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Proteomics analysis and generation antibodies for the detection of pregnancy-specific proteins in milk

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The objective of this study was to confirm pregnancy in cows by detection of early pregnancy-specific milk proteins through an approach with proteomics analysis, production of antibodies to synthetic peptides and Western analysis. Milk samples were collected from pregnant (day 30 and 50) and non-pregnant cows. After acidifying to pH 4.6 to remove casein proteins and samples were centrifuged. Following separation of 2 mg milk protein with isoelectric point using pH 3.0–10.0, pH 4.0–7.0, and pH 6.0–9.0 strips, proteins were resolved in the second dimension by SDS polyacrylamide gel electrophoresis on 8%–16% linear gradient gels. The images of stained gels were analyzed to detect differences in protein spots between non-pregnant and pregnant milk samples using an Image Master followed by MALDI TOF-MS. Analysis of the 2-DE gel image revealed a total of 39 proteins were differentially expressed in pregnant milk. Eight of these 39 spots corresponded to pregnancy-specific proteins. Peptides were synthesized based on amino acid sequence information obtained from a proteomics analysis of pregnancy-specific milk whey proteins and then used to raise antibodies. Western blot analyses showed that antibodies against lactoferrin, lactotransferrin and alpha-1G could detect the elevated expressions in milk during pregnancy. The results of this study suggest that proteomic analyses could be systematically applied to the production of antibodies and confirmation of their potential applications. The proteins identified in pregnant samples and their antibodies could be good candidate starting points for the development of pregnancy detection for cows.

Biography

Dong Il Jin is a professor in Chungnam National University, Korea. He received Ph. D in North Carolina State University, USA in 1992 by performing research on producing transgenic animals, and then move to Duke University Medical Center as a postdoctoral fellow to work in ras-signaling study using transgenic mice. Currently, his major research interest is genetic reprogramming of NT embryos as well as early pregnancy detection in animals.