 (30.2%)	93 (20.8%)	39 (8.7%)	16 (3.6%)	16 (3.6%)
AOX prescription (n=1075)	480 (44.7%)	227 21.1%)	171 (15.9%)	129 (12.0%)	41 (3.8%)	27 (2.5%)

* Results are expressed as frequencies and percentages.