E497

Influence of α_2-agonists on manual semen collection in standing white rhinoceros (*Ceratotherium s. simum*)

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The demographic crisis of the captive white rhinoceros (*Ceratotherium simum*) population has led to a multi-institutional research project in order to overcome the low reproductive rate. Safe and reliable semen collection is required for the application of reproductive technologies, such as artificial insemination. To date, the common technique to obtain semen samples in rhinoceros is electroejaculation in anesthetized animals. The objective of this study was to provide a less invasive procedure that would allow repeated deseminations in this species. Based on successful pharmacologically induced ex copula ejaculations in stallions using α-adrenergic agents, a study was conducted on two rhino bulls (age 12 and 30 years) at Zoo Salzburg and Allwetterzoo Münster. Both males were conditioned to tolerate manual stimulation of the genital region.A combination of the α_2-agonist detomidine-hydrochloride (Domosedan®) and the partial opioid agonist butorphanol (Butomidor®) was administered i.m. To evaluate the influence of these α-adrenergic agents on manual semen collection the trials were carried out double-blind. The medication and a placebo (sterile water) were applied in a random fashion. Manual stimulation consisted of genital massage in cases where no penile protrusion occurred within 15 minutes following injection in order to induce the onset of penile erection. The stimulation of the erect penis was performed by manual compression of the glans and the base of the penis. Seminal fluids were collected into pre-warmed graduated containers and assessed for standard semen parameters. Behavioral patterns and objective parameters such as erection strength, time till erection onset etc. were noted for each trial. The medication had a significant positive influence on onset and duration of penile erection in both males ($p<0.05$; t-test). Additionally a superior degree of erection was achieved using the α-adrenergic agents. Due to the sedative effect of the applied medication, both animals were less nervous and more quiet standing during the manipulation. Seminal fluids of varying quantity and quality could be obtained in the medicated trials from both individuals, but more often from the younger male. In this study, the application of α_2-agonists facilitated the manual semen collection. Further investigations are necessary to assess the reliability and to enhance this method.

Regulation of molecular changes in canine spermatozoa during interaction with homologous and heterologous oviductal epithelial cells

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Immediately after ejaculation sperm are not able to fertilize the egg. They have to undergo further processes of maturational changes, collectively called capacitation. Capacitation represents a series of cellular and molecular changes priming sperm for the acrosome reaction and fertilization. A crucial capacitational event is the phosphorylation of membrane proteins described for several mammalian species. Essential steps of the capacitation process take place in the oviductal isthmus. In this region sperm are stored in close contact to the oviductal epithelium and the progress of capacitation is coordinated with ovulation. Regulation of the tyrosine phosphorylation by the oviduct has not been examined in dog sperm yet. The aims of this work were 1) to study the effect of dog sperm binding to oviductal epithelium on tyrosine phosphorylation and 2) to investigate the specificity of regulation of molecular changes by the oviduct of different species comparing the numbers of sperm bound to heterologous (porcine) and homologous epithelium and the kinetics of tyrosine phosphorylation. Semen was collected from 4 healthy dogs of the institutes colony and washed through a Percoll gradient. For coincubation of sperm with the oviductal epithelium a complete bicarbonate Tyrode's medium has been used. Little pieces of epithelium, explants, were cut out of porcine and oestrous bitch oviducts. During 6 h of coincubation, numbers of bound sperm (counted by microvideography) and the state of tyrosine phosphorylation (using mouse anti-phospho-tyrosine 1G2 and a Cy 3-conjugated anti-mouse IgG) were determined after 3, 30, 90, 180 and 360 min. Distribution and intensity of Cy3- fluorescence were analysed by fluorescence microscopy. An increasing tyrosine phosphorylation of tail membrane proteins and a subsequent phosphorylation of head membrane proteins were observed. A significant difference ($p<0.05$) was found between sperm bound to oviductal epithelium and unbound spermatozoa. Binding occurred mainly in sperm with non- phosphorylated heads (~2 % phosphorylated), while higher proportions of phosphorylated cells were found in unbound populations (~40-60 %). The rate of tyrosine phosphorylation of tail membrane proteins increased faster in cells bound to explants than in unbound cells or cells incubated in control medium. The head phosphorylation progressed significantly during incubation in unbound spermatozoa ($p<0.05$); however, it was suppressed in bound suspensions. Canine sperm bound in similar numbers to homologous and heterologous explants. There were no significant differences with respect to kinetics of tyrosine phosphorylation between the two coincubation systems. These observations support the hypothesis that spermatozoa with non-phosphorylated heads preferentially attach to epithelial cells. It can be concluded that tyrosine phosphorylation of head membrane proteins and capacitation are delayed in spermatozoa being in closed contact with oviductal epithelium. This mechanism appears to be species-independent, as sperm bound similarly to pig and dog oviduct explants and similar phosphorylation kinetics were observed.