Online ISSN: 2249-4626
Print ISSN: 0975-5896

Global Journals

of Science Frontier Research: G

Bio-Tech & Genetics

Sex Regulation in Mammals

Polycystic Ovary Syndrome

Highlights

Potent Antibiotic Producing

Vitro Regeneration System

Discovering Thoughts, Inventing Future

VOLUME 13 ISSUE 2 VERSION 1.0

Global Journal of Science Frontier Research: G
Bio-Tech & Genetics

Global Journal of Science Frontier Research: G
Bio-Tech & Genetics

Volume 13 Issue 2 (Ver. 1.0)

Open Association of Research Society

Incorporation No.: 0423089
License No.: 42125/022010/1186
Registration No.: 430374
Import-Export Code: 1109007027
Employer Identification Number (EIN):
USA Tax ID: 98-0673427

Global Journals Inc.

*(A Delaware USA Incorporation with "Good Standing"; **Reg. Number: 0423089**)*

Sponsors: Open Association of Research Society
Open Scientific Standards

Publisher's Headquarters office

Global Journals Headquarters
301st Edgewater Place Suite, 100 Edgewater Dr.-Pl,
Wakefield MASSACHUSETTS, Pin: 01880,
United States of America
USA Toll Free: +001-888-839-7392
USA Toll Free Fax: +001-888-839-7392

Offset Typesetting

Global Journals Incorporated
2nd, Lansdowne, Lansdowne Rd., Croydon-Surrey,
Pin: CR9 2ER, United Kingdom

Packaging & Continental Dispatching

Global Journals
E-3130 Sudama Nagar, Near Gopur Square,
Indore, M.P., Pin:452009, India

Find a correspondence nodal officer near you

To find nodal officer of your country, please email us at *local@globaljournals.org*

eContacts

Press Inquiries: *press@globaljournals.org*
Investor Inquiries: *investers@globaljournals.org*
Technical Support: *technology@globaljournals.org*
Media & Releases: *media@globaljournals.org*

Pricing (Including by Air Parcel Charges):

For Authors:
22 USD (B/W) & 50 USD (Color)
Yearly Subscription (Personal & Institutional):
200 USD (B/W) & 250 USD (Color)

Integrated Editorial Board
(Computer Science, Engineering, Medical, Management, Natural Science, Social Science)

Dr. Bart Lambrecht
Director of Research in Accounting and FinanceProfessor of Finance
Lancaster University Management School
BA (Antwerp); MPhil, MA, PhD (Cambridge)

Dr. Carlos García Pont
Associate Professor of Marketing
IESE Business School, University of Navarra
Doctor of Philosophy (Management), Massachusetts Institute of Technology (MIT)
Master in Business Administration, IESE, University of Navarra
Degree in Industrial Engineering, Universitat Politècnica de Catalunya

Dr. Fotini Labropulu
Mathematics - Luther College
University of ReginaPh.D., M.Sc. in Mathematics
B.A. (Honors) in Mathematics
University of Windso

Dr. Lynn Lim
Reader in Business and Marketing
Roehampton University, London
BCom, PGDip, MBA (Distinction), PhD, FHEA

Dr. Mihaly Mezei
ASSOCIATE PROFESSOR
Department of Structural and Chemical Biology, Mount Sinai School of Medical Center
Ph.D., Etvs Lornd University
Postdoctoral Training,
New York University

Dr. Söhnke M. Bartram
Department of Accounting and FinanceLancaster University Management SchoolPh.D. (WHU Koblenz)
MBA/BBA (University of Saarbrücken)

Dr. Miguel Angel Ariño
Professor of Decision Sciences
IESE Business School
Barcelona, Spain (Universidad de Navarra)
CEIBS (China Europe International Business School).
Beijing, Shanghai and Shenzhen
Ph.D. in Mathematics
University of Barcelona
BA in Mathematics (Licenciatura)
University of Barcelona

Philip G. Moscoso
Technology and Operations Management
IESE Business School, University of Navarra
Ph.D in Industrial Engineering and Management, ETH Zurich
M.Sc. in Chemical Engineering, ETH Zurich

Dr. Sanjay Dixit, M.D.
Director, EP Laboratories, Philadelphia VA Medical Center
Cardiovascular Medicine - Cardiac Arrhythmia
Univ of Penn School of Medicine

Dr. Han-Xiang Deng
MD., Ph.D
Associate Professor and Research
Department Division of Neuromuscular Medicine
Davee Department of Neurology and Clinical NeuroscienceNorthwestern University
Feinberg School of Medicine

Dr. Pina C. Sanelli
Associate Professor of Public Health
Weill Cornell Medical College
Associate Attending Radiologist
NewYork-Presbyterian Hospital
MRI, MRA, CT, and CTA
Neuroradiology and Diagnostic Radiology
M.D., State University of New York at Buffalo,School of Medicine and Biomedical Sciences

Dr. Roberto Sanchez
Associate Professor
Department of Structural and Chemical Biology
Mount Sinai School of Medicine
Ph.D., The Rockefeller University

Dr. Wen-Yih Sun
Professor of Earth and Atmospheric SciencesPurdue University Director
National Center for Typhoon and Flooding Research, Taiwan
University Chair Professor
Department of Atmospheric Sciences, National Central University, Chung-Li, TaiwanUniversity Chair Professor
Institute of Environmental Engineering, National Chiao Tung University, Hsin-chu, Taiwan.Ph.D., MS The University of Chicago, Geophysical Sciences
BS National Taiwan University, Atmospheric Sciences
Associate Professor of Radiology

Dr. Michael R. Rudnick
M.D., FACP
Associate Professor of Medicine
Chief, Renal Electrolyte and Hypertension Division (PMC)
Penn Medicine, University of Pennsylvania
Presbyterian Medical Center, Philadelphia
Nephrology and Internal Medicine
Certified by the American Board of Internal Medicine

Dr. Bassey Benjamin Esu
B.Sc. Marketing; MBA Marketing; Ph.D Marketing
Lecturer, Department of Marketing, University of Calabar
Tourism Consultant, Cross River State Tourism Development Department
Co-ordinator , Sustainable Tourism Initiative, Calabar, Nigeria

Dr. Aziz M. Barbar, Ph.D.
IEEE Senior Member
Chairperson, Department of Computer Science
AUST - American University of Science & Technology
Alfred Naccash Avenue – Ashrafieh

President Editor (HON.)

Chief Author (HON.)

Dr. R.K. Dixit
M.Sc., Ph.D., FICCT
Chief Author, India
Email: authorind@computerresearch.org

Dean & Editor-in-Chief (HON.)

Contents of the Volume

i. Copyright Notice
ii. Editorial Board Members
iii. Chief Author and Dean
iv. Table of Contents
v. From the Chief Editor's Desk
vi. Research and Review Papers

1. Isolation and Characterization of Potent Antibiotic Producing Marine Actinomycetes from Tiruchendur and Kulasekarapattinam, Tamilnadu. *1-5*
2. Cercetări Privind Utilizarea Anticorpilor Monoclonali Şi Policlonali În Evidenţierea Celulelor Stem Umane Neuronale În Creierul Fetal Ovin. *7-21*
3. Is it Possible Artificial Sex Regulation in Mammals? *23-29*
4. An Efficient *in vitro* Regeneration System for Tori (*Brassica Campestris*)-7. *31-34*
5. Serum Homocysteine and Paraoxonase1 Levels in Women with Polycystic Ovary Syndrome Treated with Metformin Versus Metformin and Folic Acid. *35-40*

vii. Auxiliary Memberships
viii. Process of Submission of Research Paper
ix. Preferred Author Guidelines
x. Index

Global Journal of Science Frontier Research
Bio-Tech & Genetics
Volume 13 Issue 2 Version 1.0 Year 2013
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Isolation and Characterization of Potent Antibiotic Producing Marine Actinomycetes from Tiruchendur and Kulasekarapattinam, Tamilnadu

By Prasantha Kumari. Mantada, Girija Sankar. G, Prabhakar.T

A.S.N.Pharmacy College, India

Abstract - A set of 97 actinomycete strains which were isolated from 24 marine samples such as sea water, sea sediments, sponges, and corals obtained from Tiruchendur and Kulasekarapattinam, Tamilnadu state, India. The actinomycetes isolated from these eco systems are capable of producing antibiotics that strongly inhibit the growth of gram positive and gram negative bacteria and yeast like fungi. A total of 27 isolates representing the range of morphological diversity observed from each sample, were obtained in pure culture. However of the 27, four were found to produce antibiotic substances. KSP-SS-06-1C/1 exhibited higher activity and was, therefore selected for further studies. The purification and characterization of the substances is now in progress.

Keywords : marine actinomycetes, isolation, biochemical characterization, kulasekarapattinam, antimicrobial activity.

GJSFR-G Classification : FOR Code: 670403

Strictly as per the compliance and regulations of :

Isolation and Characterization of Potent Antibiotic Producing Marine Actinomycetes from Tiruchendur and Kulasekarapattinam, Tamilnadu

Prasantha Kumari. Mantada [α], Girija Sankar. G [σ], Prabhakar.T [ρ]

Abstract - **A set of 97 actinomycete strains which were isolated from 24 marine samples such as sea water, sea sediments, sponges, and corals obtained from Tiruchendur and Kulasekarapattinam, Tamilnadu state, India. The actinomycetes isolated from these eco systems are capable of producing antibiotics that strongly inhibit the growth of gram positive and gram negative bacteria and yeast like fungi. A total of 27 isolates representing the range of morphological diversity observed from each sample, were obtained in pure culture. However of the 27, four were found to produce antibiotic substances. KSP-SS-06-1C/1 exhibited higher activity and was, therefore selected for further studies. The purification and characterization of the substances is now in progress.**

Keywords : marine actinomycetes, isolation, biochemical characterization, kulasekarapattinam, antimicrobial activity.

I. Introduction

As a great promising for new natural products which have not been observed from terrestrial microorganisms, marine bacteria are being developed for the discovery of bioactive substances with new types of structure, with growing intensive interest. The achievements have been well reviewed (Bernan, V.S et al.,1997) ,where many novel antibiotics were obtained from actinomycetes (Okami, Y & K. Hotta,1998) .Thus actinomycetes associated with marine plants and animals are expected to be a potential source for new natural bioactive agents. Actinomycetes in marine environment are found in water, sediments, fishes, fauna, free swimming vertebrates, invertebrates, corals, coral reefs, sponges and marine snow (Alan C. Ward and Nagamani Bora, 2006).

II. Materials and Methods

a) Sites of Sampling

A total of 24 marine samples were collected from Tiruchendur and Kulasekarapattinam located in Thoothukudi district, Tamilnadu state, India.

Author α : A.S.N Pharmacy College, Tenali, Guntur (Dt), Andhra Pradesh, India-522201. E-mail : prasanthi.mantada@gmail.com

Authors σ ρ : Department of Pharmaceutical Biotechnology, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India-530013.

Tiruchendur: This area is located along the southern coast of Tamilnadu state. It extends over a distance of 28 km of the south eastern coast line border of Bay of Bengal. It is 37 km away from Thoothukudi and 85 km from Kanyakumari. Latitude - $8^0$28'60 N, Longitude-$78^0$7'0 E.

Kulasekarapattinam: It is about 51 km south of Thoothukudi and about 13 km south of Tiruchendur and about 116 km away from Kanyakumari. Latitude - $8^0$05'and $9^0$30' N, Longitude-77^0 05' and 78^0 25' E.

b) Collection and Processing of samples

The marine samples include sea water, sea sediments, sponges and corals which were collected in sterile containers containing sterile starch casein broth at depth of 5,10,15,25,30 m respectively at a distance of about 10-15 kms off the sea shore. All samples were labeled and were brought to the laboratory. They were stored at a temperature between 6 to 10°C for further use.

c) Isolation of Actinomycetes

The collected marine samples were serially diluted to ten folds with sterile sea water (1:10 w/v).To germinate actinomycetes spores, each diluted sample (10^{-6},10^{-7},10^{-8},10^{-9},10^{-10}) were heated to 35-45°C for 10 min in a shaking incubator (Grein, A. and Meyers, S.P , 1958). 1mL from each diluted sample was inoculated in 250mL Erlenmeyer flask containing 50 mL of sterile molten starch casein agar medium (g/L: Starch, 19; Casein, 0.3; KNO_3, 2; NaCl, 2; K_2HPO_4, 2; $MgSO_4.7H_20$, 0.05; $CaCO_3$, 0.02; $FeSO_4.7H_2O$, 0.01; Agar, 20) supplemented with Cyclohexamide (50 μg/mL) and Rifampicin (5μg/mL) to prevent fungal and bacterial growth respectively, (Haque et al.,1992) poured into sterile petriplates and incubated at 28°C for 14 days. After 14 days, colonies of unicellular bacteria were counted on agar plates (Dwight, R. and Fenical, W, 1991). The average colony count of each dilution was found to be 20-250 colonies.

d) Identification of Actinomycetes

Actinomycetes were recognized by their characteristic tough leathery colonies, branched vegetative mycelia, and, when present, aerial mycelia

and spore formation. Because of these criteria, only colonies with well developed and branched hyphae were included in this study.

e) Determination of Antimicrobial Activity

i. *Test Micro organisms*

Anti microbial activity of the selected isolates were tested against following organisms such as *Bacillus subtilis* (NCIM-2063), *Staphylococcus aureus* (NCIM-2901), *Escherichia coli* (NCIM-2065), *Saccharomyces cerevisiae* (NCIM-3047), *Candida albicans* (NCIM-3100), *Aspergillus niger* (NCIM-787), *Pseudomonas aeruginosa* (NCIM-2037). All the test organisms employed in the present study were supplied by The National Collection of Industrial Microorganisms, National Chemistry Laboratory, Pune, India.

f) Screening of antibiotic producing strains

i. *Primary Screening Test*

The antimicrobial activity of isolates were tested by Cross Streak Plate Method (Elliah,P. et al.,1996), (Dhanasekaran, D. and Selvamani, S, 2009) . Each actinomycete isolate was transferred from starch casein agar plate to nutrient agar plate. This is done by streaking a straight line of the actinomycete inoculum across the surface of nutrient agar medium in the plate and incubated at 28°C for 7days. Inoculum of the test micro organisms were then streaked at right angles to the actinomycetes straight line colony (Zhomghui Zheng. and Wei Zeng, 2000).Control plates were also maintained to assess the normal growth of the pathogenic organisms.

ii. *Secondary Screening Test*

The active isolates resulted from primary screening, promising isolates were tested for their extra cellular antibiotic production capabilities under submerged fermentation conditions. The production medium containing (g/L: Sucrose, 1.0; Yeast extract, 2.0; KNO_3, 3.8) was used for antibiotic production test. Well sporulated, 7 to 10 days old isolates were taken and 5ml of sterile water was added to each slant and 5ml of spore suspension was added to 45mL of respective production media and incubated at 28°C for 6 days on a rotary shaker. Then samples were collected in to sterile centrifuge tubes and centrifuged at 4000 rpm for 15 minutes to separate the fermented broth and the mycelium. The clear supernatant was used for antibiotic assay using agar diffusion method (Barry AL, Thornsberry C, 1985), employing nutrient agar for bacteria and potato dextrose agar for fungi.

g) Characterization of Actinomycetes

i. *Determination of the color of the actinomycete isolates*

Morphological characters and color determination of selected isolate was studied on different media such as Yeast extract malt extract agar (ISP-2), Oat meal agar (ISP-3),Inorganic salts starch agar medium (ISP-4), Glycerol aspargine agar medium (ISP-5) (Rathna Kala, R. and Chandrika,V,1993) in accordance with the International *Streptomycetes* Project (ISP) (Shirling, E.B. and Gottlieb, D. 1966). Color was determined using the color names lists (Pridham TG, 1964).

ii. *Morphological Characterization*

Morphological characterization consisting of macromophology and micromorphology. Micromorphology was noted by the naked eye and by observation with magnifying lens. To study aerial mycelium and its spore characteristics was identified by inclined cover slip culture technique (Kawato, N. and Shinobu, R, 1959).

iii. *Biochemical Characterization*

Actinomycetes isolates are characterized using Melanin reaction, H_2S production, Tyrosinase reaction, Starch hydrolysis, Gelatin hydrolysis, Casein hydrolysis, Nitrate reduction, Milk coagulation and peptonization according to International *Streptomycetes* Project.

iv. *Physiological and Cultural Characterization*

The ability to grow at various Growth temperature range (10-70°C), range of pH (3-10) and in different concentrations of NaCl (2-16 g/L) on medium was also tested.

v. *Chemo taxonomical characterization*

Isomers of diaminopimelic acid (DAP) in cell wall hydrolysates and whole cell sugars of actinomycete were determined by thin layer chromatography following the standard methods of (Waksman SA, Henrici AT, 1943) and (Boone KE, Pine L, 1968).

III. Results and Discussion

The selective isolation process resulted in isolation of 97 actinomycetes strains from 24 marine samples. Out of which 10 samples collected from Kulase karapattinam and 14 from Tiruchendur resulted in isolation of selective and high number of actinomycetes. Among the collected samples, Kulase karapattinam samples yielded 41 strains of actinomycetes and the Tiruchendur samples yielded 56 strains as shown in (Table 1). Among 97 actinomycete colonies isolated, 27 isolates were morphologically distinct, among 27 isolates only four isolates exhibited the anti microbial activity in the primary screening test against bacterial and fungal pathogens. Whereas, in the secondary screening test out of four active isolates KSP-SS-06-1C/1 isolate showed the potent activity against both bacterial and fungal pathogens. Hence, this strain has been taken for further character analysis. Zone of inhibitions of all four isolates were shown in (Table 2). The morphological and cultural characteristics of most

active isolate were studied on various media in accordance with International Streptomycetes Project. The growth characteristics, presence of mycelium and soluble pigments were observed (Table 3).Gram staining reveals that the active isolate was gram positive. Chemotaxonomy plays a major role in identification of actinomycetes to generic level. In this study, the active isolate contained LL-DA and absence of characteristic sugars, belongs to cell wall type-I. Different physiological characteristics are influencing the growth rate of actinomycetes. Assessment of physiological characteristics of the strain revealed that they could be grow well at 25°C and 40°C temperature, pH 7.0-10 respectively. However, the strain had maximum growth rate at a NaCl concentration of 2-7%. In this study various biochemical tests were performed and result were shown in (Table 4).

IV. Conclusion

A total of 27 isolates of actinomycetes from marine samples collected from coastal areas of Kulasekarapattinam and Tiruchendur of Tamilanadu state, India. Out of 27 isolates 4 isolates showed good antimicrobial activity against pathogenic bacteria and fungi. starch casein agar supplemented with Rifampicin and Cyclohexamide was found to be suitable for isolating actinomycetes from marine samples. Out of the 4 isolates one isolateshowed potent activity. After performing some biochemical tests, most active isolate belongs to the genus *Streptomyces*. The physiological characteristics of actinomycetes varied depending on the available nutrients in the medium and physical conditions. The present study is an attempt to identify and characterize versatile strains of Streptomyces. Further purification and characterization secondary metabolites can be carried out.

V. Acknowledgements

Author extend warm gratitude to professors in Pharmaceutical biotechnology department, University college of Pharmaceutical Sciences, Andhra University for providing facilities to carry out this work.

References Références Referencias

1. Bernan, V.S., Greenstein, M. and Maiese, W.M. (1997) Marine microorganisms as a source of new natural products. *Adv.Appl.Microbiol*.43, 57-90.
2. Okami, Y & K. Hotta (1998). Search and discovery of new antibiotics. In: Actinomycetes in biotechnology (Eds: Good fellow, M. Williams S.T., Mordarski M.). Academic press, London. pp 37-67.
3. Alan C. Ward and Nagamani Bora., *Current Opinion in Microbiology*. 2006, 9, 279.
4. Grein, A. and Meyers, S.P. (1958) Growth characteristics and antibiotic production of actinomycetes isolated from littoral sediment and material suspended in sea water. *J. Bacteriol,* 76, 457-463.
5. Haque, S.K., Sen, S.K., Pal, S.C. (1992). Sceening and identification of antibiotic producing strains of Streptomyces. Hind. Antibiot. Bull. 4:76-83.
6. Dwight, R. and Fenical, W. (1991) Distribution of actinomycetes near-shore tropical marine sediments. *Appl. Environ. Microbiol.* 57,1102-1108.
7. Elliah, P., Kalyan, D., Rao, V.S. and Rao, B.V. (1996) Isolation and characterization of bioactive actinomycetes from marine sediments. *Hindustan Antibiot. Bull*. 38, 48-52.
8. Dhanasekaran, D. and Selvamani, S. (2009) Isolation and characterization of actinomycetes in Vellar Estuary, Annagkoil, Tamilnadu. *African Journal of Biotechnology*., Vol.8(17), 4159-4162.
9. Zhomghui Zheng. And Wei Zeng. (2000). Detection of anti tumor and anti microbial activities in marine organisms associated actinomycetes isolated from the Taiwan Strait, China. *FEMS Microbiology Letters*., 188, 87-91.
10. Barry AL, Thornsberry C (1985). Susceptibility tests: diffusion test procedure. In *Manual of Clinical Microbiology*, 4th edn., eds. Ballows EA, Hawsler Jr. WJ, Shadomy HI. Washington DC: Am. Soc. Microbiol. ISBN 0-914826-65-4. pp: 978-987.
11. Rathna Kala, R. and Chandrika, V. 1993.Effect of different media for isolation, growth and maintenance of actinomycetes from mangrove sediments. *Indian J. mar. sci*., 22:297-299.
12. Shirling, E.B. and Gottlieb, D. (1966). Methods for characterization of *Streptomycetes* species. *Int. J .Syst. Bactetiol.,* 1996; 16: 313-340.
13. Pridham TG (1964). Color of *Steptomyces*: Report of an international work shop on determination of Color of *Steptomyces*. *Appl. Microbiol*., 13:43-61.
14. Kawato, N. and Shinobu, R. (1959). On *Streptomyces herbaricolor, nov SP* supplement:a simple technique for the microscopic observation .Mem. Saka Univ. Lib. Arts Educ. *J. Nat. Sci*. 8:114-119.
15. Waksman SA, Henrici AT, (1943). The nomen-clature and classification of actinomycetes. *J. Bacteriol;* 46:337-341.
16. Boone KE, Pine L, (1968). Rapid method for characterization actinomycetes by cell wall composition. *Appl. Microbiol;* 16:279-284.

Table 1 : Distribution of actinomycetes in various marine samples

Sample code	Type	Characters	Total no. of Isolates
KSP-06	Sponge	White, brain like mass, tiny spores are present	08
KSP-SS-06,1A,1B,1C	Sea Sediment	Green colored sediments	19
KSP-M1,M2,M3	Mixture	Pale Yellow colored Species	02
KSP-04	Sponge	Thick Green color *Ircinia* Species	09
KSP-SW-I,II	Sea water	Light Green color – Water	03
TCH-01,02,03	Sponge	Dark brown in color, Finger like Projections	18
TCH-04	Sponge	*Ircinia* Species	10
TCH-05,06,07	Sponge	Unknown species	08
TCH-08	Sponge	Light Grape colored *Hydella* Species	06
TCH-09,10	Corals	White Hard substance	02
TCH-11,12,14	Sponge	Unknown species, Dark Brown In color	07
TCH-13	Sponge	*Ircinia* Species, hard to cut, Tilden like smell	05

KSP-Kulasekarapattinam, TCH- Tiruchendur, Total no. of Samples-24,
Total no. of Isolates = 97

Table 2 : Antimicrobial activity of selected isolates

Test organisms	KSP-04/1	KSP-SS-06-1C/1	TCH-09/1	TCH-13/1
Bacillus subtilis	11	22.4	10.4	12.5
Staphylococcus aureus	14	21.9	11	17.5
Escherichia coli	12	20.4	10.9	13.2
Saccharomyces cerevisiae	13.7	19.5	11.5	12.0
Candida albicans	11.6	13.9	-	-
Aspergillus niger	9.8	11.7	10	9.8
Pseudomonas aeruginosa	11.8	12.8	10.5	10.3

- = No activity

Table 3 : Cultural characteristics of isolate KSP-SS-06-1C/1

Name of the medium	Growth	Aerial mycelium	Reverse color	Soluble pigment
Yeast extract malt extract medium(ISP-2)	Abundant, spreading, powdery	Abundant, powdery, grey	Pale yellow	None
Oat meal agar(ISP-3)	Moderate, spreading, powdery	Good, powdery, grey	Pale yellow	None
Inorganic salt starch agar(ISP-4)	Poor, spreading, powdery	Absent	None	None
Glycerol Aspargine agar (ISP-5)	Good, spreading, powdery	Abundant, powdery, grey	Yellow	None
Nutrient agar	Moderate, spreading, powdery	Moderate, powdery, grey	Pale yellow	None
Starch casein agar	Abundant, spreading, powdery	Abundant, powdery, grey	Pale yellow	None
Bennett's agar	Good, spreading, powdery	Moderate, powdery, grey	Yellow	None

Table 4 : Biochemical characteristics of isolate KSP-SS-06-1C/1

S.No	Reaction	Observation	Results
1.	Melanin Reaction on		
	ISP-1	No color change	Negative
	ISP-7	No color change	Negative
2.	H_2S production	No color change	Negative
3.	Tyrosinase reaction	No color change	Negative
4.	Starch Hydrolysis	Growth zone=9 mm Hydrolyzed zone=23 mm	Positive
5.	Casein Hydrolysis	No hydrolyzed zone	Negative
6.	Gelatin Hydrolysis	No hydrolyzed zone	Negative
7.	Milk coagulation and Peptonization	Coagulation followed by Peptonization	Positive Positive
8.	Nitrate Reduction	Pink color was observed	Positive
9.	Growth Temperature Range	25°C to 45°C	
10.	Chemical Tolerance(pH)	6.5 to 10	
11.	NaCl Tolerance	2% to 7%	
12.	Cell Wall Composition	LL-DAP, Glycine, Type-I	
13.	Gram staining	Stained to violet color	Positive
14.	Spore staining	No staining observed	Negative

This page is intentionally left blank

Global Journal of Science Frontier Research
Bio-Tech & Genetics
Volume 13 Issue 2 Version 1.0 Year 2013
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Cercetări Privind Utilizarea Anticorpilor Monoclonali Şi Policlonali În Evidenţierea Celulelor Stem Umane Neuronale În Creierul Fetal Ovin

By Eng. Receanu Cristina Monica

University Of Agricultural Sciences and Veterinary Medicine Cluj-Napoca Doctoral School, Romania

Introduction - Stem cells were first isolated from the internal cell mass of blastocyst and grown *in vitro*. Variants of these techniques for obtaining and cultivation are used today in laboratories around the world.

The most important publications related to stem cells have been centralized in the years 2001 and 2002 by R. Edwards.

In vitro growth of the embryo is the subject of many research teams, the first one realizing it was Heope in 1980, which won the doe produced after embryo transfer to recipient. (Gottlieb, D.I. and Huettner, J.E., 1999).

Early research on stem cells in vitro cultivation (Fig. 1) were made in 1963 by J. Paul and E. Edwards, who prepared for a group of cells growing rabbit embryo preimplantaţional in drops of NCTC 109 , 199, F10. (Theia, N.D., 2000).

A stem cell has two main properties: long-term capacity autoreînnoire without senescence and pluripotenţă and ability to differentiate into one or more specialized cell types. These cells can provide an inexhaustible source of cells for transplantation. Totipotente stem cells that have the ability to generate all types of tissue plays a critical role in human development, providing material for the development of all tissues and organs in the embryo and in all extra embryonic tissues.

GJSFR-G Classification : *FOR Code: 060410*

Strictly as per the compliance and regulations of :

Cercetări Privind Utilizarea Anticorpilor Monoclonali Şi Policlonali În Evidenţierea Celulelor Stem Umane Neuronale În Creierul Fetal Ovin

Eng. Receanu Cristina Monica

I. Introduction

Stem cells were first isolated from the internal cell mass of blastocyst and grown *in vitro*. Variants of these techniques for obtaining and cultivation are used today in laboratories around the world.

The most important publications related to stem cells have been centralized in the years 2001 and 2002 by R. Edwards.

In vitro growth of the embryo is the subject of many research teams, the first one realizing it was Heope in 1980, which won the doe produced after embryo transfer to recipient. (Gottlieb, D.I. and Huettner, J.E., 1999).

Early research on stem cells in vitro cultivation (Fig. 1) were made in 1963 by J. Paul and E. Edwards, who prepared for a group of cells growing rabbit embryo preimplantaţional in drops of NCTC 109 , 199, F10. (Theia, N.D., 2000).

A stem cell has two main properties: long-term capacity autoreînnoire without senescence and pluripotenţă and ability to differentiate into one or more specialized cell types. These cells can provide an inexhaustible source of cells for transplantation. Totipotente stem cells that have the ability to generate all types of tissue plays a critical role in human development, providing material for the development of all tissues and organs in the embryo and in all extra embryonic tissues.

At first it was thought that these adult stem cells regenerate only a very limited set of cell lines, but they demonstrated during a much greater plasticity.

II. Importance of Stem Cells

Neurodegenerative diseases are characterized by gradual and progressive damage or loss of nerve cells and neural tissue. Common are Alzheimer's disease, Parkinson's and multiple sclerosis. Neurodegenerative disorders affecting over 22 million people worldwide. Some symptomatic treatments have become possible in the past 15 years, but still not found complete treatment of these diseases.

Multiple sclerosis produce progressive brain destruction of myelin protective foil spinal neurons with progress towards irreversible disease. It is estimated that over 2.5 million people on the globe suffering from this disease. This number includes those 10,000 people in Romania. Cell replacement strategies are suitable for multiple sclerozelor because, unlike other tissues, the mammalian brain and spinal cord have a limited capacity to renew.

There is evidence that embryonic stem cells and those derived from fetal sources can generate neural cells that can be used to replace lost neurons. Mesenchymal stem cells make neurons able to migrate through the brain and spinal cord tissue seats where dysfunction is present sclerotic

a) Stem Cells - Characterization Overview

Stem cells are undifferentiated primary cells that have the ability so to differentiate into other cell types. This category includes both bone marrow cells and peripheral blood cells and embryonic cells, whose differentiation capacity up to 200 other different cell lines property which called evolutionary plasticity (Erghelegiu, Marina, 2005).

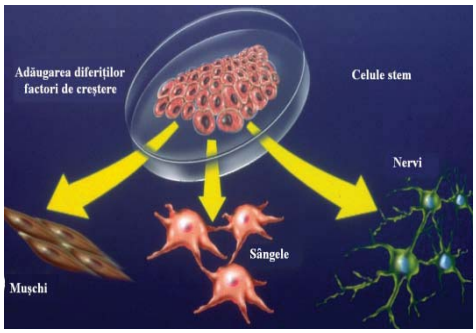

Figure 1 : Differentiation of embryonic stem cells (http://www.srsp.net/kassel/images/Pluripotent_Stem_Cells.jpg)

Author : University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca Doctoral School Faculty of Animal Production And Biotechnologies. E-mail : criss_roll@yahoo.com

Researchers believe that stem cell therapy, also called regenerative medicine, has the potential to change the face of common diseases in humans and animals by using them to replace specific tissues or organ development. Stem cell therapy experiments are currently in various medical centers around the world. There are at least 10-15 such trials in progress. The most important application of human stem cells is the generation of cells and tissues that can be used in so-called 'cell-based therapies".

b) Classification of Stem Cells

i. *Embryonic stem cells*

Embryonic stem cells from the blastocyst. Blastocyst stage of embryonic development is preimplantaţional, when the embryo consists of 150 Murin compacted blastomere arranged as spherical (Gottlieb, DI., 1999).

The blastocyst stage, embryonic cells are divided into cells forming embrioblastul (interior) and trophoblastic cells forming (from outside). Embrioblastului stem cells are cells pluripotente thus give rise to all tissues of the body except those forming the placenta and fetal envelopes. Trophoblastic cells can give rise only to the placenta and fetal envelopes (I Vintilă, 2005).

Embryonic stem cells [ES], similar to embryonic germ cells [EG], may come from the primordial germ cells which form the ova and sperm, can be isolated and grown in vitro where further proliferation and maintain their differentiation capacity (Soria , B., 2000).

The criteria defining embryonic stem cells have been developed by Weaver, CH (2006) whose studies have contributed significantly to the establishment of lists of essential characteristics of these cells.

ii. *Adult stem cells*

Adult stem cell is undifferentiated, but is in a differentiated tissue. It is able to renew the lifetime of the organism, but also generates precursors differentiated tissue. I pluripotente cellsthat can give rise to all tissues except the placenta and fetal bags.

Adult stem cells, although difficult to isolate the various organs and tissues and a small period of life, do not develop tumors, and the probability of rejecting the body is weak, especially in patients receiving organ and tissue cells harvested from their own.

c) Adult Somatic Pluripotent Stem Cells Sources And Differentiation Potency

Regarding the embryonic stem cells (Fig. 2), the main source is the embryo before the blastocyst stage. Can be obtained from the blastocyst (us) clone, is an important source of embryos resulting from insemination, in vitro "(Fraichard, A., 1995).

The 2nd source are cells derived from fertilization, in vitro"a ova with sperm are in the 'fertile banks". Researchers do not need to create embryos from sperm and ova, because it can obtain from fertilization clinics.

Sometimes couples have a child trying to create more embryos, but not all use. They can donate them for research, otherwise will be destroyed.

The 3rd source is embryonic stem cells created by SCNT (Somatic Cell Nuclear Transfer = somatic cell nuclear transfer).

This is the process by which genetic material of certain cells in the body is transferred to an egg enucleator, thus resulting in an 'embryo" produced without fertilization by a sperm. It is assumed that in fact this is a technique for cloning, which raises many questions around this subiect. Cloning is developing embryos for therapeutic use in treating diseases. The method assumes that the genetic information of a somatic cell to be transferred into an egg from which DNA was manipulated eliminat. Last source is coming from fetuses in first trimester abortions.

Adult stem cell harvesting is practiced in the medical world for over 30 years, at least in terms of their harvesting bone marrow cells (Fig. 11). 200 x 109 red blood cells are created every day in the body of haematopoietic stem cells (Eglitis, MA and Mezey, E., 1997)

For example, only 1 of 10.000-15.000 cells is bone marrow hematopoietic stem cells (blood forming).

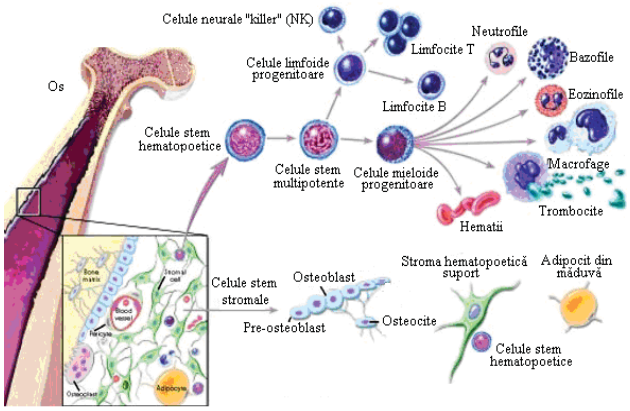

Figure 2 : differentiation of bone marrow stem cells
http://stemcells.nih.gov/info/basics/basics4.asp

The collection of the blood seems to have some advantages over harvesting of the bone marrow (Ferrari, G., 1998), meaning that you can harvest a sufficient number of stem cells, which can support more intensive meetings chemotherapy.

Basic material for the cultivation of cells in culture serve mesenchymal stem cells (CD34-), which were first identified by A. Fridenstein (et al., 1973) in the bone marrow. CD34-negative cells, which are predecessors of the bone marrow niche and leave the bloodstream. Got the blood in the appropriate

microenvironment, they enter into interaction with signal-regulatory cells mature and form a stromal framework, which can differentiate them in glial tissue and fibroblasts. (Schuldiner, 2000)

It was also shown that in the event of localized damage, stem cells mezenhimale receive signals from damaged tissues, which trigger reparative mechanisms (Tavasolli et al., 1991). Mezenhimale and hematopoietic stem cells found in cord blood. In experimental studies it was shown that during long-term cultivation of mononuclear of cord blood, endothelial-cells predecessors under the action of certain cytokines in culture form colonies of fibroblasts. (M. Nied, 1997).

III. Human Stem Cells Evidentiation using Antibodies, Antigens, Markers and Flow Cytometry Technique

a) *Antibodies*

i. *General Characterization Of Antibodies*

Antibodies are a type of protein molecules, are also known as the immunoglobulins. There are 5 types of immunoglobulins: IgG, IgA, IGD, IgE and IgM. Antibodies are produced by B lymphocytes naturally in both forms of exocytosis: the plasma membrane and secretory full. They form the B cell antigen receptor specific antibodies found in plasma and paste specific receptors flat region of immunoglobulin. Antibodies are molecules in the shape of the letter Y (Fig. 3) is composed of 2 heavy chains and 2 light chains of polypeptides These chains are linked together by covalent and necovalente (www.sinauer.com).

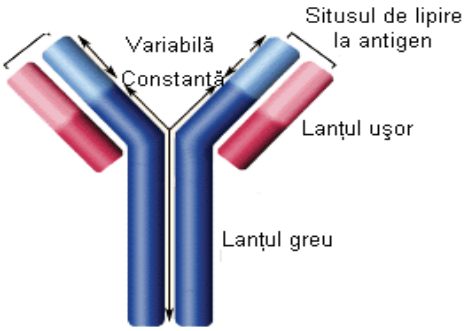

Figure 3 : The structure of an antibody (http://images.google.ro/imgres?imgurl=http://www.capemaster.net)

Primary antibodies are antibodies formed against antigens of interest (a protein, peptide, carbohydrate, or other small molecules) and are typically unconjugated. Primary antibodies that recognize and bind with high affinity and specificity of epitopii unique. A primary antibody can be very useful for the detection of biomarkers for diseases such as cancer, diabetes, Parkinson's and Alzheimer's disease and are used for the study of ADME and multi-drug resistance (MDR) of therapeutic agents (http://en.wikipedia. org / wiki / Primary _antibodies).

Specific primary antibodies used by me in my experiments were Synaptophisin, NPT II, DsRed Murin, CD Murin 31 and 20 murine Cytokeratina.

ii. *Secondary Antibodies*

A secondary antibody is an antibody that binds the primary antibody or antibody fragment. Usually they are labeled with probes that make them easy to use for detection, purification and cell sorting.

Second antibodies may be polyclonal or monoclonal and are available with specificity for whole Ig molecules or antibody fragments, such as FC (fragment crystallizable region - which is the terminal region of an antibody that interacts with receptors on the surface of a cell) or Fab regions (fragment antigen-binding - that is the region of an antibody that binds to the antigen).

Are selected by using primary antibodies, primary antibody class (eg, IgG or IgM), and the type of probe that is preferred. Identification of optimal secondary antibody is normally carried out by more tests (http://en.wikipedia.org/wiki/Secondary_antibody).

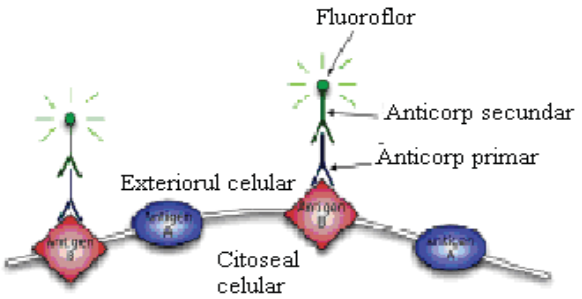

Figure 4 : Attaching the secondary antibody primary antibody http://upload.wikimedia. org/wikipedia/commons/thumb/f/fc/Primary-Secondaryantibody.svg/300px-Primary-Secondaryantibody.svg.png

Secondary antibodies specific to my use of my experiments are the Alexa 488 goat anti Flor Murin and goat anti rabbit Alexa Flor 633 Murin both goat and goat anti-rabbit anti-goat Alexa 594 anti Flor Murin.

b) *Antigens*

Antigen (Greek: anti = against and geano = to give birth, to generate) is the term that defines any substance endogenous or exogenous origin, which once reached the body, is recognized as its own and causes an immune response, aimed at neutralizing and elimination. (Http://ro.wikipedia.org/wiki/Antigen).

Due to the similarity of these stem cells have with other types of cells, the question of how to differentiate. The answer came through stem cell markers. These markers are represented such as protein receptors from the cell envelope. They have the ability to selectively bind or join with other molecules

signals. Normally, cells use these receptors and the molecules attached to them for communication and smooth the body functions.

Currently researchers use to identify specific stem populations, a combination of chemical properties of fluorescence with a single model of surface receptors. Technique called 'florescence-activated cell sorting (FACS) (Shamblott, MJ et al., 2001). Identification of stem cells is also based on the presence of genes by PCR technique (polymerase chain reaction). (Shamblott, M.J., 2001).

c) *Flow Cytometry*

Continuous-flow cytometry is a technique of counting and examination of microscopic particles such as cells and chromosomes by their suspension in a stream of fluid and passing it through an electronic device detection. This allows simultaneous analysis of physical and chemical characteristics of thousands of particles per second.

Flow cytometry is used routinely in the diagnosis of health disorders, particularly in cases of blood cancer, but has many other applications, both in research and in clinical practice (Recktenwald DJ. 1993).

► *The principle of the flow cytometry*

A beam of light (laser light, usually) a single wavelength is directed by a hydrodynamic flow of liquid concentrate. A series of detectors are directed towards the point where the stream passes through beam of light: one beam of light according to the dispersion of the forward (Forward Scatter) and the dispersion of the light beam perpendicular (Side Scatter). Each particle size from 0.2 - 150 micrometer beam of light passing through chemical substances found in fluorescent particles or attached to particles can be excited to emit light at a wavelength greater than the source of light. FSC correlates with cell volume and SSC depends on the complexity inside the particle. (Givan A. 2001).

IV. Results of National and International Research Regarding the Culture of Stem Cells

a) *Current Status of the National Research*

► The first public bank of placental blood in our country who have free access to all Romanian citizens working in Cluj-Napoca, Romania using EUROCORD Foundation. Another private bank placental blood was opened in Bucharest, the CMU, in collaboration with the Stem-Health Greece (http://www.contraboli.ro/bank-of-cells-stem-to-Cluj).

► A team of doctors including Dr. Irinel Popescu, performed the first stem cell transplant for liver disease in Romania. Patient T.L. aged 46 years, initially suffered from a liver cirrhosis and liver cancer.After four years of operation ill T.L. was cured as cirrhosis and cancer. (http://www.evenimentul.ro/ article / first-transplant-to-cell-stem-in-romania.html).

► Other investigations were performed by Daria Maria Groza in his doctoral thesis titled experimental research on behavior in vitro and in vivo stem cells in human reproduction", 2009. The aim of this PhD thesis is the practical application of current knowledge on stem cells from fetal and Annexes umbilical cord blood and experimental research on the isolation, differentiation and in utero transplantation of human stem cells obtained from animal model, using cutting-edge biotechnologies.

b) *Current Status of the International Research*

► A new stage in the use of adult stem cells is one from 1989, the discovery and use as a source of adult stem cells, extracted from umbilical cord blood. Even in that year, was successful cord blood transfusion. This was the first clinical evidence that cord blood is indeed a source of adult stem cells. (http://www.produsenaturiste.net/pages/Stimularea-pe-cale-naturala-a-celulelor-stem-adulte.html).

► In America there was an experiment of this kind. In laboratory conditions the stem cells of a cow have obtained a functional kidneys that were transplanted into animals. The research and experiences of adult stem cells raises many moral and ethical questions. (http://dictionar.romedic.ro/transplant-de-celule-stem).

V. Purpose and Objectives of Research

a) *The Aim of the Research*

International studies conducted to date in the stem cells have demonstrated their characteristics, become nowadays an important tool of regenerative medicine. In Romania, there are few studies that follow this line of inquiry, which is focused mainly on elucidating the specifics of adult stem cells.

The present study was undertaken to test the ability of monoclonal antibodies following primary Synaptophisin, NPT II, DsRed Murin, CD Murin 31 and 20 murine Cytokeratina and the secondary antibodies Alexa Flor 488 both goat and goat anti Murin anti rabbit Alexa Flor 633 the goat anti Murin and goat anti rabbit and Alexa 594 goat anti Flor Murin. They are cited in the literature (JW Goding, 1984, AM Campbell, 1984) and are used in order to highlight the existing human stem cells in fetal sheep brain.

It should be stressed that although there are research findings involving stem cells, the results are far from satisfactory. This is why we chose to perform the study.

b) *Objectives of the Research*

Research objective was to identify the most appropriate methods of recognizing human stem cells migrated into the fetal sheep brain. Methodological objectives are targeted testing several sets of monoclonal and polyclonal antibodies to identify possibilities for use in highlighting human stem cells in fetal sheep brain. To this end, the plan was compiled experimental research study taking into 8 sets of monoclonal and polyclonal antibodies, whose effectiveness has been tested on brain derived from fetal sheep. He practiced the same testing methodology, based on specific fluorescence emission principle, that red color when nerve cells and double sheep red - green for human neurons. Citometriei in continuous flow technique, we determined that the antibody is suitable to identify human stem cells.

VI. Researches Regarding Monoclonal and Policlonal Antibodies Utilization in Stem Cells Evidentiation

a) *Materials and Methods*

i. *Materials*

a. *Biological Materials*

To test monoclonal antibodies has been studied a lot of sheep and their fetuses belonging merino breed of livestock of the farm in Reno, Nevada

As organic materials were used:

-Fetal sheep brain, stem cells from existing cell lines in the laboratory cell bank in Reno, Nevada, antibodies, antigens, markers.

b. *Chemical Materials*

- Solutions of PBS (Phosphate Buffered Saline), NGS, blocking solution (buffer bloking), Ethanol, Methanol, Alcohol different concentrations (100, 95 and 75%), antigen recovery solution (antigen retrival), DAPI (4 ',6-diamidino-2-phenylindole)-fluorescent dye that binds to DNA, Hoecst, Media Dako, Dulbeco, DMEM (Dulbeco odified Eagle Medium), sterile water, alcohol medicinal and disinfection solutions.

c. *Laboratory Equipment*

Equipment used to carry out experiments was: microtome, electron microscope, confocal microscope, Olympus Bx 60 microscope (Epifluorescence microscope), PCR devices, UV lights, centrifuges, stirrer ultraturax (Helidolph), Apparatus for ice product (Bremen), autoclave (Raypa), Bath Marine (Bioblock Fisher Scientific polystat), analytical balance (320m AW Schimadzu) Biohazard waste containers (Bremen), deionizator water (SMEG WP 3000), oven (Memmet), refrigerators, freezers, fume for chemicals (Kottermann) PCR hood (sterile), microcentrifuge (Sigma 1-15), pH - meter (InoLab), UV-VISNano Drop ND-1000, Vortex (Schimadzu).

d. *Matriale Laboratory Supplies*

Glassware, blades, blades, tubes, Bisturie, gloves, pipettes (Eppendorf) with adjustable volume of 0,2-5 ml, 5-10 ml, 50-100 ml and 100-1000 ml, tubes (Eppendorf) for 1.5 ml pipette tips of different sizes (large, medium and small), the volume of 0,2-5 ml, 5-10 ml, 50-100 ml and 100-1000 ml.

ii. *Staining methods*

Specific antibody testing methods are used to reach the stage and coloring other steps must be taken first, as follows:

a. *Sampling of biological material (tissue from fetal sheep brain)*

→ *Collection*

Harvesting is the operation of taking small pieces of tissue or organ, or in the living body or from cadavers. Although often considered a trivial operation, it is actually one of the most important steps in making permanent preparation.

→ *The sampling of the brain*

Adult animals in 58-60 days pregnant were subjected to surgery, which were removed through the incision made uterine horns, fetuses intraperitoneally injecting is a certain amount of human stem cells. After a week of the operation of adult animals are slaughtered and their fetuses are collected organs of interest. They are treated and kept.

Parts of the tissues of these organs are preserved in paraffin blocks or ice. In this way can be stored for long periods of time.

b. *The inclusion of biological material in blocks of paraffin*

→ *Standing preparation*

Make a permanent preparation requires a more complicated procedure in comparison with fresh preparation. To be completed execution of a number of stages or times of work, each having a decisive influence on the quality of the preparation.

In most cases these phases are: harvesting, fixing, washing, including, cutting, coloring and installation. How to do these steps is often tailored to the needs of parts processed. Thus, these working time can be shortened or extended, simplified or complicated depending on the situation. The fact is that only one who knows how to work this time Drinking from the sample needs to obtain the final preparation of histological quality.

→ *Fixing*

For histological study is not enough evidence to be examined to be transparent and have adequate optical contrast.

Cells and tissues (especially those taken from animals) are unstable physically and chemically. Cells

and extracellular materials must be "preserved" so that structural changes and chemical composition of parts processed histologically to be minimal. This "conservation" is the subject of fixing. Simply put, fix is to stop the vital phenomena of cells and tissues as faithful preservation of their structure.

Formaldehyde is a gas, but the histology is used as a solution from 37 to 40% formaldehyde in water solution called formalin. If Formalin is used as a fixative in simple aqueous solutions, it should be diluted with a few days before, and when using the formalin neutralized can be used immediately.

→ *Money*

After setting Catchers action must be stopped by removal of parts. Otherwise, continue acting, Catchers will gradually distort parts of biological parts. The only set in which parts can be stored for longer without the major changes is Formalin. Catchers washing of the end of fixing parts water or alcohol use, depending on composition Catchers used.

Money should be made compulsory in water after fixation with dichromate, chromic acid or dinitrogen osmium. May be used for this purpose special containers with perforated walls and cork than cork.

→ *Inclusion in paraffin*

After washing, water is extracted components (dehydration) and replaced with solvents of paraffin (clarification), and the final piece is infiltrated with molten paraffin (parafinare). Standard procedure is valid for pieces with a thickness of 3-5 mm. Times for dehydration, clarification, infiltration with paraffin can be shortened or extended, as the pieces are larger or smaller.

If using fixatorii alcoholics early stages of dehydration are omitted. For the entire procedure volume of liquid must be 10 to 20 times the volume of the piece.

c. *Slicing*

After solidification of paraffin blocks containing biological parts, it can move to cutting them. The operation is executed most frequently with a rotary microtome, although it may be used microtoame tilt or sliding. Sections are obtained as thin slices, with an average thickness of 6 to 7 ìm. With a special skill can obtain ribbons consisting of a sequence of sections that may have a thickness of only 4 ìm. Sections obtained will be glued to strips of glass with a thin layer of albumin Mayer.

d. *Deparaffinization*

Paraffin is removed using Xylene. Allow slides in this solution for 10 minutes at room temperature. If necessary, repeat until the total removal of paraffin.

e. *Staining*

Histology using numerous coloring solutions, typically used at room temperature.

This extraction process is called differentiation or color fading. The success of both methods of staining depends on the colors that generally have a greater affinity to certain structures than to others. Staining is called simple when using a single color (eg simple methylene blue color), dual two solutions are used coloring (eg with hematoxylin-eozină dual color) and three solutions are used tricromică coloring (eg tricrom Masson). The most used method of staining of histological sections with hematoxylin is dual color (hemalaun) and eozin.

f. *Mounting*

After staining, sections are mounted in a substance that protects and does not affect section contrasts. The mounting medium is used Canada balsam. Canada balsam is naturally a yellow viscous liquid, which is softened by heating. By drying it becomes solid and the installation must be done properly mixed with xylene.

b) Staining Techniques

The application of immunohistochemical methods are-established some general principles concerning the purpose of methods, choice of primary antibodies, the binding agent system view.

- The purpose of primary processing methods for possible adaptation involves making immunohistochemical methods. In most cases, these techniques are performed for malignant tumors and rarely imunofenotipizarea with other injuries. This should be considered especially in harvesting, fixing and including in paraffin..
- Inhibition of endogenous peroxidase is always done with hydrogen peroxide 3%, 5 minute is usually sufficient.
- Choice of buffer solution is optional only in part, because some antibodies require saline phosphate buffer, pH 7.2 to 7.6 and other antibodies require the use of Tris buffer.
- Choice of primary antibody should be adequate for the purposes resulting from examination of preparations stained with routine methods.

Viewing will be done preferably with diaminbenzidin (DAB). DAB solution is activated with hydrogen peroxide before and in this form is active only 12 o'clock. Aminoethyl carbazole (AEC) prevent dehydration in alcohols and clarification so that installation will be done in aqueous medium and preparations are not stable.

i. *In vivo staining*

This technique is used to stain living tissues. Causing certain cells or structures to get a contrasting color, they can more easily study the morphology and position within a tissue. The main purpose of staining is to discover certain cytological details could be omitted.

By this technique can show where there are chemicals or specific chemical reactions which take place within cells or tissues.

ii. *In vitro staining*

Staining in vitro cell or tissue staining means it is no longer alive. Certain colors are often combined to have more staining power than a single dye to reveal the detail and quality than a single color. Used with restraint protocols and sample preparation, this technique can be used in making the diagnosis.

iii. *Counterstain Technique*

This is the technique that is used when using the simple technique of color are not results, making the cells or cellular structures more visible. For example, "crystal violet" color only gram-positive bacteria. Counterstain technique is used with safrarin all cells, permiting the identification of Gram-negative bacteria.

Often these techniques are called 'critical techniques", because colors are placed in living organisms. However, some dyes are toxic to organisms.

To achieve the desired results, colors are used in dilutions from 1:5000 to 1:500000. (Howey, 2000).

c) Interpretation of the Immunohistochemistry Stained Tissue

Although positive imunoreacţiile eliminates much of the examiner's subjectivity, it is necessary first of all a strict evaluation of the quality of preparation. To this effect must be taken into account a number of parameters:

- external positive control - will include with the blade to study in technical work (eg investigation of anti-CD3, external positive control section of thymus; enolază investigate the neuronal specific external positive control section of cerebral cortex).
- External Negative Control - is a section of the biopsy to study that instead of antibody solution is applied "negative control" the result will compare with that obtained on section treated with antibody. Most antibodies are supplied with the negative control solution.
- internal positive control - turns on section substrates antibody positive potential (eg anti-von Willebrand stains the cytoplasm of endothelial cells, anti-smooth muscle Actin stains miocitele smooth mioepiteliale cells and miofibroblastele).
- internal negative control - no color antibody substrates that are potentially negative (eg epithelialsquamous citokeratina 8 no color, no color desmina epithelial cells or connective).

i. *Immunohistochemistry protocol*

a. *From paraffin tissues*

1. remove paraffin, using Xylene. Allow slides in this solution for 10 minutes at room temperature. If necessary, repeat until the total removal of paraffin.
2. Are place of the blade tissue rehydration, repeated transfer of ethanol solutions of concentrations 100%, 95% and 75% for one minute at each concentration. Finally leave the water a minute.
3. Se incubated in solution of antigen retrival (antigen recovery solution) at a temperature of 93 ° C for 5 minutes, then left to cool at room temperature.
4. Leave in PBS solution.

After these steps, slides with fixed tissue are prepared for staining with primary and secondary antibodies, in order to highlight the cells of interest.

b. *From paraffin and ice tissues*

1. Rinse slides in PBS .Se of 3 x 5 minute
2. Incubate in NGS solution, which is a buffer - the buffer solution (consisting of PBS + 10% normal goat serum) for 1 hours at 4 ° C, or 15 to 30 minutes at room temperature
3. Rinse slides with PBS solution + 2% NGS, 2 x 5 minute
4. Incubated with primary antibody diluted with PBS + 2% NGS for 3 hours at room temperature or overnight at 4 ° C in a room humidifier. Primary antibody is on all slides, except the negative control strip, which is available only secondary antibody.
5. Rinse slides with PBS solution + 2% NGS, 3 x 5 minute
6. Incubated with secondary antibody, diluted in PBS + 2% NGS for 1 hours at room temperature.
7. Secondary antibody is on all slides, the amount of 2.5 ml / blade.
8. Rinse slides with PBS solution + 2% NGS, 3 x 5 minutes.
9. Rinse slides with PBS solution
10. Add a few drops of each blade DAPI dye and leave 5 minute. If there is DAPI, HOECHST solution can be used in dilution of 1 ml / 5 ml PBS
11. Rinse with PBS solution
12. Dry slides at room temperature and sealed with the strip, using Cytoseal 60 for sealing blades.

d) Importance of Staining Technique

Staining technique is the procedure by which a histological specimen is viewed in full resolution by setting it on a blade placed on the support slot located on the light microscope. Stainingul is an ancillary technique used in microscopy to enhance the contrast of the image seen in electron microscope.

The biochemistry of this technique is used for substrate and DNA specific dyes, proteins, fats and carbohydrates, to qualify and quantify the presence of specific components. This technique is widely used in biology and medicine to highlight structures in biological tissue. Staining can be used to define and examine muscle or connective tissue, organic cells or cell populations.

e) *Antibodies Test Sets*

i. *Testing of the first set of antibodies by performing a dual staining on the tissues in paraffin*

a. *Results and discussion*

In test 1, in which primary antibodies were used Synaptophisina NPT II murine and rabbit, and the secondary antibodies Alexa 488 goat anti Murin Flor and Flor Alexa 633 goat anti-rabbit, although they were strictly observed the reaction conditions, concentration and reaction sequence of recommended protocols, have not achieved expected results.

In figure 5 it can be seen that there has been coloring, but failed to identify human stem cells, so could not determine their existence in the individual brain with number 2246.

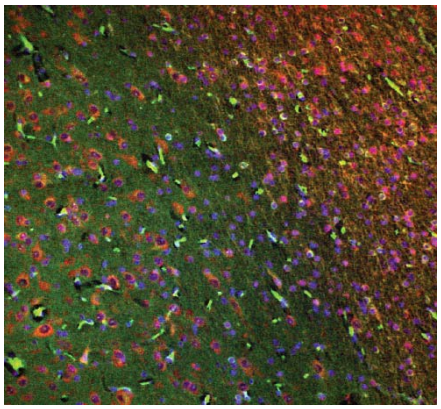

Figure 5 : Double staining with antibodies Synaptophisină primary murine and rabbit NPT II, and the secondary antibodies Alexa 488 goat anti Murin Flor and Flor Alexa 633 goat anti-rabbit (original)

The literature data were found to refer to test these antibodies in highlighting human neural stem cells exist in fetal sheep brain.

ii. *Testing of the II set of antibodies by making a single color, the tissues in paraffin*

To conduct this test in order to reflect existing human stem cells in fetal sheep brain, we used the individual with the number 2269.

There is an easy color to be able to easily distinguish between human and sheep cells, using as primary antibody Murin DsRed and the secondary antibody Alexa 488 goat anti Flor Murin. 10 blades were used.

b. *Results and discussion*

For test 2, the primary antibody were used DsRed Murin, and the secondary antibody Alexa 488 goat anti Flor Murin. In Figure 6 you can see the dye occurred, but failed to identify human stem cells, so could not determine their existence in the individual brain with number 2246.

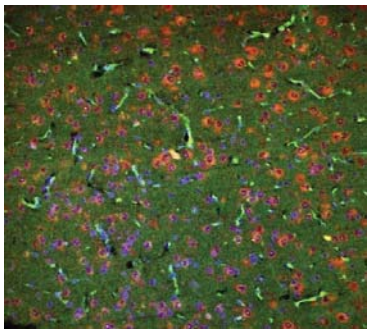

Figure 6 : Staining with primary antibody DsRed Murin and secondary antibody Alexa 488 goat anti Flor murine (original)

Neither this set of antibodies were not found in the literature data refer to test these antibodies in highlighting human neural stem cells exist in fetal sheep brain.

iii. *Testing of the III antibodies by performing a set of double color, the tissues in paraffin*

To conduct this test in order to reflect existing human stem cells in fetal sheep brain, we used the individual with the number 2267. Double staining was performed in order to lighten the difference between human and sheep cells, using as primary antibodies and CD 31 Murin Murin Cytokeratina 20 and the secondary antibodies Alexa 488 goat anti-Murin Flor and Flor Alexa 633 goat Anti Murin. 20 blades were used.

c. *Results and discussion*

Neither test has been conducted with 3 primary antibodies and CD 31 mouse and Cytokeratin 20 and the secondary antibodies Alexa 488 goat anti-mouse Flor and Alexa flor 633 goat anti mouse, did not lead to expected results. Note that in this case have been strictly observed the reaction conditions, concentration and reaction sequence of recommended protocols. In Figure 7 it can be seen that there has been coloring, but failed to identify human stem cells, so could not determine their existence in the individual brain with number 2267.

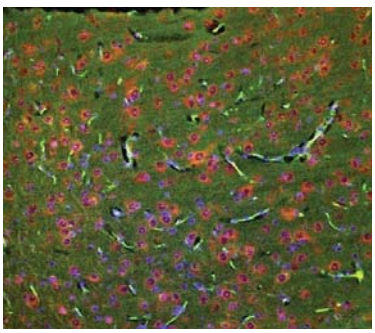

Figure 7 : Double staining with primary antibodies CD 31 and Cytokeratina Murin Murin and 20 antibodies Alexa 488 goat anti-Murin Flor and Flor Alexa 633 goat anti-murine (Original)

Neither this set of antibodies were not found in the literature data refer to test these antibodies in highlighting human neural stem cells exist in fetal sheep brain.

iv. *Testing of the IV set of antibodies, by making a double color, the tissues in paraffin*

To conduct this test in order to reflect existing human stem cells in fetal sheep brain, we used the individual with the number 2248. Double staining was performed, with 30 slides available. In order to lighten the difference between human and sheep cells were used as primary antibodies UCHL1 polyclonal, rabbit monoclonal DsRed mouse, and the secondary antibodies Alexa Flor 633 goat anti rabbit and Alexa 488 goat anti Flor mouse.

d. *Results and discussion*

In test 4, where I used UCHL1 polyclonal primary antibodies, rabbit monoclonal DsRed mouse, and the secondary antibodies Alexa Flor 633 goat anti rabbit and Alexa 488 goat anti Flor mouse. In Figure 8 you can see the dye occurred, but failed to identify human stem cells, so could not determine their existence in the individual brain with number 2248.

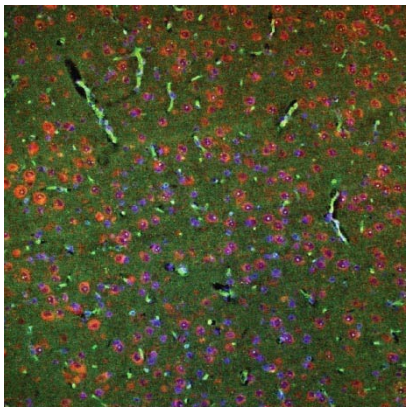

Figure 8 : Double staining with UCHL1 polyclonal primary antibodies, rabbit monoclonal DsRed Murin, and the secondary antibodies Alexa 633 goat anti rabbit Flor and Flor Alexa 488 goat anti-mouse (original)

The literature data were found to refer to test these antibodies in highlighting human neural stem cells exist in fetal sheep brain.

v. *Testing of the V set of antibodies, by making a double color, the tissues in paraffin*

To conduct this test in order to reflect existing human stem cells in fetal sheep brain, we used the individual with the number 2270. Double staining was performed, with 30 slides available. In order to lighten the difference between human and sheep cells were used as primary antibodies monoclonal DsRed Murin and Chromatogranină A Rabbit, and the secondary antibodies.

e. *Results and discussion*

For test 5, DsRed was used as primary monoclonal antibody and Chromatogranină Murin A Rabbit, and the secondary antibodies Alexa 488 goat anti Murin Flor and Flor Alexa 633 goat anti-rabbit.

Again, although they were strictly observed the reaction conditions, concentration and reaction sequence of recommended protocols, have not achieved expected results. In Figure 9 you can see the dye occurred, but failed to identify human stem cells, so could not determine their existence in the individual brain with number 2270.

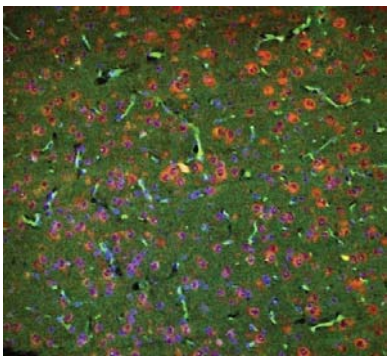

Figure 9 : Double staining with primary antibodies and DsRed monoclonal Murin Chromatogranină A Rabbit, and the secondary antibodies Alexa 488 goat anti Murin Flor and Flor Alexa 633 goat anti-rabbit (original)

Neither this set of antibodies were not found in the literature data refer to test these antibodies in highlighting human neural stem cells exist in fetal sheep brain.

vi. *Testing of the VI set of antibodies by performing a double color, the tissues in paraffin*

To conduct this test in order to reflect existing human stem cells in fetal sheep brain, we used the individual with the number 2280. Double staining was performed, with 30 slides available. In order to lighten the difference between human and sheep cells were used as primary antibodies and Oligodendrocyte Murin Chromatogranină A Rabbit, and the secondary antibodies Alexa 594 goat anti Murin Flor and Flor Alexa 633 goat anti-rabbit.

f. *Results and discussion*

For test 6, primary antibodies were used Oligodendrocyte Murin and Chromatogranină A Rabbit, and the secondary antibodies Alexa 594 goat anti Murin Flor and Flor Alexa 633 goat anti-rabbit.

Again, although they were strictly observed the reaction conditions, concentration and reaction sequence of recommended protocols, have not achieved expected results. In figure 10 it can be seen that there has been coloring, but failed to identify human stem cells, so could not determine their existence in the individual brain with number 2280.

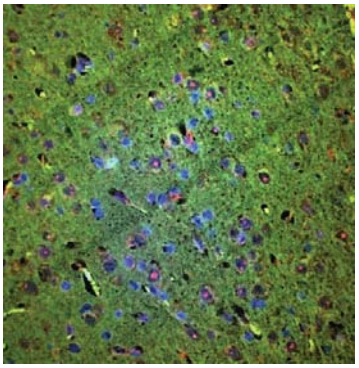

Figure 10 : Double staining with primary antibodies and Oligodenrdocyte Murin Chromatogranină A Rabbit, and the secondary antibodies Alexa 594 goat anti Murin Flor and Flor Alexa 633 goat anti-rabbit (original)

Neither this set of antibodies were not found in the literature data refer to test these antibodies in highlighting human neural stem cells exist in fetal sheep brain.

vii. *Testing of the VII antibodies by performing a set of double color, the tissues in paraffin*

To conduct this test in order to reflect existing human stem cells in fetal sheep brain, we used the individual with the number 2281. Double staining was performed in order to lighten the difference between human and sheep cells, using as primary antibodies and DsRed Murin Chromatogranina A Rabbit, and the secondary antibodies Alexa 488 goat anti Flor Murin and Alexa Flor 594 goat anti-rabbit. 20 slides were used.

g. Results and discussion

For test 7, primary antibodies were used DsRed Murin and Chromatogranină A Rabbit, and the secondary antibodies Alexa 488 goat anti Murin Flor and Flor Alexa 594 goat anti-rabbit. In figure 11 it can be seen that there has been coloring, but failed to identify human stem cells, so could not determine their existence in the individual brain with number 2281.

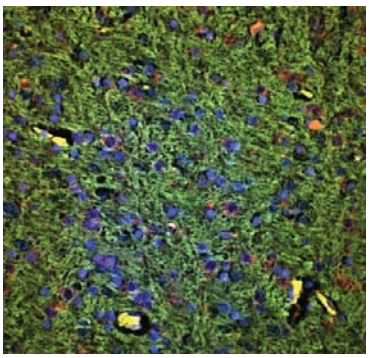

Figure 11 : Double staining with primary antibodies and DsRed Murin Chromatogranină A Rabbit, and the secondary antibodies Alexa 488 goat anti Murin Flor and Flor Alexa 594 goat anti-rabbit (original)

Neither this set of antibodies were not found in the literature data refer to test these antibodies in highlighting human neural stem cells exist in fetal sheep brain.

viii. *Testing of the VIII antibodies by performing a set of double color, the tissues in paraffin*

To conduct this test in order to reflect existing human stem cells in fetal sheep brain were used individuals in 2270, 2271, 2272, 2273, 2274. Double staining was performed, with each 4 blades available from each individual, 20 slides.

In order to lighten the difference between human and sheep cells were used as primary antibodies monoclonal DsRed Murin and Chromatogranină A Rabbit, and the secondary antibodies Alexa 488 goat anti Murin Flor and Flor Alexa 594 goat anti-rabbit.

h. Results and discussion

The test 8 was conducted with DsRed monoclonal primary antibodies and Chromatogranin A Rabbit, and the secondary antibodies Alexa 488 goat anti mouse and Alexa 594 goat anti-rabbit. The only test that led to expected results. Note that in this case have been strictly observed the reaction conditions, concentration and reaction sequence of recommended protocols. In figure 12 it can be seen that there has been coloring and stem cells could be identified uname, so it could establish their existence in the brain of all individuals analyzed, which had a positive DsRed mouse primary antibody and a rabbit with Chromatogranin A secondary antibodies Alexa 488 goat anti mouse Flor and Flor Alexa 594 goat anti-rabbit.

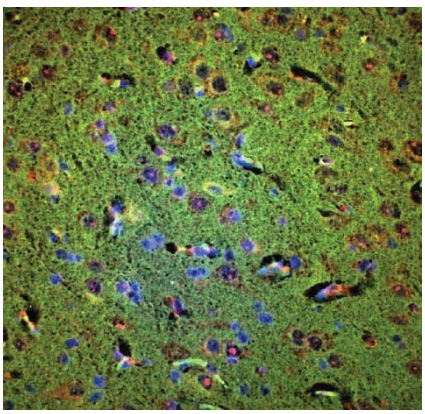

Figure 12 : Double staining with primary antibodies and DsRed monoclonal Murin Chromatogranină A Rabbit, and the secondary antibodies Alexa 488 goat anti Murin Flor and Flor Alexa 594 goat anti-rabbit (original)

V. Characterization of the Antibodies using the Flow Cytometry Technnique

Flow cytometry is a technology that allows a single cell to be measured for a variety of features, the measurements are determined only by observation of cells passing through a liquid. The instruments used for this can gather information about cells by measuring the fluorescent emission of visible light, allowing a cell sorting based on physical characteristics, biochemical and antigenic. (http://en.wikipedia.org/wiki/Flow_cytometry)

a) Materials and Methods

i. *Materials*

a. Biological materials

As organic materials were used: fetal sheep brain, stem cells from existing cell lines in the laboratory cell bank in Reno, Nevada, blood fetal sheep

b. Chemical materials

-dyes fluorescent-FITC (fluoresceinisothio-cyanate), PE (ficoeritrină), PCA (aloficocianină) antibodies: CD4, CD8, CD25, Mouse IgG 1

c. Laboratory equipment

-flow-cytometric apparatus continue

d. Matriale Laboratory supplies

-Glassware, tubes, gloves, pipettes (Eppendorf) with adjustable volume of 0,2-5 ml, 5-10 ml, 50-100 ml and 100-1000 ml, tubes (Eppendorf) of 1.5 ml pipette tips of different sizes (large, medium and small), the volume of 0,2-5 ml, 5-10 ml, 50-100 ml and 100-1000 ml.

b) Results and Discussion

Following application of flow cytometric technique to identify human stem cells from fetal sheep brain, some of the foundings are:

1. Is observed on CD4-positive population ov marked with P1 representing 9 events, respectively, and 0.41% of total CD 25 FITC-positive population ov marked P2, representing 77 events, ie 3.50% of total cells (Fig. 13). Starting from the premise that the antibodies used were created to recognize human stem cells, we can say that 3.91% of analyzed cells are human stem cells from individuals in 2164 (Annex. 1).

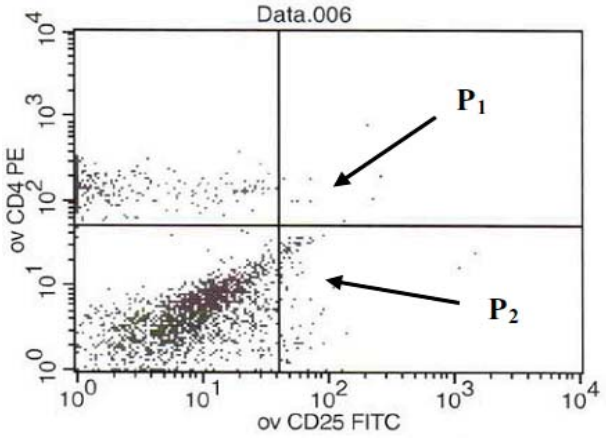

Figure 13 : Results of analysis of CD4 and ov ov CD25 antibody (original)

2. Positive population is observed on ovCD8 marked P1 representing 40 events, respectively, and 1.07% of total CD4-positive population ov FITC marked P2, 767 events representing respectively 20.49% of total cells (Fig. 14). Starting from the premise that the antibodies used were created to recognize human stem cells, we can say that 21.56% of analyzed cells are human stem cells from individuals in 2165 (Annex 1).

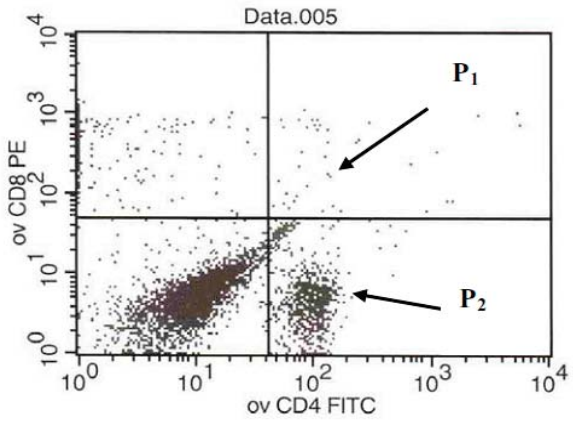

Figure 14 : Results of analysis of antibodies ov ov CD8 and CD4 (original)

3. Positive population is observed on ovCD8 marked P1 representing 84 events, respectively, and 1.31% of total CD4-positive population ov FITC marked P2, representing the 2083 events, respectively 32.38% of total cells (Fig. 15). Starting from the premise that the antibodies used were created to recognize human stem cells, we can say that 33.69% of analyzed cells are human stem cells from individuals in 2166 (Annex 2)

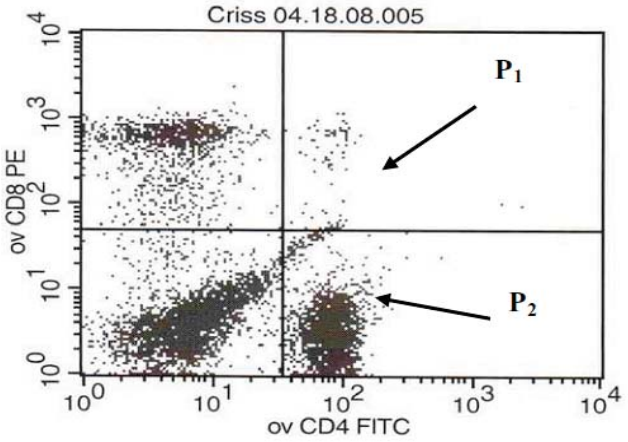

Figure 15 : Results of analysis of antibodies ov ov CD8 and CD4 (original)

4. Positive population is observed on ovCD4 marked P1 representing 45 events, respectively, and 0.68% of total positive population ov CD 25 FITC marked P2, representing 202 events, ie 3.06% of total cells (Figure 16) Starting from the premise that the antibodies used were created to recognize human stem cells, we can say that 3.74% of analyzed cells are human stem cells from individuals in 2169 (Annex 2).

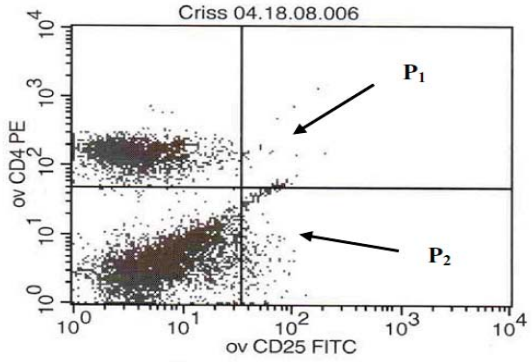

Figure 16 : Results of analysis of CD4 and ov ov CD25 antibody (original)

VI. Statistical Intepretation of the Data Regarding the Total Amount of the Cells, Sheep Neurons and Human Stem cells Identiffied by the used Antibodies

Average number of cells shown in fetal sheep brain recorded at between 761.70 when disclosure was made with the set of antibodies VII and 610.50 when highlighting kit antibody was performed with V. There was variability between 30.68% for set VI antibody and 15.74% for set II antibodies (Table 3)

Table 3 : Averages and dispersion parameters for all cells highlighted in the 8 sets of antibodies used

Issue	n	$\overline{X}$	±	$s_{\overline{X}}$	V%
Set I of antibodies	20	674,95	±	1,79	1,19
Set II of antibodies	10	691,50	±	1,72	0,79
Set III of antibodies	20	757,10	±	0,79	0,47
Set IV of antibodies	30	675,03	±	1,67	1,36
Set Vof antibodies	30	614,33	±	1,28	1,14
Set VI of antibodies	30	680,60	±	2,08	1,67
Set VII of antibodies	20	744,05	±	1,67	1,01
Set VIII of antibodies	20	764,05	±	1,21	0,71

The average number of neurons in the brain of fetal sheep sheep highlight recorded values between 614.33 when evidence was made with the set of antibody V and shown as 764.05 when performed with a set of antibodies VIII. There was variability between 0.47% for set III antibody and 1.67% for set VI antibody (Table 4)

Table 4 : Averages and dispersion parameters for sheep neurons highlighted in the 8 sets antibody

Issue	n	$\overline{X}$	±	$s_{\overline{X}}$	V%
Set I of antibodies	20	225,90	±	1,74	39,43
Set II of antibodies	10	192,40	±	1,59	56,97
Set III of antibodies	20	123,90	±	1,65	35,39
Set IV of antibodies	30	160,60	±	0,93	27,30
Set Vof antibodies	30	167,23	±	1,48	32,72
Set VI of antibodies	30	213,47	±	0,98	34,99
Set VII of antibodies	20	175,50	±	1,31	39,66
Set VIII of antibodies	20	158,90	±	1,19	22,87

Human stem cells were identified only when testing was performed with the set VIII antibody. He obtained an average of 4.50 cells and a very high coefficient of variability, 48.29% respectively. If we refer to the total number of cells in the brain evidenced by Kit antibody VIII (Table 1), found that the number of identified human stem cells is only 5.91% of them.

*Table 5 :*The averages and dispersion parameters for the human stem cells emphasized with VIII[th] set of antibodies

Specificare Issue	n	$\overline{X}$	±	$s_{\overline{X}}$	V%
Setul VIII de anticorpi	20	4,80	±	0,39	36,04

VII. Conclusions

1. After conducting the experiment to test the possible use of staining techniques (staining) of neural stem cells in sheep tissue with a series of monoclonal antibodies, the result the following conclusion: Set 1 of antibodies, consisting of primary antibodies Synaptophisin and NPT II, and the secondary antibodies Alexa 488 and Alexa 633, not suitable for use to reflect neural stem cells in sheep tissue by staining technique developed and perfected in the laboratory of the Department of Biotechnology University Reno, Nevada.

2. Neither set of antibodies 2, consisting of DsRed Murin primary antibody and secondary antibody Alexa 488 goat antimurin, not suitable for use to reflect neural stem cells in sheep tissue by staining technique developed and perfected in the laboratory of the Department of Biotechnology, University Reno , Nevada, USA.

Again assume that their structure does not lend itself to the formation of a COMPEX stem cells producing fluorescence, visible confocal microscope.

3. The results were also negative for antibodies set 3, consisting of primary antibodies CD 31 murine and 20 Murin Cytokeratina and secondary antibody Alexa 488 goat anti-Murin, not suitable for use to reflect neural stem cells from sheep tissue by staining technique developed and perfected in the laboratory of the Department of Biotechnology, University Reno, Nevada.

Our results show that neither CD 31 murine primary antibody structure and Cytokeratina 20 Murin and secondary antibody Alexa 488 goat anti-Murin is not compatible with stem cells to form a COMPEX producing fluorescence, visible confocal microscope.

4. Set 4 antibody, UCHL1 up of primary polyclonal antibodies, monoclonal rabbit DsRed Murin, and the secondary antibodies Alexa 633 goat anti-rabbit and Alexa 488 goat anti Murin not suitable for use to reflect neural stem cells in sheep tissue The staining technique developed and perfected in the laboratory of the Department of Biotechnology, University Reno, Nevada.

Suppose that the structure does not lend itself to the formation of a COMPEX stem cells producing fluorescence, visible confocal microscope.

5. Neither set of antibodies 5, 6 and 7 were positiv for what I have searche for.

8. The results were positive for antibodies set 8, consisting of primary DsRed monoclonal antibodies and Chromatogranină Murin A, and the secondary antibodies Alexa 488 goat anti-rabbit and Alexa 594 goat anti Murin, which do not lend themselves to use stem cells to highlight neural tissue of sheep by the dye technique developed and perfected in the laboratory of the Department of Biotechnology, University Reno, Nevada.

The results show that the structure of DsRed rabbit primary antibody with secondary antibody Alexa 488 goat anti rabbit is compatible with stem cells to form a COMPEX that produces visible fluorescence confocal microscope.

9. Mention that for the first time that this type of testing was efectuatpe neural stem cells.

10. Este should continue and expand research to identify other antibodies whose structure have availability for evidence of human neural stem cells.

11. Citometriei technique in continuous flow were determined antibodies that can identify existing human stem cells in fetal sheep brain, and the quantity they are found in each individual analysis.

12. The overall analysis of data obtained by flow fluorocitometrie may find the following: the highest percentage of grafting human stem cells in fetal sheep brain in the amount of 33.69% was obtained from the individual with the number 2166 and the lowest percentage of engrafting of human stem cells in fetal sheep brain was the amount of 1,74% , was obtained from the inndivids with numbers 2270, 2271, 2272, 2273, 2274.

References References References

1. A. M. Campbell, 1984, Monoclonal Antibody Technology: the production and characterization of rodent and human hybridomas''. Ed. Elsevier. Amsterdam-NewYork-Oxford. 33-211

2. Bittner S. , B. Dietz, M. Miski-Oglu, P. Oria Iriarte, A. Richter,and F. Schäfer 2009, Phys. Rev. A 80, 023825 (2009) [9 pages]. Experimental test of a two-dimensional approximation for dielectric microcavities;
3. Bittner S., R. E. Christian Schöfer2, Klara Weipolt-shammer2, Silva Ivanova1, Berthold Streubel1, Erwin Hauser3, Michael Freilinger3, Harald Höger4, Adelheid Elbe-Bürger5 and Franz Wachtler2, 1999, Journal of Anatomy and Embryology;
4. Donnall Thomas Lecture: Normal and Neoplastic Stem Cells *Biology of Blood and Marrow Transplantation*, Volume 14, Issue 8, Pages 849-858 I. Weissman;
5. Edward R. Pressman, R., Dixon, DP & Walbot, V, 2000, Plant glutathione S-transferases: enzymes with multiple functions in sickness and in health. Trends In Plant Science 5(5): 193-198;
6. Eglitis , M. A., Kantoff, E Gilboa, and WF Anderson , 1997, Gene expression in mice after high efficiency retroviral-mediated gene transfer, Science, Vol 230, Issue 4732, 1395-1398;
7. Evans M. J. & M. H. Kaufman, 1981, Establishment in culture of pluripotential cells from mouse embryos, Nature. 1981 Jul 9;292(5819):154-6;
8. Feldman, A. (2000). *Existential approaches to action research*. An invited paper at the Annual Symposium of the National Academy of Educational Researchers, November 2000, Ponte Verde, FL;
9. Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, Mavilio F.H. Muscle regeneration by bone marrow-derived myogenic progenitors, The EMBO journal 16(14), 1997 Jul 16;
10. Feldman B., W Poueymirou, VE Papaioannou, TM DeChiara, and M Goldfarb , 1995, Requirement of FGF-4 for postimplantation mouse development, Science, Vol 267, Issue 5195, 246-249;
11. Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, Mavilio F., 1998, bMuscle regeneration by bone marrow-derived myogenic progenitors, Science2,79(5356):1528-30;
12. Fraichard A, Chassande O, Plateroti M, Roux JP, Trouillas J, Dehay C, Legrand C, Gauthier K, Kedinger M, Malaval L, Rousset B, and Samarut JFraichard A, 1997, The T3R alpha gene encoding a thyroid hormone receptor is essential for post-natal development and thyroid hormone production. The EMBO journal 16(14):4412-20, 1997 Jul 16;
13. Friedenstein A. et al., Bones and cartilage, developmental and evolutionarz skeletal biology, 1973.
14. Gossler A., AL Joyner, J Rossant, and WC Skarnes , 1989, Mouse embryonic stem cells and reporter constructs to detect developmentally regulated genes, Vol. 244. no. 4903, pp. 463 – 465;
15. Gibbons HM, Hughes SM, Van Roon-Mom W, Greenwood JM, Narayan PJ, Teoh HH, Bergin PM, Mee EW, Wood PC, Faull RLM, Dragunow M (2007) Cellular composition of human glial cultures from adult biopsy brain tissue. Journal of Neuroscience Methods 166:89-98;
16. Gossler A., R. Balling, 1992, The molecular and genetic analysis of mouse development and Serotonergic Neurons after Transplantation, Department of Gynecology and Obstetrics, Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA. Derivation of Hepatocytes from Bone Marrow Cells in Mice After Radiation-Induced Myeloablation, vol 31. nr 1, pg 4-7, for fermenting solutions. U. S. Patent 2,188,192. 1956] 33 San Raffaele-Telethon Institute for Gene Therapy (TIGET), 20132 Milan, Italy. Science. 1998 Mar 6;279(5356):1528-30;
17. Gottlieb D.I. and Huettner J.E., 1999, An in vitro pathway from ES cells to neurons and Glia. Cells Tissues Organs, 165:165-172;
18. Groza I., D.D. Ciupercescu, Emoke Pall, Elen Gocza, 2006, In vitro production embryoid bodies from a mouse embryonic stem cell line and differentiation protocols towards cardiomyocyte and neuron lineages, Buletin Medicină Veterinară, Vol. 63, pag 264-269;
19. Groza I., Ciupe Simona, D.D. Ciupercescu, Emoke Pall, Daria Groza, 2006, Researches reggarding the collection of mouse embryos and their use to obtain the stem cells, Buletin Simpozion Ştiinţific, Bucureşti, pag 228- 238;
20. Jennifer Nichols, Ian Chambers, Tetsuya Taga, Austin Smith 2000, Physiological rationale for responsiveness of mouse embryonic stem cells to gp130 cytokines;
21. J. W. Goding, 1984, Monoclonal antibodies: Principles and Practice. Production and application of Monoclonal Antibodies in Cell Biology, Biochemistry and Immunology''. Third Edition. Academic press. USA. 116-212;
22. Kohler, G. & Milstein, C., 1975, Continuous cultures of fused cells secreting antibody of predetermined specificity. *Nature* 256 (5517): 495. doi:10.1038/256495a0;
23. Kunath K., A. von Harpe, H. Petersen, D. Fischer, K.H. Voigt, T. Kissel, U.Bickel The structure of PEG-modified poly (ethylene imines) influences biodistribution and pharmacokinetics of their complexes with NF-kappaB decoy in mice Pharm. Res. 19 (2002) 810-817;
24. Lichner Zuzsanna, Emoke Pall, Elen Gorcza, 2008, Investigation the role of stem cells specific MMU-MIR 290-295 Cluster, First Internatioonal Conference of Functional Annotation of the Mammalian Genome, Rottach- Egern, Germany;

25. Miller, J.S. (2005) Eicosanoids Influence *In Vitro* Elongation of Plasmatocytes from the Tobacco Hornworm, *Manduca sexta*. *Archives of Insect Biochemistry andPhysiology*59:42-51;
26. Miller, J.S. and Stanley, D.W. (2004) Lipopoly-saccharide Evokes Microaggregation Reactions to Hemocytes Isolated from Tobacco Hornworms, *Manduca sexta*. *Comparative Biochemistry and Physiology* 137: 285-295;
27. Moore, Samantha Allison, Investigations of laser-induced neuronal guidance, 2004, Doctoral dissertation, The University of Texas at Austin, vol 4. pg 78- 80;
28. Nicholas A., Malcolm R. Alison1, Richard Poulsom2, Rosemary Jeffery2, Amar P. Dhillon3, Alberto Quaglia3, Joe Jacob1, Marco Novelli5, Grant Prentice4, Jill Williamson6 &, Wright1,2, 1996, Cell differentiation: Hepatocytes from non-hepatic adult stem cells;
29. Nieda M, Nicol A, Denning-Kendal P et. Al. Endotelian cell precursors are normal component of human umbilical cord blood. Br J Haematol 1997; 98; 775-777
30. Pall Emoke, Groza I., Şoritau Olga, Cătană Laura, Cenariu M., 2008, Modulation of cardiac differentiation of mouse embryonic stem cells under the influence of some growth factors, Lucrări Ştiinţifice, vol 51, ISSN 1454-7406, Medicină Veterinară, Ed. Ioan Ionescu de la Brad, Iaşi, pag 117-122;
31. Pall Emoke, I., Groza, Olga , Simona Ciupe, Laura Cătană, 2008, Isolation, culture and identification of bone cells from newborne rats, Bulletin of University of Agricultural Sciences and Veterinary Medicine, 65 pISSN -1843-5378;
32. Cheever3, Peter J. Murray2, Thomas A., 1999, Arginase-1–Expressing Macrophages Suppress Th2 Cytokine–Driven Inflammation and Fibrosis;
33. Recktenwald DJ. 1993. Introduction to Flow Cytometry: Principles, Fluorochromes, Instrument Set-Up, Calibration. J Hematotherapy 2(3): 387-94.
34. Ross JS. 1996. DNA Ploidy and Cell Cycle Analysis in Pathology. New York: Igaku-Shoin
35. Shamblott MJ, Axelman J, Wang S, Bugg EM, Littlefield JW, Donovan PJ, Blumenthal PD, Huggins GR, Gearhart JD, Derivation of pluripotent stem cells from cultured human primordial germ cells Proc Natl Acad Sci U S A. 1998 Nov 10;95(23):13726-31. Proc Natl Acad Sci U S A. 1998 Nov 10;95(23):13726-31;
36. Soria B, Andreu E, Berna G, Fuentes E, Gil A, Leon-Quinto T, Martin F, 2000, Engineering pancreatic islets. Pflugers Arch 2000; 440(1):1-18. (PMID 10863992);
37. Spangrude GJ, Cho S, Guedelhoefer O, Vanwoerkom RC, Fleming WH. (2006). Mouse models of hematopoietic engraftment: limitations of transgenic green fluorescent protein strains and a high-performance liquid chromatography approach to analysis of erythroid chimerism. Stem Cells, 24(9), 2045-51;
38. Strübing C., G. Krapivinsky, L. Krapivinsky, D. Clapham, 1995, TRPC1 and TRPC5 Form a Novel Cation Channel in Mammalian Brain Neuron, Volume 29, Issue 3, Pages 645-655;
39. Tanaka, S. and S. Ohnishi 1998 Some biological aspects of freshwater stingrays collected from Chao Phraya, Mekong, and Ganges River systems. Adaptability and Conservation of Freshwater Elasmobranchs;
40. Tanskanen, A. J. and R. L. Winslow (2006). "Integrative Structurally-detailed model of calcium dynamics in the cardiac diad." SIAM Journal of Multi-Scale Modeling and Simulation;
41. Thomson S., Joseph Itskovitz-Eldor, Sander S. Shapiro, Michelle A. Waknitz, Jennifer J. Swiergiel, Vivienne S. Marshall, Jeffrey M. Jones , 1984, Embryonic Stem Cell Lines Derived from Human Blastocysts, Vol. 282. no. 5391, pp. 1145 – 1147;
42. Tsieh S. 2002. Flow Cytometric Analysis of Hematologic Neoplasms: A Color Atlas & Text. Philadelphia: Lippincott Williama & Wilkins. 276p
43. Véronique Royer, Stéphane Fraichard and Hervé Boutin A novel putative insect chitinase with multiple catalytic domains: hormonal regulation during metamorphosis;
44. Weaver C. H., FB Petersen, FR Appelbaum, WI Bensinger, O Press, P Martin, B Sandmaier, HJ Deeg, JA Hansen and M Brunvand ,2006, High-dose fractionated total-body irradiation, etoposide, and cyclophosphamide followed by autologous stem-cell support in patients with malignant lymphoma,Journal of Clinical Oncology, Vol 12, 2559-2566;
45. ***http://www.evenimentul.ro/articol/primul-trans-plant-de-celule-stem-in-romania.html
46. ***http://www.produsenaturiste.net/pages/Stimularea-pe-cale-naturala a celulelor stem adulte.html
47. ***http://celulestem-lifeline.blogspot.com/2009/05/celulele-stem-din-sangele-din-cordonul. html
48. ***http://www.faqs.org/health/bios/56/E-Donnall-Thomas.html

This page is intentionally left blank

Global Journal of Science Frontier Research
Bio-Tech & Genetics
Volume 13 Issue 2 Version 1.0 Year 2013
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Is it Possible Artificial Sex Regulation in Mammals?

By A.I. Ibraimov

Kazakh National Medical University, Kyrgyzstan

Abstract - In spite of, impressive breakthrough in modern genetics and molecular biology the problem of artificial sex regulation of mammals still remains unsolved. Moreover, the very problem of sex origin of eukaryotes in the process of evolution still has not settled. Existing theories and hypothesizes mainly concern the maintenance and biological reasonability of sexual mode of replication. Their theoretic foundation is based on Darwin's and Mendel's ideas that sex was originated due to natural selection and genes. On the basis of other model of genesis and sex evolution of eukaryote, it was suggested the idea of artificial sex regulation of mammals. Seemingly, the sex differentiation (SD) in animals and human is determined by the amount of constitutive heterochromatin region (cHR) in the Y chromosomes of the undifferentiated embryonic gonads (UEG) via cell thermoregulation. It is assumed the medulla and cortex tissue cells in the UEG differ in vulnerability to the increase of the intracellular temperature because of their anatomical position in genital ridges. If the amount of the cHR on Y chromosome is enough for efficient elimination of redundant metabolic heat from rapidly growing UEG cells the medulla tissue survives. Otherwise it doomed to degeneration and a cortex tissue will remain in the UEG. For artificial regulation of the SD it is proposed to remove a layer of cortex in the UEG.

Keywords : sex differentiation; sex regulation; cell thermoregulation; constitutive heterochromatin; Y chromosome; genital ridges; non coding DNAs.

GJSFR-G Classification : FOR Code: 270205

Strictly as per the compliance and regulations of :

Is it Possible Artificial Sex Regulation in Mammals?

A.I. Ibraimov

Abstract - In spite of, impressive breakthrough in modern genetics and molecular biology the problem of artificial sex regulation of mammals still remains unsolved. Moreover, the very problem of sex origin of eukaryotes in the process of evolution still has not settled. Existing theories and hypothesizes mainly concern the maintenance and biological reasonability of sexual mode of replication. Their theoretic foundation is based on Darwin's and Mendel's ideas that sex was originated due to natural selection and genes. On the basis of other model of genesis and sex evolution of eukaryote, it was suggested the idea of artificial sex regulation of mammals. Seemingly, the sex differentiation (SD) in animals and human is determined by the amount of constitutive heterochromatin region (cHR) in the Y chromosomes of the undifferentiated embryonic gonads (UEG) via cell thermoregulation. It is assumed the medulla and cortex tissue cells in the UEG differ in vulnerability to the increase of the intracellular temperature because of their anatomical position in genital ridges. If the amount of the cHR on Y chromosome is enough for efficient elimination of redundant metabolic heat from rapidly growing UEG cells the medulla tissue survives. Otherwise it doomed to degeneration and a cortex tissue will remain in the UEG. For artificial regulation of the SD it is proposed to remove a layer of cortex in the UEG.

Keywords : sex differentiation; sex regulation; cell thermoregulation; constitutive heterochromatin; Y chromosome; genital ridges; non coding DNAs.

I. Introduction

In many mammals, including us, the genetic sex is determined at fertilization by sex chromosomes carried by the father's sperm, X in the case of female and Y in the case of male. At the early stages of embryonic development a pair of undifferentiated embryonic gonads (UEG) and both rudimentary female and male reproductive system develops in the embryo. As result of this all embryos are potentially bisexual.

For the time being the mechanisms of the sex differentiation (SD) are not known. At present the balance hypotheses, worked out by Bridges (1939) and Goldschmidt (1955) are generally accepted. According to these hypotheses, the interaction of genes, located in the sex chromosomes and autosomes, underlie the SD. Their theoretic foundation is based on Darwin's and Mendel's ideas that sex was originated due to natural selection and genes.

However, almost nothing is known about concrete mechanisms and types of gene interaction at the SD. The problem also becomes complicated especially as: a) the number, localization, products, and types of these gene interactions are not determined; b) the role of the sex chromosomes in the embryo SD remains not completely clear; c) there are no ideas as regards the possible role of a great amount of constitutive heterochromatin region (cHR) of the Y chromosome in the SD.

Indeed it is hard to believe that having impressive breakthrough in modern genetics and molecular biology the reasons and mechanisms of sex origin are still unknown. This probably has to do with the fact that in the basis of all hypothesizes and theories on sex biology lies idea on all-powered role of natural selection and genes in eukaryotic organisms' evolution. Although they help to explain reasonably and justify such widespread propagation of sexual reproduction in the world of eukaryotes; nevertheless, these approaches had little help in the development of theories and hypothesizes explaining of sex origin (Ibraimov 2012). Moreover they were not able to show ways of their experimental check. As Ridley (2010) begrudgingly admitted: 'Sex is not used simply for want of an alternative. Nothing, in an evolutionary sense, forces organisms to reproduce sexually'.

We offer the idea that probably sex and sexual reproduction of eukaryotic organisms are the result of the long evolution of non coding DNAs (ncDNAs), which led to the origin of mitotic chromosome, mitosis, meiosis, sex determination and differentiation mechanism. It is supposed that sex and sexual reproduction were a direct consequence of the origin of unicellular eukaryote, but not are result of emergence of some specific structural genes determining the sex development. Genes pertaining to sex appeared later, but again not for determination and differentiation of sex, but for development of the secondary sexual characters with the help of hormones (Ibraimov 2008; 2009; 2010; 2011; 2012).

The overwhelming majority of modern biologists associate development and life evolution with structural, functional and frequency changes of genes. If the role of non-informative, i.e. ncDNAs in

Author : Kazakh National Medical University after name S.D. Asfendyarov, Tole bi str., 88, Almaty, KZ-050012, Kazakhstan. Institute of Balneology and Physiotherapy, Bishkek, KG-720 040, Kyrgyzstan.
E-mail : ibraimov_abyt@mail.ru

these processes is admitted, then only as a secondary one. The possible role of ncDNAs in the development and the evolution of eukaryotes, except for maybe satellite DNAs and chromosomal heterochromatic regions (HRs), is not considered seriously. Mostly, they are considered as "surplus" DNAs.

However, recently there has appeared data about the possible role of ncDNAs in individual development and evolution (Ibraimov 2003, 2004, 2008, 2009, 2011, 2012; Ibraimov and Tabaldiev 2007), including the sex differentiation of higher eukaryotes (Ibraimov 2008). In particular, on the basis of the cell thermoregulation concept there was proposed a hypothesis of a possible sex differentiation mechanism. Seemingly, the SD in animals and human is determined by the amount of constitutive heterochromatin region (cHR) in the sex chromosomes of the undifferentiated embryonic gonads (UEG) via cell thermoregulation. If the amount of the cHR on Y chromosome is enough for efficient elimination of heat difference between the nucleus and cytoplasm in rapidly growing UEG cells the medulla tissue survives. Otherwise it doomed to degeneration and a cortex tissue will remain in the UEG (in more detail see Ibraimov 2008). Thus, the sex differentiation is an immediate consequence of cell thermoregulation, but not a result of some specific structural gene emergences determining sex development. In other words, the basis of SD at the stage of indifferent primordial gonads is mainly physical processes rather than chemical.

II. What are the Approaches for Artificial Regulation of Sex?

Currently the following approaches are practiced: chemical (hormonal), physical and cellular methods of artificial regulation of sex. The disadvantage of the first approach, as we know, is the reversibility of the sex after the termination of the sex hormones. The second approach starts from the classical embryo-genetic studies by Jost (1972) on removing of the future gonad (genital ridges) in early embryos of rabbits: removal of genital ridges before the formation of the gonads led to the development of embryos as females.

Scientists tried to separate the sperm by size, weight, mobility in an electric field and by other methods. Despite the large number of experiments carried out, the problem of regulating sex in a mammal by physical separation of sperm has still not been solved.

It is possible to receive organisms with desired sex from animals using cell engineering such as transplantation and cloning. Currently, a method of dividing embryos has been developed. Embryos of pets can be divided into 4, 8 or more parts. At the same time one part of the embryo is planted into the uterus of the female, the remaining parts are frozen and stored for a long time in liquid nitrogen. After the birth of the offspring transplants of other parts of the divided embryo can be done. In this case, the sex will be the same as in the birth of the animal.

The essence of our idea is to transform the embryo with XX karyotype in the male fetus, which will be able to produce gametes of only one type (X sperm) in the future, and it is based on the following assumptions:

1. Sex-determining ability of Y chromosome is not due to the presence there in *SRY* / *Sry* and other not yet identified genes, but due to the fact that it has a large block of cHR.
2. It is the presence of cHR in Y chromosome that ensures the survival of medulla at the stage of indifferent primordial gonads from heat degeneration through the mechanisms of cell thermoregulation.
3. A two-layer structure of the genital ridges –medulla inside and cortex outside - has profound biological significance: the emergence of a male depends on the survival of medulla, and not on the presence of *SRY* and others genes that has to do with sex determination.
4. Location of the medulla in the depth of genital ridges puts her in a difficult position in terms of the effective removal of metabolic heat, as it is surrounded by a layer of adipose tissue (mesentery) and cortex. Rise in the temperature of the medulla tissue above the physiologically acceptable levels may cause it to heat death, with all the ensuing consequences. Medulla survival depends on timely removal of excess heat through the layers of the mesentery and the cortex outside the genital ridges.
5. There are mammals that determine male sex without the *SRY* gene.
6. Along with genetic factors (the presence of the sex chromosomes of a particular type, *SRY* and other hypothetic genes) on the formation of sex in some vertebrates and invertebrates environmental factor, such as temperature has a significant impact as well.
7. Production of gametes does not depend on the type of gonads, if testis is formed, germinal cells develop into sperm cells, and if the ovary is formed, then the same cells form eggs.
8. For the normal functioning of mammalian organisms, as well as for other homoiotherms, body temperature needs to be maintained within very narrow limits (temperature homeostasis), which is provided by organ-based mechanisms of physiological thermoregulation. Obviously, the ultimate goal of any thermoregulation is to maintain a constant temperature inside the cells. However, while maintaining the equal temperature between

nucleus and cytoplasm, the role of organ-based physiological thermoregulation is limited and mechanisms of intracellular thermoregulation must be turned on through a layer of condensed chromatin, which is located inside the nuclear membrane (nuclear envelope).

9. As known, complex systems function poorly. It is hard to imagine that such an important process as sex determination in mammals has been assigned to *SRY* and other, more than a hundred, genes. Perhaps Nature invented the simplest way, to determine sex through the physical survival of the medulla, while giving each species of mammals a defined window of time (f.e.: on 10.5-12th day of embryonic development in the mouse).

Thus, our idea of artificial regulation of sex is based on the lately discovered cell thermoregulation (CT) phenomenon, the essence of which is in the following: the peripheral layer of condensed chromatin (CC) of nucleus, being the densest domains in a cell, apparently conducts heat between the cytoplasm and nucleus when there is a difference in temperature between them. The assumed heat conductivity effect of CC, consisting of highly repetitious DNA (the main component of cHR), is stipulated by its principal features: condensed state during the interphase, association with the lamina and the inner nuclear membrane, replication at the end of the S period of a cell cycle, formation of the chromocenter, genetic inertness, and wide variability in the quantitative contents both within and between species (Ibraimov2003; 2004). The reality of the CT existence is shown at the organism level. In particular, it turned out that the individuals in the population differ from one another by the heat conductivity of their bodies (BHC). At that the BHC value depends on amount of the constitutive heterochromatin in their genomes (Ibraimov and Tabaldiev2007; Ibraimov et al., 2010 a,b).

As known, the sexual development in the mammals is a process consisting of at least three stages: the 1ststage is the chromosome determination of sex (XX or XY); the 2nd stage is SD (the development of testicles or ovaries); the 3st stage is the development of the secondary sexual characteristics. In case the testicles are formed, they excrete the testosterone hormone, circulating over embryo, and causing the development of the somatic cells by the male type.

On the contrary, the lack of testosterone results in invariable development of the somatic cells by the female type.

Now let's try to ground our assumption. (1). The heat conductive effect of the CC especially strongly increases in conditions of mulicellularity (Ibraimov 2003, 2004); (2). By the 3rd week of the embryo development in human, the cHRs is completely formed (Prokofyeva-Belgovskaya 1988), and they are able to exert their heat conductive effects in the cells. (3). In mammals it has been shown that at equivalent gestational ages, males are developmentally more advanced than females. Medulla, being located in the very middle of the genital ridges closed to aorta and surrounded with cortex and mesentery probably experiences the greatest problems with removal of the excessive metabolic heat in comparison with cortex. Obviously, the cortex having a relative advantage in supporting the intracellular temperature homeostasis than the medulla, other things being equal, has more chances to preserve and further develop in to the female tissue.

It is possible that the medulla of the UEG is more vulnerable to the temperature increase than the cortex tissue. The following data testify to this: a) the clinical consequences of cryptorchidism in boys; b) location of scrotum outside the body in the mammals. In other words, the direction of development of the genital ridges towards the male or female side is not something strictly fixed, it will depend on the environment in which it is being implemented. In our opinion, most likely this is the temperature influence on the cells of the genital ridges, and seemingly on which it depends, whether the medulla tissue will remain or not. If the medulla tissue is not provided with the timely and efficient removal of excessive metabolic heat, it will be doomed to degeneration having making for the cell from the cortex. Just in this meaning we understand the role of cHR in SD, as efficient means of medullar tissue protection against "heat death".

It could be possible to test our hypothesis experimentally (Fig. 1). At genital ridges with the karyotype XX to remove its cortical layer preserving the medulla tissue. If our hypothesis is true then a male with a female genotype (XX) will be developed, which at usual crossing results only in females. Such experiments could give an answer to two interrelated question: 1) what does the SD depend on, either on the gene balance or on the "dose" of the cHR on Y chromosome?; 2) why does at genotype XX the medulla tissue preliminarily degenerate?, either from the "heat death" or from the impact of the gene products, produced by the cortex cells, on the medulla tissue?

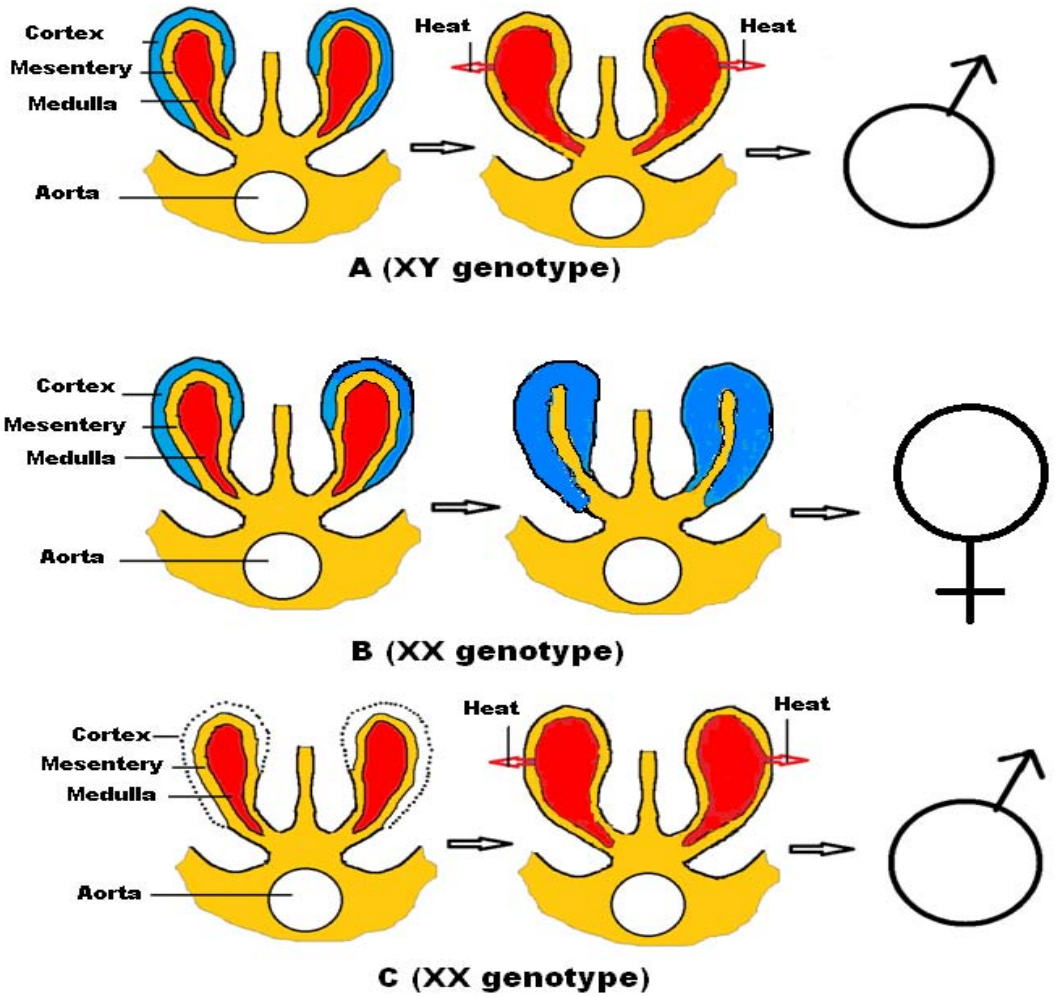

Figure 1

A. Genital ridges of the embryo with the genotype XY. Due to the presence of a Y chromosome with large block of constitutive heterochromatin (cHR) in medulla cells metabolic heat effectively deduces into the surrounding genital ridges space through the mechanisms of cell thermoregulation that allows medulla and subsequent development of the testis to survive.

B. Genital ridges of the embryo with the genotype XX. Due to lack of Y chromosome with large cHR in fetal cells excess metabolic heat is not deduced in the surrounding genital ridges space, which will lead to thermal degeneration of medulla and preservation of the tissue of cortex only with subsequent development of the ovary.

C. Genital ridges of the embryo with the genotype XX with remote cortex. After surgical removal of the cortex from the genital ridges medulla cells can survive despite the absence of Y chromosome in its nuclei. Excess metabolic heat of medulla cells from such embryos will be deduced in the surrounding genital ridges space due to lack of cortex layer, which, along with the mesentery, prevented the retraction of heat energy from the medulla tissue that, ultimately, allow it to survive and helps the development of the testis.

Theoretically, the problem in realizing our ideas may be associated mainly with *Sry* gene. In mammals, it has been known for 20 years that the Y chromosomal gene *Sry* is necessary for male sex differentiation, and many others genes have since been implicated in testis development. Thus, Y chromosome analysis revealed that a hypothetical factor responsible for the formation of the sex, in fact exists – it is gene *SRY* / *Sry* (sex region of Y-chromosome) and is localized on the short arm of Y chromosome. *SRY* – a gene that controls male sexual development; produces testis-determining factor (*TDF*), a product that triggers the formation of testes from undifferentiated embryonic gonad tissue. Its protein - a transcription factor, contains *HMG*-domain and belongs to the *SOX*-family proteins. *SRY* is not present on the X-chromosome gene. For the determination of the male

phenotype also protein to another gene *Sox*-family - *SOX9*, localized to an autosome is need. *SRY* and *SOX9* genes are expressed together in the gonad ridges of males. Expression screens such as microarray analyses have resulted in hundreds of candidate genes that show sex-specific expression patterns. However, it has been difficult to place these genes into a network of gene regulation and function. Even for *Sry*, it is still not known how its expression is regulated, what proteins might interact with it, and which genes it regulates (for more details see Wilhelm et al., 2007).

However, while *Sry* is found only in mammals, *Sox9* is present in all vertebrates. Thus, *Sox9* is more ancient gene and apparently plays a more general role in sex determination than *Sry*. In mammals, *Sox9* activates related gene *Sry*. Thus, *Sry* may act primarily as a "switch" that activates the gene *Sox9*, and protein *Sox9* initiates an evolutionarily conserved pathway for the formation of the testes. But in any case, the final answer to the question whether you can do without the *Sry* gene during artificial regulation of sex in mammals comes after the relevant experiments.

Returning to the classical experiments by Jost (1972), it should be noted that the author did not conduct experiments on the removal of the cortex to the genital ridges, so it seems to us highly likely to change the sexual differentiation of the fetus. From available sources of information, we could not find information as well as conducted experiments there any one presented on all to removal the cortex or medulla of the genital ridges.

III. The Hypothetic Mechanism of Sex Regulation in Mammals

We believe that the impact of cHR Y chromosome on sex regulation starts with the formation of genital ridges and continue until the end of ontogeny, through the protection of *SRY* and other genes localized on this chromosome, from the harmful effects of excess heat through mechanisms of cell thermoregulation. Role of cHR on the Y chromosome at the stage of differentiation of sex is, apparently, in the preservation of medulla cells from heat degeneration due to their location in the back of the depth of the body of genital ridges (within the mesentery and the cortex). As it is well known, medulla cells of mammals begin operating earlier than the cortex cells. If medulla maintains its viability (for Nature allocated for this type short time in the early stages of embryogenesis), then it will continue to exist, and cortical tissue subjected to thermal degeneration.

Survival of the medulla in the genital ridges, where the embryo has XY karyotype, is explained by the fact that, due to the presence of cHR on the Y chromosome cells efficiently derive excess heat into the surrounding space. The thing is that removals of excess metabolic heat outside the cell are fraught with difficulties, particularly from the nucleus. Latter possesses two ways to remove the excess heat: increase its size, it is not possible because of the numerous organelles in the cytoplasm or increase the thermal conductivity of the layer of condensed chromatin (CC) around the nucleus. Next, through a dense layer of CC thermal energy is deduced in the extracellular space through the cell skeleton (microfilaments, intermediate filaments, microtubules). We believe that, in general, the second option is implemented (see more Ibraimov, 2003, 2004).

Apparently, the heat diverting property of cHR on the Y chromosomes in the cells has its own characteristics. It seems very likely that the *SRY* and other genes, potentially affecting the production of testosterone, are not localized on the Y chromosome by chance. Indeed, when the medulla cells begin functioning, as a result of the accelerated metabolism heat might build up above physiologically acceptable level, which is fraught with negative consequences ("heat death" of the cells). If the layer around the nucleus of the CC is sufficiently dense, then the cell can avoid the heat death, through the timely removal of excess heat in the extracellular space. Such cells have the possibility of having cHR on the Y chromosome, and perhaps for this reason medulla tissue of embryo with XY genotype survive. It is known that transgenic XX mice carrying a genomic fragment containing the *Sry* gene develop as males, which are sterile in spermatogenesis (Koopman et al., 1991). Therefore, we argue that the presence of that *SRY* and other genes in the genome are not sufficient for the survival and development of the medulla. The availability of cHR on the Y chromosome is necessary, as it provides effective removal of excess metabolic heat from the medulla through mechanisms of cell thermoregulation.

Perhaps, following analogy is appropriate here. As it is well known, high pressure mercury lamps are used as a source of ultraviolet light for fluorescence microscopes. For normal operation of such lamps timely removal of excess heat outside the glass bulb is very important. Different heat diverting devices are invented for this aim. Recently, manufacturers of fluorescent microscopes began to supply its products with special cooling fins for these lamps to keep the temperature below 230° C. We believe that the survival of the medulla in the body of genital ridges depends on the timely removal of excess metabolic heat from the nucleus of cells. Perhaps, *SRY* and other genes located on Y chromosome are more sensitive to increased intracellular temperatures than genes located on autosomes. Apparently, Nature solved this engineering task by making cHR on Y chromosome as the heat removal, which in contact with the *SRY* and other genes outputs thermal energy outside the cell nucleus. It is no

accident that *SRY* produced protein binding to the minor groove of the DNA induces a sharp bend of 60–85°. Biochemical analysis of *SRY* protein expressed in human XY sex-reversed patients revealed that DNA binding and bending are integral parts of *SRY function* (Harley et al., 1992; Pontigga et al., 1994).

IV. Conclusion

According to Lyon (1992)"...the X-chromosome is clearly involved at some point in the sex-determination pathway". The point that I am trying to convey is that: a) the SD is one of the most important examples of how the physical state of the DNA molecular (in this case the level of compacting of the peripheral layer of CC around the cell nucleus) influences to the cell differentiation; b) X-inactivation is not involved in the SD; c) X-chromosome is not being inactivated, but it is heterochromatinized in order to compensate the lacking in the female karyotype the largest block of the cHR in the interest of the cell thermoregulation. Thus it would be more correct to speak about compensation of the heterochromatin dosage, and not about the dosage (double) of genes (Ibraimov 2003, 2004, 2008, 2010, 2011, 2012).

The reasons why the cortex tissue in the genital ridges is not exposed to the heat degeneration as the medullar tissue because of the problems connected with the CT in addition to the above mentioned, are also explained by the fact that: a) it has been shown that at equivalent gestational ages, males are developmentally more advanced than females, even before the gonads form. So, as for example a detailed analysis of XX v. XY developmental differences in mice has shown that XY fetuses are indeed larger than XX fetuses prior to gonad SD (Burgoyneetal.1995). The human testicles are formed earlier (6^{th}–12^{th}week) than the ovaries (14^{th}–16^{th}week); b) by the time of the ovaries formation, the problems with heat conductivity out from the deep parts of the organs and embryo tissues become considerably less complicated, as by this time the circulation system of fetus begins to function, i.e. a distant transfer of heat energy in a body (Ibraimov and Tabaldiev 2007), that eliminates the danger of the cell heat degeneration including the cortex tissues; c) with mammals and human the development towards the male sex is determined by availability of a testicle, to be more precise by a hormone secreted by it – testosterone, and the development towards the female sex is not induced, i.e. it is not the consequence of availability of the embryo ovary. It rather should be considered as the consequence of the testicle absence (Ohno et al.,1971); d) random X-inactivation of either the maternally - or paternally – inherited X chromosome most likely justifies to the participation of the facultative heterochromatin in the CT, and not to the dosage compensation of genes; and last c) homoiotherms keep the constant temperature in a body not so much they possess a 4-chamber heart and lung respiration (as a result the tissues obtain more oxygen) for acceleration of heat production, as their ability to efficiently remove the excessive heat from the organism. This is promoted by high conductivity of their bodies which are provided, in addition with the cHR, with the availability of G+ and Q+ bands in the chromosomes which intensify the compactization of the peripheral CC layer in the interphase nuclei, thus accelerating the liquidation of the appeared temperature difference between the nucleus and cytoplasm in the cell (Ibraimov 2003, 2004; Ibraimov and Tabaldiev 2007). So, the cause of the differential survivability of the medulla and cortex cells is the CT (preservation of the intracellular temperature homeostasis), which is determined by the cHRs on Y chromosome.

V. Acknowledgements

The research described in this paper was made possible, in part, by grant of Ministry of Education and Science of the Republic of Kazakhstan.

References Références Referencias

1. Bridges CB (1925) Sex in relation to chromosomes and genes. Amer Nat 59: 127-137.
2. Burgoyne PS, Thornhill AR, Kalmus Boudreau S, Darling SM, Bishop CE, Evans EP (1995) The genetic basis of XX-XY differences present before sex differentiation in the mouse. Philos Trans Roy Soc Lond Biol, 350: 253-261.
3. Goldschmidt R (1955) Theoretical Genetics. Berkeley and Los Angeles: University of California Press.
4. Harley VR, Jackson DI, Hextall PJ, Hawkins JR, Berkovitz GD, Sockanathan S, Lovell-Badge R, Goodfellow PN (1992) DNA binding activity of recombinant SRY from normal males and XY females. Science 255: 453–456.
5. Ibraimov AI (2003) Condensed chromatin and cell thermoregulation. Complexus 1: 164-170.
6. Ibraimov AI (2004) The origin of condensed chromatin, cell thermoregulation and multicellularity. Complexus 2: 23-34.
7. Ibraimov AI, Tabaldiev SK (2007) Condensed chromatin, cell thermoregulation and human body heat conductivity. J Hum Ecol 21(1): 1-22.
8. Ibraimov AI (2008) Possible mechanism of the sex differentiation and its artificial regulation. Int J Hum Genet 8(3): 283-290.
9. Ibraimov AI (2009) Noncoding DNAs and origin of sex. Int J Hum Genet 9(1): 39-47.
10. Ibraimov AI (2010) Noncoding DNAs in Development and Evolution. In: Bhasin MK, Susanne C (Eds.): Anthropology Today: Trends and

Scope of Human Biology. Delhi: Kamla-Raj Enterprises, pp. 199- 224.
11. Ibraimov AI, Kazakova AK, Moldotashev IK, Sultanmuratov MT, Abdyev KS (2010a) Variability of Human Body Heat Conductivity in Population. I. Methodological and Theoretical Approaches. J Hum Ecol 32(1): 1-22.
12. Ibraimov AI, Kazakova AK, Moldotashev IK, Sultanmuratov MT, Abdyev KS (2010b) Variability of Human Bod Heat Conductivity in Population. II. Diseases of Civilization. J Hum Ecol 32(2): 69-78.
13. Ibraimov AI (2011) Origin of modern humans: a cytogenetic model. Hum Evol 26(1-2): 33-47 Ibraimov AI (2011) Evolution without genes. Int J Genet 3(1): 50–61.
14. Ibraimov AI (2012) on the origin of sex. Global Journal of Science Frontier Research: C Biological Sciences 12(8) (Ver. 1.0), 1-6.
15. Jost A. A new look at the mechanisms controlling sex differentiation in mammals. Johns Hopkins Med J 130: 38–53, 1972.
16. Koopman P, Gubbay J, Vivian N, Goodfellow P, Lovell-Badge R (1991) Male development of chromosomally female mice transgenic for *Sry*. Nature 351: 117–121.
17. Lyon MF (1992) Some milestones in the history of X chromosome inactivation. Annu Rev Genet 26: 17-28.
18. Ohno S, Tettenbroun U, Dofuku R (1971) Molecular biology of sex differentiation. Hereditas 69: 107-124.
19. Pontiggia A, Rimini R, Harley VR, Goodfellow PN, Lovell-Badge R, Bianchi ME (1994) Sex-reversing mutations affect the architecture of SRY-DNA complexes. EMBO J 13: 6115–6124.
20. Prokofyeva-Belgovskaya AA (1986) Heterochromatic Regions of Chromosomes (in Russian). Moscow: Nauka.
21. Ridley M (2001) the Cooperative Gene. The Free Press, New York, pp 108-111.
22. Wilhelm D, Palmer S, Koopman P (2007) Sex determination and gonadal development in mammals. Physiol Rev 87: 1–28.

This page is intentionally left blank

Global Journal of Science Frontier Research
Bio-Tech & Genetics
Volume 13 Issue 2 Version 1.0 Year 2013
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

An Efficient *in vitro* Regeneration System for Tori (*Brassica campestris*)-7

By Sabrina Shameen Alam, Laila Khaleda & Mohammad Al-Forkan

University of Chittagong, Bangladesh

Abstract - An efficient *in vitro* regeneration system for Tori (*Brassica campestris*) -7 was developed from calli of hypocotyls and cotyledonary leaves. The optimum medium for callus induction was found with 0.5 mgl^{-1} 2, 4- dichloro-phenoxyacetic acid (2,4-D). The best shooting medium contained 3.0 mgl-1 6, benzyl amino purine (BAP), 0.1 mgl^{-1} naphtalene acetic acid (NAA), and 5.0 mgl^{-1} $AgNO_3$. Maximum number of shoots were produced when 0.5 mgl^{-1} kinetin was used, whereas the combined effect of 2.0 mgl^{-1} BAP, 0.1 mgl^{-1} NAA, and 5.0 mgl^{-1} $AgNO_3$ regenerated calli the most. The effect of $AgNO_3$ was found for callogenesis at 0.5 mgl^{-1} and for regeneration at 5.0 mgl^{-1}. *In vitro* regenerated shoots of Tori-7 developed roots on medium with 1.0 mgl^{-1} indole 3- butyric acid (IBA). The developed efficient *in vitro* regeneration protocol can be used as a baseline for Agrobacterium-mediated genetic transformation of the studied plants.

Keywords : *agrobacterium, in vitro regeneration, and tori-7.*

GJSFR-G Classification : *FOR Code: 270805*

Strictly as per the compliance and regulations of :

An Efficient *in vitro* Regeneration System for Tori (*Brassica campestris*)-7

Sabrina Shameen Alam [α], Laila Khaleda [σ] & Mohammad Al-Forkan [ρ]

Abstract - An efficient *in vitro* regeneration system for Tori (*Brassica campestris*) -7 was developed from calli of hypocotyls and cotyledonary leaves. The optimum medium for callus induction was found with 0.5 mgl^{-1} 2, 4- dichlorophenoxyacetic acid (2,4-D). The best shooting medium contained 3.0 mgl^{-1} 6, benzyl amino purine (BAP), 0.1 mgl^{-1} naphtalene acetic acid (NAA), and 5.0 mgl^{-1} $AgNO_3$. Maximum number of shoots were produced when 0.5 mgl^{-1} kinetin was used, whereas the combined effect of 2.0 mgl^{-1} BAP, 0.1 mgl^{-1} NAA, and 5.0 mgl^{-1} $AgNO_3$ regenerated calli the most. The effect of $AgNO_3$ was found for callogenesis at 0.5 mgl^{-1} and for regeneration at 5.0 mgl^{-1}. *In vitro* regenerated shoots of Tori-7 developed roots on medium with 1.0 mgl^{-1} indole 3-butyric acid (IBA). The developed efficient *in vitro* regeneration protocol can be used as a baseline for *Agrobacterium*-mediated genetic transformation of the studied plants.

Keywords : *agrobacterium, in vitro regeneration, and tori-7.*

I. Introduction

Mustard (including rape) is one the most important crops and accounts 3.08% of the total production of major crops in Bangladesh. The production of oil seed in 1994-95 was 307,602 metric tons in the country, from which 179,524 metric tons of oil was produced that can supply only 27% of the daily per head oil need. Hence, we need to increase annual *Brassica* production. Improvement of *Brassica* production rate can be achieved through *in vitro* tissue culture of mustard. Tissue culture or *in vitro* micropropagation technique has been applied for *Brassicas* for a long time observed by Ali *et al* (2007) and John *et al* (1991). The combination of 2.4-D, BAP, NAA and $AgNO_3$ was found to be the best medium for callus initiation and growth for *Brassica napus* by Ali *et al,* (2007). The use of $AgNO_3$ was reported by Khan et al (2003) in *Brassica napus* and Ali *et al* (2007) for plant regeneration. However, the best condition for Bangladeshi *Brassicca* local variety (Tori-7) regeneration system is not known. The present study was designed to screen out the varietal response of Tori-7 regarding its response to specific cultural conditions in comparison with and finally, to establish an efficient genotype-independent *in vitro* cultural system for the initiation and development of embryogenic calli with an ultimate goal of plant regeneration that can be used in future as a baseline for *Agrobacterium*–mediated genetic transformation for quality improvement.

Authors α σ ρ : Department of Genetic Engineering & Biotechnology, University of Chittagong, Chittagong-4331, Bangladesh.
E-mail : alforkancu@hotmail.com

II. Materials and Methods

Tori-7 (*Brassica campestris)* seeds were collected from Bangladesh Agricultural Research Institute (BARI), Hathazari Substation, Chittagong. The explants collected from *in vitro* grown seedlings used for the experiments were: hypocotyls and cotyledons with petiole. Hypocotyl segments of 0.5-1.0 cm and cotyledonary leaves with petioles were used for the experiments. MS (Murashige and Skoog, 1962) medium was prepared with 3% (w/v) sucrose and solidified with 0.4% (w/v) agar. Only half strength MS medium was used for seed germination whereas, MS media supplemented with different PGRs (Plant Growth Hormones) such as 2, 4-D, BAP and additives such as proline and casein hydrolysate (CH) were used for callus induction. Shoots were developed by using media with BAP and the effect of hormones and additives were checked. Initiation of roots were tried on media supplemented with NAA and IBA. The plantlets with sufficient rooting system were taken out of the culture vessels and the roots were washed under tap water. The *in vitro* grown rooted plants were then transferred into small pots. Hardening was carried out by periodical exposure of the plants to natural environment.

III. Results and Discussions

Cotyledons and hypocotyls, both formed large calli on MS medium supplemented with 0.5 mg l^{-1} 2, 4-D. The increasing concentration of 2, 4-D greatly reduced the percentage of callus formation as well as size of the callus (Fig.1), though this effective concentration of 2,4-D does not agree with many previous reports such as Cardoza and Stewart (2003), who found the best callus induction using 1.0 mg l^{-1} 2, 4-D. Quain and Zhang (2004) and Khan *et al.* (2002) reported best callus induction medium using 2,4-D at 1.5 mg l^{-1} and 2.0 mg l^{-1}, respectively. These differences with the optimal concentration of 2, 4-D of current investigation can be assumed as a result of varietal differences and variations in the exo- and endogenous environments. The use of BAP on callogenesis was found to increase the size and frequency of callus formation. Withdrawal of BAP from the medium reduced

the percentage of hypocotyl callus formation even when 2, 4-D concentration was increased to 1.0 mg l^{-1}. The explants became brown that did not produce any significant callus (Fig. 2). On the other hand, withdrawal of $AgNO_3$ from the medium caused the explants become brown and dead (data not shown). The use of $AgNO_3$ was described to improve the growth of the callus by Noman *et al.* (2008) that supports the result. About 12% of the hypocotyl calli and 1-2% of the cotyledonary explants produced roots within 20 days of culture on medium containing 0.5 mg l^{-1} IBA and 0.5 mg l^{-1} IAA (Fig. 3). Hypocotyls produced callus of embryogenic nature on proline containing medium though cotyledons responded less in proline and CH contacting media (Fig. 3). The increasing BAP reduced shooting (Fig.4). When NAA at 0.1 mg l^{-1} was used in combination with BAP, the percentage of shoot formation raised to 80% from 47.8% for hypocotyl explants. George and Rao (1980) observed maximum regeneration from cotyledon explants in *Brassica juncea* on medium supplemented with BAP and NAA rather than BAP only, which supports the present findings. About 20% cotyledonary calli started shooting within 10-12 days. The medium without $AgNO_3$ but with NAA gave shoots at a percentage of 65-80% (data not shown). Presence of CH in media reduced shooting frequency.

Spontaneous root generation occurs sometimes on MS medium with hormonal supplements for the induction of shoots (Fig.5). Tori-7 responded only on media containing IBA (Fig.5). This finding again suggests the genotypic and environmental difference may cause the variety to respond.

On the basis of observation and results taken, Tori-7 was better in terms of producing more shoots but the subsequent growth and establishment was difficult that is supported by Dunwell (1981), Dietert *et al.* (1982) and Glimelius (1984) who concluded the difficulties of regeneration of *Brassica campestris* than other *Brassicas*.

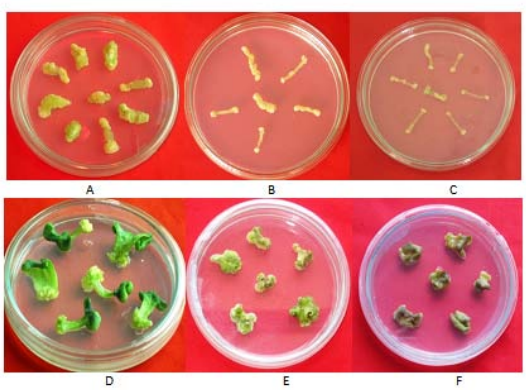

Fig. 1 : Decreasing level of callus formation in hypocotyls (A-C) and cotyledons (D-F) with increasing concentrations of 2,4-D from 0.5 mg l^{-1} to 2.0 mg l^{-1}

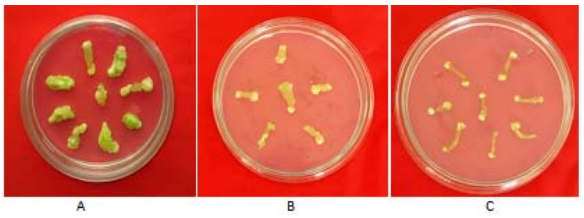

Fig. 2 : Combined action of BAP and $AgNO_3$ increases callus formation, (A) media with both BAP and $AgNO_3$, (B) media with BAP but no $AgNO_3$ and (C) media with $AgNO_3$ but no BAP

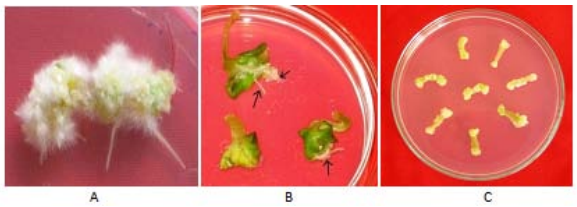

Fig. 3 : Effect of hormones; initiation of root from hypocotyl (A) and cotyledon (B) explants on callus induction medium containing both IBA and IAA, and proline: callus appearing at the edges of hypocotyls (C)

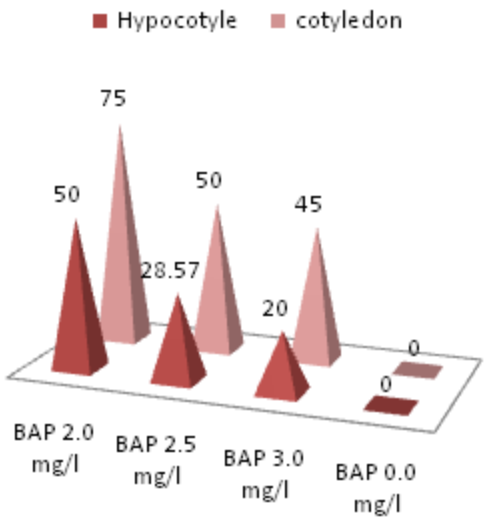

Fig. 4 : Change in the percentage of shoot formation from Tori-7 calli on different media; changing BAP concentration changes shooting frequency

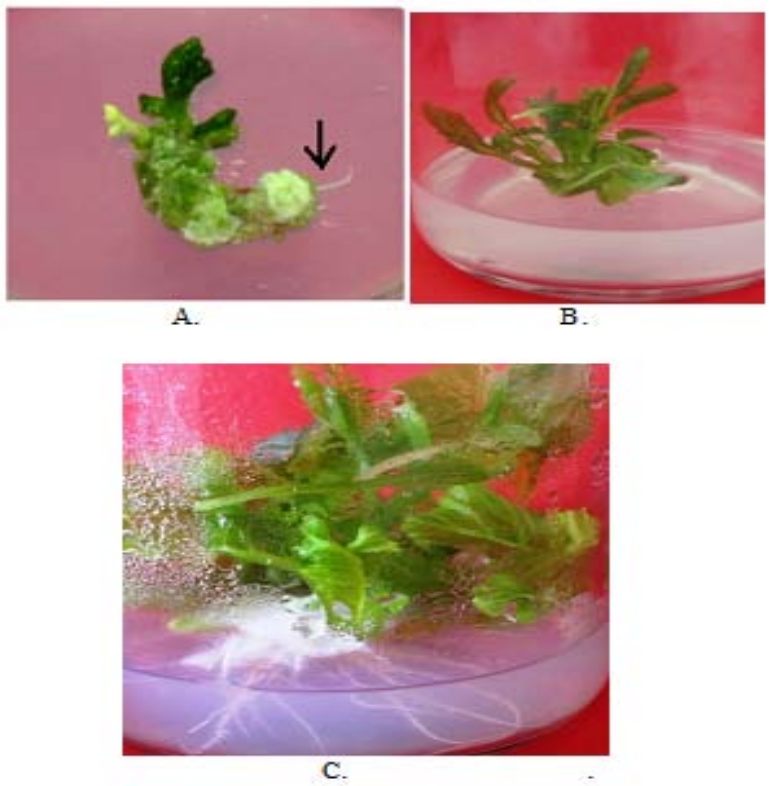

Fig. 5 : Different stages of regeneration; spontaneous root formation on shooting medium unattached to shoots (A), shoot elongation from Tori-7 hypocotyls on medium containing kinetin (B) and formation of rooting system of Tori-7 on IBA containing medium (C)

IV. Conclusion

The present study has described an efficient in vitro regeneration system for a local *Brassicas* variety namely Tori-7. The optimum medium for callus induction was found with 0 .5 mgl^{-1} 2, 4-D, where as the best shooting medium was 3.0 mgl^{-1} BAP, 0.1 mgl^{-1} NAA, and 5.0 mgl^{-1} $AgNO_3$. Maximum number of shoots were produced when 0.5 mgl^{-1} kinetin was used, whereas the combined effect of 2.0 mgl^{-1} BAP, 0.1 mgl^{-1} NAA, and 5.0 mgl^{-1} $AgNO_3$ regenerated calli the most. *In vitro* regenerated shoots of Tori-7 developed roots on medium with 1.0 mgl^{-1} indole 3-butyric acid (IBA). The developed efficient *in vitro* regeneration protocol will be supportive for increasing the local productivity.

References Références Referencias

1. Ali, H., Ali, Z., Mehmood, S. and Ali, W., 2007, *In vitro* regeneration of *Brassica napus L.* cultivars (Star, Cyclone and Westar) from hypocotyls and cotyledonary leaves. *Pak. J. Bot.*, 39: 1251-1256.
2. Cardoza, V. and Stewart, C.N., 2003. Increased *Agrobacterium*-mediated transformation & rooting efficiencies in canola (*Brassica napus* L.) from hypocotyl segment explants. *Plant Cell Rep*., 21: 599-604.
3. Dietert, M.F., Barron, S.A. and Yoder, O.C., 1982. Effects of genotype on *in vitro* culture in the genus *Brassica. Plant Sci. Let*., 26: 233-240.
4. Dunwell, J.M., 1981. *In vitro* Regeneration from excised leaf discs of *Brassica* species. *J. Exp. Bot*., 32: 789-799.
5. George, L. and Rao, P.S., 1980. *In vitro* regeneration of mustard plants (*Brassica juncea* var. RAI-5) on cotyledon explants from non-irradiated irradiated and mutagen treated seed. *Ann. Bot.*, 46: 107-112.
6. Glimelius, K., 1984. High growth rate and regeneration capacity of hypocotyls protoplasts in some *Brassicaceae*. *Physiol. Plant.*, 61: 38-44.
7. John, E. H., Kiran, K.S. and Maurice, M.M., 1991. Efficient shoot regeneration of *Brassica campestris* using cotyledonary explants cultured *in vitro*. *Plant Cell Rep*., 9: 549-554.
8. Khan, M. R., Rashid, H. and Azra, 2002. Effects of various growth regulators on callus formation and regeneration in *Brassica napus* cv. *Oscar. Pak. J. Bio. Sci*., 5: 693-695.
9. Khan, M. R., Rashid, H., Ansar, M. and Chaudry, Z., 2003. High frequency shoot regeneration and *Agrobacterium*-mediated DNA transfer in canola (*Brassica napus*). *Plant Cell Org. and Tiss. Cult.* 75: 223-231.

10. Murashige, T. and Skoog, F., 1962. A revised rapid growth and bioassay with tobacco tissue culture. *Plant Physiol*., 15: 473-497.
11. Noman, A.S.M., Islam, M.S., Siddique, N.A. and Hossain, K., 2008. High Frequency Induction of Multiple Shoots from Nodal Explants of Vitex negundo using Silver Nitrate. *Int. J. of Agr. & Biol.,* 10, 633-637.
12. Quain, H. and H. Zhang, 2004. Factors affecting the callus induction and GUS transient expression in indica rice. *Pak. J. Bio. Sci*., 7: 615-619.

Global Journal of Science Frontier Research
Bio-Tech & Genetics
Volume 13 Issue 2 Version 1.0 Year 2013
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Serum Homocysteine and Paraoxonase1 Levels in Women with Polycystic Ovary Syndrome Treated with Metformin Versus Metformin and Folic Acid

By Dr. Tarek H. Abed Tawfik Al-Khyat, Dr. Melal Mohammed Al-Jeborry & Qusay Mohsin Kadium

University of Babylon, Iraq

Abstract - Background: Polycystic ovary syndrome (PCOS) is the most common reproductive endocrinopathy and currently considered to be a part of the metabolic syndrome. Women with PCOS cluster risk factors associated with risk of atherosclerosis. These risk factors include insulin resistance, dyslipidemia, hyperandrogenemia, obesity, endothelial dysfunction and oxidative stress.

Aim of the study: Study of the relationship between homocysteine and paraoxonase 1 in PCOS in two different regimes of treatment with Metformin and Folic acid in order to elucidate some risk factors that may lead to atherosclerosis and vascular diseases in the corresponding patients.

Patients and Methods: Fifty patients with PCOS were enrolled in this study that subdivided into two groups according to type of treatment received i.e. G1 (comprising 25 patients) treated with Metformin only, G2 (comprisin25 patients) with Metformin and Folic acid. Serum Homocysteine (Hcy), Paraoxonase 1(PON1) Apolipoprrptein A-1(Apo A-1) and Apolipoprotein B(Apo B) were determind by using enzyme-linked immunosorbent assay (ELISA). Reduced Glutathion (GSH) and Malondialdehyde (MDA) were determined by using colorimetric method.

GJSFR-G Classification : FOR Code: 320307p, 100499

Strictly as per the compliance and regulations of :

Serum Homocysteine and Paraoxonase1 Levels in Women with Polycystic Ovary Syndrome Treated with Metformin Versus Metformin and Folic Acid

Dr. Tarek H. Abed Tawfik Al-Khyat [α], Dr. Melal Mohammed Al-Jeborry [σ] & Qusay Mohsin Kadium [ρ]

Abstract - Background: Polycystic ovary syndrome (PCOS) is the most common reproductive endocrinopathy and currently considered to be a part of the metabolic syndrome. Women with PCOS cluster risk factors associated with risk of atherosclerosis. These risk factors include insulin resistance, dyslipidemia, hyperandrogenemia, obesity, endothelial dysfunction and oxidative stress.

Aim of the study: Study of the relationship between homocysteine and paraoxonase 1 in PCOS in two different regimes of treatment with Metformin and Folic acid in order to elucidate some risk factors that may lead to atherosclerosis and vascular diseases in the corresponding patients.

Patients and Methods: Fifty patients with PCOS were enrolled in this study that subdivided into two groups according to type of treatment received i.e. G1 (comprising 25 patients) treated with Metformin only, G2 (comprisin25 patients) with Metformin and Folic acid. Serum Homocysteine (Hcy), Paraoxonase 1(PON1) Apolipoprptein A-1(Apo A-1) and Apolipoprotein B(Apo B) were determind by using enzyme-linked immunosorbent assay (ELISA). Reduced Glutathion (GSH) and Malondialdehyde (MDA) were determined by using colorimetric method.

Results: The results indicated a significant increase in the serum levels of Hcy($p<0.01$), PON1 and GSH($p<0.05$) anda significantdecreasein thelevels of MDA and Apo B/Apo A-1 ratio($p<0.01$)in PCOS group after Metformin only(G1-A).But, after Metformin with Folic acid treatment (G2-A)Hcy, Apo B/Apo A-1 ratio and MDA levels were significantly lower ($p<0.01$) and PON1 as well as GSH levels were significantly higher ($p<0.01$) in comparison with those before treatment. Whereas, a significant decreasein level ofHcy, MDA ($p<0.01$) and Apo B/Apo A-1 ratio($p<0.05$) anda significantincrease in PON1($p<0.01$) and GSH levels ($p<0.05$) in (G2-A)as compared with (G1-A). The results revealed a significant positive correlation between Hcywith MDA and Apo B/Apo A-1 ratio as well as between PON1 and GSH. And a significant negative correlation between HcywithGSH, PON1and also, between PON1 with MDA and Apo B/Apo A-1 ratio in patients groups.

Conclusion: Folic acid administration to PCOS patients tends to lower homocysteine levels in the serumof the corresponding patients and alleviate the oxidative stress in those patients. Besides, increase of PON1 prevents atherogenic effects by changing Apo B/Apo A-1 ratio.

Authors α ρ : Dept. of Clinical Biochemistry, College of Medicine, University of Babylon, Hilla, Iraq.

Author σ : Dept. of Obstetrics and Gynecology, College of Medicine, University of Babylon, Hilla, Iraq. E-mail : qusaym64@yahoo.com

I. Introduction

Polycystic ovarian syndrome (PCOS) is one of the most common female endocrine disorders and is a complex, heterogeneous disorder of uncertain aetiology, but it can be classified as a genetic disease [1]. Itis one of the most common causes of anovulatory infertility with a prevalence between 6% and 10% based on the U.S. National Institute of Health (NIH) criteria and as high as 15% when the broader Rotterdam criteria are applied [2]. Typically, PCOS is first identified during the early reproductive years. The clinical expression varies but commonly includes oligo-ovulation or anovulation, hyperandrogenism (either clinical or biochemical) and the presence of polycystic ovaries [3]. Theaetiology ofPCOS is not fully known, but many environmental and genetic factors may cause this disease, such as diet, pollution and sedentary lifestyle. Stress may contribute to its development [4].

Homocysteine (Hcy) is an intermediate product formed during the breakdown of the amino acid methionine, and may undergo remethylation to methionine (folate and cobalamin is involved in Hcyremethylation) or trans-sulphuration to cystathionine then to cysteine (vitamin B6 is involved in Hcy trans-sulphuration) [5]. Excess Hcy in the blood stream may cause injures to arterial vessels due to its irritant nature, and result in inflammation and plaque formation by impaired endothelial function, increased oxidative stress, alterations of lipid metabolism, increasing platelet adhesiveness, activation of the coagulation system and stimulating vascular smooth muscle cell proliferation [6].

PON1 is a member of family of proteins that also includes PON2 and PON3. that share considerable structural homology and are located adjacently on chromosome 7 in humans. All the three proteins prevent oxidative stress and fight inflammation [7]. PON1 has different anti-atherogenic features such as protection from free radical induced oxidation of cholesterol in arterial wall and protection against harmful effects of oxidized LDL. PON1 is synthesized primarily in the liver and a portion is secreted into the plasma, where it is

associated with apoA-I incorporation in the high-density lipoprotein (HDL) particles [8].

The ApoB/ApoA-I ratio represents the balance of pro-atherogenic and anti-atherogenic lipoproteins, which is the better parameter than routine lipid measurements in predicting risk of cardiovascular diseases(CVD), might also be potential biomarkers in predicting risk of CVD in patients with PCOS[9]. Various studies had demonstrated that this ratio was associated with the incidence metabolic syndrome (MS), insulin resistance (IR) and increased free androgen index and visceral adiposity [10].

Oxidative stress, defined as the rate of Reactive Oxygen Species (ROS) (and/or Reactive Nitrogen Species (RNS) production) and the rate of their removal by cellular defense mechanisms [11].In humans, oxidative stress is thought to be involved in the development of an environment unsuitable for normal female physiological reactions [12]. This, in turn, can lead to a number of reproductive diseases including endometriosis, PCOS, and unexplained infertility. It can also cause complications during pregnancy, such spontaneous abortion, recurrent pregnancy loss, preeclampsia, and intrauterine growth restriction [13].

Metformin is an insulin-sensitizing drug from the biguanide class. It is orally administered drug used to lower blood glucose concentrations in patients with non-insulin dependent diabetes mellitus (NIDDM), and is now also used in the treatment of polycystic ovary syndrome [14]. Metformin had many benefits such as, decrease in body mass index (BMI), androgens in both lean and obese women activity for around 20% andimprovement of menstrual cycles (ovulation rates). Normal menstrual cycles achieved within 3 months of starting treatment in some groups of patients, reduce early pregnancy losses and reduce risk of miscarriage,a 10-fold reduction in gestational diabetes (31% to 3%) and prevention or delay of onset of diabetes, lowering the blood insulin levels, increasing insulin sensitivity and lowering glucose level[15]. However, serum vitamin B12 and Folic acid levels are known to decrease during Metformin therapy probably due to malabsorption. Hence, Hcy levels might increase [16]. Folic acid may help reduce certain complications of PCOS as Folic acid supplements are "likely effective" for the treatment of high Hcy levels. Folate is involved in Hcy remethylation to methionine.

Women with PCOS may be able to improve their chances of getting pregnant by taking Folic acid every day [17] and besides possibly reducing infertility associated with PCOS, Folic acid may also improve pregnancy outcomes with PCOS. As with all pregnant women, pregnant women with PCOS need to get sufficient Folic acid to prevent neural tube defects. Both low Folic acid levels and PCOS are linked to miscarriage. Pregnant women with PCOS may thus help reduce their risk of miscarriage by taking Folic acid [18].

In the present study, the relationship between homocysteine, paraoxonase 1 and oxidative stress levels was elucidated in different regimes of treatment with Metformin and Folic acid in Iraqi patients inflicted with polycystic ovarian syndrome and the effect of those treatments regime on ApoB/Apo A-1 ratios in the corresponding patients.

II. Patients and Methods

This study was conducted in Babylon Maternity and Pediatric Teaching Hospital and in the laboratory of Biochemistry Department, College of Medicine, University of Babylon in the period starting from November 2012 to June 2013. Fifty patients with PCOS were enrolled in this stady. The patients were divided into two groups according to type of treatments received. The first group (G1) included 25 patients with PCOS received Metformin only, their age ranged between (18 - 30) years. The second group (G2) included 30 patients who received Metformin with Folic acid, their age ranged between (19 - 30) years. Full history was taken for all the patients which includes: age, address, length, weight, past history of diseases, obstetrical history, smoking, family history of disease, medical history. No drugs were prescribed to those patients that may interfere with the measured parameters (fertility drugs, oral anti-diabetic agents and oral contraceptive pills).

Seven to ten milliliters of blood were obtained from those patients. Blood samples were collected in tubes without anticoagulants and were left for 15 minutes at room temperature to clot. After that, the blood samples were centrifuged at 1500 xg for approximately 10 minutes. The serum was isolated and divided into five aliquots using eppendrof tubes and stored at (-20°C) until time of use.

Serum Hcy and PON1, were determined using ELISA kit provided by CUSABIO, China. Whereas, Apolipoprotein A-1 and B were determined using ELISA kit provided byEAGLE Biosciences, USA. The determination of Serum Hcy, PON1, Apo A-1 and Apo B is based on sandwich principle., using ELISA technique .Serum GSH concentration was determined by using a modified procedure utilizing Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid) or DTNBwhen Sulfhydryl group of (GSH) reduces disulfide chromogen DTNB and change it to an intensely yellow compound which is proportional to total GSH and measured spectro-photometrically at 412 nm. The method of determination of serum MDA was based on the reaction with thiobarbituric acid (TBA) at 90-100°C and pH 2-3 for 15 minutes to form pink color product; forming an MDA-TBA_2 adduct that absorbs strongly at 532 nm.

All statistical analysis was performed by using SPSS version 18 for windows. Data were expressed as Mean ± SD. The normality of the distribution of all

variables was assessed by the ANOVA, LSD and Pearson correlation analysisthat have been used to determine the significant difference between PCOS patients groups. P values less than (0.05) is considered significant while valuesless than (0.01) is considered highlysignificant.

III. Results and Discussion

The results in table (1) show non-significant differences in the age among different groups. This is due to the selection of subjects who are nearly within the same age. In fact, this is an important aspect for the comparison of other parameters especially those parameters which vary with age.

The body mass index (BMI) is a measure for human body shape based on an individual's mass and height. This anthropometric parameter is an important measure of obesity. Elevated BMI was associated with insulin resistance [19]. In our study, neither Metformin therapy nor Metformin and Folic acid therapy changed the BMI significantly. Our results were in agreement with at least two studies that failed to demonstrate the non-significant BMI reduction during the treatment with Metformin only [15,20] and also, during the treatment with Metformin plus Folic acid [15,21]. The small number of patients in the studies and differences in the treatment periods may explain these discrepancies.

In this study, A significant increase in Hcy ($p<0.01$), a significantly low in Apo B/Apo A-1 ratio and MDA levels ($p<0.01$) were observed in PCOS patients treated with Metformin only while there were significant increase in PON1 and GSH ($p<0.05$) in the corresponding patients. The results revealed a high significant decrease in Hcy, Apo B/Apo A-1 ratio and MDA levels ($p<0.01$)in patients on Metformin plus Folic acid but, there weresignificant increase in PON1 and GSH($p<0.01$)in the corresponding patients. Table (1).

Some investigators found that Metformin tends to decrease Folic acid which in turn leads to increase of Hcy in the PCOS patients [22, 23]. in spite of the benefits of such treatment in PCOS patients to improve fertility, normalization of menstrual cycles and reduction of thecal androgen production, there were some harms in using this drug alone[15]. For this reason Folic acid was administrated for PCOS patients with Metformin to improve antioxidant status and to lower Hcy levels. Previous studies that are done by Palomba [21] and Kilicdag [20] revealed a significant decrease in Hcy levels after adding Folic acid to Metformin in PCOS patients.

Elevated Hcy levels can damage endothelial cells, impairing the release of nitric oxide (NO) and leading to a net increase in the production of superoxide ($O_2^{\bullet}$-) which lead the formation of atherosclerosis plagues and myocardial infarction (MI). It also leads to structural changes in lipoproteins especially LDL molecules [6]. This may give the idea about the correlation between Hcy, oxidative stress and Apo B/ApoA-I ratio as shown in table (2).

The significant increase in PON1 associated with Folic acid administration to Metformin indicate the essential role of this vitamin in the improving lipid status since the latter is involved in the incorporating of Apo A-1 in HDL-particles[8]. This was confirmed by the negative correlation between PON1 and Apo B/ApoA-I ratio in PCOS patients after treatment with Metformin and Folic acid in the present study.

PON1 may acts synergistically with Folic acid to lower Hcy levels since PON1 can hydrolyze Hcy-thiolactone back to Hcy and Hcy may be then converted either back to methionine (this reaction which needs folate and vitamin B12 as co-factors), or condensed with serine to form cystathionine in a reaction that is dependent on vitamin B6 as reported by Yilmaz [24]. This conclusion can be attributed to negative correlation between PON1 and Hcy in PCOS patients treated with Metformin and Folic acid as reported in table (2).

The results revealed a significant decrease inApoB/ApoA-I ratio after treatment with of Metformin plus Folic acid in PCOS patients because Metformin therapy improves glycemic control and may decrease oxidative stress related oxidation of LDL [25] as well as the protective effects of Folic acid supplementation on endothelial dysfunction by direct effect on free-radical oxidation of LDL lipids and prevent the structural and functional modification of Apo A-1in HDL due to nitration [9,26]. In fact, the decrease in Apo B/ApoA-I ratio levels in this study is more important because it is a potential biomarkers in predicting risk of CVD in patients with PCOS [9].

In the present study, the addition Folic acid to Metformin had further effect on oxidative stress parameters (decrease MDA and increase GSH) due to the fact that Metformin was able to regulate ovarian oxidative stress by decrease androgen level via reducing pituitary gonadotropin secretion [23, 27]. And Folic acidhad direct antioxidant role and free radical scavenging activity[28].

The unique idea in our work is the relationship among Hcy, PON1, oxidative stress and Apo B/Apo A-1 ratio since previous studies lack the correlations among those parameters and the results in table 2 confirmed such relations among different parameters.

Finally administration of a combined treatment of Metformin and Folic acid is quite essential for the lowering of serum levels of Hcy, oxidative stress and improving apolipoprotein status in PCOS patients to exclude further complications associated with this disease.

Table 1 : The anthropometric and clinical characteristicsof PCOS groups before and after treatment

Variable	Group	N	Mean ± SD	Range	*P* Value
Age (year)	G1-B	25	22.84 ± 3.85	18 – 30	
	G2-B	25	23.92 ± 3.54	19 – 30	P_1 >0.05
BMI (kg/m^2)	G1-B	25	29.25 ± 5.99	21.77– 44.96	P_1> 0.05
	G1-A		29.01 ± 6.01	21.91 –45.54	P_2> 0.05
	G2-B	25	30.56 ± 5.83	22.14– 47.38	P_3> 0.05
	G2-A		28.73 ± 5.67	19.56– 46.09	P_4> 0.05
Hcy (nmol/ml)	G1-B	25	13.96 ± 0.68	12.69 – 15.50	P_1>0.05
	G1-A		16.12 ± 1.03	14.69 -19.93	P_2<0.01
	G2-B	25	14.30±0.79	12.78 – 16.03	P_3<0.01
	G2-A		12.75±0.77	11.50 – 14.21	P_4<0.01
PON1 (mlU/ml)	G1-B	25	163.44±16.54	125.42 – 191.44	P_1>0.05
	G1-A		170.51±7.71	155.49- 183.13	P_2<0.05
	G2-B	25	165.12± 10.62	143.36- 182.06	P_3<0.01
	G2-A		188.15±12.2	167.00 – 211.92	P_4<0.01
GSH (μM)	G1-B	25	22.23±5.18	11.99 – 31.26	P_1>0.05
	G1-A		25.22±5.25	16.12 – 34.01	P_2<0.05
	G2-B	25	20.95±4.91	12.26 – 30.15	P_3<0.01
	G2-A		28.05±4.60	18.97 – 36.86	P_4<0.05
MDA (μM)	G1-B	25	5.89±0.91	4.23 – 8.09	P_1>0.05
	G1-A		5.03±0.85	3.87 – 6.79	P_2<0.01
	G2-B	25	6.10±0.57	5.15 – 7.73	P_3<0.01
	G2-A		4.39±0.69	3.65 – 6.87	P_4<0.01
Apo B/Apo A-1 ratio	G1-B	25	11.16±2.72	5.59– 16.81	P_1>0.05
	G1-A		9.25±1.52	5.47 – 12.20	P_2<0.01
	G2-B	25	10.31±1.96	6.39 – 14.06	P_3<0.01
	G2-A		7.94±1.61	5.19 – 10.67	P_4<0.05

G1-B= patients group before treated with Metformin only, G1-A= patients group after treated with Metformin only, G2-B= patients group before treated with Metformin and Folic acid, G2-A= patients group after treated with Metformin and Folic acid, SD= standard deviation, N= number of patient, BMI= body mass index, HCY= Homocysteine, PON1= Paraoxonase1, CAT= Catalase, GSH= Glutathione, MDA= Malondialdehyde, Apo B/Apo A-1 ratio=Apolipoprotein B/ Apolipoprotein A-1 ratio, P_1= between G1-B and G2-B, P_2= between G1-B and G1-A, P_3= between G2-B and G2-A, P_4= between G1-A and G2-A.

Table 2 : The Pearson correlations between different parameters in PCOS groups before and after treatment

		G1-B		G1-A		G2-B		G2-A	
		Hcy nmol/l	PON1 mlU/ml	Hcy nmol/l	PON1 mlU/ml	Hcy nmol/l	PON1 mlU/ml	Hcy nmol/l	PON1 mlU/ml
Age (year)	r	- 0.106	- 0.040			0.275	- 0.249		
	P-value	0.615	0.850			0.182	0.230		
BMI	r	0.156	- 0.388	0.141	- 0.315	0.0.157	- 0.140	0.371	- 0.184
(kg/m^2)	P-value	0.457	0.055	0.501	0.125	0.454	0.504	0.068	0.378

MDA (μM)	r	0.517	- 0.751	0.424	- 0.703	0.788	- 0.697	0.506	- 0.611
	P-value	0.008	0.000	0.034	0.000	0.000	0.000	0.010	0.001
GSH (μM)	r	- 0.682	0.751	- 0.529	0.688	- 0.701	0.765	- 0.850	0.890
	P-value	0.000	0.000	0.044	0.000	0.000	0.000	0.000	0.000
Apo B/ Apo A-1 ratio	r	0.553	- 0.554	0.413	- 0.554	0.634	- 0.752	0.752	- 0.680
	P-value	0.004	0.004	0.040	0.004	0.001	0.000	0.000	0.000
Hcy nmol/l	r		- 0.463		- 0.418		- 0.537		- 0.843
	P-value		0.020		0.038		0.006		0.000

G1-B= patients group before treated with Metformin only, G1-A= patients group after treated with Metformin only, G2-B= patients group before treated with Metformin and Folic acid, G2-A= patients group after treated with Metformin and Folic acid, BMI= body mass index, HCY= Homocysteine, PON1= Paraoxonase1, GSH= Glutathione, MDA= Malondialdehyde, Apo B/Apo A-1 ratio=Apolipoprotein B/ Apolipoprotein A-1 ratio and r= pearson correlations coefficient

References Références Referencias

1. Nardo, L.G., Patchava, S. and Laing, I. (2008). Polycystic ovary syndrome: Pathophysiology, molecular aspects and clinical implications. Panminerva Med. 50(4): 267-278.
2. Oates, J. and S. Abraham (2010). LIEWELLYN-JONES Fundamentals of obstetrics and gynaecology 9th edition. Elsevier limited. China. 223-224.
3. Presannakumari, B. (2011). Postgraduate gynecology 1st edition. Ajanta offset and Packagings Ltd. New Delhi. 140-154.
4. Keith, E. (2011). Dewhurst's Textbook of Obstetrics and Gynaecology. John Wiley & Sons. UK. 517.
5. De la, C. M., Usandizga, R., Sancha, M., Magdaleon, F., Herranz, A. and Carillo, E. (2003). Homocysteine, Folic acid and B-group vitamins in obstetrics and gynecology. Eur. J. Obstet. Gynecol. Reprod. Biol.107:125–134.
6. Holven, K.B., Aukrust, Pa., RetterstOl, K., Otterdal, K., Bjerkeli, V. Ose, L., Nenseter, M. S. and Halvorsen, B.(2008). The Antiatherogenic Function of HDL Is Impaired in Hyperhomocysteinemic Subjects. J. Nutr. 138: 2070–2075.
7. Draganov, D.I., Teiber, J. F. Speelman, A., Osawa, Y., Sunahara, R. and La Du, B.N. (2005). Human paraoxonases (PON1, PON2 and PON3) are lactonases with overlapping and distinct substrate specificities. Journal of Lipid Research. 46: 1239-1247.
8. Loscalzo, J. (2008). Paraoxonase and Coronary Heart Disease Risk Language Misleads, Linkage Misinforms, Function Clarifies. Circ. Cardiovasc. Genet.1:79-80.
9. Zhang, J. Fan, P., Liu, H., Bai1, H., Wang, Y. and Zhang, F. (2012). Apolipoprotein A-I and B levels, dyslipidemia and metabolic syndrome in south-west Chinese women with PCOS. Hum Reprod. 27(8):2484-2493.
10. Lee, Y.H., Choi, S.H., Lee, K.W. and Kim, D.J. (2011). Apolipoprotein B/A1 ratio is associated with free androgen index and visceral adiposity and may be an indicator of metabolic syndrome in male children and adolescents. Clin. Endocrinol (Oxf). 74:579–586.
11. Hiroyuki, T., Shintaro K., and Matsushima, S. (2011). Oxidative stress and heart failure. Sapporo. 8630-8638.
12. Webster, R.P., Roberts, V.H. and Myatt, L. (2008): Protein nitration in placenta – functional signify-cance. Placenta. 29:985–994.
13. Al-Gubory, K.H., Fowler, P.A., Garrel, C. (2010). The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. Int. J. Biochem. Cell Biol. 42:1634–1650.
14. Eurich, D.T., McAlister, F.A. and Blackburn, D.F. (2007). Benefits andharms of antidiabetic agents in patients with diabetes and heart failure: systematic review". BMJ., 335 (7618): 497.
15. Lashen, H. (2010).Role of Metformin in the management of polycystic ovary syndrome. Ther. Adv. Endocrinol Metab. 1(3): 117–128.
16. Xu, L., Huang, Z., Xiaoying He, X., Wan, X., Fang, D. and Li, Y. (2013).Adverse effect of Metformin therapy on serum vitamin B12 andfolate: Short-term treatment causes disadvantages. Medical Hypotheses. 81(2):149-151.
17. Altmäe, S., Stavreus-Evers, A., Ruiz, J.R., et al. (2010). "Variations in folate pathway genes are associated with unexplained female infertility". Fertil. Steril. 94 (1):130–137.
18. Goh, Y.I., Koren, G. (2008). "Folic acid in pregnancy and fetal outcomes". J. Obstet. Gynaecol. 28 (1): 3–13.
19. Schatchter, M., Raziel, A., Friedler, S., Strassburger, D., Orna Bern, O. and Ron-El, R. (2003). Insulin resistance in patientswith polycystic ovary syndrome

is associated withelevated plasma homocysteine. Hum. Repr. 18(4):721-727.
20. Kilicdag, E.B., Bagis,T. Tarim, E. Aslan, E. Erkanli, S. Simsek, E. Haydardedeoglu, B. and Kuscu, E. (2005). Administration of B-group vitamins reduces circulating homocysteine in polycystic ovarian syndrome patients treated with Metformin:a randomized trial. Human Reproduction. 20(6):1521–1528.
21. Palomba, S., Falbo, A., Giallauria, F. and Russo, R. (2010). Effects of Metforminwith or without supplemen-tation withFolate on Homocysteinelevels and vascular endotheliumof women with polycystic ovary syndrome.Diabetescare. 33(2):246-251.
22. Kilicdag, E.B., Bagis,T. Tarim,E. Aslan,E. Erkanli,S. Simsek,E. Haydardedeoglu, B. and Kuscu, E. (2005). Homocysteine levels in polycystic ovarian syndrome treated with Metformin versus rosiglitazone. Human Reproduction. 20(4):894–899.
23. Yilmaz, M., Bukan, N., Ayvaz, G., Karakoç, A., Törüner, F., Çakir, N. and Arslan, M. (2005). the effects of rosiglitazone and Metformin on oxidative stress and homocysteine levels in lean patients with polycystic ovary syndrome. Human Reproduction. 20(12):3333–3340.
24. Yilmaz, N. (2012). Relationship between paraoxonase and homocysteine: crossroads of oxidative diseases. Arch. Med. Sci.8 (1):138-153.
25. Bailey, C.J. (2008). Metformin: effects on micro and macrovascular complications in type 2 diabetes. Cardiovasc. Drugs Ther. 22: 215–224.
26. Nakano, E., Higgins, J.A. and Powers, H.J. (2001). Folate protects against oxidative modification of human LDL. Br. J. Nutr. 86:637-639.
27. Elia, E., Sander, V., Luchetti, C.G., Solano, M.E., Girolamo, G.Di., Gonzalez, C. and Motta1, A.B. (2006). The mechanisms involved in the action of Metformin in regulating ovarian function in hyperandrogenized mice. Molecular Human Reproduction. 12(8):475–481.
28. Racek, J., Rusnakova, H., Trefil, L. and Siala, K.K. (2005). The influence of folate and antioxidants on homocysteine levels and oxidative stress in patients with hyperlipidemia and hyperhomocysteinemia. Physiol. Res. 54:87–95.

Global Journals Inc. (US) Guidelines Handbook 2013

www.GlobalJournals.org

FELLOWS

FELLOW OF ASSOCIATION OF RESEARCH SOCIETY IN SCIENCE (FARSS)

Global Journals Incorporate (USA) is accredited by Open Association of Research Society (OARS), U.S.A and in turn, awards "FARSS" title to individuals. The 'FARSS' title is accorded to a selected professional after the approval of the Editor-in-Chief/Editorial Board Members/Dean.

- The "FARSS" is a dignified title which is accorded to a person's name viz. Dr. John E. Hall, Ph.D., FARSS or William Walldroff, M.S., FARSS.

FARSS accrediting is an honor. It authenticates your research activities. After recognition as FARSB, you can add 'FARSS' title with your name as you use this recognition as additional suffix to your status. This will definitely enhance and add more value and repute to your name. You may use it on your professional Counseling Materials such as CV, Resume, and Visiting Card etc.

The following benefits can be availed by you only for next three years from the date of certification:

FARSS designated members are entitled to avail a 40% discount while publishing their research papers (of a single author) with Global Journals Incorporation (USA), if the same is accepted by Editorial Board/Peer Reviewers. If you are a main author or co-author in case of multiple authors, you will be entitled to avail discount of 10%.

Once FARSB title is accorded, the Fellow is authorized to organize a symposium/seminar/conference on behalf of Global Journal Incorporation (USA). The Fellow can also participate in conference/seminar/symposium organized by another institution as representative of Global Journal. In both the cases, it is mandatory for him to discuss with us and obtain our consent.

You may join as member of the Editorial Board of Global Journals Incorporation (USA) after successful completion of three years as Fellow and as Peer Reviewer. In addition, it is also desirable that you should organize seminar/symposium/conference at least once.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time.This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

The FARSS can go through standards of OARS. You can also play vital role if you have any suggestions so that proper amendment can take place to improve the same for the benefit of entire research community.

As FARSS, you will be given a renowned, secure and free professional email address with 100 GB of space e.g. johnhall@globaljournals.org. This will include Webmail, Spam Assassin, Email Forwarders,Auto-Responders, Email Delivery Route tracing, etc.

The FARSS will be eligible for a free application of standardization of their researches. Standardization of research will be subject to acceptability within stipulated norms as the next step after publishing in a journal. We shall depute a team of specialized research professionals who will render their services for elevating your researches to next higher level, which is worldwide open standardization.

The FARSS member can apply for grading and certification of standards of their educational and Institutional Degrees to Open Association of Research, Society U.S.A.
Once you are designated as FARSS, you may send us a scanned copy of all of your credentials. OARS will verify, grade and certify them. This will be based on your academic records, quality of research papers published by you, and some more criteria. After certification of all your credentials by OARS, they will be published on your Fellow Profile link on website https://associationofresearch.org which will be helpful to upgrade the dignity.

The FARSS members can avail the benefits of free research podcasting in Global Research Radio with their research documents. After publishing the work, (including published elsewhere worldwide with proper authorization) you can upload your research paper with your recorded voice or you can utilize chargeable services of our professional RJs to record your paper in their voice on request.

The FARSS member also entitled to get the benefits of free research podcasting of their research documents through video clips. We can also streamline your conference videos and display your slides/ online slides and online research video clips at reasonable charges, on request.

The FARSS is eligible to earn from sales proceeds of his/her researches/reference/review Books or literature, while publishing with Global Journals. The FARSS can decide whether he/she would like to publish his/her research in a closed manner. In this case, whenever readers purchase that individual research paper for reading, maximum 60% of its profit earned as royalty by Global Journals, will be credited to his/her bank account. The entire entitled amount will be credited to his/her bank account exceeding limit of minimum fixed balance. There is no minimum time limit for collection. The FARSS member can decide its price and we can help in making the right decision.

The FARSS member is eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get remuneration of 15% of author fees, taken from the author of a respective paper. After reviewing 5 or more papers you can request to transfer the amount to your bank account.

MEMBER OF ASSOCIATION OF RESEARCH SOCIETY IN SCIENCE (MARSS)

The ' MARSS ' title is accorded to a selected professional after the approval of the Editor-in-Chief / Editorial Board Members/Dean.

The "MARSS" is a dignified ornament which is accorded to a person's name viz. Dr. John E. Hall, Ph.D., MARSS or William Walldroff, M.S., MARSS.

MARSS accrediting is an honor. It authenticates your research activities. After becoming MARSS, you can add 'MARSS' title with your name as you use this recognition as additional suffix to your status. This will definitely enhance and add more value and repute to your name. You may use it on your professional Counseling Materials such as CV, Resume, Visiting Card and Name Plate etc.

The following benefitscan be availed by you only for next three years from the date of certification.

MARSS designated members are entitled to avail a 25% discount while publishing their research papers (of a single author) in Global Journals Inc., if the same is accepted by our Editorial Board and Peer Reviewers. If you are a main author or co-author of a group of authors, you will get discount of 10%.

As MARSS, you will be given a renowned, secure and free professional email address with 30 GB of space e.g. johnhall@globaljournals.org. This will include Webmail, Spam Assassin, Email Forwarders,Auto-Responders, Email Delivery Route tracing, etc.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time.This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

The MARSS member can apply for approval, grading and certification of standards of their educational and Institutional Degrees to Open Association of Research, Society U.S.A.

Once you are designated as MARSS, you may send us a scanned copy of all of your credentials. OARS will verify, grade and certify them. This will be based on your academic records, quality of research papers published by you, and some more criteria.

It is mandatory to read all terms and conditions carefully.

AUXILIARY MEMBERSHIPS

Institutional Fellow of Global Journals Incorporation (USA)-OARS (USA)

Global Journals Incorporation (USA) is accredited by Open Association of Research Society, U.S.A (OARS) and in turn, affiliates research institutions as "Institutional Fellow of Open Association of Research Society" (IFOARS).

The "FARSC" is a dignified title which is accorded to a person's name viz. Dr. John E. Hall, Ph.D., FARSC or William Walldroff, M.S., FARSC.

The IFOARS institution is entitled to form a Board comprised of one Chairperson and three to five board members preferably from different streams. The Board will be recognized as "Institutional Board of Open Association of Research Society"-(IBOARS).

The Institute will be entitled to following benefits:

The IBOARS can initially review research papers of their institute and recommend them to publish with respective journal of Global Journals. It can also review the papers of other institutions after obtaining our consent. The second review will be done by peer reviewer of Global Journals Incorporation (USA) The Board is at liberty to appoint a peer reviewer with the approval of chairperson after consulting us.

The author fees of such paper may be waived off up to 40%.

The Global Journals Incorporation (USA) at its discretion can also refer double blind peer reviewed paper at their end to the board for the verification and to get recommendation for final stage of acceptance of publication.

The IBOARS can organize symposium/seminar/conference in their country on behalf of Global Journals Incorporation (USA)-OARS (USA). The terms and conditions can be discussed separately.

The Board can also play vital role by exploring and giving valuable suggestions regarding the Standards of "Open Association of Research Society, U.S.A (OARS)" so that proper amendment can take place for the benefit of entire research community. We shall provide details of particular standard only on receipt of request from the Board.

The board members can also join us as Individual Fellow with 40% discount on total fees applicable to Individual Fellow. They will be entitled to avail all the benefits as declared. Please visit Individual Fellow-sub menu of GlobalJournals.org to have more relevant details.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time. This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

After nomination of your institution as "Institutional Fellow" and constantly functioning successfully for one year, we can consider giving recognition to your institute to function as Regional/Zonal office on our behalf.
The board can also take up the additional allied activities for betterment after our consultation.

The following entitlements are applicable to individual Fellows:

Open Association of Research Society, U.S.A (OARS) By-laws states that an individual Fellow may use the designations as applicable, or the corresponding initials. The Credentials of individual Fellow and Associate designations signify that the individual has gained knowledge of the fundamental concepts. One is magnanimous and proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice.

Open Association of Research Society (US)/ Global Journals Incorporation (USA), as described in Corporate Statements, are educational, research publishing and professional membership organizations. Achieving our individual Fellow or Associate status is based mainly on meeting stated educational research requirements.

Disbursement of 40% Royalty earned through Global Journals : Researcher = 50%, Peer Reviewer = 37.50%, Institution = 12.50% E.g. Out of 40%, the 20% benefit should be passed on to researcher, 15 % benefit towards remuneration should be given to a reviewer and remaining 5% is to be retained by the institution.

We shall provide print version of 12 issues of any three journals [as per your requirement] out of our 38 journals worth $ 2376 USD.

Other:

The individual Fellow and Associate designations accredited by Open Association of Research Society (US) credentials signify guarantees following achievements:

- The professional accredited with Fellow honor, is entitled to various benefits viz. name, fame, honor, regular flow of income, secured bright future, social status etc.

- In addition to above, if one is single author, then entitled to 40% discount on publishing research paper and can get 10%discount if one is co-author or main author among group of authors.
- The Fellow can organize symposium/seminar/conference on behalf of Global Journals Incorporation (USA) and he/she can also attend the same organized by other institutes on behalf of Global Journals.
- The Fellow can become member of Editorial Board Member after completing 3yrs.
- The Fellow can earn 60% of sales proceeds from the sale of reference/review books/literature/publishing of research paper.
- Fellow can also join as paid peer reviewer and earn 15% remuneration of author charges and can also get an opportunity to join as member of the Editorial Board of Global Journals Incorporation (USA)
- • This individual has learned the basic methods of applying those concepts and techniques to common challenging situations. This individual has further demonstrated an in–depth understanding of the application of suitable techniques to a particular area of research practice.

Note :

"

- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone.

"

Process of submission of Research Paper

The Area or field of specialization may or may not be of any category as mentioned in 'Scope of Journal' menu of the GlobalJournals.org website. There are 37 Research Journal categorized with Six parental Journals GJCST, GJMR, GJRE, GJMBR, GJSFR, GJHSS. For Authors should prefer the mentioned categories. There are three widely used systems UDC, DDC and LCC. The details are available as 'Knowledge Abstract' at Home page. The major advantage of this coding is that, the research work will be exposed to and shared with all over the world as we are being abstracted and indexed worldwide.

The paper should be in proper format. The format can be downloaded from first page of 'Author Guideline' Menu. The Author is expected to follow the general rules as mentioned in this menu. The paper should be written in MS-Word Format (*.DOC,*.DOCX).

The Author can submit the paper either online or offline. The authors should prefer online submission.Online Submission: There are three ways to submit your paper:

(A) (I) First, register yourself using top right corner of Home page then Login. If you are already registered, then login using your username and password.

(II) Choose corresponding Journal.

(III) Click 'Submit Manuscript'. Fill required information and Upload the paper.

(B) If you are using Internet Explorer, then Direct Submission through Homepage is also available.

(C) If these two are not conveninet , and then email the paper directly to dean@globaljournals.org.

Offline Submission: Author can send the typed form of paper by Post. However, online submission should be preferred.

Preferred Author Guidelines

MANUSCRIPT STYLE INSTRUCTION (Must be strictly followed)

Page Size: 8.27" X 11'"

- Left Margin: 0.65
- Right Margin: 0.65
- Top Margin: 0.75
- Bottom Margin: 0.75
- Font type of all text should be Swis 721 Lt BT.
- Paper Title should be of Font Size 24 with one Column section.
- Author Name in Font Size of 11 with one column as of Title.
- Abstract Font size of 9 Bold, "Abstract" word in Italic Bold.
- Main Text: Font size 10 with justified two columns section
- Two Column with Equal Column with of 3.38 and Gaping of .2
- First Character must be three lines Drop capped.
- Paragraph before Spacing of 1 pt and After of 0 pt.
- Line Spacing of 1 pt
- Large Images must be in One Column
- Numbering of First Main Headings (Heading 1) must be in Roman Letters, Capital Letter, and Font Size of 10.
- Numbering of Second Main Headings (Heading 2) must be in Alphabets, Italic, and Font Size of 10.

You can use your own standard format also.
Author Guidelines:

1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

1. GENERAL

Before submitting your research paper, one is advised to go through the details as mentioned in following heads. It will be beneficial, while peer reviewer justify your paper for publication.

Scope

The Global Journals Inc. (US) welcome the submission of original paper, review paper, survey article relevant to the all the streams of Philosophy and knowledge. The Global Journals Inc. (US) is parental platform for Global Journal of Computer Science and Technology, Researches in Engineering, Medical Research, Science Frontier Research, Human Social Science, Management, and Business organization. The choice of specific field can be done otherwise as following in Abstracting and Indexing Page on this Website. As the all Global

Journals Inc. (US) are being abstracted and indexed (in process) by most of the reputed organizations. Topics of only narrow interest will not be accepted unless they have wider potential or consequences.

2. ETHICAL GUIDELINES

Authors should follow the ethical guidelines as mentioned below for publication of research paper and research activities.

Papers are accepted on strict understanding that the material in whole or in part has not been, nor is being, considered for publication elsewhere. If the paper once accepted by Global Journals Inc. (US) and Editorial Board, will become the copyright of the Global Journals Inc. (US).

Authorship: The authors and coauthors should have active contribution to conception design, analysis and interpretation of findings. They should critically review the contents and drafting of the paper. All should approve the final version of the paper before submission

The Global Journals Inc. (US) follows the definition of authorship set up by the Global Academy of Research and Development. According to the Global Academy of R&D authorship, criteria must be based on:

1) Substantial contributions to conception and acquisition of data, analysis and interpretation of the findings.

2) Drafting the paper and revising it critically regarding important academic content.

3) Final approval of the version of the paper to be published.

All authors should have been credited according to their appropriate contribution in research activity and preparing paper. Contributors who do not match the criteria as authors may be mentioned under Acknowledgement.

Acknowledgements: Contributors to the research other than authors credited should be mentioned under acknowledgement. The specifications of the source of funding for the research if appropriate can be included. Suppliers of resources may be mentioned along with address.

Appeal of Decision: The Editorial Board's decision on publication of the paper is final and cannot be appealed elsewhere.

Permissions: It is the author's responsibility to have prior permission if all or parts of earlier published illustrations are used in this paper.

Please mention proper reference and appropriate acknowledgements wherever expected.

If all or parts of previously published illustrations are used, permission must be taken from the copyright holder concerned. It is the author's responsibility to take these in writing.

Approval for reproduction/modification of any information (including figures and tables) published elsewhere must be obtained by the authors/copyright holders before submission of the manuscript. Contributors (Authors) are responsible for any copyright fee involved.

3. SUBMISSION OF MANUSCRIPTS

Manuscripts should be uploaded via this online submission page. The online submission is most efficient method for submission of papers, as it enables rapid distribution of manuscripts and consequently speeds up the review procedure. It also enables authors to know the status of their own manuscripts by emailing us. Complete instructions for submitting a paper is available below.

Manuscript submission is a systematic procedure and little preparation is required beyond having all parts of your manuscript in a given format and a computer with an Internet connection and a Web browser. Full help and instructions are provided on-screen. As an author, you will be prompted for login and manuscript details as Field of Paper and then to upload your manuscript file(s) according to the instructions.

To avoid postal delays, all transaction is preferred by e-mail. A finished manuscript submission is confirmed by e-mail immediately and your paper enters the editorial process with no postal delays. When a conclusion is made about the publication of your paper by our Editorial Board, revisions can be submitted online with the same procedure, with an occasion to view and respond to all comments.

Complete support for both authors and co-author is provided.

4. MANUSCRIPT'S CATEGORY

Based on potential and nature, the manuscript can be categorized under the following heads:

Original research paper: Such papers are reports of high-level significant original research work.

Review papers: These are concise, significant but helpful and decisive topics for young researchers.

Research articles: These are handled with small investigation and applications

Research letters: The letters are small and concise comments on previously published matters.

5.STRUCTURE AND FORMAT OF MANUSCRIPT

The recommended size of original research paper is less than seven thousand words, review papers fewer than seven thousands words also.Preparation of research paper or how to write research paper, are major hurdle, while writing manuscript. The research articles and research letters should be fewer than three thousand words, the structure original research paper; sometime review paper should be as follows:

Papers: These are reports of significant research (typically less than 7000 words equivalent, including tables, figures, references), and comprise:

(a)Title should be relevant and commensurate with the theme of the paper.

(b) A brief Summary, "Abstract" (less than 150 words) containing the major results and conclusions.

(c) Up to ten keywords, that precisely identifies the paper's subject, purpose, and focus.

(d) An Introduction, giving necessary background excluding subheadings; objectives must be clearly declared.

(e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition; sources of information must be given and numerical methods must be specified by reference, unless non-standard.

(f) Results should be presented concisely, by well-designed tables and/or figures; the same data may not be used in both; suitable statistical data should be given. All data must be obtained with attention to numerical detail in the planning stage. As reproduced design has been recognized to be important to experiments for a considerable time, the Editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned un-refereed;

(g) Discussion should cover the implications and consequences, not just recapitulating the results; conclusions should be summarizing.

(h) Brief Acknowledgements.

(i) References in the proper form.

Authors should very cautiously consider the preparation of papers to ensure that they communicate efficiently. Papers are much more likely to be accepted, if they are cautiously designed and laid out, contain few or no errors, are summarizing, and be conventional to the approach and instructions. They will in addition, be published with much less delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and to make suggestions to improve briefness.

It is vital, that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

Format

Language: The language of publication is UK English. Authors, for whom English is a second language, must have their manuscript efficiently edited by an English-speaking person before submission to make sure that, the English is of high excellence. It is preferable, that manuscripts should be professionally edited.

Standard Usage, Abbreviations, and Units: Spelling and hyphenation should be conventional to The Concise Oxford English Dictionary. Statistics and measurements should at all times be given in figures, e.g. 16 min, except for when the number begins a sentence. When the number does not refer to a unit of measurement it should be spelt in full unless, it is 160 or greater.

Abbreviations supposed to be used carefully. The abbreviated name or expression is supposed to be cited in full at first usage, followed by the conventional abbreviation in parentheses.

Metric SI units are supposed to generally be used excluding where they conflict with current practice or are confusing. For illustration, 1.4 l rather than 1.4 × 10-3 m3, or 4 mm somewhat than 4 × 10-3 m. Chemical formula and solutions must identify the form used, e.g. anhydrous or hydrated, and the concentration must be in clearly defined units. Common species names should be followed by underlines at the first mention. For following use the generic name should be constricted to a single letter, if it is clear.

Structure

All manuscripts submitted to Global Journals Inc. (US), ought to include:

Title: The title page must carry an instructive title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) wherever the work was carried out. The full postal address in addition with the e-mail address of related author must be given. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining and indexing.

Abstract, used in Original Papers and Reviews:

Optimizing Abstract for Search Engines

Many researchers searching for information online will use search engines such as Google, Yahoo or similar. By optimizing your paper for search engines, you will amplify the chance of someone finding it. This in turn will make it more likely to be viewed and/or cited in a further work. Global Journals Inc. (US) have compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Key Words

A major linchpin in research work for the writing research paper is the keyword search, which one will employ to find both library and Internet resources.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy and planning a list of possible keywords and phrases to try.

Search engines for most searches, use Boolean searching, which is somewhat different from Internet searches. The Boolean search uses "operators," words (and, or, not, and near) that enable you to expand or narrow your affords. Tips for research paper while preparing research paper are very helpful guideline of research paper.

Choice of key words is first tool of tips to write research paper. Research paper writing is an art.A few tips for deciding as strategically as possible about keyword search:

- One should start brainstorming lists of possible keywords before even begin searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in research paper?" Then consider synonyms for the important words.
- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
- One should avoid outdated words.

Keywords are the key that opens a door to research work sources. Keyword searching is an art in which researcher's skills are bound to improve with experience and time.

Numerical Methods: Numerical methods used should be clear and, where appropriate, supported by references.

Acknowledgements: Please make these as concise as possible.

References

References follow the Harvard scheme of referencing. References in the text should cite the authors' names followed by the time of their publication, unless there are three or more authors when simply the first author's name is quoted followed by et al. unpublished work has to only be cited where necessary, and only in the text. Copies of references in press in other journals have to be supplied with submitted typescripts. It is necessary that all citations and references be carefully checked before submission, as mistakes or omissions will cause delays.

References to information on the World Wide Web can be given, but only if the information is available without charge to readers on an official site. Wikipedia and Similar websites are not allowed where anyone can change the information. Authors will be asked to make available electronic copies of the cited information for inclusion on the Global Journals Inc. (US) homepage at the judgment of the Editorial Board.

The Editorial Board and Global Journals Inc. (US) recommend that, citation of online-published papers and other material should be done via a DOI (digital object identifier). If an author cites anything, which does not have a DOI, they run the risk of the cited material not being noticeable.

The Editorial Board and Global Journals Inc. (US) recommend the use of a tool such as Reference Manager for reference management and formatting.

Tables, Figures and Figure Legends

Tables: Tables should be few in number, cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g. Table 4, a self-explanatory caption and be on a separate sheet. Vertical lines should not be used.

Figures: Figures are supposed to be submitted as separate files. Always take in a citation in the text for each figure using Arabic numbers, e.g. Fig. 4. Artwork must be submitted online in electronic form by e-mailing them.

Preparation of Electronic Figures for Publication

Even though low quality images are sufficient for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit (or e-mail) EPS (line art) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings) in relation to the imitation size. Please give the data for figures in black and white or submit a Color Work Agreement Form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution (at final image size) ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs) : >350 dpi; figures containing both halftone and line images: >650 dpi.

Color Charges: It is the rule of the Global Journals Inc. (US) for authors to pay the full cost for the reproduction of their color artwork. Hence, please note that, if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a color work agreement form before your paper can be published.

Figure Legends: Self-explanatory legends of all figures should be incorporated separately under the heading 'Legends to Figures'. In the full-text online edition of the journal, figure legends may possibly be truncated in abbreviated links to the full screen version. Therefore, the first 100 characters of any legend should notify the reader, about the key aspects of the figure.

6. AFTER ACCEPTANCE

Upon approval of a paper for publication, the manuscript will be forwarded to the dean, who is responsible for the publication of the Global Journals Inc. (US).

6.1 Proof Corrections

The corresponding author will receive an e-mail alert containing a link to a website or will be attached. A working e-mail address must therefore be provided for the related author.

Acrobat Reader will be required in order to read this file. This software can be downloaded

(Free of charge) from the following website:

www.adobe.com/products/acrobat/readstep2.html. This will facilitate the file to be opened, read on screen, and printed out in order for any corrections to be added. Further instructions will be sent with the proof.

Proofs must be returned to the dean at dean@globaljournals.org within three days of receipt.

As changes to proofs are costly, we inquire that you only correct typesetting errors. All illustrations are retained by the publisher. Please note that the authors are responsible for all statements made in their work, including changes made by the copy editor.

6.2 Early View of Global Journals Inc. (US) (Publication Prior to Print)

The Global Journals Inc. (US) are enclosed by our publishing's Early View service. Early View articles are complete full-text articles sent in advance of their publication. Early View articles are absolute and final. They have been completely reviewed, revised and edited for publication, and the authors' final corrections have been incorporated. Because they are in final form, no changes can be made after sending them. The nature of Early View articles means that they do not yet have volume, issue or page numbers, so Early View articles cannot be cited in the conventional way.

6.3 Author Services

Online production tracking is available for your article through Author Services. Author Services enables authors to track their article - once it has been accepted - through the production process to publication online and in print. Authors can check the status of their articles online and choose to receive automated e-mails at key stages of production. The authors will receive an e-mail with a unique link that enables them to register and have their article automatically added to the system. Please ensure that a complete e-mail address is provided when submitting the manuscript.

6.4 Author Material Archive Policy

Please note that if not specifically requested, publisher will dispose off hardcopy & electronic information submitted, after the two months of publication. If you require the return of any information submitted, please inform the Editorial Board or dean as soon as possible.

6.5 Offprint and Extra Copies

A PDF offprint of the online-published article will be provided free of charge to the related author, and may be distributed according to the Publisher's terms and conditions. Additional paper offprint may be ordered by emailing us at: editor@globaljournals.org .

Before start writing a good quality Computer Science Research Paper, let us first understand what is Computer Science Research Paper? So, Computer Science Research Paper is the paper which is written by professionals or scientists who are associated to Computer Science and Information Technology, or doing research study in these areas. If you are novel to this field then you can consult about this field from your supervisor or guide.

TECHNIQUES FOR WRITING A GOOD QUALITY RESEARCH PAPER:

1. Choosing the topic: In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourses to accomplish the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

2. Evaluators are human: First thing to remember that evaluators are also human being. They are not only meant for rejecting a paper. They are here to evaluate your paper. So, present your Best.

3. Think Like Evaluators: If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

4. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

5. Ask your Guides: If you are having any difficulty in your research, then do not hesitate to share your difficulty to your guide (if you have any). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work then ask the supervisor to help you with the alternative. He might also provide you the list of essential readings.

6. Use of computer is recommended: As you are doing research in the field of Computer Science, then this point is quite obvious.

7. Use right software: Always use good quality software packages. If you are not capable to judge good software then you can lose quality of your paper unknowingly. There are various software programs available to help you, which you can get through Internet.

8. Use the Internet for help: An excellent start for your paper can be by using the Google. It is an excellent search engine, where you can have your doubts resolved. You may also read some answers for the frequent question how to write my research paper or find model research paper. From the internet library you can download books. If you have all required books make important reading selecting and analyzing the specified information. Then put together research paper sketch out.

9. Use and get big pictures: Always use encyclopedias, Wikipedia to get pictures so that you can go into the depth.

10. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right! It is a good habit, which helps to not to lose your continuity. You should always use bookmarks while searching on Internet also, which will make your search easier.

11. Revise what you wrote: When you write anything, always read it, summarize it and then finalize it.

12. Make all efforts: Make all efforts to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in introduction, that what is the need of a particular research paper. Polish your work by good skill of writing and always give an evaluator, what he wants.

13. Have backups: When you are going to do any important thing like making research paper, you should always have backup copies of it either in your computer or in paper. This will help you to not to lose any of your important.

14. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several and unnecessary diagrams will degrade the quality of your paper by creating "hotchpotch." So always, try to make and include those diagrams, which are made by your own to improve readability and understandability of your paper.

15. Use of direct quotes: When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

16. Use proper verb tense: Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

17. Never use online paper: If you are getting any paper on Internet, then never use it as your research paper because it might be possible that evaluator has already seen it or maybe it is outdated version.

18. Pick a good study spot: To do your research studies always try to pick a spot, which is quiet. Every spot is not for studies. Spot that suits you choose it and proceed further.

19. Know what you know: Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

20. Use good quality grammar: Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straight forward. put together a neat summary.

21. Arrangement of information: Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

23. Multitasking in research is not good: Doing several things at the same time proves bad habit in case of research activity. Research is an area, where everything has a particular time slot. Divide your research work in parts and do particular part in particular time slot.

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

26. Go for seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

Administration Rules Listed Before Submitting Your Research Paper to Global Journals Inc. (US)

Please carefully note down following rules and regulation before submitting your Research Paper to Global Journals Inc. (US):

Segment Draft and Final Research Paper: You have to strictly follow the template of research paper. If it is not done your paper may get rejected.

- The **major constraint** is that you must independently make all content, tables, graphs, and facts that are offered in the paper. You must write each part of the paper wholly on your own. The Peer-reviewers need to identify your own perceptive of the concepts in your own terms. NEVER extract straight from any foundation, and never rephrase someone else's analysis.

- Do not give permission to anyone else to "PROOFREAD" your manuscript.

- Methods to avoid Plagiarism is applied by us on every paper, if found guilty, you will be blacklisted by all of our collaborated research groups, your institution will be informed for this and strict legal actions will be taken immediately.)
- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.

Criterion for Grading a Research Paper (Compilation) by Global Journals Inc. (US)

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals Inc. (US).

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

INDEX

A

Actinomycetes · 1, 2, 3, 4, 5
Aeruginosa · 3, 6
Apolipoprptein · 40

B

Begrudgingly · 25

C

Callogenesis · 36
Chromosomes · 25, 27, 28, 31, 32
Cyclohexamide · 2, 5
Cystathionine · 41, 43

D

Diaminopimelic · 4
Dyslipidemia · 40, 45

E

Embryogenesis · 31
Embryonic · 25, 27, 29, 30
Endocrinopathy · 40
Escherichia · 3, 6

G

Glycerol · 4, 7
Goldschmidt · 25, 32
Gonadotropin · 43

H

Heterochromatinized · 32
Homocysteine · 40, 41, 44, 45, 46
Homoiotherms · 28, 32

I

Immunohistochemistry · 15

M

Macromophology · 4
Malondialdehyde · 40, 44, 45

P

Paraoxonase · 40, 44, 45
Pharmaceutical · 1, 5
Phenoxyacetic · 36

R

Remethylation · 41, 42

S

Staphylococcus · 3, 6
Streptomycetes · 4, 5

T

Thermoregulation · 25, 27, 28, 29, 30, 31, 32

Global Journal of Science Frontier Research

Visit us on the Web at www.GlobalJournals.org | www.JournalofScience.org
or email us at helpdesk@globaljournals.org

ISSN 9755896