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Progress Report

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Title: Isolation of a luteotropic substance produced by the blastocyst and early pregnancy diagnosis in cattle.

For period: Jan. 1, 1989-June 30, 1989

1. In our previous report we have demonstrated luteotropic activity in granules obtained from fetal cotyledons of 100-150 days of gestation. Preliminary steps in purification indicated that there were two active protein fractions one at 30-35 KD and a larger protein at 68,000 KD.

2. We are currently further purifying these proteins by the following two different strategies:

A. Isolation and purification of the 30-35KD proteins.

SDS polyacrilamide gel (3 mm) electrophoresis separated the active fraction into four bands (12, 29, 32 and 35 KD). Each of the four bands was eluted and injected with adjuvant into rabbits to produce specific antibodies. Total homogenates and acid extracts of fetal cotyledon separated on 12% polyacrylamide gel were used to test the specificity of the antibody. To date only the 12 KD generated antibodies have been tested using Western blot analysis. Results (Fig. 1) indicate that the 12 KD generated antibodies reacted with not only the 12 KD band but with other proteins. This means that the 12 KD fraction is a fragment of a larger protein or is a member of a family of proteins with similar antigenic properties. The antibody was therefore judged not to be useful for further investigation.

B. Isolation and purification of the higher molecular weight protein.

Since both LH and hCG can be successfully purified on ConA columns, we applied this technic to purify the higher mol. wt. placental luteotropic protein. Columns packed with 50 ml ConA Sepharose were calibrated with authentic bLH or hCG to determine the optimal elution conditions and column capacity. To determine the rate of extraction ovine pituitary extracts were used as controls. Granules obtained from a large amount of initial placental material (2.5 Kg) were then loaded on the column. The column was then eluted with 0.3 M α -methyl-mannoside. The luteotropic activity of the extracts will be determine by bioassay and radioreceptor assay. Depending on the yield from the control extraction, we will decide whether to use this method for futher purifications.

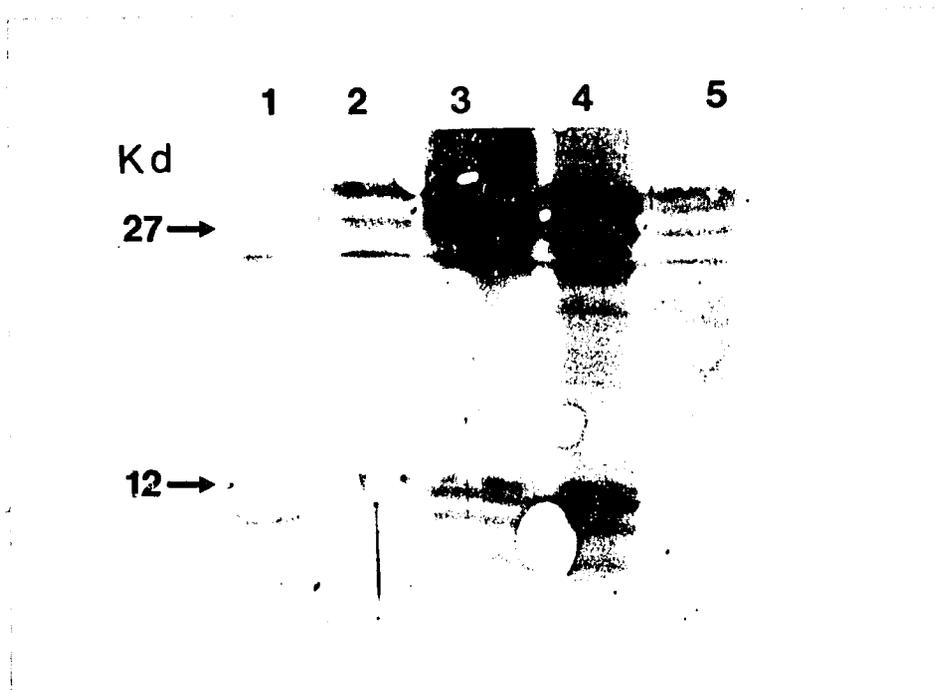


Fig. 1. Maternal and fetal tissues were homogenized in TRIS buffer or acidic granule extracts were separated on 12% acrylamide gel followed by Western blot analysis using anti-12K antibodies.

The left lane represents molecular markers. Lanes 1 to 3 were homogenates and lanes 4 and 5 were granule extracts. The crown-rump length of the fetuses were: lane 1 - 17 cm, maternal caruncle; lane 2 - 14 cm, fetal cotyledon; lane 3 - 14 cm, maternal; lane 4 - 17 cm maternal; lane 5 - 13 cm fetal.

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