Comparative Genomic Identification and Expression Profiling of CatSper Genes in the Reproductive Tract of the Bull

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Abstract

Cation channel of sperm (CatSper), are sperm-specific calcium channels involved in the regulation of sperm function and male fertility of many species. We hypothesised that CatSper channels are present in bull sperm and play a role in hyperactivation and rheotaxis. Results found all four CatSper genes to be conserved in the bovine genome, and have a variation in expression along the bull reproductive tract. This study also shows that hyperactivation and rheotaxis are dependent on the entrance of extracellular calcium, potentially via CatSper channels in bull sperm.

1. Introduction

Cation channel of sperm (CatSper) are weakly voltage-dependent, Ca²⁺ selective, pH-sensitive ion channels that control the entry of positively charged calcium ions into sperm cells¹. CatSper channels localize to the principal piece of the sperm flagellum. CatSper channels are involved in the regulation of sperm function and male fertility of many species including human, mouse and equine. Hyperactivation, characterised by increased sperm flagellar bend amplitude and asymmetry, has been shown to be dependent upon the presence of CatSper channels in mice².We hypothesised that CatSper channels are present in bull sperm and they play a role in hyperactivation and rheotaxis.

The aim of this study was to use a comparative genomics approach to identify and characterise the evolutionary orthologs of CatSper genes in the bovine genome and to investigate the effect of CatSper agonists and antagonists as well as extracellular calcium on bull sperm hyperactivation and rheotaxis.

2. Materials and Methods

2.1 Bioinformatic identification of bovine CatSper orthologs

Orthology searches using the basic local alignment search tool (BLAST) was performed for known human and mouse CatSper gene sequences (CatSper 1-4) in the bovine genome (version: bosTau8). The bioinformatic tool, BLAST-Like Alignment Tool (BLAT), was used to determine the chromosomal position of the four orthologous bovine genes identified. Phylogenetic analysis was also performed to investigate the evolutionary relationships between the four novel bovine CatSper genes and their evolutionary orthologs using MEGA software. A multiple sequence alignment for all genes was performed using T-coffee and annotated using Jalview.

2.2 Tissue collection and quantitative real-time polymerase chain reaction (qPCR)

To characterise the expression profile of these novel genes, bull reproductive tract tissues, including parenchyma testis, rete testis and the different segments of the epididymis (caput, corpus, cauda) were collected from sexually-mature beef bulls (n=4) within 10 min of slaughter. All tissue samples were immediately snap frozen in liquid nitrogen and transported to the laboratory for RNA extraction and cDNA synthesis. Quantitative real-time polymerase chain reaction was performed using a 20 µL reaction mix containing 10 µL SYBR green PCR MasterMix.

2.3 Sperm preparation

Frozen-thawed bull sperm was treated with one of, or a combination of; high extracellular pH (7.25, 7.75, 8.25, 8.75 and 9.25), 5mM caffeine (CatSper agonists), 5µM nöbebradil (CatSper antagonist) and 2mM ethylene glycol tetraacetic acid (EGTA; chelates calcium) for 10 min prior to the assessment of hyperactivation and rheotaxis.

2.4 Hyperactivation and rheotaxic response

Hyperactivation, characterised by high amplitude, asymmetrical beating pattern of the sperm tail, was assessed using a phase-contrast microscope (100 motile sperm assessed per treatment).

In order to assess sperm rheotactic response sperm were loaded into the starting well (50 µL at a concentration of 20 x 10⁶ sperm/mL) of a specialised microfluidic device (channel size of 300 µm wide, 100 µm deep and 30nm in length) with a flow rate of 30µm/sec. The number of sperm which swam passed the 10mm mark in the channel at 10 min were assessed. All experiments were replicated a minimum of three times.

2.5 Statistical analysis

Functional data were analysed using one way ANOVA, while qPCR Data were analysed using univariate ANOVA (SPSS, v. 22).

3. Results
3.1 Bioinformatic analysis

CatSper 1–4 genes were found to be present on Chromosome 29, 21, 7, and 2 respectively in the bovine genome. Phylogenetic analysis showed that orthologous genes can be confidently predicted with bootstrap values generally in excess of 90%. A bootstrap value of 100 indicates that the sequences below that node consistently cluster together even with multiple resamplings of the data. They are thus likely to be orthologs because their similarity is systemic and internally consistent rather than dependent on a few similar sites in the alignment.

3.2 Expression of CatSper 1-4 channels along bull reproductive tract

A significant effect of tissue location was detected for expression of all four of the CatSper genes, with CatSper 1-4 upregulated in the parenchyma testis compared to the three segments of the epididymis (P<0.01). For CatSper 1-4 the rete testis had significantly higher expression than the caudal and corpus epididymis (P<0.01) however, no difference in expression level between it and the caput epididymis or the parenchyma testis (P>0.05) was detected. The caudal epididymis had lower expression of all four CatSper genes than the parenchyma and rete testis (P<0.01).

Fig 1: Variation in expression of CatSper 1-4 along the bull reproductive tract.

3.3 Hyperactivation and rheotaxic response

Initially, caffeine and pH 8.75 were found to be equally effective, however, after a 10 min incubation period caffeine had the greatest hyperactivating effect (P<0.05). Mibefradil inhibited the induction of hyperactivation by caffeine (P<0.01) and decreased progression of sperm in the microfluidic device in comparison to the caffeine treated sperm (P<0.001). When extracellular calcium was removed by the addition of EGTA, there was a significant reduction in hyperactivation or sperm progression when compared to the control and caffeine treated sperm (P<0.001). Interestingly, when caffeine was added back to the media containing EGTA, there was no increase in hyperactivation or in sperm progression along the device.

Fig 2: Effect of EGTA on hyperactivation in bull sperm, showing a significant increase in hyperactivation in cells treated with caffeine. However, when extracellular calcium is removed there is a significantly reduced percentage of hyperactivated sperm (p<0.001).

4. Discussion and conclusions

The present study was designed to examine if CatSper 1-4 are expressed along the bull reproductive tract and to perform functional assessments. Here we reveal the presence of CatSper 1-4 channels in bull testicular tissue, and show that they potentially play a role in hyperactivation and rheotaxis.

Using a comparative genomics approach, we found homologs of four CatSper channel genes in the Bos Taurus genome on various chromosomes, and revealed that the bovine CatSper genes present in similar synthetic sequence to those of humans and mice. Expression analysis at the gene level showed that all four CatSper channels are expressed highest in testis tissue, telling us they are potentially incorporated into sperm at early formation.

Sperm treated with caffeine, show a significant increase in percentage hyperactivation and rheotactic response, however, when we treat sperm with mibebradil (CatSper antagonist) and EGTA (chelates calcium) there is a significantly reduced percentage of hyperactivated sperm and number of sperm to progress along the channel. These findings lead us to hypothesise that caffeine operates via CatSper and requires extracellular calcium to induce hyperactivation and rheotactic response.

In conclusion this study is the first report on the identification and initial characterisation of CatSper genes in the bovine. The testes expression and location-specific changes in mRNA abundance support an evolutionary conserved role for these channels in bull reproduction. This study demonstrates that CatSper channels hyperactivation and rheotactic response in bulls.

5. References