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Nucleic acids and protein content in relation to growth and regression of buffalo *(Bubalus bubalis)* corpora lutea

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Abstract

Information on nucleic acids and protein content of buffalo corpus luteum (CL) in relation to growth, development and regression is not available. An experiment was thus conducted to investigate the variation and relationship between nucleic acids and protein content in CL during different developmental stages and to determine the qualitative differences in protein constituents in any of these stages. Buffalo corpora lutea of different developmental stages viz., developing (day 5–10, $n = 16$), developed (day 11–17, $n = 12$) and regressed (day 18–21, $n = 10$) stages were collected from non-pregnant and -pathological genitalia ($n = 38$). The DNA, RNA and protein content in tissue extracts were determined and the proteins in pooled samples were analyzed by polyacrylamide gel electrophoresis.

Developing stage CL had more total and per gram tissue level of DNA and RNA with significant positive relationship with total and per gram RNA and protein contents. Although there was no significant difference in total weight, a significant decrease in total DNA as well as per gram level of DNA and RNA was observed in developed stage compared to developing stage CL. The total protein content in developed stage CL was compared to developing and regressed stage CL. Non-denaturing PAGE analysis of CL proteins of different stages showed five protein bands of 210, 190, 82, 68 and 66 kDa and one that migrated with the dye front in all the stages however, not shown any differences in banding pattern. Denaturing PAGE showed 15 bands viz., 205, 66, 53, 42, 35, 27, 24, 22, 20, 18, 17, 14, 9, 7.5 and 6.5 kDa. Out of these 66 and 53 kDa bands appeared with maximum intensity in all the three stages of CL. Comparison of bands between the three stages revealed five 57, 31, 27, 19 and 16 kDa stage-specific bands in regressed stage CL.

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The present study indicated that the DNA, RNA and protein content of buffalo CL varied with the stages of development and regressed stage CL contained some unique protein bands which were not observed either in developed or developing stage CL.

Keywords: Corpus luteum; Buffalo; DNA; RNA; Protein

1. Introduction

Buffalo suffers from a number of inherent reproductive problems mainly due to our inadequate knowledge of the basic reproductive physiology with regard to regulation of ovarian functions pertaining to folliculogenesis, corpus luteum formation, structure, function and regression (Guraya, 1987). Corpus luteum secreted progesterone is essential for bringing about the critical biochemical mechanisms in play that are important for conditioning of endocrine mileau and blastocyst–endometrium interaction. Besides progesterone, corpus luteum also secretes a number of protein and peptide including oxytocin, neurophysin, relaxin, gonadotrophin releasing hormone like substance, follicle regulatory protein, proteases and their inhibitors, progesterone binding protein, acute-steroid-regulatory-protein, plasminogen activator, growth factors and their binding proteins. All the proteins are found important for the corpus luteum function and are studied either in small laboratory animals (ferrete: Mead et al., 1988 and Huang et al., 1993 rat: Fiedler et al., 1999), cattle (Fields et al., 1987), sheep (Schams et al., 1999) and pig (Fujimori et al., 1988). However, information on nucleic acids that serve as a better index for the major biochemical changes taking place (Hafs and Armstrong, 1968; Mares et al., 1962) and the protein content during development and regression in buffalo corpus luteum are not available. The present investigation is therefore designed to study the variations in nucleic acid and protein content, to elucidate the interrelationships between these constituents and also to determine the qualitative differences in soluble proteins during developing, developed and regressed stages of corpus luteum.

2. Materials and methods

2.1. Collection of samples

Buffalo whole genitalia containing ovaries with corpora lutea (CL) of different developmental stages were collected immediately after slaughter in ice-cooled temperature from the local abattoir in Bangalore District of Karnataka state, India and transported to the laboratory. The uterine horns palpated for enlargement were dissected open to check the pregnancy and presence of any gross pathological condition. Three pregnant and eight pathological genitalia obtained while screening were not included in this study. Non-pregnant, unilaterally symmetrical tracts ($n = 38$) with no pathological indication were selected for this study. Corpora lutea were dissected out of the ovary, cleaned from adhering tissues and classified into developing (day 5–10, $n = 16$), developed (day 11–17, $n = 12$) and regressed corpus luteum (day 18–21, $n = 10$) based on blood vascularization, size and consistency