In-vitro progesterone production by ovarian interstitial cells from hypophysectomized hamsters*

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Summary. Collagenase-dispersed interstitial cells from 5-day hypophysectomized hamsters produced progesterone (81 ± 7 pg/10 000 viable cells/2 h incubation) and responded to ovine LH stimulation *in vitro* with a dose-dependent increase in progesterone. FSH and prolactin had no effect. The interstitial cells did not produce detectable levels of oestradiol, oestrone, androstenedione, 17α -hydroxyprogesterone or 20α -dihydroprogesterone although 17α -hydroxyprogesterone production rose to 26 ± 5 pg/10 000 cells/2 h in response to 25 ng LH/ml. Isoproterenol (500 ng/ml) and epinephrine (500 ng/ml) stimulated progesterone production and this response was blocked by concurrent administration of 6×10^{-6} M-propranolol which had no effect on LH-stimulated progesterone production. Simultaneous LH and catecholamine stimulation did not produce an additive effect. Incubation in medium containing 10% serum from hypophysectomized animals did not affect progesterone production. The addition of 10^{-6} M-D-ala⁶-LHRH to interstitial cells resulted in a significant reduction of baseline steroidogenesis. These results suggest long-term retention of functional LH receptors and integrity of the steroidogenic pathway through progesterone despite chronic gonadotrophin deprivation and may indicate a role for the interstitium in priming follicular growth following periods of anoestrus.

Introduction

Ovaries from hamsters hypophysectomized for as long as 20 days produce progesterone *in vitro* although serum progesterone concentrations are very low (≤ 500 pg/ml). Administration of LH *in vivo* increases progesterone production by ovaries *in vitro* (Taya & Greenwald, 1980). The presence of Δ^5 - 3β -hydroxysteroid dehydrogenase was demonstrated histochemically only in the ovarian interstitium of long-term hypophysectomized hamsters (Taya, Saidapur & Greenwald, 1980). Interstitial cells contain lipid droplets indicating the presence of steroid precursor (Taya *et al.*, 1980). These data suggest that the interstitium is the site of steroid synthesis in hypophysectomized animals.

The low steroid production *in vivo* by the interstitium of hypophysectomized hamsters may be due to the presence of a circulating inhibitor that is normally suppressed by gonadotrophins (Taya & Greenwald, 1980). Since gonadotrophin releasing hormone (GnRH) has been shown to inhibit steroidogenesis in rat granulosa cells (Hsueh & Ling, 1979), porcine granulosa cells (Massicotte, Veilleux, Lovoie & Labrie, 1980) and rat luteal cells (Clayton, Harwood & Catt, 1979), it is possible that GnRH or a GnRH-like factor is responsible for the suppression of

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progesterone production *in vivo* in hypophysectomized hamsters. It is also possible that the interstitium is producing hormone that is rapidly converted to metabolites (Taya & Greenwald, 1980) and we have found significant concentrations of 17 α -hydroxyprogesterone and 20 α -dihydroprogesterone in ovary and serum of hamsters during the oestrous cycle (Hubbard & Greenwald, 1982) and pregnancy (Shaha & Greenwald, 1982).

In the rat and the guinea-pig there is extensive adrenergic innervation of the interstitium (Burden, 1972) and catecholamines stimulate luteal progesterone production in the sheep (Jordan, Caffrey & Niswender, 1978) and cow (Condon & Black, 1976). It is therefore feasible that catecholamines modulate interstitial cell steroidogenesis. The present study was designed to examine further the steroidogenic capabilities of ovarian interstitial cells from hypophysectomized hamsters.

Materials and Methods

Golden hamsters (*Mesocricetus auratus*) were maintained on a 14 h light:10 h dark schedule with lights on from 05:00 to 19:00 h. At least 3 consecutive 4-day cycles were monitored before using the animals.

Hamsters were hypophysectomized between 09:00 and 11:00 h on Day 1 of the oestrous cycle (day of ovulation) by the parapharyngeal approach under ketamine anaesthesia (Ketaject: Bristol Laboratories, Syracuse, New York). At necropsy, the sella turcica was examined with a dissecting microscope for fragments of pituitary and animals in which any gross remnants were present were rejected. Hamsters were decapitated at 09:00 h on Days 5–7 after hypophysectomy and trunk blood was saved for steroid hormone determinations.

Interstitial cell preparation

Ovaries were excised immediately, placed in Hank's balanced salts solution pH 7.4 (Parker, 1961) containing 25 mM-Hepes (Sigma, St Louis, Missouri) at room temperature and trimmed of fatty tissue. Two to six ovaries were minced, placed in a 10 ml flask containing 5 mg collagenase/ml (Type V, 205 i.u./mg, Sigma) in a total volume of 2 ml medium and incubated in a Dubnoff metabolic incubator at 37°C with shaking. After 30 min, fresh enzyme was added to the tissue and incubation continued for 1.5 to 2 h with intermittent aspiration of tissue through a Pasteur pipette to facilitate dispersion of cells. Cells were washed twice in medium and centrifuged at room temperature for 7 min at 50 g. Cells were counted in a haemocytometer and cell viability was assessed by the trypan blue dye exclusion method. Approximately 75 000–100 000 viable interstitial cells (see 'Discussion') were incubated in a total volume of 1 ml Hank's medium and treated as described below. After incubation at 37°C for 2 h, tubes were snap-frozen in a solid CO₂-ethanol bath and stored at –20°C until further use. Before steroid determinations, tubes were thawed and the contents were sonicated for 5 sec with a Kontes microultrasonic cell disruptor at a setting of 313 W/6.45 cm².

Effects of gonadotrophin administration

Interstitial cells were incubated for 2 h with various concentrations of ovine LH (NIAMDD-oLH-22), ovine FSH (NIAMDD-oFSH-13) or ovine prolactin (NIAMDD-oP-11). Progesterone determinations were made in all experiments and in some experiments oestrone, oestradiol, androstenedione, 17 α -hydroxyprogesterone or 20 α -dihydroprogesterone were measured.

Roles of protein synthesis and microfilaments

To determine whether progesterone production represented de-novo synthesis of steroid, interstitial cells were incubated with 10^{–4} M-cycloheximide (Sigma), a protein synthesis inhibitor,

with or without LH (25 ng/ml). In addition, 10 or 20 μ g cytochalasin B/ml (purified from *Helminthosporium dermatioideum* (Sigma)), a microfilament function inhibitor, were added in 0.5% dimethylsulphoxide final concentration to assess the role of microfilaments in progesterone production.

Effects of catecholamines, serum, and GnRH

Cells were incubated with 500 ng epinephrine/ml (Parke-Davis, Detroit, Michigan) or 500 ng isoproterenol/ml (Elkins-Sinn Inc., Cherry Hills, New Jersey) alone or in combination with 25 ng LH/ml or 6×10^{-6} M-propranolol (Inderal: Ayerst Laboratories, New York), a β -adrenergic antagonist.

The presence of a serum inhibitor of steroidogenesis was examined by incubating interstitial cells in medium containing 10% serum from hypophysectomized hamsters. Serum was previously pooled and was extracted 3 times with charcoal, heat treated at 50°C for 1 h, charcoal extracted and heat treated, or untreated.

The antisteroidogenic properties of GnRH on the interstitial cells were examined by adding GnRH (Beckman Instrument Co., Bioproducts Div., Palo Alto, California) or D-ala⁶-LHRH (Vega Biochemicals, Tuscon, Arizona), an analogue of GnRH that has approximately 36 times the ability of GnRH to release LH (Banik & Givner, 1975).

In one experiment, control or hypophysectomized hamsters received 400 ng GnRH i.p. 30 min before decapitation to verify the biological activity of the GnRH and as another means of establishing the completeness of hypophysectomy.

Radioimmunoassays (RIA)

Steroids. The antisera used in the assays were for progesterone (Surve, Bacso, Brinkerhoff & Kirsch, 1976), androstenedione (provided by Dr J. Resko, Oregon Regional Primate Research Center, Beaverton, Oregon), oestrone (Wright, Collins & Preedy, 1973), oestradiol (Wright *et al.*, 1973), 17 α -hydroxyprogesterone (Steranti Research Ltd, Hertfordshire, England) and 20 α -dihydroprogesterone (provided by Dr C. N. Pang & Dr J. Hilliard, University of California at Los Angeles). The radioimmunoassay procedures were identical to those described previously (Terranova, Connor & Greenwald, 1978; Johnson, 1979). Sensitivities of the assays were 5 pg for progesterone, 17 α -hydroxyprogesterone and 20 α -dihydroprogesterone, 4 pg for androstenedione and 2 pg for oestrone and oestradiol. Cross-reactivities for 17 α -hydroxyprogesterone did not exceed 1% for all steroids analysed (Steranti Research Ltd). The inter-assay and intra-assay coefficients of variation for steroid assays were all <7.0%.

LH. LH was determined by using the NIAMDD kit. The method used was that of Bast & Greenwald (1974) and the antiserum was anti-rat LH serum 3. The reference preparation used for standards was rat LH-RP-1 (0.03 $\times$ NIH-LH-S1). The iodination preparation was LH-I-6. The assay sensitivity was 350 pg and the intra- and inter-assay coefficients of variation were 5.33 and 9.33% respectively.

Statistics

Specific differences were determined by Duncan's Multiple Range test (Steel & Torrie, 1960) following one-way analysis of variance. Differences were adjudged significant if $P < 0.05$.

Results

Effect of gonadotrophins

Collagenase-dispersed interstitial cells were $85 \pm 1.2\%$ (mean $\pm$ s.e.m., $n = 12$) viable before incubation and $69.6 \pm 1.2\%$ (mean $\pm$ s.e.m., $n = 5$) viable following a 2 h incubation. Steroid

values are expressed as pg/10 000 viable cells based on preincubation viability estimates. Interstitial cells produced baseline progesterone values of 81 ± 7 pg/10 000 viable cells/2 h and there were no detectable amounts of oestradiol, oestrone or androstenedione. Serum concentrations of 17 α -hydroxyprogesterone were undetectable; baseline levels of 17 α -hydroxyprogesterone from interstitial cells were undetectable but rose to 26 ± 5 pg/10 000 cells/2 h in response to 25 ng LH/ml. Concentrations of 20 α -dihydroprogesterone were undetectable in serum or interstitial cells from hypophysectomized hamsters.

Interstitial cells responded to LH stimulation *in vitro* with a dose-dependent increase in progesterone values which remained level at 100 ng LH/ml (Table 1). Neither FSH (200 or 1000 ng/ml) nor prolactin (125 or 250 μ g/ml) stimulated steroid production by interstitial cells *in vitro*.

Table 1. Effect of LH on in-vitro progesterone production by interstitial cells from hypophysectomized hamsters

Treatment	Progesterone (pg/10 000 cells/2 h)
None	81 ± 7 (6)
1 ng LH	160 ± 12 (5)
10 ng LH	$367 \pm 18^*$ (6)
25 ng LH	$562 \pm 67^*$ (6)
100 ng LH	$2252 \pm 292^*$ (3)
200 ng LH	$2417 \pm 292^*$ (3)

Values are mean $\pm$ s.e.m. with the no. of observations in parentheses.

* Significantly different from control value, $P < 0.05$.

Role of protein synthesis and microfilaments

The addition of 10^{-4} M-cycloheximide slightly reduced baseline progesterone values and completely blocked LH-induced progesterone secretion (Table 2). Cytochalasin B treatment had no effect on baseline production but significantly reduced LH-stimulated progesterone secretion (Table 3).

Table 2. Effect of cycloheximide and LH on in-vitro progesterone production by interstitial cells from hypophysectomized hamsters

Treatment	Progesterone (pg/10 000 cells/2 h)
None	81 ± 7 (6)
25 ng LH	$562 \pm 67^*$ (6)
10^{-4} M-cycloheximide	45 ± 16 (5)
10^{-4} M-cycloheximide + 25 ng LH	55 ± 10 (6)

Values are mean $\pm$ s.e.m. with the no. of observations in parentheses.

* Significantly different from control value, $P < 0.05$.

Effects of catecholamines, serum and GnRH

Epinephrine and isoproterenol increased progesterone production (Table 4). Propranolol at 6×10^{-6} M, a dose that inhibits catecholamine action without anaesthetizing membrane effects (Jordan *et al.*, 1978), blocked the response of interstitial cells to epinephrine and isoproterenol but had no effect on LH-induced progesterone production (Table 4). Simultaneous administration of catecholamines and LH did not produce an additive increase in progesterone

Table 3. Effect of cytochalasin B and LH on in-vitro progesterone production by interstitial cells from hypophysectomized hamsters

Group	Treatment	Progesterone (pg/10 000 cells/2 h)
1	None	60 ± 16 (6)
2	25 ng LH	453 ± 43 ^a (5)
3	10 µg cytochalasin B	59 ± 2 (3)
4	20 µg cytochalasin B	43 ± 1 (5)
5	10 µg cytochalasin B + 25 ng LH	192 ± 10 ^b (5)
6	20 µg cytochalasin B + 25 ng LH	139 ± 22 ^b (6)

Values are mean ± s.e.m. with the no. of observations in parentheses.

^a Significantly different from values for all other groups, $P < 0.05$.

^b Significantly different from control value, $P < 0.05$.

Table 4. Effect of catecholamines and LH on in-vitro progesterone production by untreated or propranolol-treated interstitial cells from hypophysectomized hamsters

Group	Treatment	Progesterone (pg/10 000 cells/2 h)
1	None	57 ± 14 (6)
2	25 ng LH	568 ± 59 ^a (6)
3	500 ng epinephrine	415 ± 26 ^b (6)
4	500 ng isoproterenol	540 ± 37 ^a (6)
5	6 × 10 ⁻⁶ M-propranolol	54 ± 8 (6)
6	LH + epinephrine	574 ± 23 ^a (6)
7	LH + isoproterenol	562 ± 20 ^a (6)
8	LH + propranolol	650 ± 50 ^a (6)
9	Epinephrine + propranolol	53 ± 8 (6)
10	Isoproterenol + propranolol	42 ± 5 (5)

Values are mean ± s.e.m. with the no. of observations in parentheses.

^a Significantly different from values for Groups 1, 3, 5, 9 and 10, $P < 0.05$.

^b Significantly different from control value, $P < 0.05$.

secretion, indicating that both substances were stimulating progesterone production by the same mechanism in the same cell type.

The addition of untreated, charcoal-extracted, heat-treated or charcoal extracted-heat treated serum from hypophysectomized hamsters had no effect on baseline or LH-induced progesterone production. Only the addition of 10⁻⁶ M-D-ala⁶-LHRH significantly reduced the baseline levels of progesterone (Table 5).

Table 5. Effect of GnRH and D-ala⁶-LHRH on in-vitro progesterone production by interstitial cells of hypophysectomized hamsters

Treatment	Progesterone (pg/10 000 cells/2 h)
None (control)	45 ± 7 (8)
10 ⁻⁶ M-GnRH	51 ± 6 (6)
10 ⁻⁷ M-GnRH	40 ± 7 (7)
10 ⁻⁶ M-D-ala ⁶ -LHRH	25 ± 1* (5)
10 ⁻⁷ M-D-ala ⁶ -LHRH	52 ± 7 (6)

Values are mean ± s.e.m. with the no. of observations in parentheses.

* Significantly different from control value, $P < 0.05$.

Treatment of hypophysectomized hamsters with 400 ng GnRH 30 min before killing had no effect on interstitial cell steroidogenesis or serum progesterone concentrations (<1 ng/ml). In addition, serum LH concentrations were undetectable. Injection of 400 ng GnRH to intact hamsters on the morning of Day 1, 30 min before decapitation, resulted in serum LH concentrations of 144.3 ± 26.2 ng/ml (14.3 ± 2.5 ng/ml for intact control animals).

Discussion

Although the interstitial cell preparations used in these experiments were not highly purified, several lines of evidence support the contention that progesterone is produced by interstitial cells and not by contaminating follicular cells. Corpora lutea are not maintained following hypophysectomy in the hamster. Histochemical studies demonstrated that by Day 6 after hypophysectomy, only the interstitium contained Δ^5 - 3β -hydroxysteroid dehydrogenase; the enzyme was lacking from the small preantral follicles (Taya *et al.*, 1980). Finally, ^{125}I -labelled hCG binds only to interstitial cells and theca in ovaries from cyclic hamsters and not to granulosa cells of preantral follicles (Oxberry, 1981).

Although 25 ng LH did not produce maximal progesterone production by interstitial cells, this dose was used in these studies because it consistently resulted in at least a 7-fold increase in progesterone production and allowed for the determination of possible additive effects of LH and catecholamines.

It was possible that progesterone was produced by hypophysectomized hamsters but was rapidly converted to metabolites. The progestagen, 17α -hydroxyprogesterone represents a steroidogenic endpoint in the hamster ovary similar to that of 20α -dihydroprogesterone in the rat (Hubbard & Greenwald, 1982). Thus, if progesterone produced by interstitial cells was being converted *in vivo*, 17α -hydroxyprogesterone would be a likely end product. However, 17α -hydroxyprogesterone and 20α -dihydroprogesterone were undetectable in serum of hypophysectomized hamsters. Our study shows that interstitial cells from hypophysectomized hamsters produce 17α -hydroxyprogesterone only in response to LH and indicates that progesterone is not produced *in vivo* and metabolized. However, other metabolites of progesterone could have been involved.

The addition of serum from hypophysectomized hamsters to incubations of interstitial cells had no effect on progesterone synthesis indicating a lack of circulating inhibitor of steroidogenesis in the hypophysectomized animal. However, D-ala⁶-LHRH did effectively inhibit baseline progesterone production (Table 5). While serum concentrations of GnRH are not great enough to cause an inhibition of progesterone production *in vivo* it is possible that a local GnRH-like factor is present in preantral follicles or uterus. A uterine factor may also involve one or more prostaglandins.

The necessity of protein synthesis for trophic hormone stimulation of steroidogenesis has been documented for rat adrenal (Davis & Garren, 1968), rat ovaries (Azhar, Menon & Menon, 1980) and ovine corpora lutea (Silavin, 1979). The interstitial cells of hypophysectomized hamsters therefore appear to require protein synthesis in order to produce progesterone in response to LH in the same manner as do other steroidogenic tissues.

Microfilaments appear to be important cellular elements in trophic hormone stimulation of steroidogenesis. There is evidence suggesting a role for microfilaments in transport of cholesterol to mitochondria in steroidogenic tissue, including ovine corpus luteum (Silavin, Moss & Niswender, 1980), rat luteal cells (Azhar & Menon, 1981) and adrenal tumour cells (Mrotek & Hall, 1977). Cytochalasin B inhibits trophic hormone stimulation of steroidogenesis without affecting baseline steroid production and blocks LH-induced progesterone production in hamster interstitial cells, indicating a role for microfilaments in the steroidogenesis of these cells.

Catecholamines stimulated progesterone production in interstitial cells and propranolol

blocked this effect without affecting LH-induced progesterone production (Table 4). The effects of LH and catecholamines were not additive, suggesting that both substances stimulate steroidogenesis via the same mechanism and, in addition to retention of LH binding sites, adrenergic receptors are also retained in the interstitium of the long-term hypophysectomized hamster. The presence of adrenergic innervation of the interstitium has been shown in the rat and guinea-pig (Burden, 1972). It is unclear what role catecholamines may play alone or in combination with gonadotrophins in interstitial cell steroidogenesis during the cycle or pregnancy.

Interstitial cells have morphological and steroidogenic properties similar to those of other steroid-producing cells except that they retain the gonadotrophin receptor–adenylate cyclase mechanism and steroidogenic enzymes for long periods despite lack of gonadotrophin. These characteristics may be of particular importance to animals that are seasonal breeders and whose ovaries become quiescent during the non-breeding season or hibernation. In bats, the first ovarian event to occur before the end of hibernation is hypertrophy of interstitial cells (Oxberry, 1979). Similarly, administration of PMSG to hypophysectomized rats leads to interstitial cell hypertrophy (Carithers & Green, 1972), suggesting increased activity of the interstitial gland. Thus, the ability of interstitial cells to produce steroid after gonadotrophin deprivation may be necessary to 'prime' follicular growth in the ovary at the onset of the breeding season.

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References

- Azhar, S. & Menon, K.M.J. (1981) Receptor-mediated gonadotropin action in the ovary. Action of cytoskeletal element-disrupting agents on gonadotropin-induced steroidogenesis in rat luteal cells. *Biochem. J.* **194**, 19–27.
- Azhar, S., Menon, M. & Menon, K.M.J. (1980) Receptor mediated gonadotropin action in the ovary. Modulation of progesterone response in isolated rat ovarian cells by gonadotropin, cholera enterotoxin and cyclic nucleotides: requirement for RNA and protein synthesis. *Acta endocr., Copenh.* **95**, 528–539.
- Banik, U.K. & Givner, M.L. (1975) Ovulation induction and antifertility effects of an LHRH analogue (AY-25,205) in cyclic rats. *J. Reprod. Fert.* **44**, 87–89.
- Bast, J.D. & Greenwald, G.S. (1974) Serum profiles of follicle stimulating hormone, luteinizing hormone and prolactin during the estrous cycle of the hamster. *Endocrinology* **94**, 1295–1299.
- Burden, H.W. (1972) Adrenergic innervation in ovaries of the rat and guinea-pig. *Am. J. Anat.* **133**, 455–462.
- Carithers, J.R. & Green, J.A. (1972) Ultrastructure of rat ovarian interstitial cells. II. Response to gonadotropin. *J. Ultrastruct. Res.* **39**, 251–261.
- Clayton, R.N., Harwood, J.P. & Catt, K.J. (1979) Gonadotrophin-releasing hormone analogue binds to luteal cells and inhibits progesterone production. *Nature, Lond.* **282**, 90–92.
- Condon, W.A. & Black, D.L. (1976) Catecholamine-induced stimulation of progesterone by the bovine corpus luteum *in vitro*. *Biol. Reprod.* **15**, 573–578.
- Davis, W.W. & Garren, L.D. (1968) On the mechanism of action of adrenocorticotrophic hormone. The inhibitory site of cycloheximide in the pathway of steroid biosynthesis. *J. biol. Chem.* **243**, 5153–5157.
- Hsueh, A.J.W. & Ling, N.C. (1979) Effect of an antagonistic analog of gonadotropin releasing hormone upon ovarian granulosa cell function. *Life Sci.* **25**, 1223–1230.
- Hubbard, C.J. & Greenwald, G.S. (1982) Cyclic nucleotides, DNA, and steroid levels in ovarian follicles and corpora lutea of the cyclic hamster. *Biol. Reprod.* **26**, 230–240.
- Johnson, D.C. (1979) Maintenance of functional corpora lutea in androgenized female rats treated with PMSG. *J. Reprod. Fert.* **56**, 263–269.
- Jordan, A.W., Caffrey, J.L. & Niswender, G.D. (1978) Catecholamine-induced stimulation of progesterone and adenosine 3',5'-monophosphate production by dispersed ovine luteal cells. *Endocrinology* **103**, 385–392.
- Massicotte, J., Veilleux, R., Lavoie, M. & Labrie, F. (1980) An LHRH agonist inhibits FSH-induced cyclic AMP accumulation and steroidogenesis in porcine granulosa cells in culture. *Biochem. Biophys. Res. Commun.* **94**, 1362–1366.
- Mrotek, J.J. & Hall, P.F. (1977) Response of adrenal tumor cells to adrenocorticotropin: site of inhibition by cytochalasin B. *Biochemistry, N.Y.* **16**, 3177–3181.
- Oxberry, B.A. (1979) *The reproductive biology of the female vespermillionid bat, Antrozous pallidus*. PhD. dissertation, University of Arizona.

- Oxberry, B.A. (1981) Autoradiographic analysis of gonadotropin binding in the hamster ovary throughout the estrous cycle. *Biol. Reprod.* **24**, Suppl. 1, p. 118a, Abstr. 192.
- Parker, R.C. (1961) *Methods of Tissue Culture*, p. 57. Paul B. Hoeber, Inc., New York.
- Shaha, C. & Greenwald, G.S. (1982) *In vivo* and *in vitro* production of progestins by the corpus luteum of pregnancy of the hamster. *Biol. Reprod.* **26**, 854–860.
- Silavin, S.L. (1979) *Regulation of steroidogenesis in the ovine corpus luteum*. Ph.D. dissertation, Colorado State University, Fort Collins.
- Silavin, S.L., Moss, G.E. & Niswender, G.D. (1980) Regulation of steroidogenesis in the ovine corpus luteum. *Steroids* **36**, 229–241.
- Steel, R.G. & Torrie, J.H. (1960) *Principals and Procedures of Statistics*. McGraw-Hill, New York.
- Surve, A.H., Bacso, I., Brinkerhoff, J.H. & Kirsch, S.J. (1976) Plasma levels of progesterone in pseudo-pregnant rabbits actively immunized with a progesterone protein conjugate. *Biol. Reprod.* **15**, 343–349.
- Taya, K. & Greenwald, G.S. (1980) *In vitro* and *in vivo* ovarian steroidogenesis in the long-term hypophysectomized hamster. *Endocrinology* **106**, 1093–1098.
- Taya, K., Saidapur, S.K. & Greenwald, G.S. (1980) Interstitium: site of steroid synthesis in the ovary of the long-term hypophysectomized hamster. *Biol. Reprod.* **22**, 307–318.
- Terranova, P.F., Connor, J.S. & Greenwald, G.S. (1978) *In vitro* steroidogenesis in corpora lutea and non-luteal ovarian tissues of the cyclic hamster. *Biol. Reprod.* **19**, 249–255.
- Wright, K., Collins, D.C. & Preedy, J.R.K. (1973) Comparative specificity of antisera raised against estrone, estradiol-17 β and estriol using 6-O-carboxymethyloxine bovine serum albumin derivatives. *Steroids* **21**, 755–769.

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