Radioimmunoassay of Pregnancy-Associated Glycoprotein 1 (PAG-1) isolated from Zebu (Bos Indicus) Placenta: Preliminary Results

Introduction

Bos indicus is used interchangeably with the term zebu which originates from the Tibetan word Zen or Zebra, which means “the hump of the camel”.

Zebu cattle is an important source of animal protein in many countries, being more effective in extracting nutrients from low quality roughages and having a remarkable ability to tolerate high temperatures.

Reproductive differences between domestic (Bos taurus) and zebu cattle have been described. Zebu cattle reach puberty later (15 to 40 months of age) than Bos taurus x Bos indicus crossbreeds or purebred taurine cattle. Different authors reported also longer pregnancy lengths in some breeds of zebu cattle as well as in crossbreed Bos taurus x Bos indicus.

Till now, in zebu cattle, no specific studies were made in order to describe placental production of hormones and pregnancy-associated proteins during normal and/or pathologic gestations.

Production of new reagents isolated from zebu placenta can be useful for the development of homologous radioimmunoassay (RIA) systems. In veterinary practice, these RIA may be used for both pregnancy diagnosis and follow-up of fetal well-being.

Aim

The aim of this study was to analyze the characteristics of a new preparation of zebu PAG-1 in the development of a new homologous PAG radioimmunoassay system.

Material and Method ~1

Preparation of antigen

Placenta → Extraction → Acid precipitation → Ammonium sulfate precipitation (0-40% and 40-80% A.S.) → Anion exchange chromatography (DEAE Sephadex A25 column) → Cation exchange chromatography (CM Ceramic column) → Lyophilization → Immunization of rabbits

Material and Method ~2

Radiolabelling and antiserum production

- Purified zebu PAG-1 (0.04 M NaCl fraction of DEAE Sephadex A25 column, CM ceramic column peak XI) was radiolabelled by lactoperoxidase method.
- Antiserum against zebu PAG-1 was raised in rabbits (250 μg of lyophilized antigen were injected at 2-week intervals).
- First bleeding was made 1 week after the third immunization.

Results and Discussion

- In the presence of antibody in excess, 73.6% of labeled zebu PAG-1 was bound.
- The optimal dilution of the antiserum issued from the first bleeding (1-week after the third injection of antigen) was 1:100000.
- These first results indicate that new reagents prepared from zebu placenta are now available for the development of a homologous RIA system in this species.

Perspectives

- Further investigations are in progress to produce new reagents for the development of a highly sensitive radioimmunoassay method for PAG detection in peripheral blood of pregnant zebu females.
- This new system could be used in experimental and/or farm conditions in order to improve the knowledge about the endocrine physiology of zebu females.

General References

Sousa, NM et al. (2000b). 27th Conference of the IETS (abstract accepted).

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