OMMEGA Publishers
Answering Intelligence
Journal of Stem Cell and Regenerative Biology
OPEN ACCESS
ISSN: 2471-0598

Research article
DOI: 10.15436/2471-0598.19.2408

Characterization of Induced Pluripotent Stem Cells Null for 20alpha-Hydroxysteroid Dehydrogenase

Rivka Ofir[2*], Tatiana Rabinski[2], Margalit Krup[1], Ran Taube[1], Liat Hershkovitz[1], Yacob Weinstein[1]

[1] The Shraga Segal Department of Microbiology, Immunology & Genetics, Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva , Israel
[2] Regenerative Medicine and Stem Cell Research Center, Ben Gurion University of the Negev, Beer Sheva, Israel

***Corresponding author:** Rivka Ofir, Regenerative Medicine and Stem Cell Research Center, Ben Gurion University of the Negev, Beer Sheva, Israel, Fax number: 972 8 6582727, Tel: 972 523448982; Email: rivir@bgu.ac.il

Abstract

Development of the adrenal cortex involves expression of the thyroid hormone receptor-β1 (TRβ1) in the inner cortical cell population. Expression of TRβ1 peaked at juvenile ages in the inner zone that includes cells expressing 20-α-hydroxysteroid dehydrogenase, a marker of the X-zone in mice. It is not clear which are the cells that populate these zones and whether there are zone specific stem cells. In order to begin unveiling the potential contribution of cells expressing 20HSD to these processes, we established a stem cells model *in vitro*. We describe here an *in vitro* system to test whether the gene encoding the enzyme 20alpha-hydroxysteroid dehydrogenase (20HSD), responsible for metabolizing sex hormones precursors and corticosteroids, have impact on pluripotent stem cells characteristics such as reprogramming of mouse embryonic fibroblasts (MEFs) into induced pluripotent stem cells (miPSCs) and differentiation of miPSCs *in vitro* and *in vivo* into three germ layers. miPSCs were generated from MEFs null for 20HSD gene and wild type (WT) MEFs. Colonies of miPSCs null for 20HSD appeared faster than WT colonies; the proliferation index of 20HSD null miPSCs was 30 % higher than that of WT miPSCs. The newly generated miPSCs express pluripotent markers and differentiate *in vivo* into three germ layers as expected from iPSCs. Expression of genes known to be involved in tissue regeneration and/or related to 20HSD activity showed higher expression in differentiated 20HSD null miPSCs *in vitro* when compared to differentiate WT miPSCs.

The results imply that reprogramming of MEFs is more efficient when 20HSD gene is deleted as compared to WT MEFs; the resulted 20HSD null miPSCs proliferate faster. Deletion of 20HSD has impact on the levels of expression of several genes upon embryoid body formation *in vitro*.

Introduction

Development of the adrenal cortex involves expression of the thyroid hormone receptor-β1 (TRβ1)[1] in the inner cortical cell population. Expression of TRβ1 peaked at juvenile ages in the inner zone that include cells expressing 20-α-hydroxysteroid dehydrogenase, a marker of the X-zone in mice[1]. The X-zone is sexually dimorphic: regression in males occur at 4 weeks of age and persist in females into adulthood. Although the definitive adult cortex develops from surface capsular cells that migrate into the gland, it is possible that the x-zone might represent another source of cells involved in stress response. Triiodothyronine (T3) treatment of males delay the regression of the TRβ1-positive zone and might regulate progesterone and 11-deoxycorticosterone levels via 20α HSD positive cells[1,2] .

The enzyme 20HSD, (also termed AKR1C18) plays a central role in the termination of pregnancy in rodents[3,4]. The enzyme belongs to the aldo-keto reductase (AKR) family of proteins and catalyzes the NADPH-dependent reduction of progesterone to 20alpha-hydropregn-4en-3-one (20alpha-hydroxyprogesterone), which is biologically inactive and cannot maintain pregnancy[5]. The enzyme also reduces other steroids with a C20 keto group (e.g. pregnenolone or 17-deoxycorticosterone)[6]. In mice, 20HSD is expressed in the adrenals, brain, kidneys, thymus, T cells and bone marrow[6-12]. Four different 20HSD enzymes (AKR1C1, AKR1C2, AKR1C3 and AKR1C4) are expressed

Received date: January 19, 2019
Accepted date: February 18, 2019
Published date: February 23, 2019

Citation: Ofir, R., et al. Characterization of Induced Pluripotent Stem Cells Null for 20alpha-Hydroxysteroid Dehydrogenase (2019) J Stem Cell Regen Biol 4(1): 1-5.

in human tissues[10]. AKR1C18 in rodent and AKR1C1 and AKR1C2 in human reduce progesterone and deoxycorticosterone to inactive steroids[10-13]. Mice deficient for 20HSD sustain high progesterone levels during the whole period of pregnancy and display a delay in the parturition of several days[6], indicating that expression of 20HSD activity is mandatory for the induction of parturition through reduction of progesterone blood concentration. In addition to progesterone, 20HSD reduces and catabolises 11-deoxycorticosterone (DOC). DOC is an intermediate in the biosynthesis of corticosterone and glucocorticoids. Mice null for 20HSD show higher blood concentration of corticosterone (at least 2 fold higher concentration; personal communication). Corticosteroids and glucocorticoids seem to affect almost every tissue and organ system in the human body including mesenchymal stem cells[14]. Dexamethasone, a synthetic glucocorticoid similar to the natural glucocorticoid hydrocortisone, promotes osteogenic, adipogenic, and chondrogenic differentiation of human mesenchymal cells[15]. Interestingly, it has been shown that progesterone induces adult mammary stem cell expansion[16].

In mice, expression of 20HSD within the adrenal is restricted to the X-zone, a transient zone between the adrenal cortex and the medulla of yet unknown function[6]. Large X-zone was detected in the adrenals of young females while a regressed X-zone was found in males older than 5 weeks. Treatment with androgens induced rapid involution of the female adrenal X-zone that was associated with the disappearance of the 20HSD positive cells by apoptosis[6]. Adrenal receptor is essential for removal of X-zone cells during puberty. *In vivo*, testosterone and DHT treatment for 7 days leads to X-zone regression, whereas withdrawal of the androgenic block is accompanied by growth of a secondary X-zone[6], namely, regeneration of 20HSD positive cells in the X-zone. Whether the X-zone re-appears as result of X-zone specific stem cells differentiation, or as result of other adrenal specific cell, is not known. In human and mouse there is a transient developmental zone between the functional cortical zones and the adrenal medulla of the adrenal: the fetal or X zone, respectively[17]. It is not clear which are the cells that populate these zones and whether there are zone specific stem cells.

In order to begin unveiling the potential contribution of cell expressing 20HSD to these processes, we established a stem cells model *in vitro*. This model enabled us to assess the impact of 20HSD enzyme on stem cells behaviour including the potential of 20HSD null mouse embryo fibroblasts (MEFs) to be reprogrammed into mouse induced pluripotent stem cells (miPSCs) and to spontaneously differentiate *in vitro* and *in vivo*.

Materials and Methods

Reprogramming: The generation of 20HSD knockout (- /-) mice was published previously[6]. These mice were used for preparing 20HSD primary mouse embryonic fibroblasts (MEFs). BalB/c mice (purchased from Harlen Jerusalem, Israel) were used to prepare WT MEFs. All animal experiments were carried out in accordance with an approved Ben-Gurion University Institutional Animal Care and Use Committee (IACUC) protocol. For reprogramming, 40,000 cells/well in 24 well plates were transduced with lentivirus containing human Oct4, Sox2, Klf4, and c-MYC produced in 293T cells as described before[18]. After viral transduction, cells were allowed to recover between 24 and 48 hours before being transferred to mitomycin C treated MEFs feeder plates (36,000 cells per cm^2). Transduced cells were cultured in a defined mouse stem cell media-mESC medium [DMEM-Biological Industries (Cat. 01-056-1A), Pen strep amph 1%- Biological Industries (Cat.03-033-1B), FBS15%-Foetal Bovine Serum Biological Industries (Cat.04-002-1A), 350 000 units of LIF - LIF ESGRO Millipore (Cat. ESG1107 for 50 ml medium), NEAA- Biological Industries (Cat.01-340-1B), 0.1mM 2-mercaptoethanol 100ul Gibco (Cat.31350-010 for 50 ml)]. Medium was filtered with Nalgene 0.22um Pore Size Filter (Cat.566-0020).miPSCs colonies were picked after 3 and 5 weeks culture and subcultures clone by clone on mitomycin C pretreated MEFs in Greiner six-well tissue culture plates (Cat.657160), coat with 2 ml of Gelatin 0.1% purchased from Sigma (Cat.G1890). Each single picked colony was maintained and expanded following routine ES cell passaging and culturing protocols and were established as one miPSCs line. The cells were detached by TrypLE Express (Gibco;Cat. 12604) and cultured in 37^{O}C and 5% CO_2.

Immunocytochemistry: miPSCs grown on mitomycin C treated MEFs in 24 well tissue culture plates were washed with PBS, and fixed with cold ethanol at −20°C for 15 minutes. After the cells were air-dried, they were incubated with blocking solution (PBS containing 0.5% BSA) for 1 hour at room temperature (RT) following by incubation with primary antibody in mESC medium at 4°C overnight: Oct3/4 (Santa Cruz Biotechnology Inc., 1:200), SSEA1 (Santa Cruz Biotechnology Inc., 1:200), NF66 (Abcam, 1:25), SMA (Abcam, 1:20). The cells were washed and incubated with secondary antibody in PBS containing 0.5% BSA for 1 hour at RT. 2nd antibody for Oct3/4 and SSEA1 was Alexa 488 goat anti-mouse KPL 202-03-18-06 DyLight 488 Labeled and 2nd antibody for NF66 and SMA was goat anti Rabbit-FITC Santa Cruz Biotechnology Inc. The cells were washed and visualized under a fluorescent microscope (Olympus IX70).

Teratoma Assay: The mice were NOD SCID (Harlan Jerusalem, Israel). miPSCs were grown to near confluence, harvested using TrypLE Express treatment, washed in PBS, and re-suspended in PBS supplemented with 30% Matrigel (BD Biosciences). Methoxyflurane-anesthetized mice were intramuscularly injected in the hind leg with 50 µl of approximately 1×10^6 cells at one site only. Prior to teratoma removal, mice were sacrificed by Methoxyflurane. Teratomas were surgically removed, fixed in 4% formalin, and embedded in paraffin. Sections were mounted onto slides, stained with hematoxylin eosin (H&E), and examined for the presence of structures of the three germ layers.

Embryoid bodies (EB) assay: miPSCs were grown in ultra-low attachment plates in suspension culture for 8 days with DMEM / F12 (1:1) containing 20% knockout serum (Invitrogen), 4.5 g / L L-glutamine, 1% nonessential amino acids, 0.1mM 2-mercaptoethanol, 50 U / mL penicillin, and 50 ug / mL streptomycin. EBs formed were seeded on 0.25 % gelatin-coated tissue culture dish for another 8-20 days to allow spontaneous differentiation before analysis of specific proteins for ectoderm marker NF66, mesoderm marker SMA[18]. For directed differentiation, 7 days old EBs were seeded on gelatin and treated with Ascorbic Acid (AA, 100uM) or Retinoic Acid (RA, 100nM) for additional 7 days.

Proliferation Index: 5,000, 10,000 and 20,000 miPSCs were cultured for 24hrs, 72hrs, 96hrs and 120hrs. Proliferative index was calculated by using MTT [3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] colorimetric assay and the output was in optical density (OD).The MTT assay is based on the capacity of the mitochondrial enzyme, succinate-dehydrogenase of viable cells, to transform the MTT tetrazolium salt into a blue colored product, MTT formazan, and is proportional to the number of living cells present. Replication index (RI) was calculated from the formula RI = OD at 120h culture / OD at 24h culture. The average of RI for each seeded cell number was calculated. The results showed 1.3-fold increase in formazan activity in 20HSD null miPSCs as compared to WT miPSCs.

Quantitative Real-Time PCR Analysis: RNA was extracted by the RNeasy Plus Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Genomic DNA was removed from the RNA samples by using 50 units of RNase-free DNaseI at 37°C for 1 h. RNA (20 µg) was converted to cDNA using the Thermo Scientific Verso cDNA kit (Thermo Fisher Scientific Inc.) following the manufacturer's protocol. The cDNA was used for quantitative real-time PCR amplification with TaqMan chemistry (Applied Biosystems) using mouse Gja1, Plaur, Serpinb1a, Gatm, Ninj1, Tpt1, Fetub, Cited1, Oxtr, Notch3,Vnn3 predesigned TaqMan Gene Expression Assay from Applied Biosystems (Assay ID Mm00439105_m1, Mm00440911_m1, Mm01610780_m1, Mm00491879_m1, Mm00479014_m1,Mm03009502_g1, Mm00445538_m1, Mm00455934_m1, Mm01182684_m1, Mm01345646_m1, Mm00496417_m1, respectively). Values were normalized relative to GAPDH (Assay ID Mm99999915_g1). All results from three technical replicates were normalized to GAPDH and expressed as relative expression ratios calculated (relative quantity, RQ) using the comparative method and based on the data that were created by the ABI PRISM 7700 Sequence Detection System (using version 1.6 software).

Results and discussion

Characterization of 20HSD null mouse induced pluripotent stem cells (miPSCs)

MEFs are routinely used for generating miPSCs[18]. MEFs null for 20HSD and WT MEFs, were infected with a polycistronic reprogramming cassette consisting of Oct4, Sox2, Klf4 and c-Myc[18]. 20HSD null miPSCs colonies appeared earlier than WTmiPSCs colonies; this phenomenon may be explained by higher proliferation index of 20HSD null miPSCs as compared to WTmiPSCs (Figure- 1A). These results suggest that in cells null for 20HSD, the balance between proliferation and differentiation is more towards proliferation. 20HSD null miPSCs express the pluripotent transcription factor Oct3/4 and SSEA1 proteins (Figure -1B). The process of spontaneously differentiation *in vitro* involves embryonic bodies (EBs) formation in suspension and plating on gelatin-coated plates. 20HSD null EBs barley stick to the plate and only few cells differentiate out of the EB "ball" (Figure-2A); WT EBs stick to the plate and differentiate into germ layers (Figure- 2B, WT miPSCs). *In vivo*, both miPSCs formed teratoma in nude mice, with three germ layers (Figure-3, 20HSD null miPSCs).The results imply that 20HSD, and probably relevant steroid products, have impact during differentiation of miPSCs *in vitro*. *In vivo*, no visible difference were observed between teratoma formed following injecting 20HSD null iPSCs and WT iPSCs to nude mice, suggesting that the *in vivo* milieu compensate on the missing activity of 20HSD and enable the differentiation of 20HSD null miPSCs into teratoma.

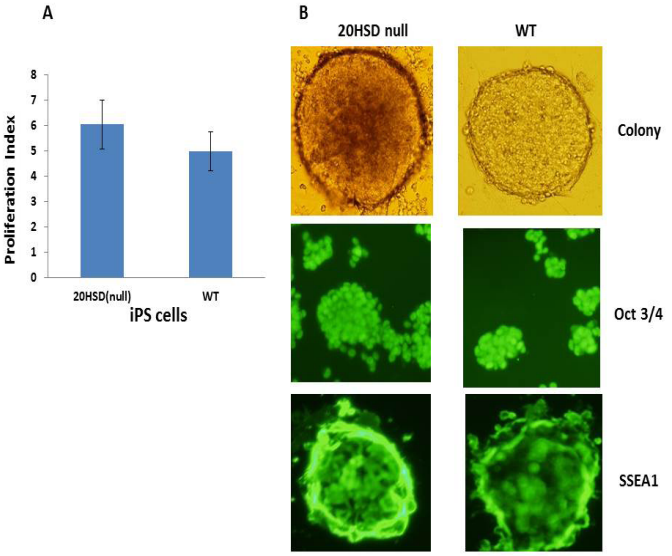

Figure 1: miPSCs proliferation and differentiation
A. Proliferation of WT and 20HSD null miPSCs: 5,000, 10,000 and 20,000 cells were cultured for 24hrs, and 120hrs. Proliferation was measured using MTT and the index of proliferation was calculated by dividing the MTT values for 120hrs culture by that of 24hrs culture (see material and methods). The average of index of proliferation is presented in A. The proliferation index of 20HSD null miPSCs is 30% higher than that of WT miPSCs.
B. WT and 20HSD null miPSCs are pluripotent: Staining of miPSCs colonies with Oct 3/4 (nuclear transcription factor) and SSEA1 (membrane marker). Both serve as pluripotent markers.

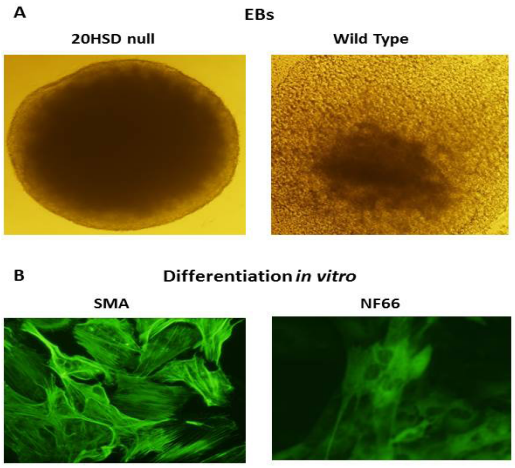

Figure 2: EB formation and *in vitro* differentiation of 20HSD null miPSCs and of WT miPSCs.
A. 1000 cells in 20ul media were used to form EBs using the hanging drop methods; following 7 days in suspension, the cells were seeded on gelatin coated plats. We realized that EBs of 20HSD null miPSCs did not stick to the plate and very few cells differentiate outside of the EB.
B. EBs of WT miPSCs were plated on gelatin coated plate for 14 days before staining with SMA and NF66 as differentiation markers of mesoderm and ectoderm, respectively.

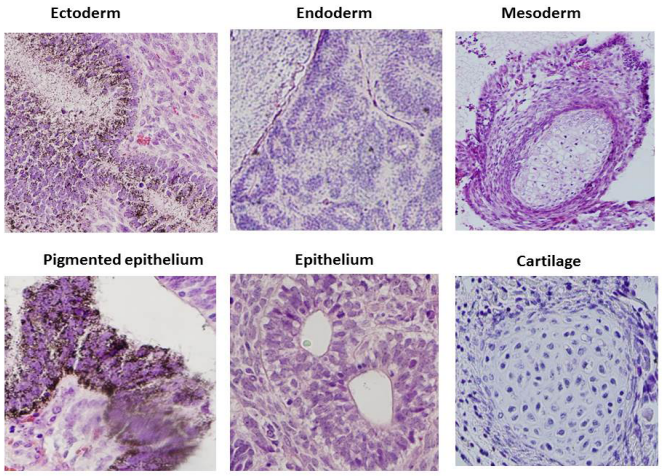

Figure 3: *In vivo* differentiation of 20HSD null miPSCs to form teratoma with 3 germ layers. Million cells mixed with matrigel were injected into nude mice and fixed sections of the teratoma were stained with H&E. WT miPSCs showed the same pattern of *in vivo* differentiation (data not shown).

Expression of genes related to tissue regeneration

List of genes involved in regeneration of the adrenal x-zone cells (expressing 20HSD) was established[6].

The expression patterns of genes from the following groups were analyzed:

Normal adrenals; adrenals from mice implanted with-Dihydrotestosterone (DHT) releasing implants for 7 days (namely, adrenal with involute x-zone); adrenals from mice 14 days after the removal of the testosterone releasing implants (adrenals with regenerated x-zone). A data set of genes induced/ repressed in this process of adrenal X-zone degeneration/ regeneration was established (unpublished data) based on the model published before[6]. This dataset highlights several pathways according to David and GO software including GO:0042246 ~ tissue regeneration pathway that contain genes involved in regeneration, tissue development, developmental growth and wound healing. Comparisons between two groups were performed: I. Transcripts enriched in involute x-zone vs. regenerated x-zone, potentially include genes that are direct or indirect target of testosterone. II. Transcripts enriched in regenerated x-zone *vs.* involute x-zone, potentially include genes involved in regeneration. To identify genes that may explain the phenotypic differences between WT miPSCs and 20HSD null miPSCs, we decided to study several genes from these lists. The following candidates for regeneration were validated by qRT-PCR: Gja1, Plaur, Gatm , Ninj1, Serpinb1a, Tpt1,Fetub, Cited1,Oxtr, Notch3,Vnn3; Gapdh served as control. There was no difference in the levels of expression of the genes Gja1, Gatm, Ninj1, Tpt1, Cited1, Oxtr, Notch3, Vnn3 when RNA from differentiated WT miPSCs was compared to RNA of differentiated 20HSD null miPSCs (data not shown). The levels of Fetub (cysteine protease inhibitors) transcription in 20HSD null differentiated miPSCs as compared to differentiated WT iPSCs were 14, 13, 5 fold higher in spontaneous differentiation (EB), Retinoic Acid induced differentiation (RA) and Ascorbic Acid induced differentiation (AA), respectively (Figure-4A). Fetub is expressed during embryogenesis in multiple tissues and antagonizes the action of Transforming growth factor-b1/b2 (TGFb1/TGFb2) in cell cultures; TGF-b play an important role in the maintenance of pluripotency of embryonic stem cells[19]. The results here suggest that in differentiated WT iPSCs, 20HSD or its products inhibit the transcription of fetub, a TGF-b cytokine antagonist.

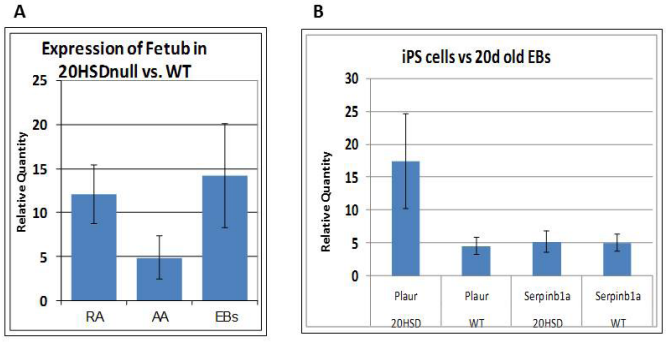

Figure 4: qRT-PCR of RNA isolated from undifferentiated and differentiated 20HSD null and WT miPSCs: Spontaneous differentiation lead to EB formation in suspension for 14 days (EBs). For directed differentiation, 7 days old EBs was seeded on gelatin and treated with Ascorbic Acid or Retinoic Acid (AA or RA, respectively) for additional 7 days. Results are presented as relative quantities (RQ) +/- STDEV.

A. Fetub gene expression. Comparison between differentiated 20HSD null miPSCs and differentiated WT miPSCs. The graph present results from 3 different experiments.

B. Serpinb1A and Plaur gene expression. Comparison between pluripotent miPSCs (WT and 20SDH) and differentiated miPSCs (20 days old EBs; WT and 20SDH null). The graph present results from 4 different experiments +/- STDEV.

The levels of expression of Plaur (Plasminogen Activator, Urokinase Receptor) and Serpinb1a (Serine / Cysteine Proteinase Inhibitor; Plasminogen Activator Inhibitor), are higher in iPSCsstate than in differentiated state. For Plaur transcripts the ratios between miPSCs state to differentiated state were 17 and 4, for 20HSD null cells and WT cells, respectively and for Serpinb1a transcripts the ratios between miPSCs state to differentiated state were 6 and 5 for 20HSD null cells and WT Cells, respectively (Figure-4B). Interestingly, over expression of Plaur and Serpin, enhances tumor cell migration and invasion and are linked to acquisition of stem cell properties[20], in line with our results that these genes showed higher expression in the stage of pluripotent stem cells as compared to differentiated cells; this property is more pronounced in 20HSD null miPSCs. In conclusion, the results here indicate that *in vitro* miPSCs null for 20HSD stay more pluripotent as compared to WT miPSCs. *in vivo*, both WT and 20HSD null iPSCs differentiate similarly into teratoma as result of compensation of the whole animal milieu. Future studies may identify the pathways involved in regeneration of 20HSD positive cells in the X-zone.

References

1. Huang, C.C., Kraft, C., Moy, N., et al. A Novel Population of Inner Cortical Cells in the Adrenal Gland That Displays Sexually Dimorphic Expression of Thyroid Hormone Receptor-β1. (2015) Endo 156(6): 2338-2348. Pubmed| Crossref | Others
2. Wondisford, F.E. A Direct Role for Thyroid Hormone in Development of the Adrenal Cortex. (2015) Endo 156(6):

1939-1940.
Pubmed| Crossref | Others

3. Piekorz, R.P., Gingras, S., Hoffmeyer, A., et al. Regulation of progesterone levels during pregnancy and parturition by signal transducer and activator of transcription 5 and 20alpha-hydroxysteroid dehydrogenase. (2005) Mol Endo 19(2): 431- 440.
Pubmed| Crossref | Others
4. Wiest, W.G. On the function of 20 alpha-hydroxypregn-4-en-3-one during parturition in the rat. (1968) Endo 83(6): 1181-1184.
Pubmed| Crossref | Others
5. Wiest, W.G. Conversion of progesterone to 4-pregnen-20alpha-ol-3-one by rat ovarian tissue *in vitro*. (1959) J Biol Chem 234: 3115-3121.
Pubmed| Crossref | Others
6. Hershkovitz, L., Klammer, S., Krup, M., et al. Adrenal 20alpha-hydroxysteroid dehydrogenase in the mouse catabolises progesterone and 11-deoxycorticosterone and is restricted to the X-zone. (2007) Endo 148(3): 976-988.
Pubmed| Crossref | Others
7. Aflalo, E., Ofir, R., Apte, R.N., et al. Characterization of mouse thymocyte subpopulations by the enzymatic marker 20-alpha-hydroxysteroid dehydrogenase: differential responses to IL-1 and IL-2. (1987) Clin Exp Immunol 70(3): 578-584.
Pubmed| Crossref | Others
8. Keller, J.R., Weinstein, Y., Hursey, M., et al. Interleukins 2 and 3 regulate the *in vitro* proliferation of two distinguishable populations of 20-alpha-hydroxysteroid dehydrogenase-positive cells. (1985) J Immunol 135(3): 1864-1871.
Pubmed| Crossref | Others
9. Pelletier, G., Li, S., Labrie, F., et al. Localization of 20alpha-hydroxysteroid dehydrogenase mRNA in mouse brain by in situ hybridization. (2004) Brain Res Mol Brain Res 12(2): 143-146.
Pubmed| Crossref | Others
10. Penning, T.M., Burczynski, M.E., Jez, J.M., et al. Human 3alpha-hydroxysteroid dehydrogenase isoforms (AKR1C1-AKR1C4) of the aldo-keto reductase superfamily: functional plasticity and tissue distribution reveals roles in the inactivation and formation of male and female sex hormones. (2000) Biochem J 351(1): 67-77.
Pubmed| Crossref | Others
11. Weinstein, Y., Lindner, H.R., Eckstein, B. Thymus metabolises progesterone- possible enzymatic marker for T lymphocytes. (1977) Nature 266(5603): 632-623.
Pubmed| Crossref | Others
12. Weinstein, Y., Isakov, Y. Effects of testosterone metabolites and of anabolic androgens on the bone marrow and thymus in castrated female mice. (1983) Immunopharmacol 5(3): 239-250.
Pubmed| Crossref | Others
13. Higaki, Y., Usami, N., Shintani, S., et al. Selective and potent inhibitors of human 20alpha-hydroxysteroid dehydrogenase (AKR1C1) that metabolizes neurosteroids derived from progesterone. (2003) Chem Biol Interact 144: 503-513.
Pubmed| Crossref | Others
14. Feldman, B.J. Glucocorticoids influence on mesenchymal stem cells and implications for metabolic disease. (2009) Pediatr Res 65(2): 249-251.
Pubmed| Crossref | Others
15. Jaiswal, R.K., Jaiswal, N., Bruder, S.P., et al. Adult human mesenchymal stem cell differentiation to the osteogenic or adipogenic lineage is regulated by mitogen-activated protein kinase. (2000) J Biol Chem 275(13): 9645-9652.
Pubmed| Crossref | Others
16. Joshi, P.A., Jackson, H.W., Beristain, A.G., et al. Progesterone induces adult mammary stem cell expansion. (2010) Nat 465(7299): 803-807.
Pubmed| Crossref | Others
17. Keegan, C.E., Hammer, G.D. Recent insights into organogenesis of the adrenal cortex. (2002) Trends Endo Metabo 13(5): 200-208.
Pubmed| Crossref | Others
18. Sommer, C.A., Stadtfeld, M., Murphy, G.J., et al. Induced pluripotent stem cell generation using a single lentiviral stem cell cassette. (2009) Stem cells 27(3): 543-549.
Pubmed| Crossref | Others
19. Park, K.S. TGF-Beta Family Signaling in embryonic stem cells. (2011) Int J Stem Cells 4(1): 18-23.
Pubmed| Crossref | Others
20. Pavón, M.A., Arroyo-Solera, I., Céspedes, M.V., et al. uPA/uPAR and SERPINE1 in head and neck cancer: role in tumor resistance, metastasis, prognosis and therapy. (2016) Oncotarget 7(35): 57351-57366.
Pubmed| Crossref | Others