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PP033

PROTEIN TYROSINE PHOSPHORYLATION OF HUMAN SPERM HEAD DURING CAPACITATION: IMMUNOLOCALIZATION AND RELATIONSHIP WITH THE ACQUISITION OF SPERM FERTILIZING ABILITY

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The capacitation-related increase in global tyrosine phosphorylation (TP) of sperm proteins, mainly distributed along the flagellum, does not necessarily reflect the acquisition of sperm fertilizing ability (Biol Reprod, 79:649-56, 2008). This study aimed to localize TP of head proteins in human spermatozoa during capacitation and to explore its relationship with the acquisition of the sperm ability to undergo acrosome reaction (AR) and to fuse with the oocyte in response to progesterone (P). With immunofluorescence, TP-immunoreactivity (IR) could be revealed in the acrosomal region, only in fixed/unpermeabilized samples, whereas, fixation and permeabilization, although strongly improved IR of the principal piece, completely abolished head IR. No IR could be detected in unfixed spermatozoa. The head TP-IR appeared to be sub-superficial when assessed by transmission electron-microscopy, using pre-embedding procedure and a sensitive peroxidase system (Envision®).

The increase in head TP was an early event during capacitation. The percentage of positive spermatozoa significantly increased from 18.7±5% to 54.6±6% at 1h-capacitation. At this time, the P-induced ARs were significantly increased (15.6±1.5% vs. 4.6±0.7%), whereas sperm/oocyte fusion, evaluated with the hamster egg penetration test, was as poor as in uncapacitated spermatozoa (penetrations/oocyte=0.8±0.4 vs. 0.1±0.06). At 5h-capacitation, both head TP and P-induced ARs were not significantly increased over 1h, whereas penetrations/oocyte significantly increased (4±1.1). Seminal plasma (SP) inhibited head TP, P-induced AR and oocyte fusion. None of these effects were prevented by the cAMP analogue, dbcAMP, which, instead, prevented SP inhibition of tail TP, as recently reported (Biol Reprod, 79:649-56, 2008).

In conclusion, head TP is a subsuperficial event early occurring during capacitation and tightly related to the sperm responsiveness to P in terms of AR but not in enhanced oocyte fusion, a later capacitation-related event.

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PP034

CAR EXHAUST POLLUTION DAMAGES HUMAN SPERM CHROMATIN AND DNA.

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In the past decades, many studies have shown that environmental pollution impairs sperm quality. Several occupational surveys reveal that exposure to inorganic lead (Pb) and other heavy metals (cadmium, Cd, aluminium, etc.) reduces sperm count, decreases the fertility rate and increases spontaneous abortion. The mechanism of male reproductive toxicity has not been fully characterised. A direct effect on the testicular function is supported by the ability of Pb and Cd to accumulate in reproductive organs, including mature spermatozoa. It has also been shown that Pb competes or even replaces the zinc of human protamine, thus negatively interfering with sperm chromatin condensation. In addition, heavy metals are able to increase the oxidative stress which not only oxidases cell membranes, but may damage sperm DNA. Given these potential genotoxic effects of heavy metals, we taught of interest to evaluate sperm chromatin/DNA integrity in workers exposed to car exhaust who have been reported to have sperm parameter abnormalities. To accomplish this, sperm chromatin packaging and DNA fragmentation were evaluated in 18 tollgate workers and in 17 unexposed age-matched controls. All of them underwent lifestyle interview, LH, FSH, testosterone, methaemoglobin (MHb), sulphaemoglobin, carboxyhaemoglobin and lead measurement and semen analysis evaluation. Sperm chromatin and DNA integrity were evaluated by flow cytometry following propidium iodide staining and TUNEL assay, respectively. Statistical analysis was carried out by unpaired Student's t test. Tollgate workers had normal LH, FSH, testosterone and sperm count. Their total and progressive motility were significantly lower compared to controls. In the subset of men with asthenozoospermia, MHb inversely correlated with total motility. Tollgate workers had a significantly higher percentage of spermatozoa with damaged chromatin and DNA fragmentation, a late sign of apoptosis, compared to controls. A significant direct correlation was found between spermatozoa with fragmented DNA and the length of occupational exposure, suggesting a time-dependent relationship. In conclusion, this study showed that car exhaust exposure has a genotoxic effect on human spermatozoa. This may be of relevant importance not only for the reproductive function of the men exposed, but also for the offspring health.

PP035

ALTERED ULTRASTRUCTURE OF THE SPERM MIDDLE-PIECE IN OTHERWISE NORMAL TAILS IS ASSOCIATED TO SEVERE ASTHENOZOOSPERMIA

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Mitochondria in the sperm middle piece deliver adenosine triphosphate (ATP) necessary for sperm movement. In the human sperm middle piece they are arranged in a helix of 11-13 gyri, with two mitochondria per girus. The contribution of an altered middle piece to human asthenozoospermia is undefined. Neither the prevalence of structural defects of the sperm middle piece among infertile men nor their possible genetic origin is at present known. This prompted to undertake a retrospective quantitative ultrastructural evaluation of ejaculates from the male partner of subfertile couples featuring different degree of asthenozoospermia. Fifthly five ejaculates were so far analyzed. Exclusion criteria were vitality < 50%, and diffuse ultrastructural degenerative changes including membrane fragmentation associated to disorganization of tail structures. Twenty-five ejaculates showed <10% forward motility (FM) and 30 ejaculates had a FM >10% (FM>10). Among the 25 severely asthenozoospermic samples 15 cases included total sperm tail defects of genetic origin [primary cilia diskinesia (PCD) and dysplasia of the fibrous sheath (DFS)] (systematic defects). Three cases with FM=0% showed a total agenesia of the middle piece, in one case only associated to DFS. NM were reduced in severe asthenozoospermia with and without systematic tail defects compared to FM>10 (28%±25; 18%±13; 42%±21 respectively); the difference was greatly significative in the group with no systematic defects compared to FM>10 (p<0.01). TDM and PDM were not significantly different among groups, while % mitochondria with normal inner structure was significantly lower in the group with no systematic defects compared to FM>10 (26%±19; 54%±22; p<0.01). In the whole study group, forward motility was significantly correlated with %normal middle piece (r=0.53; p<0.0001) and with % mitochondria with normal inner structure (r=0.57; p<0.0001). Mitochondrial defects, mostly consisting in altered inner structure, are significantly increased in severe asthenozoospermia not associated to systematic sperm tail defects, suggesting that isolate defects of mitochondria in the sperm middle piece may be responsible for asthenozoospermia in selected cases. Whether or not they represent an intrinsic or an acquired reversible defect, and their possible association with definite biochemical defects is a matter of ongoing investigations.

PP036

DIFFERENT TESTOSTERONE LEVELS ARE ASSOCIATED WITH EJACULATORY DYSFUNCTION

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Objectives. To evaluate possible contribution of testosterone and hypogonadism in the control of the ejaculatory reflex, comparing subjects with premature ejaculation (PE) or delayed ejaculation (DE) to those without ejaculatory dysfunction.

Material and Methods. A consecutive series of 2437 (mean age 51.9±13.0 years) male patients with sexual dysfunction was studied. Several hormonal and biochemical parameters were studied, along with SIEDY structured interview. Hypogonadism were defined when total testosterone (TT) was lower than 10.4 nmol/l.

Results. Among the patients studied, 714 (25.9%) and 121 (4.4%) reported PE and DE, respectively In the youngest age band (25-40 years), subjects with PE reported higher FT and free testosterone (FT) levels when compared to the other groups (subjects with DE or those without PE and DE; p<0.05 for both). Conversely, in the oldest age band (55-70 years), lower TT and FT levels were observed in DE subjects. Accordingly, patients with PE showed the lowest (12%) and subjects with DE the highest (26%) prevalence of hypogonadism. These differences were confirmed even after adjustment for confounders such as age and libido (HR= 0.75[0.57-0.99] and 1.83[1.14-3.94] for PE and DE respectively; both p< 0.05).

Conclusions. Our data seem to suggest that testosterone plays a facilitator role in the control of ejaculatory reflex. Both central and peripheral mechanisms have been advocated to explain this association. Clinical studies are currently in progress to further establish the role of testosterone in the ejaculatory dysfunction, attempting to revert DE by androgen administration.