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Human low density lipoprotein as a substrate for in vitro steroidogenesis assays with fathead minnow ovary explants.

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Abstract:

Gonad explant in vitro steroidogenesis assays are used as part of a multifaceted strategy to detect endocrine active chemicals capable of altering steroid hormone synthesis. An in vitro steroidogenesis assay used in our laboratory involves exposing fathead minnow (FHM) gonad explants to medium 199 supplemented with 25-hydroxycholesterol for 12 hours, and then measuring steroid production. Data from this assay has been used to support the development of computational models of steroidogenesis and the impacts of steroidogenesis inhibition. However, it is thought that the 25-hydroxycholesterol used in the assay may bypass steriodogenic acute regulatory protein (StAR), which is known to be an important, rate limiting step in the production of steroid hormones, as well as a target of environmental contaminants, possibly leading to inaccurate model predictions. Therefore, experiments were undertaken to determine whether commercially-available human low density lipoprotein (LDL), could be used as a viable alternative substrate in the assay. Using standard, non-stimulated conditions, steroid production by ovary explants was not detectable after 12 h of incubation in medium supplemented with LDL. Adding human chorionic gonadotropin to the LDL containing medium failed to stimulate detectable steroid production. However, after supplementing with 8-bromo cyclic adenosine monophosphate (8 Br-cAMP), steroid production using LDL as a substrate was similar to that obtained using 25-hydroxycholesterol as a substrate (without 8 Br-cAMP). To further elucidate the impact of 8 Br-cAMP treatment on the FHM ovary tissue in culture, we examined with in vitro expression of key steroidogenesis-related genes following 8 Br-cAMP treatment. The 8 Br-cAMP significantly increased the abundance of transcripts coding for cyp19a1a (aromatase) and significantly decreased the abundance of transcripts coding for follicle stimulating hormone receptor. On-going work is aimed at evaluating the sensitivity of the modified in vitro steroidogenesis assay protocol (i.e., 8 Br-cAMP stimulated, LDL as substrate) for detecting the effects of steroidogenesis inhibiting compounds, compared to the conventional assay and utilizing the modified protocol to support further model development.