Recombinant human follicle stimulating hormone production in chinese hamster ovary cell line using fusion of the DHFR/MTX and IR/MAR Gene amplification methods

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Follicle stimulating hormone (FSH) is an effective gonadotropin used in infertility treatment. Production of Recombinant Human FSH (rhFSH) has been attempted by methotrexate (MTX) induction of inserted gene, located beside the dihydrofolate reductase (DHFR) locus. However, inefficiency disadvantages of this method have been reported, so far. Here, we present a DHFR/ MTX fusion method that applies a DNA fragment containing mammalian replication initiation region (IR) and matrix attachment region (MAR). The ORF regions of alpha and beta subunits of hFSH gene were cloned in a shuttle vector and used for co-transfection of CHO-DHFR cells along with the "IR/MAR gene amplification reagent. Then, co-selection of clones using MTX and blasticidin S, followed by a precise drugs increasing dosage profile to stimulate gene amplification have been performed. The expression of gene construct entailing rhFSH in CHO-DHFR cell line was verified by SDS PAGE and western blot. The construct copy number as well as mRNA or protein expression levels were determined by real time-PCR and ELISA analyses, respectively.

Stable CHO-DHFR clones with significantly higher rhFSH expression level were established. Enhanced amplification of the copy number of a co-transfected expression vector was obtained, leading to high rhFSH protein production efficacy. Collectively, our data shows IR/MAR-Dhfr fusion, together with MTX and blasticidin S co-selection, is a reliable methodology for isolation, and high-yield production of rhFSH, as compare to the sole application of either the DHFR/MTX or IR/ MAR methods.