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# The effect of IGFBP-4 on IGF-2 stability in bovine cumulus-oocyte cells during in vitro maturation

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The insulin-like growth factor 2 (IGF-2) is essential for oocyte maturation, cumulus cell steroidogenesis and oocyte viability. Nonetheless, free IGF-2 demonstrated a short lifespan. Six high-affinity binding proteins (IGFBP-1, -6) regulate the biological functions of IGF-2 by prolonging its lifespan and regulating its bioavailability on the target cells. From the IGFBPs, especially IGFBP-4 inhibited IGF-2, and it has been linked with the appearance of follicle atresia. To gain an inside on the regulation of IGFBP-4 on IGF-2 during cumulus-oocyte cells (COCs) maturation, the *in vitro* maturation medium (TCM 199 based, supplemented with 1 mg/ml of fatty acid free bovine serum albumin, /Sigma-Aldrich, Taufkirchen, Germany/; 10 I.U./ml equine chorionic gonadotropin and 5 I.U./ml human chorionic gonadotropin /Suigonan® 80/40 I.U./ml lyophilizate and injection solution, MSD Animal Health, Unterschleissheim, Germany/) was supplemented with recombinant human IGF-2 (rhIGF-2; 50 ng/ml, R&D systems, Bio-techne, Abingdon, United Kingdom) alone or in combination with recombinant bovine IGFBP-4 (rbIGFBP-4; 2,000 ng/ml, InVivo Biotech Services, Hennigsdorf, Germany) in the presence or absence of COCs. Bovine COCs were collected from abattoir-derived ovaries. Groups of 25 COCs were randomly assigned to each experimental group and set to *in vitro* maturation for 24 h at 38°C, 5% $CO_2$. Three biological repetitions were performed. The IGFBP-4 binding capacity was evaluated by taking samples directly after medium preparation (0 h) and after 3, 6, and 24 h of *in vitro* maturation. Free IGF-2 concentrations were measured using a competitive radioimmunoassay (Mediagnost®, Reutlingen, Germany). The rhIGF-2 was bound to rbIGFBP-4 by 49.8 ± 24.6% directly at medium preparation (0 h), 74.7 ± 21.3% after 3 h, 55.4 ± 33.9% after 6 h, and 44.3 ± 40.5% remained bound after 24 h of incubation. Similarly occurred in the absence of COCs, IGF-2 was bound to IGFBP-4, 49.8 ± 24.6, 72.2 ± 23.5, 48.5 ± 38.1 and 32.5 ± 58.2% at 0, 3, 6, and 24 h respectively. The concentrations of rhIGF-2 added to the medium and incubated in the presence of COCs declined 13.2 ± 8.6, 16.0 ± 10.8 and 77.2 ± 6.2% after 3, 6 h, and 24 h incubation respectively. Contrary, the concentrations of rhIGF-2 without COCs remained stable throughout 24 h. We conclude that rbIGFBP-4 was able to bind rhIGF-2 with a maximum binding capacity around 3 h after incubation and decreased at the final stage of IVM. The binding capacity was not affected by the presence of COCs. Moreover, COCs used the free available rhIGF-2 in the medium. Finally, rhIGF-2 demonstrated to be stable for 24 h under

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