JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

LEYDIG CELLS AS A MODEL OF MALE REPRODUCTIVE SYSTEM

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ARTICLE INFO

Received 25. 10. 2013
Revised 20. 11. 2013
Accepted 16. 12. 2013
Published 1. 2. 2014

Review

OPEN ACCESS

ABSTRACT

During the past decades, a large anount of information concerning the infertility, which can be caused by malfunction at the level of sperm or production of testosterone was published. It is about androgene which is from 95 percent synthetized in testes. It plays significant role in development of individual´s sexual signs and is also the starter of spermatogenesis. The main mechanism ensuring the production of this important hormone is the process determined as a steroidogenesis. This process runs in cells located in testes and are known as Leydig cells (LC). Several types of LC are classified as for example fetal, adult, stem, progenitor or immature cells. There are mutual differences, but their common feature is a production of androgenes. Mitochondria and endoplasmic reticulum have irreplaceable position within LC and they, together with relevant enzymes and cascades of reactions, ensure the metamorphosis of cholesterol up to testosterone. With rising age the activity of steroidogenesis declines what is, however, natural. But there are many cases when this process in cells of developing individual is impaired by external or internal factors. Their identification and consequent elimination is for sufficient production of testosterone very important. This review contains on overview of Leydig cell biology and testosterone synthesis.

Keywords: Infertility, testosterone, Leydig cells, steroidogenesis

INTRODUCTION

Regulation reproductive system of males

Male reproductive system is controlled through interplay of the nervous and endocrine systems. Control center of sexual function is hypothalamic-pituitary system. The Leydig cells produce testosterone in response to hormonal stimulation by the pituitary gonadotropin luteinizing hormone. Testosterone is bound to the ABP (androgen – binding protein), which is produced by the Sertoli cells. Increased secretion of LH stimulates the cells to increased secretion of testosterone and low levels of this hormone, stimulates the secretion of LH and the cycle is repeated. This process is known as negative feedback **(Svechnikov *et al.*, 2008).**

Leydig cells

Somatic testicular Leydig cells are an important part development of male reproductive organs and male reproduction. This chapter will describe and characterized distinct stages development Leydig cells and their most important functions.

Description

Leydig cells are situating in testis, they have spherical shape and they are between seminiferous tubules. This cells contain a lot of amounts smooth endoplasmic reticulum and mitochondria. One of the basic function is production of androgen. Main androgen (male sex hormone) is testosterone. We can classify two distinct generations of Leydig cells. The first type are fetal Leydig cells and second are adult Leydig cells. Despite their differences in morphological and biochemical properties, fetal and adult Leydig cells share the same principal function to produce androgens **(Habert and Picon, 1982).**

Fetal Leydig cells (FLC)

This type of cells start to appear in the mesenchyme of the developing in rats prenatal tests at 14.5 weeks. These cells then secrete testosterone and other androgens, which regulate not only the masculinization of internal and external male genital, but also neuroendocrine functions **(Habert and Picon, 1982).**
In rats, FLCs number increase from $2,5x10^4$ to about $1,2x10^5$, but then markedly decrease during several weeks after birth. Moreover, it has been suggested that the FLCs may have first evolved from slight modifications of fetal adrenal cells. This hypothesis is supported by the finding that adrenal cortex and gonads are both derived from the common adrenal-gonadal primordium before they separate into separate organs **(O'Shaughnessy *et al.*, 2006).**
FLCs possess well-developed steroidogenic machinery expressing the key staroidogenic enzymes, such as steroidogenic acute regulatory (StAR) protein, cytochrome P450scc, 3β-hydroxysteroid dehydrogenase-isomerase (3βHSD), cytochrome P450c17, and 5α-reductase (5α-R) required for androgen synthesis. Testosterone produced by FLCs is converted to 5α- dihydrotestosterone (DHT) by the enzyme 5α-R. DHT is a more potent androgen than testosterone and controls proper development of male external genitalia (e.g., the penis and scrotum). FLCs dysfunction may lead to various malformations in the male reproductive tract of humans and animals **(Hughes and Acerini, 2008).**
FLCs do not require for their development and initial formation male sex hormone, testosterone, luteinizing hormone (LH). On the other hand, adult Leydig cells, which also produce large amounts of testosterone, are much more influenced by LH (**Baker and O'Shaughnessy, 2001; Migrenne *et al.*, 2001).**
Some hypothesis present the relationship between fetal Leydig cells and adult Leydig cells, while others theories characterized as two different cells populations with distinct gene expression **(Dong *et al.*, 2007).**

Adult Leydig cells

Adult Leydig cells originate postnatally and they are not derived from preexisting fetal Leydig cells, but most likely from undifferentiated peritubular-like stem cells. This cells produce testosterone required for development of external genitalia and onset of spermatogenesis. Four distinct types of cells have been identified and characterized as involved in the sequence of events leading to adult Leydig cells: the stem Leydig cells (SLCs), progenitor Leydig cells (PLCs), immature Leydig cells (ILCs) and adult Leydig cells (ALCs) **(Chen *et al.*, 2009).**

Stem Leydig cells (SLCs)

The (SLCs) are undifferentiated cells that are capable of indefinite self-renewal but also of differentiation to steroidogenic cells. Spindle-shaped cells are seen in the testicular interstitium, primarily in the peritubular layer. The Leydig stem cells proliferate neonatally and transform to progenitor cells by day 14 postpartum in rodents **(Chen *et al.*, 2009).**

Progenitor Leydig cells (PLCs)

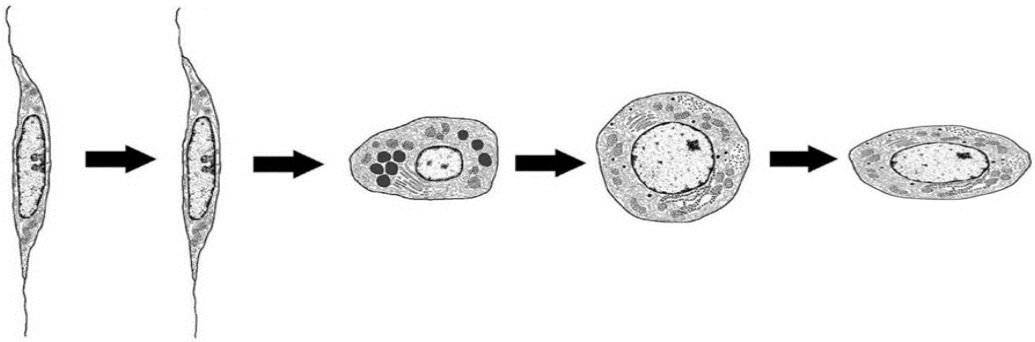
The PLCs are small spindle-shaped cells that are similar in appearance to the stem cells. They contain only small amounts of smooth endoplasmic reticulum, the organelle that houses several steroidogenic enzymes (e.g., 3β-hydroxysteroid dehydrogenase, cytochrome P450c17, 17β-hydroxysteroid dehydrogenase) and produce low but detectable levels of androgens **(Hardy et al., 1990).**
PLCs gradually enlarge and reduce their proliferative capacity. By postnatal day 28, the PLCs transform from spindle-shaped to round immature Leydig cells **(Chen *et al.*, 2009).**

Immature Leydig cells (ILCs)

During transformation prom PLC to ILC, the smooth endoplasmic reticulum (ER) complement of the cells expands greatly, conferring an ultrastructure with similarities to that of adult Leydig cells. Concurrent with the expansion of smooth (ER) the levels of 3β-HSD, P450scc and P450c17 increase, and the cells develop a capacity for steroidogenesis. 3β-HSD and 5-α-reductase activity is higher as in ALCs. A distinguishing characteristic of immature Leydig cells is their numerous cytoplasmic lipid droplets **(Shan and Hardy, 1992).**

Adult Leydig cells

The ILC population doubles only once from day 28 to day 56 producing the total adult population of approximately 25 million Leydig cells per testis. The activities of androgen metabolizing enzymes decline by day 56 as ILC differentiate into ALCs **(Steinberger and Ficher, 1969).**
In Leydig cells of 90 day old adults, testosterone production is 150 times greater than that by PLCs at 21 days of age and 5 times greater than that by ILCs at 35 days of age **(Shan *et al.*, 1993).**
Compared to ILCs that ALCs have a greater abundance of smooth ER. ALCs do not normally proliferate, but can regenerate if the original population is eliminated. Thus, the adult population of Leydig cells is completely regenerated within 7 weeks of its elimination by ethane dimethanesulfonate (EDS), an agent that kills ALCs specifically **(Sharpe *et al.*, 1990).**
Testosterone produced by adult Leydig cells is very important for development sexual organs and the onset of spermatogenesis. If is decrease levels this hormone, it is not consequence reduced number of Leydig cells but incorrect steroidogenic function **(Chen *et al.*, 1994).**



CELLULAR ATTRIBUTES	STEM LEYDIG CELL	PROGENITOR LEYDIG CELL	IMMATURE LEYDIG CELL	ADULT LEYDIG CELL	AGED LEYDIG CELL
Mitotic activity	Low	high	Once	Probably none	Probably none
Morphology	Spindle	Spindle	Round with large lipid droplets	Round with small lipid droplets	Round with small lipid droplets
Smooth ER	Low	Low	High	Highest	High
Testosterone Metabolism	None	High	Highest	Low	Low
Testosterone Synthesis	None	Low	Intermediate	High	Intermediate
Age when cell is typical	7 days	21 days	35 days	90 days	20 months
LH Receptor Number	None	Low	Intermediate	High	Intermediate
Androgen Receptor Number	?	High	High	Low	?

Figure 1 Stages of adult Leydig cell development and aging in the rat. Stem Leydig cells, progenitor Leydig cells, immature Leydig cells, adult Leydig cells and aged Leydig cells **(Chen *et al.*, 2009).**

Function

Steroidogenesis in Leydig cells is regulated mainly by LH, a glycoprotein produce by the anterior pituitary. Binding of LH to G-protein coupled receptor stimulates adenylate cyclase activity, resulting in increased cyclic adenosine monophospate (cAMP) formation. cAMP stimulates the cAMP-dependet protein kinase (PKA). The delivery of cholesterol molecules into the inner mitochondrial membrane is generally accepted to be the rate determining step in steroidogenesis **(Stocco and Clark, 1996).**
In LC steroidogenesis, the substrate cholesterol in converted to testosterone through a series of steroidogenic steps catalyzed by different proteins, including the enzymes StAR protein, P450scc, 3β-HSD, P450c17 and 17β-HSD. The expression and activities of these proteins are affected by the age. Reduction in the activities of these steroidogenic protein directly reduces testosterone synthesis in aged Leydig cells **(Payne and Hales, 2004).**
The cholesterol transport mechanism is a complex process, involving an interaction between the steroidogenic acute regulatory (StAR) protein and benzodiazepine receptor (PBR). StAR is functioning as an initiator of cholesterol transport and PBR as a gate for cholesterol entry into mitochondria. It has high affinity to bind cholesterol from cytosolic donors and transfers it from the outer into the inner mitochondrial membrane (**Hauet *et al.*, 2005).**
When cholesterol reaches the inner mitochondrial membrane, it is immediately converted into pregnenolone. This reaction catalyzed by the cytochrome P450scc enzyme CYP11A1 which decreases during male aging. Pregnenolone then leaves the mitochondria to the smooth endoplasmic reticulum, where it is converted by 3β-HSD to progesterone. This steroid is then metabolized by P450c17 to

androstenedione, which is converted to testosterone by 17β-HSD **(Payne and Hales, 2004).**

Mechanisms responsible for steroidogenic deficits in Leydig cells

Testosterone biosynthesis in testicular Leydig cells is mainly regulated by luteinizing hormone secreted from the pituitary gland. LH secretion is induced by gonadotrophin releasing hormone (GnRH) released from the hypothalamus. Hypothalamic-pituitary dysfunction may also result in a decrease in growth hormone (GH) secretion. It was reported that GH secretion decreases approximately 14% per decade of adult life. In addition to its important role in Leydig cell development, GH is an important factor in Leydig cell steroidogenesis, acting alone or synergistically with LH to modulate Leydig cell testosterone synthesis **(Childs, 2000).**
Another hormone affected by hypothalamic-pituitary axix is thyroid hormone. A role for thyroid hormone in Leydig cell steroidogenesis was supported by experimental evidence demonstrating that treatment of aged male rats with thyroxine reversed the decreases in Leydig cell volume, steroidogenic capacity and serum testosterone levels **(Kim *et al.*, 2002).**
In conjunction with this observation it was also demonstrated that thyroid hormone increased StAR gene expression and steroid production in Leydig cells **(Manna *et al.*, 2001).**
Aged Leydig cells have many deficiencies in steroidogenic pathway including decrease of the LH, reducing number of receptors LC and reducing cAMP production. It was also demonstrated that the peripheral benzodiazepine receptor (PBR) to be involved in cholesterol transfer possibly through its interaction with the StAR protein is decreased in aged rat Leydig cells **(Culty *et al.*, 2002).**
The delivery of free cholesterol to the inner mitochondrial membrane is required to initiate the steroidogenic process. Studies with male rats indicated that StAR protein in Leydig cells decreased during aging and that mitochondrial cholesterol transfer is defective **(Luo *et al.*, 2001).**

Luteinizing hormone (LH) and cAMP production

A number of studies have addressed the issue of whether changes in the steroidogenic ability of Leydig cells from aged rats is caused by extrinsic factors, intrinsic factors, of both. The most obvious possible explanation that reduced testosterone production is the result of reduced levels of LH. Is not the case because the *in vitro* long-term culture of old cells with LH failed to raise the relatively low levels of testosterone production by these cells to the significantly higher levels of the young. The response of aged Leydig cells to LH clearly is reduced, resulting in reduced cAMP production by old cells in response to LH **(Chen *et al.*, 2002).**
This result suggested that the reduced steroidogenic ability of old cells results from reduced cAMP production which presumably occurs as a consequence of the relative insensitivity of aged cells to LH. Although the molecular mechanisms by which cAMP is reduced in aged cells has not been determined, there is evidence that decreased cAMP production is result defects in the coupling of the LH receptor to adenylyl cyclase through G- proteins. This may be the cause of reduced cAMP production **(Chen *et al.*, 2004).**
Production cAMP depend on LH bound to LH receptors on Leydig cells. Then activate G-protein, which stimulates adenylyl cyclase and conversion of ATP to cAMP. At the level of the LH receptor, radioligand binding studies revealed that the number of hormone binding sites decreased significantly with age, suggestive of decreased levels of receptor at the plasma membrane. This might contribute to the decreased hormone induced cAMP synthesis, but it should be stressed that approximately 10% of LH receptor occupancy is required to elicit biological response, indicative of reserve receptors **(Hsueh *et al.*, 1977).**
The luteinizing hormone receptor is a glycoprotein member of the superfamily of G protein-coupled receptors (GPCRs). The G proteins are heterotrimers consisting of α, β and γ subunits. In their inactive state, the G α subunits bind GDP. Binding of LH to the LHR activates stimulatory G proteins, which promotes the release of GDP by the α subunit and subsequent binding of GTP, conveying an ON signal **(Defau *et al.*, 1980).**
The active G α subunit subsequently binds and activates the effector, adenylyl cyclase, which generates cAMP from the cellular ATP pool. Subsequent hydrolysis of GTP by the GTPase domain of the G α subunit results in cessation of the hormonal signal, relegating in to an OFF position **(Oldhan and Hamm, 2008).**

Mitochondrial cholesterol transport

The primary point of post-receptor control during the acute stimulation of steroidogenesis by LH is the conversion of cholesterol to pregnenolone on the inner mitochondrial membrane by P450scc/CYP11A1 **(Jefcoate, 2002).**
The stores of cholesterol within steroidogenic cells may be supplied in the form of lipoprotein and recognized by the membrane bound scavenger receptor class B typ 1 (SR-B1) – lipoprotein receptor **(Azhar and Reaven, 2002).**
Cholesterol is subsequently stored in cytoplasmic lipid droplets in the form of cholesteryl esters. Once sequestered in lipid droplets, cholesterol transport in response to hormonal stimulation can be divided into two phases. The first phase consists of mobilization of cholesteryl esters by cholesterol esterases, e. g., carboxyesterase, and subsequent transfer to the mitochondrial outer membrane. The second phase of cholesterol transfer involves the movement of cholesterol from the outer mitochondiral membrane to the mitochondrial matrix, where the P450scc enzyme resides **(Shen *et al.*, 2003).**
The two principal proteins identified with cholesterol transport across the mitochondrial membrane are the peripheral-type benzodiazepine receptor (PBR), recently renamed Translocator Protein, and steroidogenic acute regulatory protein (StAR). Evidence for StAR important role in steroidogenesis has come in part from studies congenital lipoid adrenal hyperplasia, an autosomal recessive disease in which synthesis of adrenal and gonadal steroids is severaly impaired. This disease, which is characterized by minimal steroid production, has been reported to be the result of mutations in the StAR. Studies also show defects in mitochondrial cholesterol transport and has demonstrated decrease in number of StAR in Leydig cells **(Bose *et al.*, 1997).**
Exist many biological factors affecting steroidogenesis in the blood or testis are altered during aging. One group of these biological factors are the cytokines, interleukin (IL-1, IL-6), interferon (INFγ), transforming growth factor (TGF-β1) and tumor necrosis factors (TNFs). IL-1 inhibited LH or cAMP-stimulated testosterone synthesis by repressing expression of P450c17 and P450scc. IL-6 has effects similar to IL-1 **(Diemer *et al.*, 2003).**
INFγ inhibited the expression of P450scc and P450c17 in porcine and inhibited StAR expression in rat Leydig cells. Leydig cells. Studies on TGF expression in testis indicated that TGF-β1 continuously increased during aging and that this increase may reduce LH receptor number, cAMP formation and P450c17 activity **(Herrmann *et al.*, 2002).**
TNF is a potent inhibitor of Leydig cell steroidogenesis that was demonstrated to reduced expression of the steroidogenic enzymes P450scc, 3β-HSD and P450c17 **(Hong *et al.*, 2004).**
An additional negative modulator of steroidogenesis may be increased expression of the cyclooxygenase-2 (COX-2) enzyme. LH, in addition of its stimulation of cAMP synthesis, promotes the release of intracellular arachidonic acid. The released arachidonic acid is subsequently metabolized by cellular lipoxygenases, epoxygenases or cyclooxygenases. Lipooxygenase and epoxygenase arachidonic acid metabolites are reported to stimulate steroidogenesis through enhanced expression of the StAR protein. However, metabolism of arachidonic acid by the COX-2 enzyme has been shown to tonically inhibit StAR expression and steroidogenesis **(Wang *et al.*, 2005).**
Recent experiment showed that the levels of COX-2 protein in Leydig cells increased with age, an increease inversely related to decreases in StAR protein, blood testosterone concentrations and testosterone biosynthesis. From 3 to 30 months of age, COX-2 protein in aged Leydig cells increased by 346% and StAR protein decreased to 33% over that of the young LC and blood testosterone concentration and testosterone biosynthesis decreased to 41 and 33%, respectively. These results suggest an inverse relationship between an age related increase in COX-2 protein and the observed decreases in StAR protein expression and testosterone production in Leydig cells **(Wang *et al.*, 2003).**

Factors affecting the function of Leydig cells

Leydig cells are the primary site of androgen biosynthesis in males. For ongoing steroidogenesis in LC is typical for sensitivity to various environmental factors. Steroidogenesis is a potential target for drugs, chemicals, natural products, and environmental contaminants that adversely impact reproductive development and fertility. For example, fungicides, pesticides, and phthalates elicit reproductive tract abnormalities. The *in vitro* evaluation of steroidogenesis is important for screening potential reproductive and developmental toxicants. **(Sanderson, 2006).**
An early example of an environmental contaminant that specifically interferes with steroid biosynthesis is mitotane. This is the analogue of the insecticide DDT. It inhibits cholesterol side-chain cleavage (CYP11A1) activity in Leydig cells **(Cai *et al.*, 1995).**
A similar effect has also TCDD. It is a dioxin class of halogenated aromatic hydrocarbons which, for its toxic properties causes various defects in rodents. In rats exposed to TCDD in vivo for 7 days, testicular testosterone secretion was decreased by 30-75% relative to testes from control rats. TCDD are clearly capable of causing endocrine disruption and reproductive development **(Kleeman *et al.*, 1990).**
Besides these chemicals, there are also studies that investigated the effect of magnetic field on Leydig cells. In previous experiments was found that in vitro exposure to sinusoidal 50 Hz magnetic fields was able to stimulate the testosterone production of mouse Leydig cell in a 48 hour primary culture. The exact mechanism of action of the magnetic field on Leydig cell is unknown. A possible mechanism of action may be associated with the alterations in cAMP content induced by the applied field **(Forgács *et al.*, 1998).**
Schimmelpfeng *et al.* (1995) found increased cAMP content after 5 minutes of exposure to 50 Hz magnetic field in mouse Leydig cells. It is possible that the testosterone production was increased due to an elevated cAMP level.

CONCLUSION

In the rat, two distinct generations of Leydig cells have been identified, namely Fetal Leydig cells and adult Leydig cells. Adult Leydig cells originate within the rat testis by day 56 postnatally. Their formation is the product of active proliferation and differentiation of undifferentiated stem cells to form progenitor Leydig cells, the differentiation of these cells to form steroidogenically active immature Leydig cells. Leydig cell testosterone synthesis is divided into three functional modules. Steroidogenic machinery, consisting of the P450 and HSD enzymes responsible for the conversion of cholesterol to testosterone. Cholesterol transfer apparatuses, including the enzymes responsible for hydrolysis of stored cholesteryl esters, movement of cholesterol to the mitochondria, and transfer of cholesterol across mitochondrial membranes by the StAR-PBR transfer complex and LHreceptor-adenylyl cyclase signaling circuit. Reduced LH signaling would be expect to affect cAMP production, cholesterol transport via StAR and PBR, and the steroidogenic enzymes.

Acknowledgments: This work was co-funded by European Community under project no 26220220180: Building Research Centre „AgroBioTech" and APVV-0304-12.

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