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# Influence of IL-1β on Hamster Blastocyst Hatching via Regulation of Hatching Associated Proteases

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**Abstract :** Blastocyst hatching is an indispensable process for successful implantation. One of the major reasons for implantation failure in IVF clinic is poor quality of embryo, which are not development/hatching-competent. Therefore, attempts are required to develop or enhance the culture system with a molecule recapitulating the autocrine/paracrine factors containing the environment of in-vivo endometrial milieu. We have tried to explore the functional molecules involved in the hamster hatching phenomenon. Blastocyst hatching is governed by several molecules that are entwined and regulate this process, among which cytokines are known to be expressed and are still least explored. Two of such cytokines we have used for our study are IL-1β and its natural antagonist IL-1ra to understand the functional dynamics of cytokines involved in the hatching process. Using hamster, an intriguing experimental model for hatching behavior, we have shown the mRNA (qPCR) and protein (ICC) expression of IL-1β, IL-1ra and IL-1 receptor type 1 throughout all the stages of morula, blastocyst and hatched blastocyst. Post-asserting the expression, the functional role is shown by supplementation studies, where IL-1β supplementation showed enhancement in hatching level (IL-1β treated: 84.1 ± 4.2% vs control: 63.7 ± 3.1 %, N=11), further confirmed by the diminishing effect of IL-1ra on hatching rate (IL-1ra treated: 27.5 ± 11.1% vs control: 67.9 ± 3.1%). The exogenous supplementation of IL-1ra decreased the survival rate of embryos and affected the viability in dose-dependent manner, establishing the importance of IL-1β in blastocyst cell survival. Previously, the cathepsin L and B were established as the proteases that were involved in the hamster hatching process. The inducing effect of IL-1β was correlated with enhanced mRNA level, as analyzed by qPCR, for both CAT L (by 1.9 ± 0.5 fold) and CAT B (by 3.5 ± 0.1) fold which was diminished in presence of IL-1ra (Cat L by 88 percent and Cat B by 94 percent. Moreover, using a specific fluorescent substrate-based assay kit, the enzymatic activity of these proteases was found to be increased in presence of IL-1β (Cat L by 2.1 ± 0.1 fold and CAT B by 2.3 ± 0.7 fold) and was curtailed in presence of IL-1ra. These observations provide functional insights with respect to the involvement of cytokines in the hatching process. This has implications in understanding the hatching biology and improving the embryo development quality in IVF clinics.

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