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Comparison of Extraction Methods for Removal of Insulin-Like Growth Factor Binding Proteins in Ovarian Follicular Fluid Samples of Cattle

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Story in Brief

Three methods of extraction were compared for their efficiency in removing insulin-like growth factor binding proteins (IGFBPs) from bovine follicular fluid. Pools of follicular fluid collected from small (1-5 mm) and large (> 8 mm) bovine follicles were subjected to three extraction methods: 1) formic acid-acetone extraction at 25° C with no incubation, 2) acid-ethanol extraction at 25° C with 30 min incubation, and 3) acid-ethanol extraction at -20° C for 2 h. Methods 1 and 3 were the most efficient procedures for removal of IGFBP-3 whereas Methods 2 and 3 were the most efficient procedures for removal of IGFBP-2. Method 2 was nearly two-fold less efficient than the other methods at removing IGFBP-3, whereas Method 1 was five-fold less efficient than the other methods at removing IGFBP-2.

(Key Words: Insulin-like Growth Factor, Binding Proteins, Follicular Fluid, Cattle.)

Introduction

Over 99% of insulin-like growth factors (i.e., IGF-I and -II) are bound by IGFBPs in blood and follicular fluid (for reviews see Spicer and Echternkamp, 1995; Hossner et al., 1997). These IGFBPs interfere with the measurements of IGF-I and -II, and thus must be removed from the biological sample before assay via an extraction procedure (Plaut et al., 1991; Holly and Cwyfan-Hughes, 1994; Spicer et al., 1997). Numerous extraction procedures have been developed and usually involve an acidic solvent to dissociate the IGF from the IGFBPs and precipitate the larger IGFBPs. The efficiency of a specific extraction seems to be influenced by the type of biological fluid and species from which it is derived. Therefore, we set out to compare three widely used extraction methods for removal of IGFBPs from bovine follicular fluid.

Materials and Methods

Ovaries of beef and dairy cattle were obtained at slaughter and follicular fluid collected from follicles 1 to 5 mm in diameter and from follicles 8 mm and greater in diameter. Aliquots of follicular fluid were stored at -20° C until used. Follicular fluid samples were thawed and extracted using three methods. Method 1: formic acid-acetone extraction procedures in which samples (50 m l) were added to tubes containing 25 m l of 8 M formic acid (0.5% Tween-20) and 175 m l of acetone, the contents vortexed for 10 sec and the samples were immediately centrifuged at 2500 x g for 15 min. Method 2: acid-ethanol extraction procedure in which samples (100 m l) were added to tubes containing 400 m l of acid-ethanol (12.5% 2 N HCl and 87.5% ethanol), the contents vortexed for 30 sec, incubated at 25° C for 30 min, and the samples centrifuged at 2000 x g for 30 min. Method 3: acid-ethanol extraction procedure as in Method 2 except that the incubation was for 2 h at -20° C.

To evaluate the presence of IGFBP-3 and -2 in the various samples, one-dimensional SDS-PAGE, under non-reducing conditions, was performed as described previously (Stewart et al., 1996). Briefly, proteins were separated on a 12% polyacrylamide separating gel and a 4% stacking gel, with 2 m l of unextracted follicular fluid and 22 m l of buffer loaded per lane or 10 m l of the various extraction samples, and 14 m l of buffer loaded per lane. After electrophoresis of samples, proteins were electrophoretically transferred to nitrocellulose, and ligand blotted for IGFBP activity with use of [^{125}I]IGF-II. Band intensity on autoradiographs was characterized by scanning densitometry.

Results and Discussion

All three extraction procedures resulted in removal of over 93% of IGFBP-3, with Methods 1 and 3 being the most efficient procedures for removal of IGFBP-3 (Figure 1). Method 2 was nearly two-fold less efficient than the other methods at removing IGFBP-3 (Figure 1). Methods 2 and 3 (acid-ethanol) were the most efficient procedures for removal of IGFBP-2, removing over 97% of the IGFBP-2. Method 1 (acid acetone) was five-fold less efficient than the other methods at removing IGFBP-2, with 84% removal of the IGFBP-2 (Figure 1). Previous studies have reported [3] 95% of the total IGFBPs are removed after a 16 h incubation at 4° C with acid-ethanol (Echternkamp et al., 1990). Also in agreement with previous work (Holly and Cwyfan-Hughes, 1994), IGFBP-3 appears to be more easily removed than IGFBP-2 with the formic acid-acetone extraction. Thus, the best of the three extraction methods reported here for removal of both IGFBP-2 and -3 is the 2 h incubation at 4° C with acid-ethanol.

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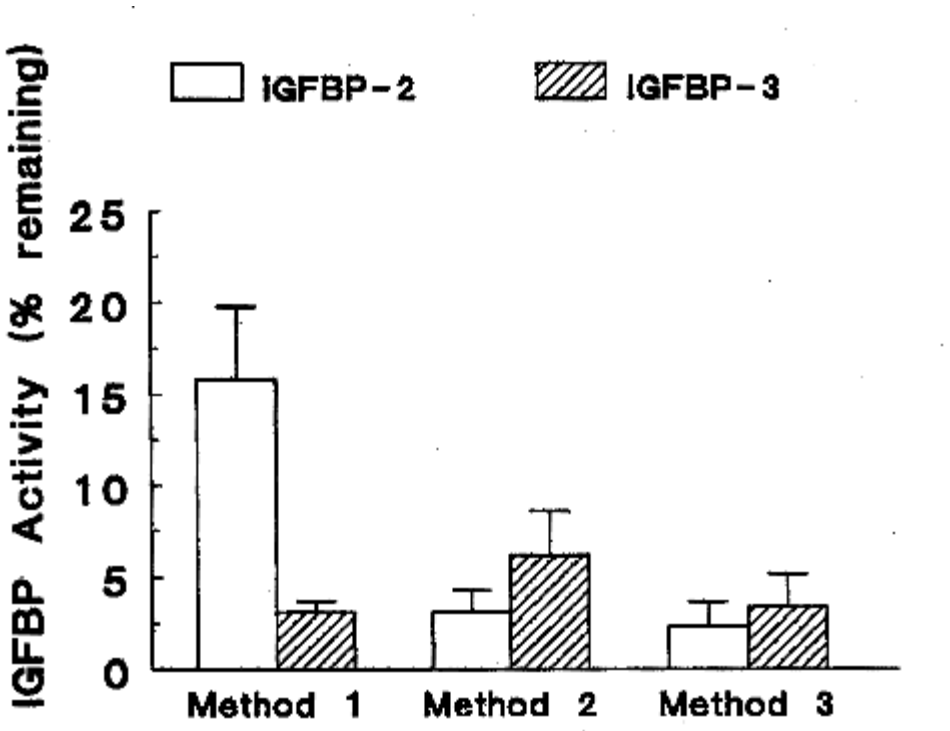

Figure 1. Effect of various extraction methods on removal of IGFBP-2 and IGFBP-3 from bovine follicular fluid. Values are means of four to six follicular fluid samples and expressed as percentage of the total IGFBP-2 or IGFBP-3 in the original samples as assessed by ligand blotting. Method 1 = formic acid-acetone extraction; Method 2 = 30 min acid-ethanol extraction at 25° C; and Method 3 = 2 h acid-ethanol extraction at -20° C.

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