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Effects of cholesterol and cAMP on progesterone production in cultured luteal cells isolated from pseudopregnant cat ovaries

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ABSTRACT

The present study was designed to incubate luteal cells isolated from pseudopregnant cats and to investigate the effects of cholesterol and cAMP on luteal progesterone production. Corpora lutea were collected from the cats on days 10 and 15 of pseudopregnancy. Luteal cells were isolated from the ovaries by collagenase digestion. Steroidogenic luteal cells were stained for 3β-hydroxysteroid dehydrogenase (3β-HSD) activity. Cells (2×10^4) staining positive for 3β-HSD were cultured for up to 7 days. The cells were treated with 22(R)-hydroxycholesterol (22R-HC) and dibutyryl cyclic AMP (dbcAMP) on days 1, 3 and 7.

Treatment of cells with 22R-HC resulted in a dose-dependent increase ($p < 0.001$) in progesterone production. When 22R-HC was used at a concentration of 10 μg/ml, it resulted in 2.7- and 5.1-fold increases in progesterone production on days 3 and 5, respectively. When the dose was doubled (20 μg/ml), treated cells produced four times more progesterone on days 3 and 7, and three times more on day 5. By day 7, progesterone production increased up to 9.1 times more than the control.

Incubation of cells with both concentrations of dbcAMP (0.1 mM and 1 mM) resulted in significant stimulations of progesterone on days 5 and 7 ($p < 0.001$). However, on day 3, only higher doses of dbcAMP (1 mM) resulted in significant stimulation ($p < 0.05$). Progesterone production was increased up to 2- and 2.9-fold of the control when cells were treated with lower concentration of dbcAMP (0.1 mM) on days 5 and 7, respectively. Incubation of cells

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with 1 mM concentrations of dbcAMP induced a 3.2-fold increase on day 5 and a 5-fold increase on day 7.
In conclusion, a successful incubation was performed for long-life culturing of luteal cells collected from pseudopregnant cats. The method works well and allows for optimal growth and development of cells in the culture. The present study also demonstrated that incubating cat luteal cells with 22R-HC and dbcAMP induces a significant increase in luteal progesterone synthesis.

1. Introduction

The female cat is described as a seasonally polyestrous, induced ovulator (Tsutsui and Stabenfeldt, 1993). If ovulated oocytes are not fertilized or if pregnancy fails for other reasons, the queen undergoes pseudopregnancy, which lasts about 40 days (Paape et al., 1975). The normal gestation period for a queen is approximately 65 days (Jemmet and Evans, 1977). Ovulation is followed by the formation of corpora lutea both in pregnancy and pseudopregnancy, with corpora lutea reaching peak progestational activity within 10–15 days and then declining in activity, with basal progesterone values observed by day 30–35 (Paape et al., 1975; Shille and Stabenfeldt, 1979). Corpora lutea persist throughout pregnancy and continue to secrete progesterone until reaching term (Verstegen et al., 1993).

Queen placentas do not produce progesterone in sufficient amounts to support pregnancy (Verstegen et al., 1993). As ovariectomy at any stage of pregnancy will cause peripheral progesterone levels to fall and the animal to subsequently abort, we have therefore decided to collect corpora lutea from pseudopregnant cats as a source of luteal tissue to study the physiology of the corpus luteum in cats.

The corpus luteum is a complex endocrine gland formed by a heterogenous population of cells that differ in steroidogenic capability, size and appearance of cell organelles (O'Shea, 1987; Arikan and Yigit, 2002). Pregnenolone is converted into progesterone by the action of 3β-hydroxysteroid dehydrogenase (3β-HSD) in the endoplasmic reticulum. Thus, steroidogenic cells can be identified by testing for 3β-HSD activity (Payne et al., 1980).

Synthesis of progesterone by luteal cells requires cholesterol, which can be derived from either de novo cellular synthesis or plasma lipoproteins (O'Shaughnessy and Wathes, 1985b). High-density lipoprotein (HDL) is the predominant cholesterol carrier in cats (Pazak et al., 1998). It is reported that incubation of mid- or late-cycle porcine luteal cells with 22R-HC (25 g/ml), a membrane-permeable progesterone precursor, resulted in 3-fold increases in progesterone production related to basal secretion in both mid- and late-cycle cultures, lasting 24 h (Brannian et al., 1995). In another short culturing study, Musicki et al. (1994) reported that the incubation of rat luteal cells with 22R-HC resulted in a dose-dependent increase in progesterone production when cells were treated with 22R-HC in concentrations from 1 to 10 μg/ml. In contrast, the higher doses of 22R-HC (30 μg/ml) did not induce an additional increase in progesterone production (Musicki et al., 1994).

It has been previously reported that treatment of luteal cells with dbcAMP, a membrane-permeable cAMP analogue, may stimulate steroid production in *in vitro* cell cultures of human (Carrascol et al., 1996), rat (Tekpetey and Armstrong, 1991), sheep (Borowczyk et al., 2007) and bovine samples (O'Shaughnessy and Wathes, 1985a; Grazul-Bilska et al., 1996). However, reported effects vary depending on both the dose of dbcAMP used and the breed studied.

There has been lack of study on the culturing of feline luteal cells. Therefore, the objectives of this experiment were to: (1) evaluate a cell-culturing protocol for luteal cells isolated from pseudopregnant queens and (2) evaluate the effects of 22R-HC and dbcAMP on luteal progesterone production.