

论著

HBV DNA重组质粒转染小鼠卵母细胞的研究

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摘要 背景与目的: 乙型肝炎是危害人类健康的全球性疾病。为了探索乙肝病毒通过卵母细胞垂直传递的可能性, 对HBV DNA重组质粒能否转染小鼠卵母细胞进行了研究。 材料与方法: 将小鼠卵母细胞与pBR322-HBV DNA重组质粒进行共培养后, 分别提取卵母细胞DNA及制备小鼠卵母细胞中期染色体。用PCR、Southern、斑点杂交及FISH技术证实HBV DNA能否转染小鼠卵母细胞。结果: PCR试验在受检样本中观察到HBV DNA阳性条带。Southern试验在受检PCR产物中观察到明显的阳性杂交信号。用每次实验的最后3次洗液进行斑点杂交未发现HBV DNA阳性信号, 排除了PCR和Southern阳性结果来自洗液污染的可能性。荧光原位杂交在1 000个卵母细胞中的36个中期相内发现HBV DNA杂交信号。结论: HBV DNA序列能够通过卵母细胞的透明带和细胞膜, 进入卵母细胞内并整合到卵母细胞染色体上。此类卵母细胞与正常精子受精时, 有可能作为载体将HBV DNA带进胚胎。

关键词 [HBV DNA](#); [转染](#); [小鼠](#); [卵母细胞](#); [整合](#); [染色体](#)

Study on Transfection of Mouse Oocytes with Recombinant Plasmid, pBR322-HBV

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Abstract **BACKGROUND & AIM:** Hepatitis B is a public health problem of worldwide importance. To explore feasibility of Hepatitis B virus(HBV) vertically transmitting via oocytes, the transfection of mouse oocytes with recombinant plasmid, pBR322-HBV was studied. **MATERIAL AND METHODS:** Genomic DNA was isolated and the metaphases were prepared, respectively from mouse oocytes co-cultured with pBR322-HBV DNA plasmids. PCR, Southern blot, Dot hybridization and FISH were performed to demonstrate if the mouse oocytes were transfected by pBR322-HBV plasmid. **RESULTS:** PCR detected positive bands in the tested samples, and then Southern blot revealed clear hybridization signal in above PCR products. Final washing solutions were collected for Dot hybridization and no signal for HBV DNA was observed, which excluded the possibility that contamination of washing solutions gave rise to positive results of PCR and Southern blot. FISH demonstrated that 36 of 1 000 metaphases presented positive signals. **CONCLUSION:** HBV DNA sequences were able to pass through the zona and oolemma to enter into oocytes and integrate into their chromosomes. It suggested that HBV DNA sequences might be brought into embryo via oocytes as vectors when they were fertilized with normal spermatozoa.

Keywords [HBV DNA](#) [transmission](#) [mouse](#) [oocyte](#) [integration](#) [chromosomes](#)

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