

CELL CULTURE OF TESTICULAR GLANDS FOR TESTOSTERONE, ESTRADIOL, AND PROGESTERONE PRODUCTION

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Introduction. Testicular cells are categorized into two main groups: germ cells and stromal cells. Germ cells, located within the seminiferous tubules, develop into sperm. Stromal cells, which include Sertoli and Leydig cells, support germ cell development and hormone production, respectively.

The testis consists of seminiferous tubules, which comprise the bulk of testicular mass and contains three types of cells, spermatogonia, spermatocytes and Sertoli cells, and interstitial space between these tubules containing the androgen-producing Leydig cells and other cell types.

Leydig cells, also known as interstitial cells of the testes and interstitial cells of Leydig, are found adjacent to the seminiferous tubules in the testicle and produce testosterone in the presence of luteinizing hormone (LH). There are two types of cell division: mitosis and meiosis. Most of the time when people refer to “cell division”, they mean mitosis, the process of making new body cells. Meiosis is the type of cell division that creates egg and sperm cells.

Leydig cells represent a majority of testicular interstitial cells. They produce androgens for normal spermatogenesis and other androgen-target organs beyond the testis. Leydig cells also regulate testicular macrophage and lymphocyte numbers. For instance, testosterone is synthesized in Leydig cells in the adult testes. It showed that mouse fetal Leydig cells prepared from neonatal testes produce androstenedione, which thereafter is converted to testosterone by the cells within the seminiferous tubules. Testicular macrophages are the largest leukocyte population in the testis. Their environment is characterized by the seemingly contradictory needs for tolerance against the autoantigenic germ cells and the capacity to mount pro-inflammatory innate immune responses against invading pathogens.

Leydig cells are another type of interstitial cells, which are responsible for androgen biosynthesis (mostly testosterone and androstenedione), and consequently the regulation of secondary sex characteristics.

The Sertoli cells express functional receptors for FSH, whereas the Leydig cells express LH receptors. Thus, these two receptors are mutually exclusive. The LH-stimulated Leydig cell androgen production is essential for the development and maintenance of the male reproductive tract and spermatogenesis.

Human chorionic gonadotrophin (hCG) is a hormone produced by the placenta during pregnancy. It's sometimes called the pregnancy hormone because of its unique role in supporting a pregnancy. HCG is found in your urine or blood around 10 to 11 days after conception (when a sperm fertilizes an egg). In men or adolescent boys, HCG helps the production of testosterone and sperm. HCG is also used in male children with cryptorchidism, a specific birth problem of the testes. Men also produce small amounts of hCG to synthesise progesterone and stimulate leydig cells in the testicles

to produce testosterone. For this reason, hCG is relevant to men's health conditions such as hypogonadism (low testosterone) and sexual dysfunction.

Aim. To determine sexual hormone production in cell culture: testosterone (T), estradiol (E₂) and progesterone (P) before and after Gonadotropine Chorionic (hCG) stimulation.

Material and Methods. Continuous cell culture of testicular gland, epithelial-like. Testicular cell culture PTP and TEB received for research from Research Institute of VIEV by Doctor of Biological Science Lev Dyakonov and Tatyana Galnbek.

Procedure of removing cells from the glass surface of flask: trypsin from bovine pancreas 0.25%, EDTA 0.02% solution in ration 1:9. Multiplicity of reseeding is 1:2; 1:3.

For testing of cultural solution for steroid hormones we used test kits producing in company, (Xema LLC, Kiyv, Ukraine). Test kits by solid phasic method for immune enzyme analysis: testosterone (T) free, estradiol (E₂) free and progesterone (P).

Solid phasic assay it is one of components of the immune system such as antigen or antibody adsorbed on solid carrier. For example on the wells of polystyrene plate.

For cultivating PTP and TEB cell culture we used media Eagla's MEM, with double amount of aminoacids and vitamins. Produced in the factory of nutrient media Research Institute of Polio Eradication.

Luteinizing hormone (LH) ELISA. A solid-phase enzyme immunoassay kit for the quantitative determination of luteinizing hormone in human serum or plasma. Shelf life - 18 months. Sample volume - 50 µL. Storage conditions +2 +8°C. Calibrators range: 5.0 - 100 IU/L. Detection range of sensitivity: LoD - 0.15 IU/L. Range of quantitative determination LoQ - 5.0 IU/L. Hook effect: absent up to 100 IU/L. (Xema LLC Company, Kyiv, Ukraine).

Follicle stimulating hormone (FSH) ELISA. A solid-phase enzyme immuno assay kit for the quantitative determination of follicle stimulating hormone in human serum or plasma. The FSH ELISA kit is an enzyme immunoassay, intended for the quantitative determination of follicle stimulating hormone in human serum or plasma. Shelf life - 18 months. Sample volume - 50 µL. Storage conditions +2 +8°C. Calibrators range: 5.0 - 100 IU/L. Detection range of sensitivity: LoD - 0.15 IU/L. Range of quantitative determination LoQ - 2.5 IU/L. Hook effect: absent up to 100 IU/L. Linearity: 5.0 - 50 IU/L.

Prolactin (PRL) ELISA. A solid-phase enzyme immunoassay kit for the quantitative determination of prolactin in human serum or plasma. Shelf life - 18 months. Sample volume - 50 µL. Storage conditions +2 +8°C. Calibrators range: 100.0 - 2000.0 mIU/L. Detection range of sensitivity: LoD - 5.0 mIU/L. Range of quantitative determination LoQ - 100.0 mIU/L.

Free Testosterone ELISA. A solid-phase enzyme immunoassay kit for the quantitative determination of free testosterone in human serum or plasma. Shelf life - 18 months. Sample volume - 25 µL. Storage conditions +2 +8°C. Calibrators range: 0.2 - 100.0 pg/mL. Detection range of sensitivity: LoD - 0.06 pg/mL. Range of quantitative determination LoQ - 0.2 pg/mL.

Estradiol (E₂) ELISA. A solid-phase enzyme immunoassay kit for the quantitative determination of estradiol in human serum or plasma. Shelf life - 18

months. Sample volume - 25 μ L. Storage conditions +2 +8°C. Detection range of sensitivity: LoD - 0.025 nmol/L. Range of quantitative determination LoQ - 0.05 nmol/L.

Progesterone (P) ELISA. A solid-phase enzyme immunoassay kit for the quantitative determination of progesterone in human serum or plasma. Shelf life - 18 months. Sample volume - 25.0 μ L. Storage conditions +2 +8°C. Calibrators range: 1.0 - 300.0 nmol/L. Detection range of sensitivity: LoD - 0.25 nmol/L. Range of quantitative determination LoQ - 0.75 nmol/L.

Results and Discussion. PTP cell culture it is from piglets testicules in age 1-30 days. Obtained in 1988 year from piglets testicules from Ukrainian Research Institute NIVI from cell culture of testicle in gnobiote piglets. It is epithelial-like cells, monolayer cell culture. For cultivation of PTP cells necessary to use Eagla's MEM media with double amount of aminoacids and vitamins. Necessary to add in media 300 mg L-glutamine for 450.0 ml in media flask, bovine serum 10% and antibiotic kanamycine - 100 IU/ml to prevent from mycoplasma contamination. Cells safety during cryoconservation and vital ability 90-95%. Procedure of removing cells from the glass surface of flask, trypsin from bovine pancreas 0.25% solution and EDTA 0.02% solution in ratio 1:9. Ratio of reseeding is 1:3. Adopted to growth in Earla's media with 5% hemohydrolisis and 10% bovine serum, pH 7.6-7.8. PTP cell culture were grown in wall culture using stacionary and roller methods. Growth properties: with reseeding with a ratio 1:4; 1:6 monolayer is forming during 2-3 days and persists during 10-12 days without changing of media. In 1.0 liter of cultural flask accumulate 50-60 million cells.

Kariological properties: (2n=38). PTP cell culture heteroploid. Cells contain from 34 up to 45 chromosomes. Modal number of cells with 40 chromosomes (52%). PTP cell culture restores original growth properties at 2-3 passages.

TEB cell culture it developed from embryonal calf testicules in age 8 month. It is epithelial-like cells, monolayer. For cultivation of PTP cells necessary to use Eagla's MEM media with double amount of aminoacids and vitamins. Add 300 mg L-glutamine for 450.0 ml media flask, bovine serum 10%, antibiotic kanamycine - 100 IU/ml to protect from mycoplasma contamination. Cells are removed from the walls of glass by 0.02% sodium salt of Ethylenediaminetetraacetic acid (EDTA), with 0.1 mg/ml. trypsin in temperature 37 $\pm$ 0.2°C, ratio 9:1. Cultivation frequency once in 5-7 days. Cells safety during cryoconservation 90-95%. Ratio of cell reseeding 1:2; 1:3. Monolayer consists from epithelial-like cells, polygonal or round cell forms. Cytoplasm is well expressed, rounded kernels, chromatin located evenly, almost in every place of microscope visible mitosis. Continious cell line has stability, morphological and genetical properties, easily of receive and cultivation.

Cell safety during cryoconservation in liquid nitrogen and vital ability 80-90%.

Caryologic characteristics: karyotype - 2n=60, modal number of chromosome 50, variability interval 49-52. polyploids 2Y, marker chromosomes: M1-M5 metacentrics with Robertsonian translocations M8, M11, M14 - submetacentrics.

Gonadotropine chorionic (hCG) it is proteinic substantion produces by placenta in pregnant women. Biological activity of Gonatropine Chorionic (hCG) similar to Luteinizing hormone (LH), produces in anterior pituitary gland and has half-life period

effect compare with LH. Therefore action is more prolonged. Gonadotropine chorionic stimulates interstitial Leydig cells and produces testosterone in testicles. The biological half-life is approximately 8 to 12 hours. Due to the slow release human chorionic gonadotropine (hCG) can accumulate when taken at short intervals (for exaple, every day). hCG intendes to activate the function of the testicles, therapeutic result depends on functional capacity of the testicles. In case of hypersecretion of Gonadotropin which is a sign of irreversible processes of primary sexual insufficiency not necessary to use hCG.

Primary dose for stimulation of normal testicular cell culture PTP and TEB is 250-300 international units (IU). hCG starts to producing in placenta with tissue of chorion implantates of embryo from 6-8 days after fertilization. If cell culture has signs of hypogonadism, in this case hCG starts for stimulation with a dose 500-1000 IU. In male, exogenous hCG stimulates spermatogenesis and production of steroid sexual hormones.

Testosterone level was significantly increased after hCG treatment. The serum testosterone levels at baseline and after 2, 4, 12, and 24 weeks of hCG treatment were 0.90 ± 1.35 ng/ml, 1.77 ± 1.31 ng/ml, 3.74 ± 2.24 ng/ml, 5.49 ± 1.70 ng/ml, and 5.58 ± 1.75 ng/ml, respectively ($p < 0.05$).

Cell cultures of calf testicular and embryonic cells continuous cultures used to study cellular function, development, and the production of biological products. Primary cultures are isolated from tissues, and continuous cultures can be propagated in the laboratory.

Piglet testicular and embryonic cell cultures are in vitro systems for studying and propagating these cells. These cultures are used in biomedical research to study reproduction, development, and other functions of the body.

Piglet testicular cells can be considered cell cultures because they are isolated directly from living tissue, piglet testicles. These cultures are used to study specific properties of the cells, such as their response to various stimuli, their behavior in the presence of viruses or their ability to proliferate and differentiate. These cell cultures can be used to study cell responses to various physiological and pathological stimuli. Cell cultures can be used to study the effects of drugs on cells and to identify new potential drug targets.

Conclusions. Continues epithelial cell culture determined to steroid hormone production. Such hormones as: testosterone, estradiol, progesterone. Further research should be focused on stimulation of cells with human chorionic gonadotropin (hCG) in different doses.

Keywords: cell culture, chronic gonadotropin, testosterone, estradiol, progesterone, continues epithelial cells.