REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION
CONFIRMATION OF CATALYTIC DOMAIN OF MATRIX
METALLOPROTEINASES -9 GENE IN SEMEN
OF MURRAH BUFFALOES

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Abstract: Matrix metalloproteinases (MMPs) form a family of structurally-related, zinc-dependent proteases which are capable of restructuring tissue components by proteolytic degradation of extracellular matrix and basement membrane compounds. MMP-2 and MMP-9 are the two major enzymes belonging to this family. These Proteases in the reproducte tract are considered to play key roles in fertilization processes. A study on biochemical and molecular characterization of seminal plasma proteins in buffalo bulls was carried out. Total RNA was isolated from the semen samples of buffalo using standard procedure. The concentration and purity of RNA was determined by 260 and 280 nm in a spectrophotometer. The ratio of \( A_{260}/A_{280} \) was 1.82 indicating the isolated RNA was reasonably pure. The integrity of RNA was assessed by agarose gel electrophoresis. Primers targeting catalytic domain of the MMP-9 were designed as the forward primer 5'-GGGCGATCTGGCA-CCACACGGATCGACTTA-3' and the reverse primer 5'-GGCGTGACAGTCTCTAAGGGAGGCACGCTGAGCA-3'. Restriction enzyme (RE) sites were incorporated viz. BamHI, XbaI and SalI along with approriate overhanging sequences on forward and reverse primers respectively for directional cloning of the amplified product. After incorporation of the RE sites the expected size of the amplicons was 1080 bp. RT-PCR was carried out. The first step was to synthesize cDNA and the second step to amplify the desired genes from cDNA by PCR. The amplified PCR product was checked by submarine gel electrophoresis using 1.5% agarose. The presence of catalytic domain of MMP-9 was observed at 1080bp, which was confirmed by the presence of MMP-9 gene in semen of Murrah buffaloes.

Key words: Catalytic domain, MMP-9 Gene, RT-PCR, Murrah buffaloes

INTRODUCTION

Seminal plasma proteins are relevant for sperm function particularly for their interactions with the various environments of the tubular genital tract and the oocyte and its vestments. Moreover, specific peptides and proteins act as signals for the immune system of the female, ultimately modulating sperm rejection or tolerance, perhaps even influencing the relative intrinsic fertility of the male and/or couple [1]. Matrix metalloproteinase isoenzyme MMP-2 as well as TIMP-1 and -2 [2] have been detected in rat