In vivo exposition to 17B-estradiol cause premature capacitation of epididymal mouse sperm

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Abstract

Estrogens play a crucial role in spermatogenesis, and estrogen receptor alpha knock-out male mice are infertile. Increasing evidence suggests that compounds with estrogen activity also significantly influence the sperm physiological processes. It has been demonstrated that estrogens significantly increase the speed of capacitation *in vitro*, however, this may lead to the reduction of reproductive potential due to the decreased ability of these sperm to undergo the acrosome reaction. Up to present the in vivo effect of estrogens on the ability of sperm to capacitate has not been investigated. Therefore, in this study, we exposed mice to 17B-estradiol (E2) at the concentration of 20 ng/ml either during puberty from the 3rd to 8th week of age (group A), or continuously from birth for a period of 12 weeks (group B) at which age the animals from both groups were sacrificed. According to our results, *in vivo* exposition to increased E2 concentrations caused premature sperm capacitation in the epididymis. The effect of E2, however, seems reversible, because in animals exposed only during puberty, the premature epididymal sperm capacitation is decreased compared to the continuously exposed group. After subsequent *in vitro* capacitation, the differences between the control and experimental groups were mainly caused by the initial difference between the numbers of capacitated sperm at the start of the capacitation. Therefore, our data implicates, that *in vivo* exposition to E2 under specific conditions leads to the premature capacitation of mouse sperm in epididymis with a potential negative impact on the sperm reproductive fitness in the female reproductive tract.

Methods

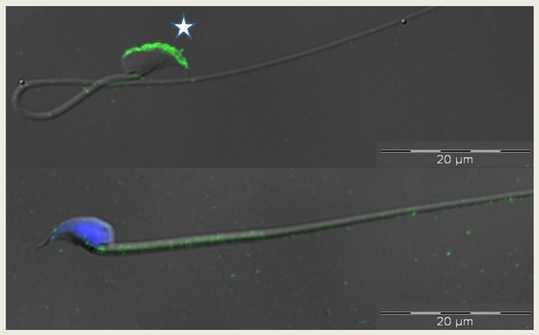

Figure 1: Immunofluorescent detection of tyrosine phosphorylation in mouse spermatozoa. Tyrosine phosphorylation (green) over the apical acrosomal region of the sperm head and in the mid and principal piece of the sperm tail. Positive sperm head labeling showed by asterisk. Nuclei are counterstained with DAPI (blue). Scale bar represents 20 µm.

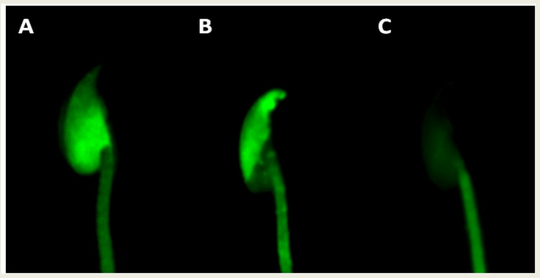

Figure 2: Patterns of fluorescence in CTC staining. A) Bright fluorescence over the entire head with a brighter line of fluorescence across the equatorial segment (pattern F, noncapacitated sperm). B) Bright fluorescence in the anterior segment of the head with absent fluorescence over the equatorial and postacrosomal segments (pattern B, capacitated sperm). C) Low or absent fluorescence over the entire head (pattern AR, acrosome-reacted sperm)

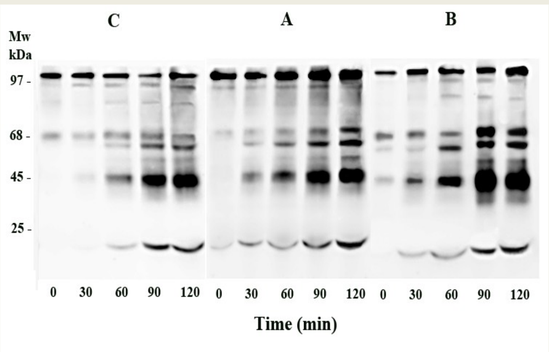

Figure 3: Western blot analysis of protein phosphorylation during in vitro capacitation detected by anti pY antibody. Control group (C) without in vivo exposition, group A after pubertal and group B after permanent exposition. The higher phosphorylation of proteins at the start of capacitation (bends around 68 and 45 kDa) in group B is visible. The highest cumulative protein phosphorylation in subsequent capacitation intervals is visible.

Main results

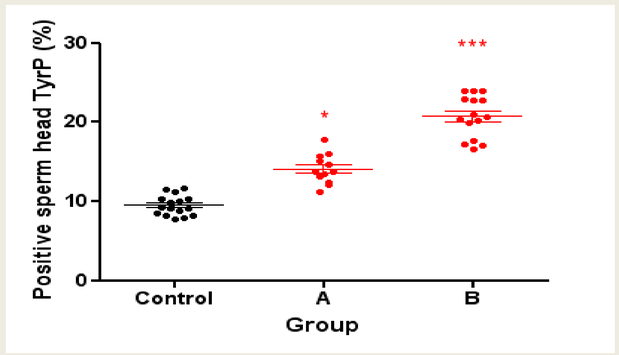

Figure 4:Number of capacitated epidymidal sperm detected by anti pY antibody. Control group without *in vivo* exposition, group A after pubertal and group B after permanent exposition. Bars denote arithmetical means of capacitated cells (%), whiskers denote standard error (SE), and points denote individual measurements. The differences have been analysed by KW-ANOVA, post hoc analysis was performed by Dunns comparisons. *p < 0.05, **p < 0.01, ***p < 0.001.

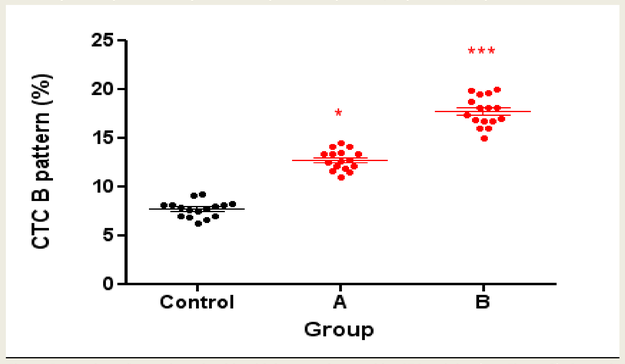

Figure 5: Number of capacitated epidymidal sperm detected by CTC staining (see Figure 3). Control group without *in vivo* exposition, group A after pubertal and group B after permanent exposition. Bars denote arithmetical means of capacitated cells (%), whiskers denote standard error (SE), and points denote individual measurements. The differences have been analysed by KW-ANOVA, post hoc analysis was performed by Dunns comparisons. *p < 0.05, **p < 0.01, ***p < 0.001.

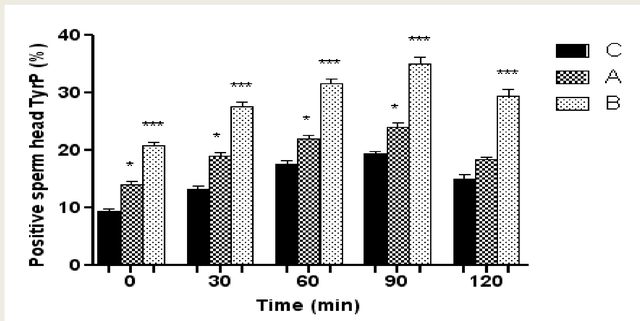

Figure 6: Number of capacitated sperm after 0, 30, 60, 90 and 120 min of in vitro capacitation. Capacitation progress was measured by anti pY antibody (Figure 1). (C) Control samples without *in vivo* exposition, (A) Experimental samples after pubertal exposition, (B) samples after permanent exposition.

Further analysis

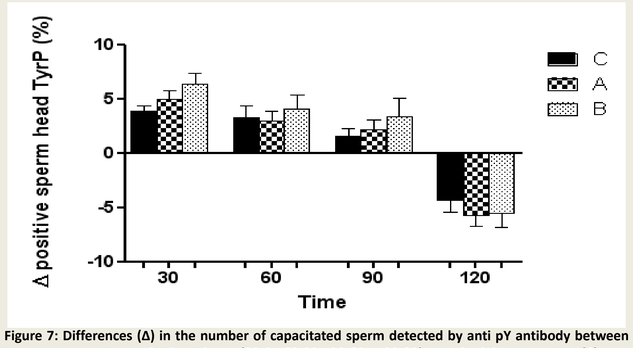

Figure 7: Differences (Δ) in the number of capacitated sperm detected by anti pY antibody between two subsequent capacitation times (30-0; 60-30; 90-60; 120-90) and individual control (C) and experimental (A, B) groups. No statistically significant differences detected by KW-ANOVA and Dunns post-hoc test. Highest difference between (Δ) control and experimental group (B) is in Δ30-0 timepoint (p=0.067).

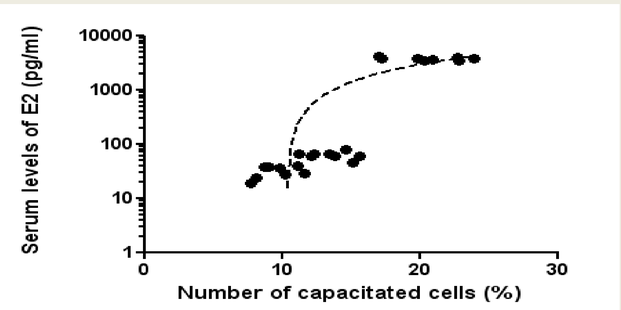

Figure 8: The correlation between the number of capacitated epidymidal sperm (time 0 of the capacitation) detected by anti pY antibody and plasma levels of E2 detected by RIA assay. The correlation coefficient r = 0.7437, p ≤ 0.001. Each data point represents the measurement of both parameters from one animal (N=24). The serum concentration of E2 is plotted on a logarithmic scale.

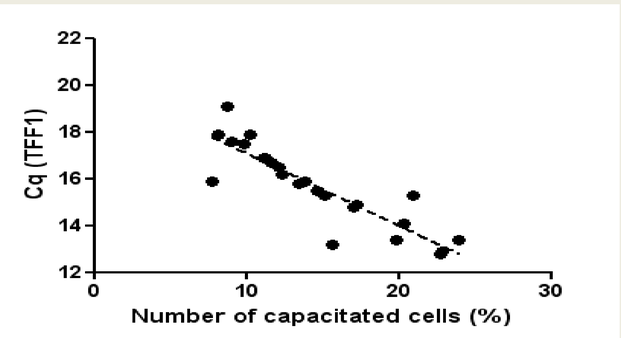

Figure 9: The correlation between the number of capacitated epidymidal sperm (time 0 of the capacitation) detected by anti pY antibody and expression of the estrogen-dependent TFF1 gene. The correlation coefficient r = -0.8814, p ≤ 0.001. The expression of the TFF1 gene is expressed as the normalised C_q values. The lowest C_q number indicates a higher expression of the TFF1 gene in testicular tissue. Each datapoint represents the measurement of both parameters from one animal (N=24).

Conclusion

Our data implies that in vivo exposition to E2 leads to premature "capacitation" of mouse sperm in cauda epididymis with a further potential negative impact on sperm reproductive fitness in the female reproductive tract. This effect is caused mainly by hyperphosphorylation of sperm proteins and premature calcium influx. These processes lead to the decreased ability of sperm to undergo acrosome reaction. Based on this evidence, in vivo exposition to E2 can lead to the reduction of the fertilizing potential in male mice with a further correlation to a possible effect of endocrine-disrupting compounds with estrogenic activity present in the environment.

Acknowledgement

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