

氏名	MD Anisuzzaman Mondal
学位の種類	博士（農学）
学位記番号	甲 第70号
学位授与の日付	平成29年3月20日
学位授与の要件	信州大学学位規程第5条第1項該当
学位論文題目	Study on the mechanism of thermotaxis and chemotaxis of bull spermatozoa (ウシ精子の走温性と走化性の作用機構に関する研究)
論文審査委員	主査 教授 濱野光市 教授 中村宗一郎 教授 藤井 博 教授 小野珠乙 教授 高坂哲也（静岡大学）

論文内容の要旨

Mammalian sperm must migrate in the female reproductive tract in order to penetrate and fertilize an ovulated egg in the ampulla. Recently, it was reported that the migration of sperm in the female reproductive tract associated with fertilization is at the very least regulated by chemotaxis and thermotaxis.

Thermotaxis that sperm migrate to higher temperature area has been confirmed in rabbit and human. In this study, I examined the migration ability of bull sperm in a temperature gradient to confirm thermotaxis and elucidate the involvement of calcium in such thermotaxis, as well as the relation between sperm capacitation and bull fertility. Thermotaxis was evaluated in a temperature gradient of 34–42 °C using a cross-type column 22-mm long, 40-mm wide, and 100- μ m deep. Significantly more sperm migrated to the high-temperature area of 39 °C in a 2 °C temperature gradient, and to 40 °C in a 1 °C temperature gradient. In calcium-free, BAPTA containing medium, and EGTA containing medium, the migrated sperm ratio in the two temperature areas was almost the same. In media containing lanthanum, ruthenium red, and 2APB, I could not confirm thermotaxis. Pre- and post-capacitated sperm migrated to the high-temperature area, expressing thermotaxis. Although the sample size of bulls was quite small, the difference in thermotaxis may have been associated with bull fertility. Sperm thermotaxis evaluation has potential as a predictor of bull fertility. The sperm from high-fertility bulls showed clear thermotaxis. Based on these results, thermotaxis of bull sperm was confirmed and the involvement of both calcium channels and intracellular stored calcium in thermotaxis was suggested. The relation between thermotaxis and bull fertility was confirmed, and bull fertility diagnosis and improvement of cow conception rate by sperm thermotaxis evaluation were suggested.

Chemotaxis was evaluated for examining the sperm migration to one end BF of the wide column using a cross column chamber with 100 μ m in depth, 10mm in

height, 40mm in width. Movement of head and flagellar of chemotaxis expressing sperm were analyzed by Computer Assisted Sperm Analysis (CASA). I confirmed bull sperm chemotaxis by recognizing of 62.7% of sperm migration toward 103 times diluted BF. Both large number of pre and post capacitated bull sperm migrated toward BF. Curvilinear velocity (VCL) and Straight-line velocity (VSL) of the sperm of the high fertility confirmed bull were significantly higher than low bull. Flagellar curvilinear ratio (FCR) of the sperm winding to BF was more significantly higher than that of pre and post winding sperm. On the basis of these results, the chemotaxis of bull sperm was confirmed, and chemotactic response to BF made increasing of VSL and VCL of bull sperm. It was suggested that chemotaxis may be a reliable tool for effectively analyzing sperm migration, and has potential as a predictor of bull fertility and chemotaxis expressing bull sperm toward BF changed the swimming direction by the change of FCR.

The results may suggest that BF potentially attracts bull sperm at a certain concentration irrespective of the capacitation status of the sperm, and contribute to the elucidation of the bull sperm motility and fertilization functions. Although the mechanism by which this attraction occurs remains unclear, our data imply that it may be related to BF-dependent changes in the sperm flagellar curvilinear ratio.