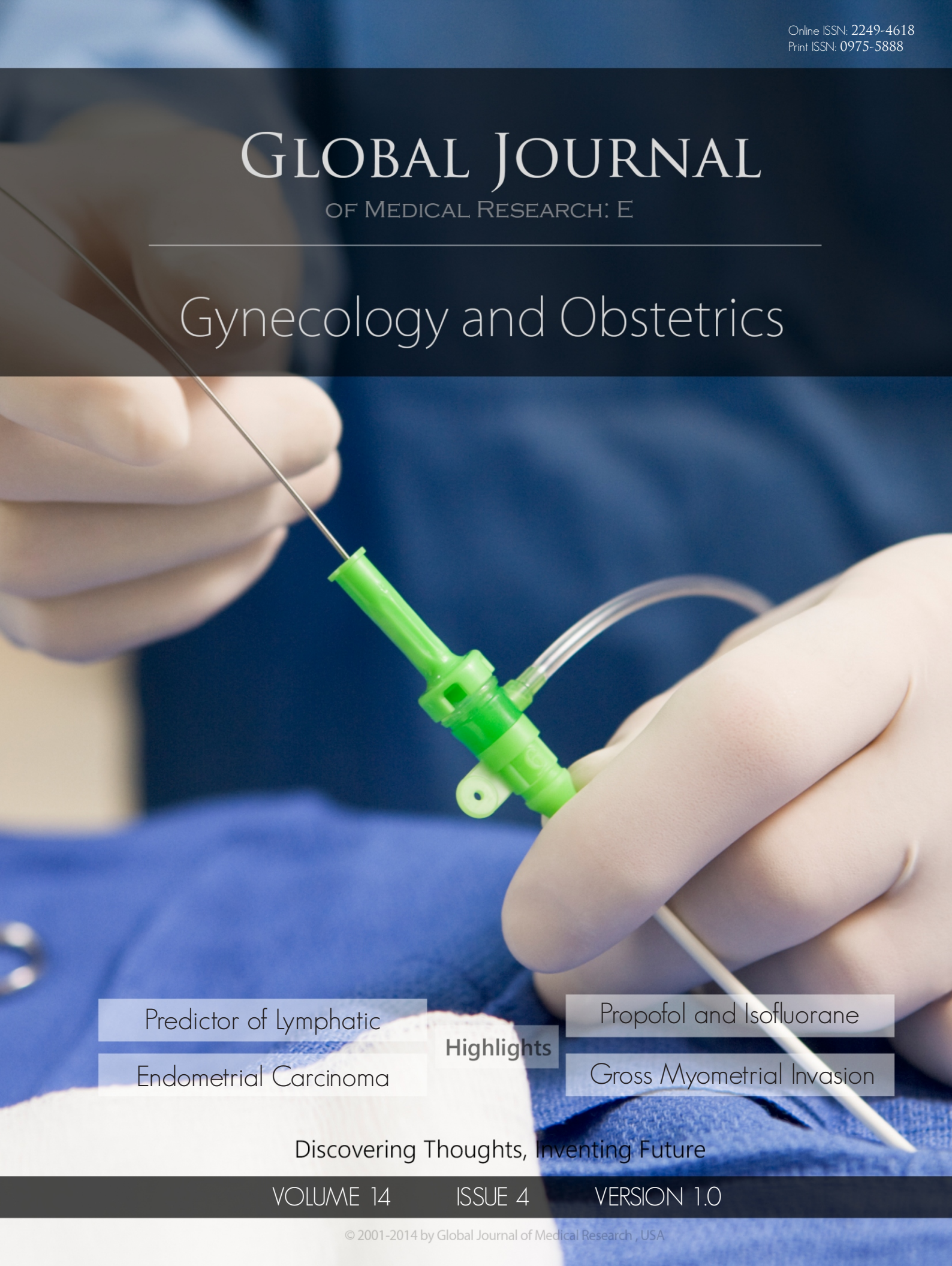
Online ISSN: 2249-4618
Print ISSN: 0975-5888

Global Journal of Medical Research: E

Gynecology and Obstetrics



Predictor of Lymphatic Endometrial Carcinoma

Highlights

Propofol and Isofluorane Gross Myometrial Invasion

Discovering Thoughts, Inventing Future

VOLUME 14 ISSUE 4 VERSION 1.0

Global Journal of Medical Research: E
Gynecology and Obstetrics

Global Journal of Medical Research: E
Gynecology and Obstetrics
Volume 14 Issue 4 (Ver. 1.0)

Open Association of Research Society

This is a special issue published in version 1.0 of "Global Journal of Medical Research." By Global Journals Inc.

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: *investors@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

Contents of the Issue

Global Journal of Medical research: E
Gynecology and Obstetrics
Volume 14 Issue 4 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

The Accuracy of Surgical Assessment of Gross Myometrial Invasion as a Predictor of Lymphatic Metastases in Women with Endometrial Carcinoma

By Andrew P.Soisson, Jessica Pittman, Mark K. Dodson, Tom Belnap, Braydon Rowley & William Sause

Saint Vincent College, United States

Abstract- Objective: The objective of this study was to determine if the surgeon can accurately predict the depth of myometrial tumor invasion in women with endometrial cancer, and if tumor invasion will correlate with node metastases.

Methods: We identified 1,943 women with endometrial carcinoma who underwent hysterectomy. Of these, 295 underwent comprehensive surgical staging including lymph node analysis. All subjects also underwent gross examination of the uterine specimen by their surgeon where the depth of myometrial invasion was recorded. Patients with grade III tumors or papillary serous and clear cell histology were excluded. The presence or absence of myometrial invasion was then correlated with the incidence of nodal involvement to determine if this system can be used to predict tumor spread at the time of hysterectomy.

Keywords: endometrial, cancer, depth of invasion.

GJMR-E Classification : NLMC Code: WP 390, WJ 190

Strictly as per the compliance and regulations of:

The Accuracy of Surgical Assessment of Gross Myometrial Invasion as a Predictor of Lymphatic Metastases in Women with Endometrial Carcinoma

Andrew P.Soisson [α], Jessica Pittman [σ], Mark K. Dodson [ρ], Tom Belnap [ω], Braydon Rowley [¥] & William Sause [§]

Synopsis: In this study, low-grade tumors with less than 50% invasion by surgeon prediction were associated with a 3% incidence of nodal metastases.

Abstract- Objective: The objective of this study was to determine if the surgeon can accurately predict the depth of myometrial tumor invasion in women with endometrial cancer, and if tumor invasion will correlate with node metastases.

Methods: We identified 1,943 women with endometrial carcinoma who underwent hysterectomy. Of these, 295 underwent comprehensive surgical staging including lymph node analysis. All subjects also underwent gross examination of the uterine specimen by their surgeon where the depth of myometrial invasion was recorded. Patients with grade III tumors or papillary serous and clear cell histology were excluded. The presence or absence of myometrial invasion was then correlated with the incidence of nodal involvement to determine if this system can be used to predict tumor spread at the time of hysterectomy.

Results: The ability of the surgeon to accurately predict the depth of myometrial invasion was 82%, sensitivity was 57%, specificity 89%, positive predictive value 62% and the negative predictive value was 88%. If this system was used as the indication for nodal evaluation the authors would have missed 3% of women with nodal metastases who had less than 50% myometrial invasion.

Conclusions: Gross evaluation of the hysterectomy specimen can accurately predict depth of myometrial invasion. However, in our analysis 3% of women with less than 50% invasion had node involvement. If the surgeon had used the absence of myometrial invasion to omit nodal assessment, these lesions would have been missed. Therefore, we feel that nodal assessment should be considered in the majority of cases.

Keywords: endometrial, cancer, depth of invasion.

I. Introduction

Endometrial carcinoma is the 4th most common cancer in females and the most common gynecologic malignancy with approximately 43,470 cases and 7,950 deaths per year diagnosed in the United States[1]. New diagnoses are rare before the age of 40 and increase ten-fold from ages 40-49. The vast majority of women with endometrial cancer are postmenopausal and the median age is 55[2,3]. Most women with endometrial carcinoma develop postmen opausal vaginal bleeding that prompts them to seek medical attention. Diagnosis of the malignancy is usually made by endometrial biopsy or dilation and curettage of the uterine cavity. Therefore, over 80% of women with endometrial adenocarcinoma are diagnosed with early stage disease that ultimately equates to a high overall cure rate[4]. Treatment options for women with stage I or II endometrial carcinoma include anti-estrogen therapy, primary radiation, and most commonly hysterectomy. Anti-estrogen therapy (progesterone) is associated with low response rates in postmenopausal women, and radiation as a primary treatment is technically difficult in many cases and is used infrequently. Therefore, surgical treatment is the most common treatment modality and is associated with high cure and good local control rates.

Surgery usually includes complete hyste rectomy, bilateral salpingo-oophorectomy,cytologic analysis of pelvic fluid for malignant cells, and retroperitoneal lymph node assessment for the presence or absence of lymphatic metastases. Despite the fact that lymph node assessment has not been shown to offer a therapeutic benefit[5,6], many believe it allows practitioners the opportunity to recommend more appropriate post-surgical adjunctive therapies, and the Society of Gynecologic Oncologists (SGO) recommends that it be performed in most cases[7]. However, not all surgeons have been trained to perform retroperitoneal lymph node assessment and the procedure is associated with some surgical morbidity and even mortality[8]. Thus, many surgeons utilize tumor grade and depth of myometrial invasion as clinical variables to predict the incidence of lymphatic metastases and therefore, an indication for lymph node assessment at the time of surgery. Myometrial invasion is a critical prognostic factor in women with low-risk factors such as

Author α σ ρ: University of Utah/Department of Obstetrics and Gynecology and The Huntsman Cancer Institute.
Author ω ¥ §: Clinical Oncology Services/Intermountain Health Corporation.

grade I an II tumors and endometriod histology. Multiple reports have shown that the surgeon's ability to measure and predict the depth of symmetrical invasion is fairly accurate[9-13], and is as good as pathologic examination by frozen section[14-16]. However, this surgical strategy has not been as extensively studied as a model to predict the true presence or absence of lymphatic metastases in a large cohort of women with endometrial cancer who have undergone complete surgical staging and who have had the uterus examined by the surgeon at the time of hysterectomy to measure the depth of myometrial invasion. The purpose of this study was to evaluate the accuracy of surgical prediction of myometrial invasion by endometrial tumor and to determine the accuracy of this method as a model for predicting lymph node involvement by tumor.

II. Materials and Methods

The state tumor registry was used to identify all women with endometrial cancer who underwent hysterectomy as their initial and primary treatment during the time period of the study. The authors created a surgical registry and abstracted the medical records, including surgical operative reports, anesthesia records, pathology reports and in-patient record of hospital stay. The collection of data and recruitment of patients for this study was approved by the institutional review board at our institution. From 1993 to 2010, 1,943 women were diagnosed with endometrial cancer and underwent hysterectomy as their primary treatment at one of 11 institutions in our geographical area. These included community and tertiary medical centers. Of these, 295 had examination under anesthesia, surgical evaluation of the pelvis and upper abdomen, oophorectomy, analysis of pelvic washings, pelvic retroperitoneal lymph node sampling, and evaluation of the uterine specimen for gross myometrial invasion by the surgeon. All cases were performed by one of three gynecologic oncologists at one of three tertiary medical centers who used the exact same methodology to evaluate the uterus and to predict the depth of tumor invasion. Only patients with endometriod histology and grade I or II tumors were included in the analysis as most gynecologic oncologists would routinely perform lymph node analysis in grade III or clear cell and papillary serous tumors.

Myometrial invasion was recorded as equal to or less than 50%, or greater than 50% invasion of the thickness of the myometrium at the maximal depth of penetration of the tumor. In each case after completing the surgical procedure, the surgeon would make a lateral incision at the 3' and 6" position of the uterine cervix and extend it to the fundus to expose the entire endometrial surface for gross examination. Lateral perpendicular full thickness incisions were then made through the endometrium and myometrium in at least two areas of the anterior and posterior surface of the uterus to assess the depth of symmetrical invasion. Depth of invasion was recorded by the surgeon in the operative report and later abstracted into a surgical database.

III. Results

The mean weight of the 295 study patients was 194 pounds and the mean body mass index was 32.3. One hundred and seven (36%) women in the study group had hypertension, 53 (18%) had adult onset diabetes mellitus (AODM), and 21 (7%) were cigarette smokers. One hundred fifty five women (52%) had grade I tumors and 140 (48%) were grade II. Women with poorly differentiated tumors (grade III) or unusual histology such as clear cell or papillary serous tumors were eliminated from the analysis. Two hundred sixty nine women (91%) had their ovaries removed at the time of surgery. All 295 women underwent hysterectomy, analysis of pelvic washings and pelvic lymph node sampling. The mean estimated blood loss was 316 ml. and 29 women underwent transfusion (10%) in the immediate postoperative period.

At the completion of the hysterectomy the primary surgeon examined the uterus and estimated the maximal depth of symmetrical invasion of the tumor. In 242 cases the surgeon accurately estimated the depth of invasion compared to histologic examination on permanent sections for an overall accuracy rate of 82%. There were 53 cases where the surgeon did not predict the true depth of symmetrical invasion. The sensitivity was calculated as 57%, specificity 89%, positive predictive value was 62%, and the negative predictive value was 88%.

The overall incidence of retroperitoneal lymph node metastases was 4% (n=12). In 62 cases the surgeon estimated that the depth of invasion was greater than 50% and of these 6 (10%) had nodal metastases and 56 (90%) had no evidence of nodal involvement by tumor. In 233 cases the surgeon estimated less than or equal to 50% symmetrical invasion and of these 6 (3%) had lymph node metastases and 227 (97%) were not involved with tumor.

IV. Discussion

Extrauterine spread of tumor cells is the most significant poor prognostic factor associated with decreased survival in women with endometrial cancer. The most common anatomic site of metastatic disease is to the pelvic and para-aortic retroperitoneal lymph nodes. Approximately 7-10% of women with endometrial adenocarcinoma will have retroperitoneal lymph node metastases, and the presence or absence of nodal metastases will impact postoperative adjuvant therapy and subsequent cure rates[4]. The relatively high likelihood of nodal spread, the impact on recommending postoperative therapies, and the relatively low morbidity associated with node sampling has prompted

the Society of Gynecologic Oncologists to recommend that retroperitoneal node sampling be performed in most women with endometrial cancer[7]. Unfortunately, not all women with endometrial cancer undergo complete surgical staging or care by gynecologic oncologists in the United States. Gynecologists and surgeons in non-tertiary centers often employ preoperative and intra-operative strategies to assess the likelihood of nodal spread, which is used to determine if nodal sampling is performed at the time of hysterectomy[17,18].

Preoperative radiographic imaging modalities evaluating retroperitoneal metastases are limited, have not been sufficiently studied in large groups of women, and are largely inaccurate[19,20]. Preoperative radiographic evaluation of myometrial invasion using ultrasonography (US), magnetic resonance imaging (MRI), and positron emission tomography (PET) are fairly comparable in their ability to accurately predict the depth of myometrial invasion. Multiple studies have demonstrated that US has an accuracy rate of approximately 65-75% and sensitivity and specificity of 70% for predicting depth of invasion compared to final histology[21-23]. Transvaginal ultrasound employing saline infusion will increase the accuracy of myometrial invasion detection slightly[24]. Magnetic resonance imaging is comparable with an accuracy, sensitivity, and specificity of 62-95%, 79-92%, and 72-100% respectively[25-29]. Finally, PET imaging appears to offer no increased accuracy compared to other radiographic modalities[30-32].

Estimation of myometrial invasion grossly by the surgeon at the time of hysterectomy has also been evaluated in multiple studies incorporating large numbers of women; these indicate that the accuracy rates, sensitivity, and specificity are comparable to or somewhat better than radiographic assessment. Reported rates for accuracy are 82-91%, sensitivity 65-84%, and specificity 83-96%[9-11,33]. Estimation of depth of invasion at the time of the hysterectomy by frozen section is comparable in accuracy to gross inspection by the surgeon. Studies using frozen section only as a predictor of myometrial invasion have accuracy rates of 70-90%[14,34-36]. Studies comparing frozen section to gross invasion in the same surgical setting show equivalent accuracy rates for the two methods[37,38]. In our study we confirm the results of prior investigations and show that surgeon assessment of myometrial invasion of less than or greater than 50% of the thickness of the myometrium is fairly accurate. Our results of 82% accuracy compare favorably to other reports.

Surgical algorithms incorporating depth of myometrial invasion as a method to assess the likelihood of retroperitoneal lymph node metastases has been less well studied. Tumor size has been shown to be a predictor of metastatic disease since 1987[39] and was used as an intraoperative factor along with depth of myometrial invasion by frozen section by Kumar and colleagues at the Mayo Clinic[40]. We chose not to use tumor size as a factor since frozen section evaluation is not rapidly available at our institution. Multiple authors have shown that depth of invasion determined at the time of surgery by the surgeon or pathologist does not accurately predict the presence or absence of retroperitoneal lymph node involvement. These reports indicate that many patients undergo lymph node resection unnecessarily and, more importantly, some do not have node assessment when they should have. Traen[41] reported on 72 women with grade I tumors who underwent complete surgical staging. In their analysis, 4% had lymph node assessment unnecessarily and 7% did not have nodal analysis when they should have. Papadia[42] reviewed data from Gynecologic Oncology Group (GOG) protocol 33 and analyzed 174 women with early stage endometrial cancer that were surgically staged. They concluded that a substantial number of cases had suboptimal management when gross inspection of the uterus and frozen section to determine the depth of myometrial invasion was used as a methodology for assessing lymph node status. In our analysis, we attempt to retrospectively evaluate a surgical system where depth of invasion is used at the time of hysterectomy to indicate whether node sampling should be performed in women with endometriod histology and grade I or II tumors. In this system, the surgeon could accurate predict depth of invasion but 3% of the subjects would not have had node assessment performed when metastases to the retroperitoneum were present. Therefore, we recommend that lymph node assessment be performed in the majority of women undergoing hysterectomy for endometrial carcinoma as we believe the incidence of significant morbidity such as vascular injury[43] associated with lymph node sampling is far less significant than not detecting lymph node metastases.

V. Conflict of Interest Statement

The authors declare there are no conflicts of interest.

References Références Referencias

1. Jemal A, Siegel R, Xu J, Ward E. Cancer Statistics, 2010. CA Cancer J Clin. 2010;60:277-300.
2. Hunn J, Dodson MK, Webb J, Soisson AP. Endometrial cancer – current state of the art therapies and unmet clinical needs: The role of surgery and preoperative radiographic assessment. Adv Drug Deliv Rev 2009; 61:890-5.
3. Erkanli S, Ayhan A. Fertility-sparing therapy in young women with endometrial cancer. I J Gynecol Care. 2010;20:1170-1187.
4. Creasman WT, Morrow CP, Bundy BN, et al. Surgical pathologic spread patterns of endometrial

cancer. A gynecologic group study. Cancer. 1987;60:2035-2041.
5. Kitchener H, Swart AMC, Qian W, Amos C, Parmar MKB. Efficacy of systematic pelvic lymphadenec tomy in endometrial cancer (MRC ASTEC trial): a randomized study. Lancet. 2009;373:125-36.
6. Benedetti P, Basile S, Maneschi F, Lissoni A, Signorelli M, Scambia G, et al. Systematic pelvic lymphadenectomy vs no lymphadenectomy in early-stage endometrial carcinoma: Randomized clinical trial. J Natl Cancer Inst. 2008;100:1707-1716.
7. Management of endometrial cancer. ACOG Practice Bulletin No. 65. American College of Obstetricians and Gynecologists. Obstet Gynecol 2005;106:413–25.
8. Lachance JA, Everett EN, Greer B, Mandel L, Swisher E, Tamimi H, Goff B. The effect of age on clinical/pathologic features, surgical morbidity, and outcome in patients with endometrial cancer. Gynecol Oncol. 2006;101:470-5.
9. Mao Y, Wan X, Lv W, Xie X. Evaluation of the accuracy of intra-operative gross examination for the surgical management of endometrial cancer. European J Obstet Gynecol and Reproductive Biology. 2008;141:179-82.
10. Franchi M, Ghezzi F, Melpingano M, Cherchi PL, Scarabelli C, Apolloni C, Zanaboni F. Clinical value of intraoperative gross examination in endometrial cancer. Gynecol Oncol. 2000;76:357-61.
11. Vorgias G, Hintipas E, Katsoulis M, Kalinogolou N, Dertimas B, Akrivos T. Intraoperative gross exami nation of myometrial invasion and cervical infiltration in patients with endometrial cancer: Decision-making accuracy. Gynecol Oncol. 2002; 85:483-86.
12. Yahata T, Aoki Y, Tanaka K. Prediction of myometrial invasion in patients with endometrial carcinoma: comparison of magnetic resonance imaging, transvaginal sonography, and gross visual inspection. Eur J Gynaecol Oncol. 2007;28:193-5.
13. Doering DL, Barnhill DR, Weiser EB, Burke TW, Woodward JE, Park RC. Intraoperative evaluation of depth of myometrial invasion in stage I endometrial adenocarcinoma. Obstet Gynecol. 1989;74:930-3.
14. Maneschi F, Sarno M, Mancione AM, Perugini A, Partenzi A. Endometrial carcinoma: intraoperative evaluation of myometrial invasion. A prospective study. Minerva Ginecol. 2008;60:267-72.
15. Altintas A, Vardar MA, Demir C, Tuncer I. Intraoperative assessment of depth of myometrial invasion in endometrial carcinoma. Eur J Gynaecol Oncol. 1999;20:329-31.
16. Frumovitz M, Slomovitz BM, Singh DK, Broaddus RR, Abrams J, Sun CC, Bevers M, Bodurka DC. Frozen section analysis as predictors of lymphatic spread in patients with early-stage uterine cancer. J Am Coll Sur. 2004;199:388-93.
17. Roland PY, Kelly FJ, Kulwicki CY, Bitzer D, Curcio M, Orr JW. The benefits of a gynecologic oncologist: a pattern of care study for endometrial cancer treatment. Gynecol Oncol. 2004;93:125-30.
18. Pearl ML, Villella JA, Valea FA, DiSilvestro PA, Chalas E. Outcomes of endometrial cancer patients undergoing surgery with gynecology oncology involvement. Obstet Gynecol. 2002;100:724-9.
19. Loubeyre P, Undurraga M, Petignat P. Non-invasive modalities for predicting lymph node spread in early stage endometrial cancer. Surg Oncol. 2011;20: 102-8.
20. Sorosky JI. Endometrial cancer. Obstet Gynecol. 2008;111:436-47.
21. Ruangvutilert P, Sutantawibul A, Sunsaneevithayakul P, Boriboonhirunsarn D, Chuenchom T. Accuracy of transvaginal ultrasound for the evaluation of myometrial invasion in endometrial carcinoma. J Med Assoc Thai. 2004;87:47-52.
22. Yahata T, Aoki Y, Tanaka K. Prediction of myometrial invasion in patients with endometrial carcinoma: comparison of magnetic resonance imaging, transvaginal ultrasonography, and gross visual inspection. Eur J Gynaecol Oncol. 2007;28:193-5.
23. Merisio BR, Piantelli G, Rolla M, Melpignano M, Nardelli GB. Preoperative transvaginal ultrasonogra phy and intraoperative gross examination for assessing myometrial invasion by endometrial cancer. J Ultrasound Med. 2008;27:349-55.
24. Takac I. Transvaginal ultrasonography with and without saline infusion in assessment of myometrial invasion of endometrial cancer. J ultrasound Med. 2007;26:949-7.
25. Nasi F, Fiocchi F, Pecchi A, Rivasi F, Torricelli P. MRI evaluation of myometrial invasion by endometrial carcinoma. Comparison between fast-spin-echo T2w and coronal-FMPSPGR gadolinium-dota-enhanced sequences. Radiol Med (Torino). 2005;110:199-210.
26. Nakao Y, Yokoyama M, Hara K, Koyamatsu Y, Yasunaga M, Araki Y, Watanabe Y, Iwasaka T. MR imaging in endometrial carcinoma as a diagnostic tool for the absence of myometrial invasion. Gynecol Oncol. 2006;102:343-7.
27. Torricelli P, Ferraresi S, Fiocchi F, Ligabue G, Jasonni VM, Di Monte I, Rivasi F. 3-T MRI in the preoperative evaluation of depth of myometrial infiltration in endometrial cancer. AJR Am J Roentgenol. 2008;190:489-95.
28. Cabrita S, Rodrigues H, Abreu R, Martins M, Teixeira L, Marques C, Mota F, de Oliveira CF. Magnetic resonance imaging in the preoperative staging of endometrial carcinoma. Eur J Gynaecol Oncol. 2008;29:135-7.
29. Vasconcelos C, Felix A, Cunha TM. Preoperative assessment of deep myometrial and cervical invasion in endometrial carcinoma: comparison of

magnetic resonance imaging and histopathologic evaluation. J Obstet Gynaecol. 2007;27:65-70.
30. Suzuki R, Miyagi E, Takahashi N, Sukegawa A, Suzuki A, Koike I, Sugiura K, Okamoto N, Inoue T, Hirahara F. Validity of positron emission tomography using fluro-2-deoxyglucose for the preoperative evaluation of endometrial cancer. Int J Gynecol Cancer.2007;17:890-6.
31. Horowitz NS, Dehdashti F, Herzog TJ, Rader JS, Powell MA, Gibb RK, Grigsby PW, Siegel BA, Mutch DG. Preoperative evaluation of FDG-PET for detecting pelvic and para-aortic lymph node metastasis in uterine corpus cancer. Gynecol Oncol. 2004;95:546-51.
32. Kitajima K, Murakami K, Yammasaki E, Fukasawa I, Inaba N, Kaji Y, Sugimura K. Acuracy of 18F-FDG PET/CT in detecting pelvic and paraaortic lymph node metastasis in patients with endometrial cancer. Am J Roentgenol. 2008;190:1652-8.
33. Doering DL, Barnhill DR, Weiser EB, Burke TW, Woodward JE, Park RC. Intraoperative evaluation of depth of myometrial invasion in stage I endometrial adenocarcinoma. Obstet Gynecol. 1989;74:930-33.
34. Kucera E, Kainz C, Reinthaller A, Sliutz G, Leodolter S, Kucera H, Breitenecker G. Accuracy of intraoperative frozen-section diagnosis in stage I endometrial adenocarcinoma. Gynecol Obstet Invest. 2000;49:62-6.
35. Furukawa N, Takekuma M, Takahashi N, Hirashima Y. Intraoperative evaluation of myometrial invasion and histologic type and grade in endometrial cancer: diagnostic value of frozen section. Arch Gynecol Obstet. 2010;28:913-7.
36. Case AS, Rocconi RP, Straughn JM, Conner M, Novak L, Wang W, Huh WK. A prospective blinded evaluation of the accuracy of frozen section for the surgical management of endometrial cancer. Obstet Gynecol. 2006;108:1375-9.
37. Altintas A, Cosar E, Vardar MA, Demir C, Tuncer I. Intraoperative assessment of depth of myometrial invasion in endometrial carcinoma. Eur J Gynaecol Oncol. 1999;20:329-31.
38. Ghaemmaghami F, Aminimoghaddam S, Modares-Gilani M, et al. Assessment of gross examination and frozen section of uterine specimen in endometrial cancer patients. Arch Gynecol Obstet. 2010;282:685-89.
39. Schink JC, Lurain JR, Wallemark CB, Chmiel JS. Tumor size in endometrial cancer: a prognostic factor for lymph node metastasis. Obstet Gynecol. 1987;70:215-9.
40. Kumar S, Medeiros F, Dowdy SC, et al. A prospective assessment of the reliability of frozen section to direct intraoperative decision making in endometrial cancer. Gynecol Oncol. 2012;127:525-31.
41. Traen K, Holund B, Mogensen O. Accuracy of preoperative tumor grade and intraoperative gross examination of myometrial invasion in patients with endometrial cancer. Acta Obstet Gynecol Scand. 2007;86:739-41.
42. Papadia A, Azioni G, Brusaca B, Fulcheri E, Nishida K, Menoni S, Simpkins F, Lucci JA. Frozen section underestimates the need for surgical staging in endometrial cancer patients. Int J Gynecol Cancer. 2009;19:1570-3.
43. Nunns D, Williamson K, Swaney L, Davy M. The morbidity of surgery and adjuvant radiotherapy in the management of endometrial carcinoma. Int J Gynecol Cancer. 2000;10:233-38.

This page is intentionally left blank

Global Journal of Medical research: E
Gynecology and Obstetrics
Volume 14 Issue 4 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Evaluation of Rocuronium Associated to Acepromazine, Propofol and Isofluorane in the Anesthesia of Female Canines Submitted to Elective Ovaryhysterectomy

By Cinthya Dessaune Neves, Lukiya Silva Campos Favarato, José Dantas Ribeiro Filho, Luiz Gonzaga Pompermayer, Erotides Capistrano da Silva, Homero Martins Leite & Letícia Correa Santos
State University of North Fluminense, Brazil

Abstract- Rocuronium is a neuromuscular blocker with potential to provide advantages in surgeries that require short-term muscular relaxation. A sample consisting of 20 female canines submitted to ovaryhysterectomy was randomly divided into two equally sized groups. All the animals received acepromazine (0,1 mg.kg-1, IV), propofol (6,0 mg.kg-1, IV) and maintenance with isofluorane. Rocuronium (0,1 mg.kg-1, IV) was administered at the moment of the skin incision to the animals in the "rocuronium group" (GR). In the "control group" (GC), saline 0,9% was administered in equivalent volume of that used to rocuronium in double-blind study. Respiratory frequency (FR) and the minute-volume (VM) were measured before the beginning of the treatment (M0), 15 minutes after the administration of pre-anesthesic medication (M1), subsequent to induction (M2), and 2, 4, 6, 8, 10, 15, 20, 25, 30, 35 and 40 minutes after the administration of rocuronium or saline.

Keywords: *rocuronium, female canines, ovary hysteric tomy.*

GJMR-E Classification : *NLMC Code: WP 390, WJ 190*

Strictly as per the compliance and regulations of:

Evaluation of Rocuronium Associated to Acepromazine, Propofol and Isofluorane in the Anesthesia of Female Canines Submitted to Elective Ovaryhysterectomy

Cinthya Dessaune Neves [α], Lukiya Silva Campos Favarato [σ], José Dantas Ribeiro Filho [ρ], Luiz Gonzaga Pompermayer [ω], Erotides Capistrano da Silva [¥], Homero Martins Leite [§] & Letícia Correa Santos [χ]

Abstract- Rocuronium is a neuromuscular blocker with potential to provide advantages in surgeries that require short-term muscular relaxation. A sample consisting of 20 female canines submitted to ovaryhysterectomy was randomly divided into two equally sized groups. All the animals received acepromazine (0,1 mg.kg^{-1}, IV), propofol (6,0 mg.kg^{-1}, IV) and maintenance with isofluorane. Rocuronium (0,1 mg.kg^{-1}, IV) was administered at the moment of the skin incision to the animals in the "rocuronium group" (GR). In the "control group" (GC), saline 0,9% was administered in equivalent volume of that used to rocuronium in double-blind study. Respiratory frequency (FR) and the minute-volume (V_M) were measured before the beginning of the treatment (M0), 15 minutes after the administration of pre-anesthesic medication (M1), subsequent to induction (M2), and 2, 4, 6, 8, 10, 15, 20, 25, 30, 35 and 40 minutes after the administration of rocuronium or saline. The other variables were systolic blood pressure, heart rate and electrocardiography, measured at M0, M1, M2 and every 10 minutes from this last moment (M3, M4, M5 and M6). The collection of samples for blood gas analysis was performed at M0, M3 and M6. A questionnaire on the muscular relaxation was responded by the surgical staff right after the procedures. The results revealed that there were only a few alterations in the respiratory dynamics. A reduction on the V_M was noted 2 minutes from the administration of rocuronium in the GR group when compared to the GC group. Both protocols produced acidemia, respiratory acidosis and hyperchloremia. No significant alteration was noted in the circulatory dynamics. The muscular relaxation was considered superior in 80% of the animals in the group treated with rocuronium when compared to the non-treated group.

Index terms: rocuronium, female canines, ovary hysteric tomy.

Author α: PhD student at State University of North Fluminense and professor at Castelo College, FACASTELO, Castelo, ES. Adress: Coronel Borges n 01, Coronel Borges, Zip code: 29306-100-Cachoeiro de Itapemirim, Espírito Santo, Brazil.
e-mail: cinthyaneves@hot mail.com.
Author σ ρ: Professor PhD at Viçosa Federal University, Veterinary Department. Viçosa, MG.
Author ω: Professor PhD at Viçosa College Union, UNIVIÇOSA, Viçosa, MG.
Author ¥: MSc. Autonomous Veterinary Physician.
Author § χ: Post-graduation students at Viçosa Federal University, Veterinary Department, Viçosa, MG.

I. Introduction

Rocuronium bromide is a mono quartenary depolarizing muscle relaxant of intermediate-term action (Tranquili et al., 2007). Due to its low potency, rocuronium needs to be administered in high doses in order to achieve a sufficient number of molecules in the neuromuscular junction, setting its fast beginning of action (Hunter 1996, Tranquili et al., 2007). Rocuronium has minimum influence on the cardiovascular system and does not promote histamine release in horses, dogs, cats and humans (Xue et al., 1998, Neves, 2007). In high doses rocuronium may cause small increase in the heart rate and significant increase in blood pressure, probably by vagolytic action (Booij, 1997, Miranda et al., 2008). According to Dugdale et al. (2002), hypertension is transient and not followed by changes in heart frequency or rhythm.

This neuromuscular blocker presents good chemical stability and minimum liver biotransformation (McCoy et al., 1996). In men, rats and dogs, its inactive metabolite 17-deacetyl-rocuronium may be found in negligible amounts (Proost et al., 2000). This is probably an advantage over vecuronium, its precursor, which presents active metabolites that may contribute to the cumulative characteristics that follow clinical doses in healthy patients and in kidney insufficient patients. (Wright et al.,1994). The main elimination pathway is hepatic-biliary (Wierda & Proost, 1995) and occurs majorly in the first 24 hours, with 65% to 75% of the metabolites being eliminated in the feces and 9% to 25% in the urine (Proost et al., 2000).

Rocuronium has a specific cyclodextrin-based reverser, sugammadex, which favors its elimination through the kidney by making the drug more water soluble. The clearing time takes about two hours after its administration with no recurarization effect (Almeida & Locks, 2011).

The present experiment aimed to evaluate the utilization of a IV bolus 0,1 mg.Kg^{-1} rocuronium dose as a muscle relaxant in elective ovaryhysterectomy

surgeries of female canines, in order to verify possible alterations over the respiratory and circulatory dynamics as well as the muscle relaxation effects, with the objective to help these proposed surgical techniques.

II. Material and Methods

With the approval of the ethics committee of Viçosa Federal University (UFV) (process 09/2007), 20 female canines with indication to elective ovaryhysterectomy from the UFV Veterinary Hospital routine were selected, with the owner's consent, all of them included in anesthetic risk class I, according to the *American Society of Anesthesiologists* (Futema, 2002). The animals presented medium weight of 15 ± 0,9 kg and medium age of 4 ± 1,12 years. They were randomly distributed into two groups of 10 subjects. Complete physical examination, hemogram and biochemical profile were performed for all the animals in the pre-surgical period, in order to ensure their good state of health. Only healthy animals were used in this experiment.

After 6 hours of water abstinence and 12 hours of fasting, all the subjects received acepromazine (IV 0,1 $mg.kg^{-1}$) and 10 minutes from that point the cephalic vein was catheterized and anesthetic induction was perfomed using propofol 6,0 $mg.kg^{-1}$ IV along with orotracheal intubation. Shortly after that, inhalatory anesthesia was initiated using isofluorane diluted in pure oxygen, in an anesthetic circuit with partial gas reinhalation with a calibrated vaporizer. The administered isofluorane concentration was the one necessary to maintain the anesthetic level compatible to the surgical procedure.

Immediately before the skin incision, the subjects in the GR group received rocuronium 0,1 $mg.kg^{-1}$ IV, while the subjects in the GC group received NaCl 0,9% solution in the same volume as the rocuronium solution.

The following variables were registered: respiratory frequency (FR), by the observation of the chest movement; minute-volume (V_M), by Wright's ventilometer[1]; systolic blood pressure (SBP), by a non-invasive method using a vascular doppler[2]; heart rate (HR) using a oximeter[3], with its sensor initially placed in the inguinal fold and in the tongue after anesthetic induction;

The electrocardiography, using a computer program in DII derivation[4]; arterial blood gasometry, performed in three samples collected from the femoral artery and measured by a portable blood gas analyzer[5]; blood electrolytes analysis (Cl^-, iCa,, Na^+, K^+) by the colorimetric method[6]; and muscular relaxation by questionnaire (Fig. 1).

Did the anesthetic protocol used in this surgery somehow interfered in the surgical procedures? Chose the option(s) that better represent your opinion about it.
( ) The muscular relaxation was equal to the commonly found in HOV anesthetic states for elective ovaryhysterectomy; ( ) The muscular relaxation was higher than the commonly found in HOV anesthetic states for elective ovaryhysterectomy; ( ) It was noted no difference regarding the easiness in exposing the suspensory ovaries ligaments; ( ) It was noted higher easiness in exposing the suspensory ovaries ligaments.

Figure 1 : Questionnaire on the muscular relaxation applied to the surgical staff after the experimental period

The respiratory frequency (FR) and the minute-volume (V_M) were measured before MPA (M0), 15 minutes after MPA (M1), and 2, 4, 6, 8, 10, 15, 20, 25, 30, 35 and 40 minutes after the administration of rocuronium or saline solution. The systolic blood pressure (SBP), heart rate (HR) and electrocardiography were measured before MPA (M0), 10 minutes after MPA (M1), after the setting of surgical anesthesia (M2), and, from that moment on, every 10 minutes (M3, M4, M5 e M6). A 40-minute fixed transoperative period was set for the surgeries. Blood samples were collected for gasometry at M0, M3 and M6. The anion gap $(Na^+ + K^+)$-$(Cl^- + HCO^-_3)$ and the measurable strong ion quotient $(Na^+ + K^+)$-(Cl^-) were obtained by calculation. After data collection was conducted statistical analysis, between groups was used for parametric analysis of variance (ANOVA) data and non-parametric Wilcoxon test data. For comparison along time within each group was used for parametric data and the Tukey test for non-parametric data, the Dunn's test with $p<0,05$. As for the muscular relaxation, descriptive analysis was performed.

III. Results and Discussion

The simultaneous evaluation of respiratory frequency (FR) and minute-volume (V_M) (Fig. 2) revealed similar responses in both GR and GC groups. After sedation with acepromazine, a significant decrease in these variables was noted due to the sensibility reduction of the chemoceptors in the carotid and aortic sheath and in the central nervous system facing the changes of carbon dioxide concentration in the blood (Cortopassi & Fantoni, 2002).

[1] Ferraris Mark 8 - Wright Ventilometer 100L.
[2] Portable Vascular Doppler – Medmega Industry and Medical Equipment Ltd.
[3] NPB 290 – Nellcor Puritan Bennett Europe BU.
[4] Computer ECG Acquisition Module –Brazilian Electronic Technology TEB.
[5] Portable Clinical Blood Gas Analyzer I –STAT.
[6] CELM E-225-D- Company for Modern Laboratory Equipment -São Paulo – Brazil.

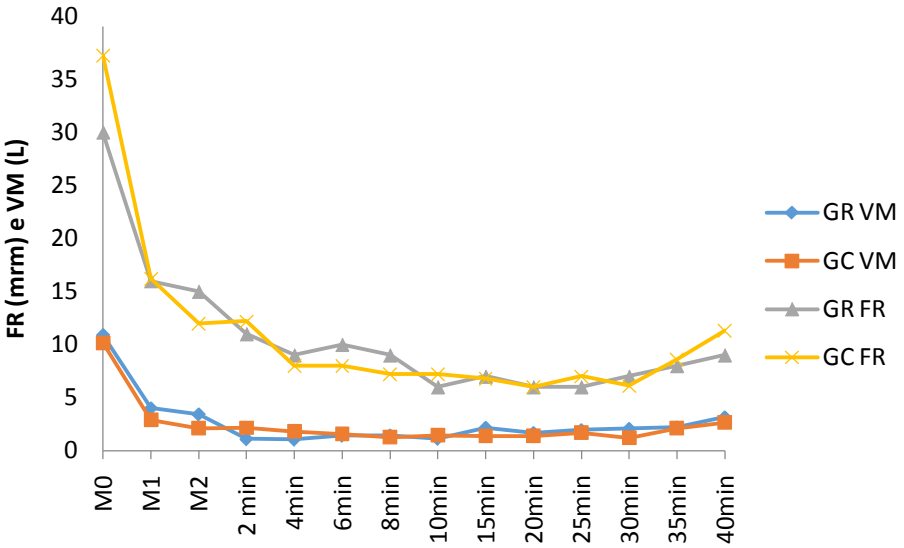


Figure 2 : Representation of FR medium values (mrm) and V_M medium values (l) featured by anesthetized female dogs with acepromazine, propofol and isofluorane, treated with rocuronium (GR) or not (GC)

*Medium values followed by equal capital letters in columns do not differ between them according to ANOVA or the non-parametric Wilcoxon test. Medium values followed by equal lowercase letters in lines do not differ between them according to Tukey's parametric test or the non-parametric Dunn's test (p<0,05). *AG (Ânion gap); **DIFm (difference between measured strong ions).*

After the administration of propofol and isofluorane for anesthetic maintenance, respectively, it was noted decrease in FR and V_M, in a dose-dependent manner, according to Short & Bufalari (1999) and Evers et al., (2006). A 0,1mg.kg^{-1} rocuronium dose did not promote respiratory arrest in any of the subjects in the GR group, nor significant reduction in FR. However, V_M was significantly and transiently affected in this group, only two minutes after the administration of rocuronium. This effect was no longer noted four minutes from the administration, demonstrating a slight reduction in intercostal muscles and/or diaphragm activities. This also demonstrates that other muscular groups were influenced by rocuronium as well, since respiratory arrest may happen when a neuromuscular blocker is administered, in a dose-dependent manner. There is a sequence of muscular paralysis beginning by facial and tail muscles, moving towards limbs and neck, followed by phonation and swallowing muscles, abdominal muscles, intercostals muscles and finally the diaphragm. Therefore, when diaphragm contraction ceases, it means that other muscular groups are under the effect of the muscular relaxant (Fuller, 2001).

The sequence of events in the recovery occurs in the reverse order of the neuromuscular blocking (Hall, 1971). Considering all that, our theory is that the dose utilized in this experiment is very close to the ideal one, if not actually ideal.

Systolic blood pressure behaved in the same manner for both groups (Tab.1). However, for the GR group, it was noted a progressive reduction nonsignificat in this variable until M4, a point where reestablishment of the values start, which may be assigned to the potentiating effect of the neuromuscular blockers on other central depressing drugs (Duke, 1995, Sano et al., 2003). The medium values of the heart rate remained stable during the whole anesthetic period in both evaluated groups, demonstrating that the protocols used in this experiment did not promote any effects on this variable. According to Xue et al. (1998) and Neves (2007), rocuronium has minimum influence on the cardiovascular system and does not lead to histamine release, which causes vascular dilation and decrease in blood pressure. Changes in pressure lead to reflex tachycardia or rhythm alterations, which were not observed in this study. Some the subjects in both experimental groups showed sinus rhythm without electrocardiogram changes during the anesthetic period.

Table 1 : Medium values of systolic blood pressure (mmHg), heart rate (beat/min) pO_2 (mmHg), pCO2 (mmHg), pH, $cHCO^-_3$ (mmol/L) , $ctCO_2$ (mmol/L), cBase (mmol/L), Cl (mEq/L), Na (mEq/L), K (mEq/L), iCa (mEq/L), anion gap (mEq/L) and difference between measured strong íons (mEq/L) featured by female dogs anesthetized with acepromazine, propofol and isofluorane, treated with rocuronium (GR) or not (GC)

Groups		M0	M1	M2	M3	M4	M5	M6
GR	PAS	129^{A,a}	107^{A,ab}	95^{A,bc}	77^{A,c}	74^{A,c}	83^{A,bc}	99^{A,bc}
GC	PAS	145^{A,a}	143^{A,a}	118,5^{A,ab}	111,5^{A,ab}	104,5^{A,ab}	99^{A,ab}	117^{A,ab}
GR	FC	115,5^{A,a}	103,2^{A,a}	114,1^{A,a}	103,8^{Aa}	102^{A,a}	105^{A,a}	108,3^{A,a}
GC	FC	136,7^{A,a}	103,3^{A,b}	117,6^{A,ab}	107^{A,ab}	102^{A,b}	94,4^{A,b}	109,7^{A,ab}
GR	SpO_2	93,1^{A,bc}	92,8^{A,c}	96,4^{A,ab}	96,3^{A,ab}	97^{A,a}	97^{A,a}	97^{A,a}
GC	SpO_2	92,8^{A,c}	93,4^{A,bc}	99^{A,a}	97^{A,ab}	97^{A,ab}	96,3^{A,bc}	95,7^{A,abc}
GR	$pO2_{(a)}$	84,5^{A,b}			437,^{A,a}			325,8^{A,ab}
GC	$pO2_{(a)}$	74,3^{B,b}			387,^{A,a}			367,4^{A,a}
GR	$pCO_{2)}$	31,03^{A,c}			79,8^{A,a}			63,83^{A,b}
GC	$pCO_{2)}$	31,06^{A,b}			69,1^{A,a}			66,43^{A,a}
GR	$pH_{(a)}$	7,39^{A,a}			7,1^{A,c}			7,17^{A,b}
GC	$pH_{(a)}$	7,39^{A,a}			7,14^{A,b}			7,17^{A,b}
GR	$cHCO^-_{3(a)}$	18,9^{A,b}			24,4^{A,a}			22,5^{A,a}
GC	$cHCO^-_{3(a)}$	18,9^{A,b}			23,2^{A,a}			21^{A,a}
GR	$ctCO2_{(a)}$	19,8^{A,b}			26,7^{A,a}			24,4^{A,a}
GC	$ctCO2_{(a)}$	19,9^{A,b}			25,4^{A,a}			23,1^{A,a}
GR	$cBase_{(a)}$	-6,1^{A,a}			-5,3^{A,a}			-6^{A,a}
GC	$cBase_{(a)}$	-6^{A,a}			-5,8^{A,a}			-6,7^{A,a}
GR	Cl^-	122,5^{A,a}			118^{A,a}			108,3^{A,a}
GC	Cl^-	117,4^{A,a}			127^{A,a}			125,4^{A,a}
GR	Na^+	143^{A,a}			141^{A,ab}			141^{A,b}
GC	Na^+	142^{A,a}			141^{A,a}			140^{A,a}
GR	K^+	3,82^{A,a}			3,88^{A,a}			3,91^{A,a}
GC	K^+	3,75^{A,a}			3,76^{A,a}			3,7^{A,a}
GR	iCa	1,33^{A,a}			1,33^{A,a}			1,24^{A,a}
GC	iCa	1,19^{A,a}			1,29^{A,a}			1,25^{A,a}
GR	*AG	5,21^{A,a}			2,22^{A,a}			13,66^{A,a}
GC	*AG	9,86^{A,a}			5,79^{A,b}			-0,51^{A,ab}
GR	**DIFm	24,12^{A,a}			26,6^{A,a}			36,22^{A,a}
GC	**DIFm	28,73^{A,a}			17,3^{A,a}			20,72^{A,a}

*Medium values followed by equal capital letters in columns do not differ between them according to ANOVA or the non-parametric Wilcoxon test. Medium values followed by equal lowercase letters in lines do not differ between them according to Tukey's parametric test or the non-parametric Dunn's test ($p<0,05$). *AG (Ânion gap); **DIFm (difference between measured strong ions).*

Evaluation of the acid-base profile revealed that $pCO_{2(a)}$ values were not significantly different in both groups during the whole experimental period, but it was noted an increase between M0 and M3 in both groups, stating post-induction respiratory acidosis. It is known that propofol and isoflourane promote respiratory depression that leads to heart rate decrease and hypoventilation, as previously seen. By its turn there may be $pCO_{2(a)}$ increase (Ferro et al., 2005), an independent respiratory variable.

The analysis of $pH_{(a)}$ medium values indicate acidosis, once this is a dependent variable that may be altered by base concentration (metabolic component) and by $pCO_{2(a)}$ (respiratory component), being inversely proportional to this last one. Since all animals in GR and GC group showed respiratory acidosis and no alterations in base concentrations it is possible to allege that the main factor was the $pCO_{2(a)}$ increase, corroborating Ferro et al., (2007).

Increase in $pCO_{2(a)}$ lead to rising bicarbonate levels, since they are interdependent. Each 10mmHg increase in $pCO_{2(a)}$ is equivalent to an increase of 1,5mEq.L$^-$ in bicarbonate levels (Tab.1). The CO_2 concentration behaved in the same manner as bicarbonate, since it maintained 1 to 2 mEq.L$^-$ higher than bicarbonate (it also represents the CO_2 concentration dissolved in plasma) (DiBartola, 2006; Tranquili et al., 2007). While CO_2 chemical metabolites elimination processes take place in the organism, CO_2 associates to water (H_2O) and forms carbonic acid

(H_2CO_3), which, by its turn, will form HCO^-_3 and H^+ ions, this last one's production higher since carbonic acid is weak and suffers low dissociation in bicarbonate. In addition, according to DiBartola (2006), $pCO_{2(a)}$ increase may be responsible for 50% of the blood bicarbonate level variation. Lately, the reason for this increase will be due to an attempt of kidney adaptation and respiratory acidosis, since kidneys control H^+ ions concentration in the organism excreting either acid or basic urine (DiBartola 2006).

The ions showed physiological medium values and did not differ between them (Tranquili et al., 2007), except by chloride, which presented itself elevated indicating hyperchloremia. It is suggested that the NaCl 0,9 % may have caused this effect (DiBartola 2006). The alterations were transient and similar in both groups, and did not influence in the recovery/deambulation of the subjects.

The response to the questionnaire on the muscular relaxation revealed that 80% of the animals treated with rocuronium showed appropriate relaxation and easiness in the maneuvers to expose the viscera, meanwhile, in the control group, this response was achieved in only 30% of the subjects.

From these observations, we are able to imply that abdominal musculature presented itself easy to manipulate, confirming the benefits mentioned by Hall (1971), who stated that the use of small doses of muscular relaxant might favor several surgical procedures in superficial anesthetic level.

IV. Conclusion

The administration of a 0,1 $mg.kg^{-1}$ rocuronium dose led to little alteration in the respiratory dynamics as well as to increase in the muscular relaxation level, thus presenting important benefits to surgical performance. Both utilized anesthetic protocols did not promote significant changes in heart rate, systolic blood pressure and electrocardiogram. Also both protocols promoted acidemia, respiratory acidosis and transient hyperchloremia, with no prejudice to anesthetic recovery.

V. Acknowledgment

The contribution of Dutra Rocha Nina for their availability and dedication to work.

References Références Referencias

1. Almeida M.C.S. & Locks G.F. 2011. Sugammadex®: Novos Questionamentos sobre a Reversão. Revista Brasileira de Anestesiologia 61 (6): 829-831.
2. Booij L.H.D.J. 1997. Neuromuscular transmission and its pharmacological blockade. Pharmacology World Science 19:1-12.
3. Cortopassi S.R.G. & Fantoni D.T. 2002. Medicação Pré-Anestésica. In: Cortopassi S.R.G. & Fantoni D.T. (Eds) Anestesia em Cães e Gatos. 1ª ed. Roca, Rio de Janeiro151-158p.
4. DiBartola S.P. 2006. Introduction to Acid-Base Disorders. In: DiBartola S.P. (Eds) Fluid, Electrolyte and Acid-Base Disorders In Small Animal Practice.1ªed. Elsevier, Missouri. 229-251p.
5. Dugdale H.A., Adams W.A. & Jones, R.S. 2002. The clinical use of the neuromuscular blocking agent rocuronium in dogs. Veterinary Anaesthesia and Analgesia 29(1):49-53.
6. Duke T. 1995. A new intravenous anesthetic agent: Propofol. Canadian Veterinary Journal 36(3):181-183.
7. Evers A.S., Crowder M. & Balser J.R. 2006. Anestésicos Gerais. In: Godman & Gilman As Bases Farmacológicas da Terapêutica. 11ªed. McGraw Hill, Rio de Janeiro 305-329.
8. Ferro P.C., Nunes N., Carareto R., Nishimori C.T.D., Paula D.P., Sousa P.S.P. & Thiesen R. 2007. Efeitos de diferentes frações inspiradas de oxigênio sobre a dinâmica respiratória em cães submetidos à infusão contínua de propofol e mantidos em ventilação espontânea. Brazilian Journal Veterinary Research of Animal Science 44:30-37.
9. Ferro P.C., Nunes N., Paula D.P., Nishimori C.T.D., Conceição E.D.V., Guerrero P.N.H. & Arruda L.M. 2005. Variáveis fisiológicas em cães submetidos à infusão contínua de diferentes doses de propofol. Ciência Rural 35(5):1103-1108.
10. Fuller, R.B. 2001. Fármacos Bloqueadores Neuromusculares. In: Muir III, W.W.; Hubbell, J.A.E.; Skarda, R.T.; Bednarski, R.M. Manual de Anestesia Veterinária, 3ed. Porto Alegre: Artmed:149-158.
11. Futema F. 2002. Avaliação pré-anestésica. In: Fantoni DT, Cortopassi, SRG, eds. Anestesia em cães e gatos. 1st ed. São Paulo: ROCA, 59-63.
12. Hall L.W. 1971. Relaxation of The Skeletal Muscles During Anesthesia. In: Wright's Veterinary Anesthesia and Analgesia. 6ªed. Bailliére Tindall, Baltimore 385-423.
13. Hunter J.M. 1996. Rocuronium: the newest aminosteroid neuromuscular blocking drug. British Journal of Anaesthesia 76:481-483.
14. McCoy E.P., Mirakhur R.K., Maddineni V.R., Wierda J.M.K.H. & Proost J.H. 1996. Pharmacokinetics of rocuronium after bolus and continuous infusion during halotane anaesthesia. British Journal of Anaesthesia 76:29-33.
15. Miranda, L.C.G, Barrucand L., Tsai, J.C., Verçosa, N. 2008. Estudo comparativo entre uma e duas doses efetivas (DE_{95}) de rocurônio para a intubação traqueal. Revista Brasileira de Anestesiologia 58(3).

16. Neves C.D. 2007. Rocurônio como miorrelaxante em cirurgias ortopédicas de cães. Monografia de Especilização em Clínica e Cirurgia Veterinárias, Universidade Federal de Viçosa, Minas Gerais. 31p.
17. Proost J.H., Eriksson L.I., Mirakhur R.K., Roest G. & Wierda J.M.K.H. 2000. Urinary, biliary and faecal excretion of rocuronium in humans. British Journal of Anaesthesia. 85(5):717-723.
18. Sano, T., Nishimura, R., Mochizuki, M. 2003.Clinical usefulness of propofol as na anesthetic induction agent in dogs and cats. Journal of Veterinary Medicine Science, 65(5):641-643.
19. Short C.E. & Bufalari A. 1999. Propofol anesthesia. The Veterinary Clinics of North American – Small Animal Practice 29(3):747-778.
20. Tranquili W.J., Thurmon J.C. & Grimm K.A. 2007. Lumb & Jones' Veterinary Anesthesia and Analgesia. 4ªed. Blackwell Publishing, Iowa 1096p.
21. Wierda J.M.K.H. & Proost J.H. 1995. The pharmacokinetics and the pharmacokinetics-dynamic relatioship of rocuronium bromide. Anaesthetic Pharmacology Review 3:192-201.
22. Wright P.M.C., Hart P., Lau M., Swharma M.L., Gruenke L. & Fisher D.M. 1994. Cumulative characteristics of atracurium and vecuronium. A simultaneous clinical and pharmacokinetic study. Anesthesiology 81:59-68.
23. Xue F.S., Liao S.Y., Tong J.H., Liu J.H., Na G. & Luo L.K. 1998. Dose-response and time-course of the effect of rocuronium bromide during sevoflurane anaesthesia. Anaesthesia 53:25-30.

Global Journal of Medical research: E
Gynecology and Obstetrics
Volume 14 Issue 4 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Effects of Adding Human Follicular Fluid and Pentoxifylline on IUI Outcome of Asthenozoospermic Patients

By Saad S. Al-Dujaily, Sabah M. Hussein & Sahar S. Raheem
University of Baghdad, Iraq

Abstract- *Background:* Although there are many motility stimulants have been found to play an important role in improving the active sperm motility and morphologically normal sperm (MNS) percentages when added in vitro with the culture medium for semen, the results of pregnancy rate following intra-uterine insemination (IUI)still not exceed 20%.

Objective: This study is a trial to improve the results of pregnancy rate following IUI by adding the follicular fluid (FF), and pentoxifylline (PX) to the culture medium used for preparation of asthenozoospermic semen samples.

Subjects, Material and methods: Ninety five infertile couples were involved in the current study (asthenozoospermic male partners with intact spouses) divided into three groups according to the sperm stimulants which were used for in vitro sperm activation followed by intrauterine insemination technique. Group one was FF-treated semen samples and consists of 40 couples, group two was FF+PX-treated semen samples and consists of 25 couples and lastly, group three was Ham's F-12 culture medium as a control group and consists of 30 couples. All the couples were followed up for determining outcome of pregnancy after IUI was done.

GJMR-E Classification : *NLMC Code: WJ 834, WJ 166*

Strictly as per the compliance and regulations of:

Effects of Adding Human Follicular Fluid and Pentoxifylline on IUI Outcome of Asthenozoospermic Patients

Saad S. Al-Dujaily [α], Sabah M. Hussein [σ] & Sahar S. Raheem [ρ]

Abstract- Background: Although there are many motility stimulants have been found to play an important role in improving the active sperm motility and morphologically normal sperm (MNS) percentages when added *in vitro* with the culture medium for semen ,the results of pregnancy rate following intra-uterine insemination (IUI)still not exceed 20% .

Objective: This study is a trial to improve the results of pregnancy rate following IUI by adding the follicular fluid (FF),and pentoxifylline (PX) to the culture medium used for preparation of asthenozoospermic semen samples.

Subjects, Material and methods: Ninety five infertile couples were involved in the current study (asthenozoospermic male partners with intact spouses) divided into three groups according to the sperm stimulants which were used for *in vitro* sperm activation followed by intrauterine insemination technique. Group one was FF-treated semen samples and consists of 40 couples, group two was FF+PX-treated semen samples and consists of 25 couples and lastly, group three was Ham's F-12 culture medium as a control group and consists of 30 couples. All the couples were followed up for determining outcome of pregnancy after IUI was done.

Results: Out of the all 95 couples, 22 pregnancies (23.15%) were resulted following IUI. The best results were obtained by group one (FF treated group) in which out of 40 cases enrolled in this group, 12 pregnancies (30%) were resulted following IUI. Regarding group two (FF+PX treated group), out of 25 cases enrolled in this group, 5 women (20%) were pregnant following IUI. Regarding group three (Ham's F-12 treated group), out of 30 cases enrolled in this group, 5 women (16.67%) were pregnant following IUI.

Conclusion: Adding FF to culture media for *in vitro* sperm activation was associated with a higher pregnancy rate following IUI than other treated culture media.

I. Introduction

Infertility is the inability of a sexually active non-contraceptive couple to achieve pregnancy through one year [1,].It is a relatively common condition affecting approximately one in ten of the population. Infertility is either primary, when no pregnancy has ever occurred, or secondary, where there has been a pregnancy, regardless of the outcome. About 67 – 71% and 29 –33% of patients have primary and secondary infertility, respectively [2]. In half of these cases, a male factor is involved, making defective sperm function the largest single, defined cause of human infertility [3]. Asthenozoospermia is one of the major causes of infertility or reduced fertility in men [4].

In vitro studies in human showed that spermatozoal accumulation into follicular fluid (FF) is significantly higher than into simple medium and that chemoattractant effect of fluid from an individual follicle correlates with the fertilizability of the egg from the same follicle [5,6].

Follicular fluid can also alter the physiology and behavior of spermatozoa by accelerating capacitation of the cell to induce sperm motility, increasing AR and signaling interaction between sperm and oocyte during fertilization. This is due to FF components which are a low molecular hydrophobic compounds, platelet activating factor and progesterone[7,8] .

On the other hand, certain metabolic stimulants induce sperm capacitation and AR via changes in the values of cyclic adenosine monophosphate (cAMP) and intracellular calcium ($[Ca^{2+}]i$). Examples of these stimulators are pentoxifylline (PX) and L-carnitine (LC) [9].

Pentoxifylline is a methylxanthine derivative which inhibits phosphodiesterase, thereby elevating the concentrations of intracellular cAMP and/or cyclic guanosine monophosphate (cGMP). It is used in ART to enhance the function of inherently poor quality spermatozoa from oligoasthenozoospermic patients[10]. Other study does not suggest any increase in teratogenicity or evidence of congenital malformations in pregnancies following the using of PX in the media of IVF cycles[9].

The Artificial Insemination (AI) is the first option treatment for infertile couples with cervical factor subfertility, mild to moderate male subfertility and unexplained infertility[11].IUI, with or without controlled ovarian hyperstimulation, is one of the treatment modalities offered most often to subfertile couples because it is less stressful, invasive and expensive than other interventions such as *in vitro* fertilization and gamete intrafallopian transfer[12-13]. The goal of the present proposal is to examine the effects of adding human FF alone or in combination with PX on the

Author α ρ: High Institute of Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrain University.
e-mail: aldujaily8@yahoo.com
Author σ: Alkindy Medical College/University of Baghdad.

medium prepared for *in vitro* sperm activation in intrauterine insemination (IUI) outcome.

II. Materials and Methods

This study was conducted at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University through the period from September 2012 to June 2013.

Clinical examination was performed by a Consultant Urologist in charge of Male Infertility Unit in the Institute including presence or absence of varicocele, cryptorchidism, hydrocele, hernia and others. Alcohol consumption and smocking habits were also reported. Those patients were classified into three subgroups according to WHO criteria of seminal fluid analysis [(1)].In this study ninety five infertile couples (asthenozoospermic male partners with intact spouses) divided into three groups according to the sperm stimulants which were used for *in vitro* sperm activation followed by intrauterine insemination techniques:

1. Group I: FF-treated semen samples. Include 40 couples.
2. Group II: FF and PX-treated semen samples. Include 25 couples.
3. Group III: Ham's F-12 culture medium as control group. Include 30 couples.

a) Preparation of follicular fluid for in vitro sperm activation

Patients underwent oocyte retrieval 36 hr after hCG administration by transvaginal ultrasound guided follicular aspiration. The first aspirated follicle was usually the one with the largest diameter, and the first follicle allowed the collection of fluid without contamination of flushing medium. Specimens that were contaminated with blood were discarded .After removal of potential oocytes for treatment , the fluid was centrifuged (500 × *g*) to eliminate granulosa cells and to monitor the contamination of red blood cells and the clear supernatant was divided into aliquots and frozen at – 20 C^{o}[(14)].The frozen samples were thawed and analyzed [(15)]. Solution of 20% hFF was prepared by the addition of 0.2 ml of hFF to 0.8 ml of Ham's F-12 medium.

b) Investigations to assess the female reproductive status

Physical examination was done following the case history. The investigations were performed to assess the normal menstruation and ovulation. Hormonal assay was done to the female namely; early follicular estradiol (E_2), FSH and LH level with serial vaginal ultra-sonographic examination for monitoring of follicular development and endometrial thickness. Their tubal patency were checked from the previous cycles by hysterosalpingography.

c) Intra-Uterine Insemination Technique

IUI was performed when 0.5 – 0.75 ml of prepared semen was aspirated into 1 ml syringe attached to endo-cervical IUI catheter (Gynetics, Belgium). In IUI room, the spouse was prepared for insemination in lithotomic position. Non lubricated Cusco's speculum was placed in the vagina to visualize the uterine cervix[(16,17)]. The specimen was slowly ejected from the syringe with gentle grasping of the cervical lips to prevent escape of the solution out of the cervix then after few minutes the catheter was removed slowly. The patient remains on supine position for 30 minutes then she allowed leaving the theater. Following the day of insemination the patient instructed to take progesterone tablets (Duphaston ® 10 mg/day) for two weeks as a luteal phase support. No use of antibiotic as it was detected that incidence of pelvic infection following IUI has been estimated to be less than o.5% [(17)]. The diagnosis of pregnancy was done either by biochemical analysis (the detection of βhCG in blood after two weeks of insemination) or by sonographic examination to assess the pregnancy status

d) Statistical analysis

The data of this study was analyzed by using SPSS (Statistical package for social sciences) versions 16 and Microsoft excel 2013. Numeric variables were expressed as mean ±SEM whereas nominal variables were expressed as numbers and percentage. The analysis was done by using one way analysis of variance (ANOVA) to compare among different groups of *in vitro* sperm activation techniques. Also, descriptive analysis was done by using Chi-square depending on the nature of the data. Differences between values of means were considered statistically significant at ($P<0.05$) [(18)].

III. Results

a) Comparison of certain sperm function parameters after activation among IUI groups

In Table 1, there was no significant ($P>0.05$) difference in the mean of sperm concentration, active sperm motility (grade A, B and A+B) and the percentage of MNS between semen samples activated *in vitro* by Ham's F12 medium only (control) and other treated media.

Table 1 : Comparison of certain sperm function parameters among IUI groups following *in vitro* activation

Certain sperm function Parameters		Group1 IUI-FF-treated group (n=40)		Group 2 IUI-PX+FF-treated group (n=25)		Group 3 IUI-Hams.F-12 treated group (n=30)		P value
Sperm Concentration ($X10^6$/ml)		18.00	1.70	24.08	2.85	23.67	2.94	0.121
Active sperm motility (%)	Grade A	25.63	2.70	27.20	3.47	25.93	3.33	0.937
	Grade B	50.25	2.73	50.40	2.94	44.83	3.75	0.381
	Grade A+B	75.63	2.47	77.60	3.54	70.77	4.56	0.399
Morphologically normal sperm (%)		60.38	2.14	57.20	2.62	60.20	3.96	0.721

Values are expressed as mean ± SE. ANOVA Test
PX: pentoxifylline
FF: follicular fluid

b) The results of pregnancy rate after in vitro sperm activation

Tables 2 and 3 reveal the effect of three different sperms stimulants (FF, FF+PX and Ham's F-12) on pregnancy rate and percentage (%) following IUI. Out of the all 95 couples, 22 pregnancies (23.15%) were resulted following IUI. The best results were obtained by group 1(FF treated group) in which out of 40 cases enrolled in this group, 12 pregnancies (30%) were resulted following IUI. Regarding group 2 (FF+PX treated group),out of 25 cases enrolled in this group, 5 women (20%) were conceived following IUI. Regarding group 3 (Ham's F-12 treated group), out of 30 cases enrolled in this group, 5 women (16.67%) were conceived following IUI.

There was a significant ($P<0.05$) difference in the rate of pregnancy between group 1 and group 2 (table 4-21), while there was no statistically significant ($P>0.05$) difference in the rate of pregnancy between group 1 and 3.

Table 2 : Comparison of pregnancy rate following IUI after *in vitro* sperm activation between group 1 and group 2

Status of pregnancy		Pregnancy Rate			
		Group 1(FF treated)		Group 2 (FF+PX treated)	
		No.	%	No.	%
Pregnant	Yes	12	30	5	20
	No	28	70	20	80
	Total	40	100	25	100
X2 = 9.474					
DF = 1	P < 0.05				

Table 3 : Comparison of pregnancy rate following IUI after *in vitro* sperm activation between group 1 and group 3

Status of Pregnancy		Pregnancy rate			
		Group 1 (FF treated)		Group 3(Ham's F-12 treated)	
		No.	%	No.	%
Pregnancy	Yes	12	30	5	16.67
	No	28	70	25	83.33
	Total	40	100	30	100
X2 = 1.656					
DF = 1	P >0.05				

IV. Discussion

The current study demonstrates that IUI by a sperm washed *in vitro* using a medium supplemented with FF results in a higher pregnancy rates per cycle (30%) in asthenozoospermic patients than by using Ham's F-12 medium alone (16.6%) even the results of certain sperm function parameters after in vitro preparation did not reveal any significant between them. This finding can be explained by the fact that the extent of hyperactivated motility produced by FF is positively correlated with the extent of zona binding, the acrosome reaction, zona-free oocyte penetration and fertilizing capacity *in vitro*[19]. Additionally, human FF is an efficient capacitating agent and only the capacitated sperm are able to migrate towards the ovum site, under thermotaxis and chemotaxis stimuli [20,21] .

It has been reported that FF may modulate spermatozoa function to be indicative of pregnancy outcome [22], this fact can verify and confirms the original results.

The contents of FF also play a crucial role in the pregnancy outcome. A lower activity of the conversion enzymes, ovarian 11 beta hydroxy steroid dehydrogenase (HSD11β) which is found in FF interconverts 'active' cortisol to 'inert' cortisone, have been associated with positive pregnancy outcomes for patients undergoing ART [23].

In this study, there was a significant ($P < 0.05$) difference in the rate of pregnancy between IUI- FF treated group (pregnancy rate was 30%) and IUI-FF+PX treated group (pregnancy rate was 20%).This finding suggests that IUI following *in vitro* sperm activation by FF containing culture media is associated with a higher pregnancy outcome than FF+PX containing media. Moreover, the proper time of IUI that performed enclosed with ovulation time can lead to increase in pregnancy rates, and is best seen in patients with pure andrological indication. At the same time, careful selection of patients might play part in achieving success with IUI. Furthermore, the use of fertility drugs like gonadotropins in the controlled ovarian stimulation followed by IUI provide better pregnancy rate [24] than the using of other ovarian stimulants.

The current study found that pregnancy rate of IUI following FF+PX (20%) was approximately similar to the results of Al-Dujaily *et al* (20.8%) using PX alone[25]. Both results had no significant different in pregnancy outcome than the pregnancy outcome following *in vitro* sperm activation with FF alone. This suggest that there is no further advantage to add PX to FF-containing culture media for the enhancement of active sperm motility, morphologically normal sperm percentages or pregnancy rate following IUI.

Thus the present study concluded that adding the FF to medium used for IUI can enhance the results of pregnancy rate of infertile couples.

References Références Referencias

1. WHO. Laboratory manual for the examination and processing of human semen.5th ed., Geneva, Switzerland. 2010.
2. Dohle GR. Male infertility in cancer patients: Review of the literature.Int.J.Urol.2010; 17(4):327-331.
3. Aitken RJ, Baker MA, Nixon B. New insights into sperm physiology and pathology. Hand book Pharmacol. 2010; (198):99-115.
4. Steiber A., Kemer J. and Hoppelm C. Carnitine: a nutritional, biosynthetic and functional perspective. Mol. Aspects Med. 2004; 25(5-6):455-73.
5. Revelli LD, Casano S, Molinaril E, Massobrio M and Rinaudo P. Follicular fluid content and oocyte quality: from single biochemical markers to metabolomics. Reprod Biol and Endocr .2009;7:7-40.
6. Leroy JL, Vanholder T, Delanghe JR, *et al.* Metabolic changes in follicular fluid of the dominant follicle in high-yielding dairy cows early postpartum. Theriogenology 2004; 62:1131-1143.
7. Wen X, Li D, Tozer AJ, *et al.* Estradiol, progesterone, testosterone profiles in human follicular fluid and cultured granulosa cells from luteinized pre-ovulatory follicles. Reprod Biol Endocrinol.2010; 8:117.
8. Guidobaldi HA, Teves ME, Unates DR, *et al.* Progesterone from the cumulus cells is the sperm chemoattractant secreted by the rabbit oocyte cumulus complex. PL J .2008; 3:30-40.
9. Abdelrazik H, Agrawal A. L-carnitine and assisted reproduction. Arch Med Sci .2009; 5: 43-47.
10. Bjorndahl L, Mohammadieh M, Pourian M, *et al.* Contamination by seminal plasma factors during sperm selection. J Androl.2005;2:170-173.
11. Verhulst SM, Cohlen BJ, Hughes E, Te Velde E, Heineman MJ. Intra-uterine insemination for unexplained subfertility. Cochrane Database of Systematic Reviews .2006; (4).
12. Casadei L, Zamaro V, Calcagni M, *et al.* Homologous intrauterine insemination in controlled ovarian hyperstimulation cycles: A comparison among three different regimens. European J. Obstet Gynecol Reprod Bio 2006;129: 155–61.
13. Andersen CY, Humaidan P, Bredkjær H , *et al.* Hormonal characteristics of follicular fluid from women receiving either GnRH agonist or hCG for ovulation induction. Hum Reprod. 2006;21(8): 2126–2130.
14. Bedaiwy M, *et al.* Effect of follicular fluid oxidative stress parameters on intracytoplasmic sperm injection outcome. Gyne Endocr. 2012; 28(1): 51–55.
15. Allen VM and Wilson RD. Pregnancy outcomes after assisted reproductive technology. J Obst Gyne. 2006;28(3):220-233.

16. Merviel P, Heraud M, Grenier N, *et al.* Predictive factors for pregnancy after intrauterine insemination. Ferti Steril. 2008;10:120-129.
17. Dickey RP, Brinsden PR and Pyrzak R. Insemination technique and insemination complications. Intrauterine insemination and ovulation induction .2010;109-118.
18. Glover T and Mitchel K. Introduction to Biostatistic. 2nd ed. 2008; p 4.
19. Cooper TG. Physiology of Sperm Maturation and Fertilization. In: Nieschlag E BH, editor. Andrology: Male Reprod Health and Dysfunction. 3rd ed: Springer-Verlag Berlin Heidelberg 2010; 61-86.
20. Bahat A, Eisenbach M. Sperm thermotaxis. Mol Cell Endocrinol 2006; 252(1-2): 115-9.
21. Eisenbach M, Giojalas LC. Sperm guidance in mammals – an unpaved road to the egg. Nat Rev Mol Cell Biol. 2006; 7(4): 276-285.
22. Machado G, Lefievre L, Ford C, *et al.* Mobilisation of Ca^{2+} stores and flagellar regulation in human sperm by S-nitrosylation: a role for NO synthesised in the female reproductive tract. Dev J. 2008;135(22):3677-3686.
23. Michael A, Gregory L, Piercy E, *et al* .Ovarian 11 beta hydroxysteroid dehydrogenase activity is inversely related to the outcome of in vitro fertilization-embryo transfer treatment cycles. Fertil.Steril.1995; 64(3):590-598.
24. Koli P, Anil M, Patil K, *et al*. Intrauterine insemination: a retrospective review on determinants of success. Int J Reprod Contracept Obstet Gynecol. 2013;2(3):311-314.
25. Al-Dujaily SS, Alnakash AH, Al-Bayati S. Effect of pentoxifylline on the outcome of artificial inseminations. Iraqi Post Med J. 2006; 5(3): 80-86.

This page is intentionally left blank

Global Journal of Medical research: E
Gynecology and Obstetrics
Volume 14 Issue 4 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Correlation of Pap Smear with Histopathological Findings in Malignant and non Malignant Lesions of Cervix

By Dr. Vijay Kumar Bodal, Dr. Rupinder Kaur Brar, Dr. Manjit Singh Bal, Dr. Balwinder kaur, Dr. Sarbhjit Kaur, Dr. Anil Kumar suri, Dr. Ranjeev Bhagat & Dr. Geetanja li

Government Medical College Patiala, India

Abstract- *Background:* Conventional cervical cytology is the most widely used cervical cancer screening test in the world. Squamous intraepithelial neoplasia (SIL) and cervical cancer remain important health problems for women worldwide.

Aim and Objective: To study various types of cervical lesions with relevant factors such as age, parity, to classify cervical lesions into malignant & benign groups and to correlate the cytological with histopathological findings.

Materials and Methods: This study was conducted on 200 cases of Pap smears and cervical biopsies, along with resected specimens. After fixation and staining, smears and cervical biopsies were processed and examined under microscope.

Keywords: *malignant, cervical cancer, pap smear, cervical biopsy.*

GJMR-E Classification : *NLMC Code: WP 475*

Strictly as per the compliance and regulations of:

Correlation of Pap Smear with Histopathological Findings in Malignant and non Malignant Lesions of Cervix

Dr. Vijay Kumar Bodal [α], Dr. Rupinder Kaur Brar [σ], Dr. Manjit Singh Bal [ρ], Dr. Balwinder kaur [ω], Dr. Sarbhjit Kaur [¥], Dr. Anil Kumar suri [§], Dr. Ranjeev Bhagat [χ] & Dr. Geetanjali [ν]

Abstract- Background: Conventional cervical cytology is the most widely used cervical cancer screening test in the world. Squamous intraepithelial neoplasia (SIL) and cervical cancer remain important health problems for women worldwide.

Aim and Objective: To study various types of cervical lesions with relevant factors such as age, parity, to classify cervical lesions into malignant & benign groups and to correlate the cytological with histopathological findings.

Materials and Methods: This study was conducted on 200 cases of Pap smears and cervical biopsies, along with resected specimens. After fixation and staining, smears and cervical biopsies were processed and examined under microscope.

Results: Age wise maximum number of patients were in fourth decade (54.50%), followed by fifth decade. On cytology, 59% were inflammatory smears and frank malignancy was reported in 10% cases. LSIL and HSIL were reported in 9% and 8.50% respectively. Maximum number of cases on biopsy was those of infections (57.50%), 27% cases were those of frank malignancy; most common being invasive squamous cell carcinoma (23%) and adenocarcinoma in 2%. Mean age among cancer cases was high (51.94±12.30 years) compared to those who did not have cervical cancer (39.53±9.66 years). Cervical cancer was seen in 39.65% of patients with having ≥3 children. 10% cases diagnosed on cytology turned out to be malignant on biopsy.

Conclusion: Pap smear followed by cervical biopsy is an effective method for detection of pre-cancerous, cancerous and non-cancerous changes in the cervix.

Keywords: malignant, cervical cancer, pap smear, cervical biopsy.

I. Introduction

Papanicolaou (Pap) smear is a simple, safe, non-invasive and effective method for detection of pre-cancerous, cancerous and non-cancerous changes in the cervix.[1] Conventional cervical cytology is the most widely used cervical cancer screening test in the world and cytology screening programmes in several developed countries have been associated with impressive reduction in cervical cancer burden.[2] Squamous intraepithelial lesions are viewed as precancerous lesions exhibiting many of the morphological characteristics of invasive carcinomas. Identification of these entities is the focus of cervical screening programs that aim to discover them and commence their treatment in order to prevent invasive disease.[3] Though data from the 20 populations based cancer registries in India indicate a steady decline in cervical cancer incidence rates over the last two decades, it still occupies second position and the risk of disease is still high.[3] Cervical carcinoma documents the remarkable effects of screening, early diagnosis, and curative therapy on the mortality rate. Death rate has declined for which the credit goes to Pap test and accessibility of cervix to colposcopy and biopsy. Though, the Pap smear is an effective screening test, yet confirmation of the diagnosis of cervical cancer or pre invasive lesions of cancer requires a biopsy of the cervix.

II. Aims and Objectives

The aims of this study were to study the changes in cervical cytology with relation to age, parity and other presenting features, to classify cervical lesions into malignant and benign groups on cytological and histopathological basis and to correlate the changes observed in cervical cytology with cervical biopsy.

III. Materials and Methods

This study was done on 200 cases of Pap smears and cervical biopsies (including hysterectomy specimens). Most of the patients with symptoms suggestive of cervical disease were selected. However, some having gynaecological symptoms other than cervical disease were also included. Few cases reporting for routine screening were also included. A detailed clinical history especially age, duration of symptoms, parity, menstrual pattern and vaginal discharge were noted. The patients in whom both Pap smear and biopsy was available, were included in the study. The fixed cervical smears were subjected to staining according to Papanicolaou's method. The cytological interpretation of the smears was made according to the New 2001 Bethesda system. After grossing and processing, cervical biopsies were subjected to histopathological examination.

Author α: Department of Pathology, Government Medical College Patiala, (Punjab) India. e-mail: vijay_bodal@yahoo.com

IV. Results

Age wise maximum number of patients were in fourth decade (54.50%), followed by fifth decade (Table-1). Duration of symptoms varied from few months to many years. Some patients presented within 1 year (79%), but few mainly cases with discharge and history of prolapse presented late (Table-2). In 200 cases, various symptoms were seen, some patients showed multiple symptoms. Majority of patients (58%) presented with vaginal discharge followed by irregular bleeding (47%). Menstrual changes were also seen in large number of patients. There was seen low usage of oral contraceptive pills in our study group (10.50%). Duration of OCP usage varied from few months to years, but long term usage was not seen in any case. On cytology, 59% were inflammatory smears and frank malignancy was reported in 10% cases, LSIL and HSIL was reported in 9% and 8.50% respectively (Table-3). Maximum number of cases on biopsy were those of infections (57.50%), among them majority had non-specific chronic cervicitis. Squamous intraepithelial lesions were seen in 25 patients. Mild dysplasias correspond to low grade squamous intraepithelial lesions, moderate and severe to high grade intraepithelial lesions. 54 cases (27%) were those of frank malignancy on biopsy (Table-4); most common diagnosis being invasive squamous cell carcinoma (23%) and adenocarcinoma in 4 cases (2%). Distribution of age was correlated with cancer cases. Most of the cancer cases were seen in the age group of 31- 45 years. The mean age among cancer cases was high (51.94±12.30 years) and (39.53±9.66 years) in cases who did not have cervical cancer (Table-6). Cervical cancer was seen in 39.65% of patients with ≥3 children. History of oral contraceptive use was present in 21(10.50%) women. Of which 14.29% had cervical cancer and 85.71 % did not have cervical cancer, showing poor correlation between oral contraceptive use and cervical cancer (p= 0.165). 20 cases diagnosed on cytology turned out to be malignant on biopsy showing strong correlation between cytology and histopathology (p<0.001). Some of the cases were obscured by blood and inflammation which were missed on cytology but proved to be malignant on biopsy.

Table 1 : showing age distribution of cervical lesions

Age group (Years)	Distribution (n=200)	
	No.	%age
18-30	29	14.50
31-45	109	54.50
46-60	41	20.50
> 60	21	10.50
Total	**200**	**100**

Table 2 : showing duration of symptoms

Duration (Years)	Distribution (n=200)	
	No.	%age
Up to 1	158	79.00
1-3	25	12.50
4-6	11	05.50
>6	06	03.00
Total	**200**	**100**

Table 3 : showing cytological diagnosis

Diagnosis	Distribution (n=200)	
	No.	%age
Unsatisfactory smear	08	4.00
Inflammatory	118	59.00
ASCUS/H	19	9.50
LSIL	18	9.00
HSIL	17	8.50
Frank malignancy	20	10.00
Total	**200**	**100**

Table 4 : showing histopathological diagnosis

Diagnosis	Distribution (n=200)	
	No.	%age
Infections	115	57.50
Carcinoma	54	27.00
Dysplasia	25	12.50
Benign tumors	06	03.00
Total	**200**	**100**

Table 5 : Showing correlation of cytological and histopathological Diagnosis

Histopathological Diagnosis	No.	Cytological Diagnosis					
		Unsatisfactory	Inflammatory	ASCUS/H	LSIL	HSIL	Ca
Infections	115	-	108	07	-	-	-
Carcinoma	54	08	-	-	12	14	20
Dysplasia	25	-	04	12	06	03	-
Benign tumors	06	-	06	-	-	-	-
Total	**200**	**08**	**118**	**19**	**18**	**17**	**20**

Table 6 : showing means age

Variable	Cervical Ca (n=54)		No Ca (n=146)	
	Mean	SD	Mean	SD
Mean	51.94	12.30	39.53	09.66
T	7.469			
Df	198			
P	< 0.001			
Significance	Highly Significant			

V. Discussion

Cancer cervix is considered to be an ideal gynaecological malignancy for screening as it meets both test and disease criteria for screening. It has a long latent phase during which it can be detected as identifiable and treatable premalignant lesions which precede the invasive disease and the benefit of conducting screening for carcinoma cervix exceeds the cost involved. [4]

Despite the success of cervical cancer screening programs, questions remain about the appropriate time to begin and end screening. This review explores epidemiologic and contextual data on cervical cancer screening to inform decisions about when screening should begin and end. The incidence and mortality rates from, cervical cancer that have had a Pap smear within 3 years have decreased since 2000.

In this study, more than half (54.50%) were aged between 31 to 45 years followed by 20.50% between 46 to 60 years. The mean age of patients with cancer in the present study was 51.94 years. This is close to that found by Biswas et al[5] and Missaoui et al.[6] Although, invasive cancer cervix is reported at all ages; it has two peaks, one at about 35 years and another above 50 years. The highest age of cervical cancer in the present study was 73 years and the lowest was 26 years. The mean age for non-cancer cases was 39.53 years. In this study, the most common symptoms was discharge per vaginum (58%) followed by irregular bleeding in 47% of the patients. Patients with cancer also presented with post-coital bleeding and in cases of older age group post menopausal bleeding was seen. Symptomatic presentation was similar to some extent as seen by Ikram et al [7].

In this study, 59% patients had the cytological diagnosis of benign/ inflammatory and carcinoma was present in 10% of the cases. This is comparable to Saha and Thapa[8] in which benign cases were 51.16% and carcinoma was diagnosed in 6.97% of the cases. Most common cancer in the present study was squamous cell carcinoma (85.18%). This study showed results similar to those seen by Ikram et al[7] (83.33%).

As regards the various histopathological varieties of SCC, the present study found an incidence of 67.39% for moderately differentiated SCC, 23.91% for well differentiated, 8.70% for poorly differentiated. Thus, the findings of the present study are consistent with that

of Missaoui et al [6] in that moderately differentiated large cell non-keratinizing variety is the commonest variety.

VI. Conclusions

It is concluded that most commonly seen problem, infection, can be controlled with good hygiene. Cervical carcinoma is seen in large number of patients. Pap is a relatively less invasive and a simple procedure to diagnose cervical lesions in developing countries. But sometimes, there can be obscuring of the cellular details by blood, especially in malignant cases. In such cases, biopsy is helpful and confirmatory.

VII. Acknowledgments

We thank the patients and technical staff of Pathology Department GMC Patiala for processing the Pap smears and cervical biopsies.

References Références Referencias

1. Khan MA, Raja FY, Ishfaq G, Tahir F, Subhan F. Pap Smear Screening for Pre-cancerous Conditions of the Cervical Cancer. Pak J Med Res 2005;44(3):111-13.
2. Sankaranarayan R, Budhuk A, Rajkumar R. Effective screening programmes for cervical cancer in low and middle income developing countries. Bulletin of WHO 2001;79:954-62.
3. National Cancer Registry Programme. Three year report of population based cancer registries 2006-2008. New Delhi: ICMR; 2010.
4. Kerkar RA, Kulkarni YV. Screening for cervical cancer: An overview. J Obstet Gynecol India.2006;56:115–22.
5. Biswas LN, Manna B, Maiti PK, Seng S. Sexual Risk Factors for Cervical Cancer among Rural Indian Women: A Case-Control Study. Int J Epidemiol 1997; 26(3):491.
6. Missaoui N, Hmissa S, Trabelsi A, Frappart A, Moncef, Mokni M et al. Cervix cancer in Tunisia: clinical and pathological study. Asian pacific J cancer prevention 2010; 11:235-8.
7. Ikram M, Talib W, Chatha S, Saed R. Carcinoma of cervix. Professional Dec Med J 2005; 12(4):392-6.
8. Saha R and Thapa M. Correlation of cervical cytology with cervical histology. Kathmandu University Medical Journal 2005; 3(3):222-24.

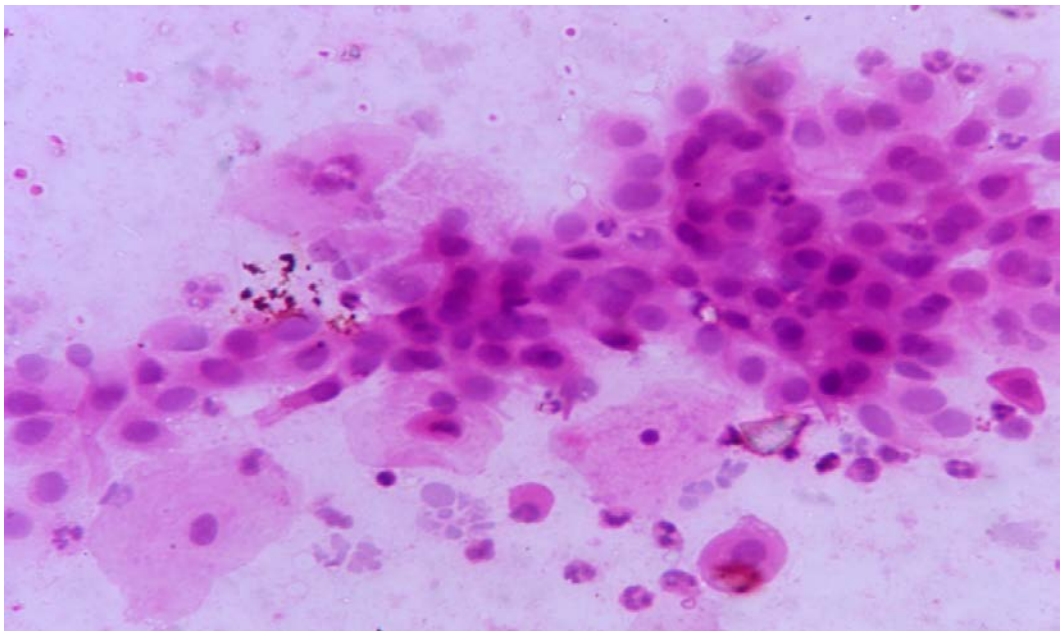


Figure 1 : Photomicrograph of HSIL showing group of hyperchromatic parabasal cells exhibiting nucleomegaly and overlapping nuclei (PAP X 400)

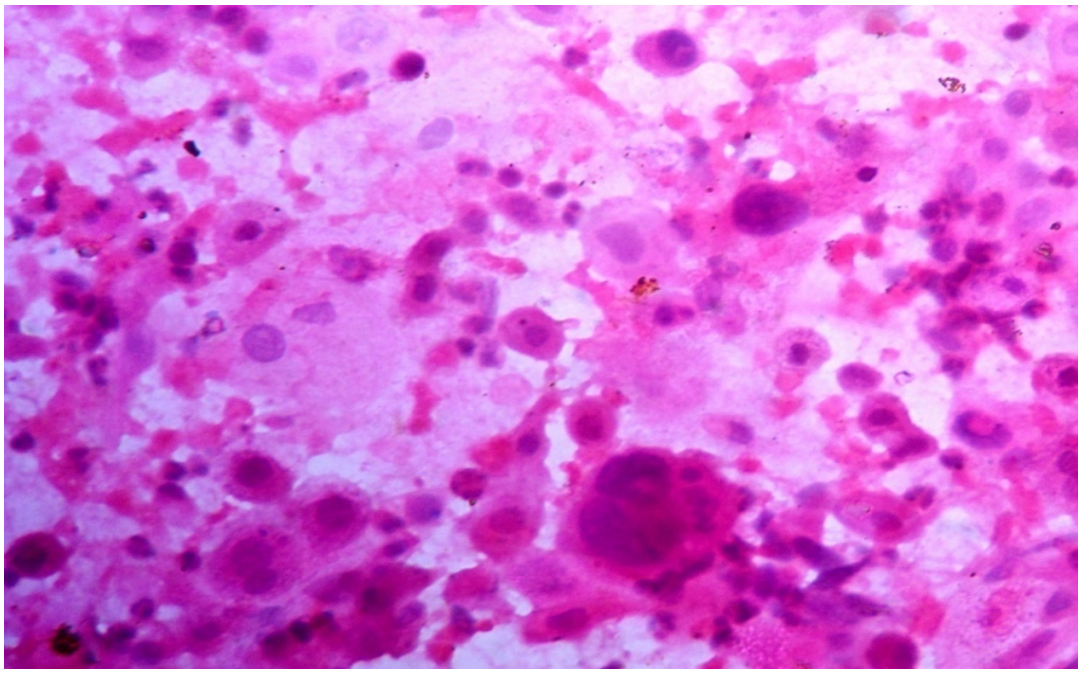


Figure 2 : Photomicrograph of **Squamous cell carcinoma** showing tumour diathesis, malignant cells with nucleomegaly, hyperchromatism and irregular nuclear margins (PAP X 400)

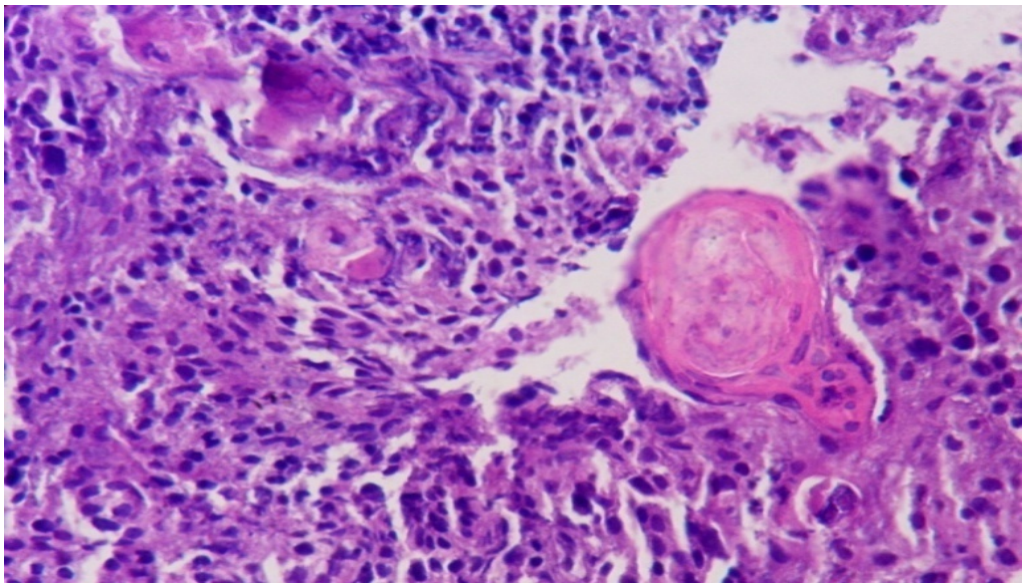


Figure 3 : Photomicrograph of **Well differentiated SCC** (H & E X 400)

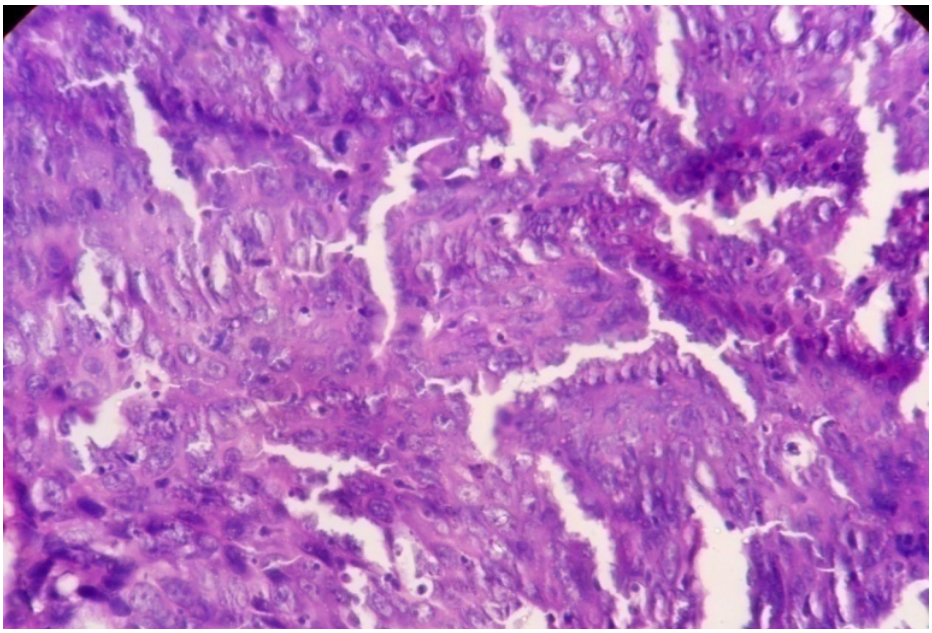


Figure 4 : Photomicrograph of **moderately differentiated SCC** (H & E X 400)

Global Journal of Medical research: E
Gynecology and Obstetrics
Volume 14 Issue 4 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Lipid Profile and Urinary Protein-Creatinine Ratio Changes in Pre-Eclampsia Cases in and around Chitradurga

By Gaurang K. Anandpara & Dinesh Javarappa

Abstract- Pre-eclampsia is defined by the new onset of elevated blood pressure and proteinuria after 20 weeks of gestation.According to current studies dyslipidemia, particularly the rise in serum triglycerides is a contributing factor in pre-eclampsia. There is a positive correlation between serum triglycerides and systolic blood pressure in pre-eclampsia cases .Urinary protein- creatinine ratio has been used to evaluate proteinuria in pre-eclampsia, this parameter is sensitive and predictive as well.The presence of proteinuria is seen as a possible indication of many complications in pregnancy, from urinary tract infection to chronic renal disease and it remains central to the diagnosis of pre-eclampsia in a hypertensive pregnancy. It has both diagnostic and prognostic implications . Extensive changes occur in the renal system in pre-eclampsia. As a part of the end organ pathology pre-eclamptic glomeruli undergo structural changes with pronounced endothelial vacuolization and hypertrophy of the cytoplasmic organelles, first defined as glomerular endotheliosis.

A case control comparative study was done with preeclampsia and normal pregnant women .both for out door patient and indoor patients of Basaveshwara Medical College Hospital & RC, Chitradurga according to the criteria.

Keywords: preeclampsia, urinary protein, urinary creatinine, urinary protein: creatinine ratio, serum lipid profile.

GJMR-E Classification : NLMC Code: WJ 303, QV 243

Strictly as per the compliance and regulations of:

Lipid Profile and Urinary Protein–Creatinine Ratio Changes in Pre-Eclampsia Cases in and around Chitradurga

Gaurang K. Anandpara [α] & Dinesh Javarappa [σ]

Abstract- Pre-eclampsia is defined by the new onset of elevated blood pressure and proteinuria after 20 weeks of gestation.According to current studies dyslipidemia, particularly the rise in serum triglycerides is a contributing factor in pre-eclampsia. There is a positive correlation between serum triglycerides and systolic blood pressure in pre-eclampsia cases .Urinary protein- creatinine ratio has been used to evaluate proteinuria in pre-eclampsia, this parameter is sensitive and predictive as well.The presence of proteinuria is seen as a possible indication of many complications in pregnancy, from urinary tract infection to chronic renal disease and it remains central to the diagnosis of pre-eclampsia in a hypertensive pregnancy. It has both diagnostic and prognostic implications . Extensive changes occur in the renal system in pre-eclampsia. As a part of the end organ pathology pre-eclamptic glomeruli undergo structural changes with pronounced endothelial vacuolization and hypertrophy of the cytoplasmic organelles, first defined as glomerular endotheliosis.

A case control comparative study was done with preeclampsia and normal pregnant women .both for out door patient and indoor patients of Basaveshwara Medical College Hospital & RC, Chitradurga according to the criteria.

Study group will be followed up every 4 weeks in first 28 weeks of gestation. Blood samples and 24 hr/random urine samples will be collected for biochemical evaluation of lipid parameters and urinary protein and urinary creatinine .

In this study,

It was found that there were significant increases in levels of Triglycerides & LDL-Cholesterol ($p < 0.001$),Slight increases in Total cholesterol levels($p < 0.001$) and significant decreases inHDL-Cholesterol levels($p < 0.001$) in cases of preeclampsia in comparison to normal pregnant women Urinary creatinine levels in cases of preeclampsia showed decreased levels ($p<0.001$) and Protein /creatinine ratio in urine showed significant increases ($p<0.001$) as compared to normal pregnant women.

Keywords: preeclampsia, urinary protein, urinary creatinine, urinary protein: creatinine ratio, serum lipid profile.

I. Introduction

Pre-eclampsia is defined as development of hypertension and proteinuria (> 300mg urinary protein in 24hrs) after 20th week gestation. Hypertension is defined as a blood pressure greater than 140/90mm Hg or a rise in blood pressure of 30/15 mm Hg from the base line confirmed by two measurements 6 hrs apart.[1]

Pre-eclampsia is a leading cause of maternal and perinatal morbidity and mortality worldwide. It is a hypertension disorder of unknown etiology characterized by proteinuria, coagulation abnormalities and different systemic manifestations [2]. Pre-eclampsia occurs in about 5-7% of pregnancies. It is known to effect the function of various organs involving-metabolism.

Several studies have shown that endothelial dysfunction is related to hyperlipidemia with elevated plasma concentration of triglycerides, phospholipids and total lipids and declination of HDL- cholesterol [3].

The most important feature in toxemia of pregnancy is hypertension which is supposed to be due to vasospastic phenomenon in kidney, uterus, placenta and brain[5].Altered lipid synthesis leading to decreased PGI_2: TXA_2 ratio is also supposed to be an important way of pathogenesis in pre-eclampsia. Thus abnormal lipid metabolism seems important in the pathogenesis of Pre-eclampsiatoo[4].

The association of serum lipid profile with gestational proteinurichypertension is highly suggestive to reflect some new diagnostic tools. Moreover, the hormonal imbalance is a prime factor for the etiopathogenesis of Pre-eclampsia and this endocrinal imbalance is well reflected in alteration of serum lipid profile [5].

The presence of proteinuria is seen as a possible indication of many complications in pregnancy, from urinary tract infection to chronic renal disease and it remains central to the diagnosis of pre-eclampsia in a hypertensive pregnancy. It has both diagnostic and prognostic implications[6] . Extensive changes occur in the renal system in pre-eclampsia. As a part of the end organ pathology pre-eclamptic glomeruli undergo structural changes with pronounced endothelial

Author α: Post graduate student, department of Biochemistry, BMCH & RC, Chitradurga. e-mail: gauranganandpara@yahoo.com.
Author σ: Professor and Head, Department of Biochemistry, BMCH & RC, Chitradurga. e-mail: drdineshj@yahoo.com.

vacuolization and hypertrophy of the cytoplasmic organelles, first defined as glomerular endotheliosis[7].

To predict proteinuria during pregnancy, estimation of urinary protein to Creatinine ratio is vital. The gold standard of for measuring proteinuria is 24hrs urine collection, but a faster screening method is needed to save time. Spot urinary protein: creatinine ratio is however preferred for the purpose. This ratio has a sensitivity of 96% and a specificity of 53%. This way of estimation of urinary protein: creatinine ratio is significant in predicting proteinuria[8].

There appears to be a lacunae in the correlation of dyslipidemia to proteinuria with respect to urinary protein :creatinine ratio in cases of pre-eclampsia. The present study had focused on enabling us to understand the probable significance of urinary protein: creatinine ratio in diagnosing pre-eclampsia in and around chitradurga..

II. Materials and Methods

a) Inclusion Criteria

Primigravida with pre-eclampsia in the age group of 18-35 yrs, without any previous history of hypertension, dyslipidemia or other organ dysfunctions.Gestational age of 20 weeks.

b) Exclusion Criteria

Multigravida and all maternal abnormalities in pregnancy.

III. Methods

Study group will be followed up from 28th weeks of gestation. Blood samples and24hr/random urine samples will be collected for biochemical evaluation of Lipid parameters and Ur. Protein.

Lipid profile is estimated by automated method, urinary protein and creatinine by Pyrogallol Red method and Jaffes method respectively.

2 ml of venous blood sample were collected from pre-eclampsia cases and normal pregnant women as per criteria into plane vaccutainers, Blood samples were analysed for lipid profile and urine was analysed for protein. The results were statistically analysed with student 'T' test.

A case control comparative study was done with Pre-eclampsia and normal pregnant women according to criteria.

IV. Results

The present study included a total number of 100 subjects consist of 50 preeclampsia cases and 50 normal pregnant women.

Table 1 narrates lipid profile in preeclampsia cases and Normal pregnant women.

Table 2 shows, the urinary protein, urinary creatinine and urinary protein – creatinine ratio.

Table 1 : Comparison of Lipid Profile Between Pre-Eclampsia Cases and Control Group

Parameters [mg/dl]	Serum TAG	Serum Total Cholesterol	Serum HDL – C	Serum VLDL- C	Serum LDL-C
Normal Pregnant Women (n=50)	132.16 ± 13.13	156.7 ± 14.96	38.52 ± 2.40	26.54 ± 2.70	91.64 ± 14.77
Preeclampsia cases(n=50)	202.04*** ± 25.29	243.5 *** ± 24.77	32.6 *** ± 3.31	40.34 *** ± 5.10	170.56 *** ± 23.80

Note :-

1. *The number in parenthesis shows the number of samples*
2. *Values are expressed as their Mean ± SD.*
3. *p-value * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.*

Table 2 : Comparison of Urinary Protein,Urinary Creatinine & Urinary Protein:Creatinine Ratio Between Pre-Eclampsia Cases and Control Group

Parameters	Urimary protein [mg/dl]	Urinary creatinine [mg/dl]	Urinary protein:creatinine ratio
Normal Pregnant Women (n=50)	4.40 ±2.00	83.34 ±17.49	0.05 ± 0.03
Preeclampsia cases(n=50)	27.56***± 17.15	50.38***± 7.92	0.55 ***± 0.35

Note :-

1. *The number in parenthesis shows the number of samples*
2. *Values are expressed as their Mean ± SD.*
3. *p-value * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.*

V. Discussion

Table 1 shows a comparative study between preeclampsia and normal pregnant women on parameter triglycerides , total cholesterol , HDL-C , LDL –C and VLDL-C .It is seen that the serum triglycerides, LDL-C , VLDL-C are significantly increased ($p < 0.001$) as compared to normal pregnant women and HDL-C is significantly decreased ($p < 0.001$) as compared to normal pregnant women and total cholesterol was slightly increased ($p < 0.001$) as compared to normal pregnant women.

Dyslipidemia is significantly evident in cases of preeclampsia according to Gohil J T [9] et al. ,Hubel[10] et.al showed in their study that triglyceride levels increase two fold in preeclamptic patients in comparison to normal pregnant women .Cuneyt Evruke et al[11] proved that in pregnancy all lipid fractions increased with increasing age of pregnancy which is secondary to increase in estrogen and progesterone levels in pregnancy, NAF Islam et al[12] have shown in their study that HDL –C levels are markedly decreased in comparison to normal pregnant women .

During pregnancy there is increase in the hepatic lipase activity and decrease in lipoprotein lipase activity .Hepatic lipase is responsible for the increased synthesis of triglycerides at the hepatic level where as the decreased activity of lipoprotein lipase is dyslipidemia mediated activation of the endothelial cells and placentally derived endothelial disturbing factors like lipid peroxides could be the possible cause of the pathogenesis of pre-eclampsia.

Table-2 shows a comparative study between Urinary Protein and creatinine in Pre-eclampsia and Normal pregnant women .Urinary creatinine levels in cases of preeclampsia decreases ($p<0.001$) as compared to normal pregnant women . GFR and renal blood flow increased predominantly during the course of the pregnancy resulting in physiological fall in the serum creatinine concentration.

The random urinary protein: creatinine ratio in preeclampsia cases is significantly increases , ($p < 0.001$) as compared to normal pregnant women is probably due to renal glomerular endotheliosis leading to impaired glomerular perfusion and filtration .

The urinary protein-creatinine ratio in pre-eclamptic pregnant women is significantly increased as compared to normal control group indicating that pre-eclamptic glomeruli undergo structural changes with pronounced endothelial vacuolization and hypertrophy of the cytoplasmic organelles leading to glomerular endotheliosis according to studies of Christopher P. Price et al.

In this study, it was found that dyslipidemia and increased protein: creatinine ratio were associated with preeclampsia suggests that lipid profile and random urinary protein: creatinine ratio may be useful in screening for preeclampsia cases .

In conclusion, Altered lipid profile with increased Triglycerides and decreased HDL levels along with increased protein–creatinine ratio in Preeclamptic patients can be used as comboparameters as an early assessment tool or early marker in the prediction of pre-eclampsia.

References Références Referencias

1. Tenhola S., Eero R., Anneli, Martikainen, and Prijo H. Blood pressure, serum lipids, fasting insulin and adrenal hormones in 12yrs old children born with maternal pre-eclampsia. J. Clin. Endocrinol Metabol. 88(3); 1217-1222: 2003.
2. Portelinha A., Belo L., Cerdeira A.S., Braga J., Tejeira E., Pinto F. et.al. Lipid levels including oxide preeclampsiaed LDL in women with history of pre-eclampsia. Hypertens. Pregnancy. 29(1); 93-100: 2010.
3. Lana K. and Wagner Diagnosis and management of pre-eclampsia. Am. Fam .Physician.70(2); 2317-2324: 2004.
4. Robson S.C. Hypertension and renal diseases in pregnancy, In: Dewhurst's Textbook of obstetrics and gynaecology for postgraduates. 6th eds. Edited

by Edmonds D.K. Blackwell Science Ltd. publications. New York. 1999: p 167-169.
5. De J., Mukhopadhyay A.K. and Saha P.K.Study of serum lipid profile in pre-eclampsia – A case controlled study. Ind. J. Clin. Biochem. 21(2);165-168: 2006.
6. Abbate M., Benigni A., Bertani T. and Remu. preeclampsia G. Nephrotoxicity of increased glomerular protein traffic. Nephrol. Dialysis Transplant. 14; 304-331: 1999.
7. Sheehan H.L. and Lynch J.B. Pathology of toxaemia of pregnancy. Edited by Williams and Williams. Baltimore. 1999: p 897-981.
8. Cote A.M., Brown M.A., Lam E., Dadels preecl-ampsia en P.V., Firopreeclampsia T., Liston R.M. et.al. Diagnostic accuracy of urinary spot protein-creatinine ratio for proteinuria in hypertensive pregnant women.BMJ.336;p 1003- 1006: 2008.
9. Gohil J T, Patel P K and Gupta Priyanka. estimation of lipid profile in subjects of preeclampsia. the journal of obstetrics and gynecology of india 2011/61 (4):399-403.
10. Carl A.Hubel, Margeret K. Mclaughlin, Rhobert Evans .fasting serum triglycerides, free fatty acids and malondialdehyde are increased in preecla-mpsia.are positively correlated and decrease within 48 hours postpartum..American journal of obstetrics and gynecology.1996/174 (3)975-982.
11. Cuneyt Evruke, S. Cansun Demir, Ibrahim F. Urunsak, F. Tuncay Ozgunen and Oktay Kad-ayifci. comparison of lipid profiles in normal and hypert-ensive pregnant women. Ann Saudi med. 2004 /24 (5)382-385.
12. NAF Islam, MAR Chowdhury, G, M, Kibria and S. Akhter. Study of serum lipid profile in preeclampsia and eclmpasia. faridpur med. Coll. J. 2010.5(2)56-59.

Global Journal of Medical research: E
Gynecology and Obstetrics
Volume 14 Issue 4 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Lipid Peroxidation During the Cryopreservation Process of Porcine Spermatozoa

By H. A. Goolsby, G. Simoni & J. Simoni+S. D. Prien
Texas Tech University, United States

Abstract- The potential advantages of sperm cryopreservation have not been fully accomplished due to the limiting detrimental effects the freezing process has on sperm structure and composition. Previous studies have suggested that cells suffer lipid peroxidation damage during the cryopreservation process, specifically indicating the damage results from mechanical stress during the preparatory and freezing processes. In this present study, sperm samples were analyzed for lipid stability throughout sample processing through evaluations for lipid peroxidation and lipid free radical concentration. Our analysis was completed in three experiments. In Exp. #1, lipid stability levels were evaluated from five separate boar ejaculates frozen using three different freezing methods to compare cryopreservation techniques. In Exp. #2, lipid peroxidation amounts for fresh post-ejaculate and albumin extended boar samples were compared. Experiment #3 involved evaluations of the semen processing to examine sample and seminal fluid alterations. Samples tested from the freezing protocol included fresh, extended, addition of a wash buffer, cooling to 17 ^{o}C, centrifugation, addition of two egg-yolk extenders, cooling to 5 ^{o}C and post-thaw values. Though there was no difference between the three freezing treatments, significant differences were noted between the fresh and extended samples ($P < 0.001$).

Keywords: lipid peroxidation, sperm, cryopreservation, porcine.

GJMR-E Classification : NLMC Code: WO 510, WJ 834

Strictly as per the compliance and regulations of:

Lipid Peroxidation During the Cryopreservation Process of Porcine Spermatozoa

H. A. Goolsby [α], G. Simoni [σ] & J. Simoni+S. D. Prien [ρ]

Abstract- The potential advantages of sperm cryopreservation have not been fully accomplished due to the limiting detrimental effects the freezing process has on sperm structure and composition. Previous studies have suggested that cells suffer lipid peroxidation damage during the cryopreservation process, specifically indicating the damage results from mechanical stress during the preparatory and freezing processes. In this present study, sperm samples were analyzed for lipid stability throughout sample processing through evaluations for lipid peroxidation and lipid free radical concentration. Our analysis was completed in three experiments. In Exp. #1, lipid stability levels were evaluated from five separate boar ejaculates frozen using three different freezing methods to compare cryopreservation techniques. In Exp. #2, lipid peroxidation amounts for fresh post-ejaculate and albumin extended boar samples were compared. Experiment #3 involved evaluations of the semen processing to examine sample and seminal fluid alterations. Samples tested from the freezing protocol included fresh, extended, addition of a wash buffer, cooling to 17 ºC, centrifugation, addition of two egg-yolk extenders, cooling to 5 ºC and post-thaw values. Though there was no difference between the three freezing treatments, significant differences were noted between the fresh and extended samples ($P < 0.001$). These findings were exemplified by the step by step analysis of the processing and freezing protocol. The lipid peroxidation amounts accumulated after each the procedural step ($P < 0.001$). Significant differences were also observed in the lipid radical levels ($P < 0.001$). The results of the pre-freezing protocol, alterations in lipid stability do not appear to be due to thermal or mechanical stress. The largest gains of both lipid parameters developed after the addition of an egg-yolk freezing extender. The results suggest further studies in alterative extenders are needed.

Keywords: lipid peroxidation, sperm, cryopreservation, porcine.

I. Introduction

Several articles have been published based on lipid peroxidation and oxygen free radicals in human and murine semen samples. Negative correlations have between associated between increased levels of malondialdehyde (MDA) and reduced motility [1-3] and poor fertilization capability [4] in human sperm. Though general conclusions may result from trials based on other species, species differences in composition have demonstrated the presence of varying protective enzymatic mechanisms against peroxidation . [5-8]

Author ρ: Texas Tech University Health Sciences Center, Lubbock, Texas. e-mail: Samuel.prien@ttuhsc.edu

Very few articles have been published on lipid radicals in relation to boar semen. However, the studies published suggested lipid radicals were more detrimental than the radical hydroxyls. [9-11] MDA and reactive oxygen species levels have been identified in samples incubated at 37 ºC [9,10], cooled liquid storage [11, 12] and post–thaw. [13]

Though peroxidation levels have been analyzed in previous reports specifically studying the post-collection and post-thawed samples, the possibility of the interaction of the cells and cryoprotectant creating radicals during pre-freezing procedures has not been investigated. Given the minimal success of freezing methodologies for porcine semen and the high susceptibility of these cells to free radical damage, the objective of this study was to investigate the possible source of damage on a molecular level evaluating the lipid stability of the phospholipid membrane during the freezing and pre-freezing process.

II. Materials and Methods

a) Semen Preparation

Semen specimens were collected artificially from boars at the Texas Tech University New Deal Farm by trained staff. During the post-collection evaluation, standard measurements including total gel-free volume, concentration, and motility were obtained. To fulfill standard breeding practices, all samples used possessed a minimum motility rate of 60 % post-collection and met the breeding requirements of the swine unit. From the filtered ejaculate, a 30 mL sample was retrieved and extended with 60 mL of a pre-heated X-Cell extender (IVM; Maple Grove, MN). The sample was then packaged for transport to one of two possible laboratories.

Experiment #1. Five semen samples from five boars were collected and processed individually for this experiment. Upon arrival, the extended sample from each boar was placed in a refrigeration unit (17 °C) for 1 h. At the end of the refrigeration, 210 mL of a wash buffer maintained at 17 ºC was added before continuing the storage in refrigeration unit for another 1.5 h. The samples were then centrifuge for 20 min at 805 x g (Sorvall RT6000 Centrifuge, Kendro Lab Products; Asheville, NC) in a 15 ºC setting. The supernant fluid was removed and discarded. The remaining pellets were reconstituted in 30 mL of a 25 % egg-yolk extender, Boarciphos A (IVM; Maple Grove, MN), also

maintained at 17 °C. The sample was allowed to equilibrate for 1.5 h in a secondary refrigeration unit set at 5 °C. With 10 min intervals between, a second 25 % egg-yolk extender with 6 % glycerol, Boarciphos B (IVM; Maple Grove, MN), cooled to 5 °C, was added three times in 10 mL increments. After the entire 30 mL was added, the sample was maintained at 5 °C for 1 h. One ml aliquots of the extended sample were placed in 1.8 mL labeled cryo-vials (Nalge Nunc International; Denmark) before being cryopreserved. Through labeling, specimens were separated into three freezing treatment groups:

Control: Liquid nitrogen mist for 10 min

A: 5 min held in a fluid vat at -10 °C followed by 4 min in a fluid vat at -25 °C (average cooling rate -4.3 °C/min)

B: 5 min held in a fluid vat at -10 °C followed by 4 min in a fluid vat at -25 °C, both vats equipped with a proprietary filter to slow cooling rates (average cooling rate -2.8 °C/min)

Freezing treatments "A" and "B" were two optimal freezing treatments of a unique freezing technology (UFT: Supachill USA; Lubbock, TX) developed in previous trials within our laboratory (references). Once frozen, samples were plunged into liquid nitrogen and stored until testing began for lipid peroxidation rates and lipid free radical amounts.

Experiment #2. For Exp. #2, we used fresh post-ejaculate boar samples (N=5) and extended post-ejaculate samples (N=5) for lipid stability comparisons from five boars. The above protocol discussed in semen processing was utilized and completed until arrival at the off-site lab. One mL aliquots were extracted from all 10 samples and placed in 1.8 mL cryo-tubes (Nalge Nunc International; Denmark). Immediately afterwards, each vial was plunged in liquid nitrogen for freezing and storage.

Experiment #3. The third phase of this trial utilized the same protocol discussed above in Exp. #1 on three semen samples from separate boars. However, the proportions of original semen sample and the various extenders used were increased. This ensured sufficient amounts for three 1 mL aliquots of semen samples and fluid to be extracted during each major step of the protocol without influencing final ratios. The semen samples represent the whole fractions available at that step. Fluid samples were created by centrifuging addition samples during each step specified below. The procedural steps represented include:

1. Ejaculation samples post-transport
2. Extended samples post-transport
3. Extended samples cooled to 17 °C for 1 h
4. Samples equilibrated with a wash buffer at 17 °C for 1.5 h
5. Samples after centrifugation for 20 min at 805 x g
6. Samples equilibrated with the first freezing extenders at 5 °C for 1.5 h
7. Samples equilibrated with the second freezing extenders at 5 °C for 1.5 h
8. Post-thaw values of optimal freezing treatment.

Due to the density of the semen pellet after centrifugation and concerns of altering final concentrations, a semen sample was not extracted for this step. While thawing the samples, fluid specimens were not removed for analysis. After extraction during each step, the aliquots were plunged and stored in liquid nitrogen.

b) Quantification of Lipid Peroxidation

After thawing at room temperature and then being vortexed, a 200 µL sample was extracted from each aliquot and utilized for this test. The semen samples were mixed with 200 µL of SDS solution, 1.5 mL of 20% AA solution (at 3.5 pH), 1.5 mL TBAR solution (1.6 g 2-thiobaritric acid with 200 mL of deionized water) and 600 µL of deionized water. After vortexing each sample, the samples were incubated in a 100 °C water bath for 2 h. The samples were then cooled for 10 min at room temperature before centrifuged at 1006 x g (CRU-500 Centrifuge, Damon/IEC; Needham Heights, MA) for 10 min. A 0.5 mL sample of fluid was again extracted from each tube and mixed with 2 mL of butanol. After a second centrifugation at 1200 x g (Sorvall RC5C Centrifuge, Kendro Lab Products; Asheville, NC) for 10 min, each fluid sample was transferred into a glass absorbance box individually for analysis at 532 nm wavelength in a spectrophometer (Coleman 575, Perkin-Elmer; Oak Brook, IL).

c) Quantification of Free Lipid Radicals

A 250 µL sample was extracted from each specimen and placed into a pre-labeled 12 x 75 mm glass tube. After diluting each sample in 2 mL of a 2:1 chloroform:heptane mixture, each tube was thoroughly vortexed. The samples were then centrifuged for 10 min at 1572 x g (CRU-500 Centrifuge, Damon/IEC; Needham Heights, MA). Fluid in the amount of 700 µL was extracted from each specimen and transfer to a new set of glass tubes. Samples were dried under a flow of normal air at ambient temperatures for 15-20 min. The dried extracts were reconstituted in 2 mL of heptane before performing a spectrophotometer (Coleman 575, Perkin-Elmer; Oak Brook, IL) analysis at a 233 nm wavelength. The lipid radical control, required for proper calculations, was processed the same as a sample except the control contained 250 µL of water instead of a sample.

d) Statistical Analysis

The analysis of the lipid peroxidation and lipid free radical amounts for the three freezing treatments was analyzed using analysis of variance (ANOVA). Boar and freezing treatment were both used as part of the statistical model. The means were separated through Fisher's Least Squared Differences (LSD).

Results of the fresh and extended values for lipid peroxidation were compared using. Though the results were significant, the sample size was too small to complete a LSD analysis.

On the procedural analysis in Exp. #3, the lipid peroxidation and lipid free radical amounts were also analyzed using the ANOVA model. Since these values represent accumulations of lipid instability over time, a linear regression model function was used to analyze each parameter.

All statistical analyses were performed using the SPSS Version 8.0 Software (SPSS, Inc.; Chicago, IL).

III. Results

a) Experiment #1

When comparing the three freezing treatments using five separate semen samples, the differences between the treatments were not significant for either lipid evaluations. However, there was an interaction between the boar and lipid peroxidation amounts ($P < 0.005$). These differences, shown in Table I, concur with the variability of semen quality between boars.

Table 1 : Averaged Lipid Peroxidation and Free Radical Amounts from Three Freezing Treatments in Experiment #1

Boar	Lipid Peroxidation (nM/mL)	SEM	Radicals (A233/mL)	SEM
Yellow (n = 3)	62.3^{b}	2.79	5.7	0.28
Pink (n = 3)	58.6^{b}	2.19	5.5	0.24
White (n = 3)	58.2^{b}	2.36	5.7	0.18
Green (n = 3)	52.2^{a}	2.19	5.9	0.10
Orange (n = 3)	50.8^{a}	2.76	5.8	0.25

Different letter superscripts indicate significant differences between the lipid peroxidation of five boars ($P < 0.02$)

b) Experiment #2

When comparing membrane lipid stability between fresh, post-ejaculation samples and extended post-ejaculation samples there was a significant difference. Lipid peroxidation rates were much higher for the fresh samples as compared to the extended samples ($P < 0.001$; Table II).

Table 2 : Averaged Lipid Peroxidation Amounts from Fresh and Extended Boar Samples in Experiment #2

Sample	n	Lipid Peroxidation (nM/mL)	SEM
Fresh 5		14.3^{a}	2.24
Extended	5	1.6^{b}	0.36

Different letter superscripts indicate significant differences between the lipid peroxidation levels of the sample types ($P < 0.001$)

c) Experiment #3

Referring to Table III, lipid peroxidation levels accumulated during the handling procedure in both specimen types was evaluated. The levels of peroxidation were significantly different within the semen samples ($P < 0.001$) and fluid samples ($P < 0.001$). Using the LSD procedure, significant differences were observed at various transition points of the procedure including steps 1 to 2, steps 4 to 6, and steps 7 to 8 in the sample ($P < 0.05$) and fluid ($P < 0.001$). Furthermore, increasing amounts of MDA were correlated between the semen and fluid levels ($r = 0.96$). These increases in peroxidation rates are emphasized in Figure I.

Table 3 : Averaged Lipid Peroxidation Amounts from Procedural Steps

Step		Malondialdehyde Amounts (nM/mL) n	Sample	SEM		n	Fluid	SEM
1: Post-collection	3	10.2^{a}	6.97	3		5.2^{w}	0.12	
2: Extended	3	22.6^{b}	4.90	3		21.4^{x}	1.77	
3: Cool to 17 °C	3	23.9^{b}	3.14	3		23.3^{x}	2.49	
4: Wash Buffer	3	23.4^{b}	2.52	3		22.6^{x}	0.67	
5: Centrifugation	0	n/a	-	3		23.5^{x}	0.94	
6: Freezing Extender at 5 °C	3	66.0^{c}	1.83	3		41.6^{y}	2.33	
7: Second Freezing Extender	3	62.2^{c}	4.42	3		50.4^{z}	3.30	
8: Frozen-Thaw Samples	3	53.2^{d}	6.74	0		n/a	-	

Different letter superscripts (a,b,c,d) indicate significant differences between lipid peroxidation levels of the procedural steps in the samples ($P < 0.05$).
Different letter superscripts (w,x,y,z) indicate significant differences between lipid peroxidation levels of the procedural steps in the fluid extracted ($P < 0.001$).

Referring to Table IV, lipid free radical levels also showed significant variations during the procedure for the samples ($P < 0.001$) and fluids ($P < 0.001$) evaluated. The amounts found from the samples and fluid extracted were again correlated ($r = 0.985$). Using the LSD procedure, a significant increase was found after the addition of the egg-yolk freezing extender in the sample ($P < 0.001$) and fluid ($P < 0.001$). The lipid free radical accumulation is presented in Figure II. In lieu of the accumulation trends of lipid instability, the reduction of motility is correlated with higher levels of both lipid peroxidation ($r = -0.913$, $P < 0.001$) and free radicals ($r = -0.940$, $P < 0.001$) as demonstrated in Table V.

Table 4 : Averaged Lipid Free Radical Amounts from Procedural Steps

Step	n	Free Radical Amounts (nM/mL) Sample	SEM	n	Fluid	SEM
1: Post-collection	3	0.1^{a}	0.13	3	0.1^{y}	0.02
2: Extended	3	0.1^{a}	0.03	3	0.1^{y}	0.01
3: Cool to 17 °C	3	0.1^{a}	0.02	3	0.0^{y}	0.0
4: Wash Buffer	3	0.0^{a}	0.0	3	0.0^{y}	0.0
5: Centrifugation	0	n/a	-	3	0.0^{y}	0.0
6: Freezing Extender at 5 °C	3	3.4^{b}	0.81	3	3.5^{z}	0.74
7: Second Freezing Extender	3	3.2^{b}	0.78	3	4.3^{z}	1.75
8: Frozen-Thaw Samples	3	3.7^{b}	0.20	0	n/a	-

Different letter superscripts (a,b) indicate significant differences between lipid free radical levels of the procedural steps in the samples ($P < 0.001$).
Different letter superscripts (y,z) indicate significant differences between lipid free radical levels of the procedural steps in the fluid extracted ($P < 0.001$).

IV. Discussion

High lipid peroxidation levels have been associated in several articles with reduced sperm functionality. [1-4] Similar results were found in the present study as well (refer to Table V). However, it has been suggested in other articles that the cause of this membrane degradation was due to the mechanical stresses of cryopreservation. Our data suggests that the changes observed are due to the pre-freezing procedures, especially the addition of egg-yolk extenders at a cooled state (refer to Tables III and IV).

Table 5 : Motility and Lipid Stability Trends from the Samples of Experiment #3

Sample	n	Motility	Lipid Peroxidation (nM/mL)	Radicals (A233/mL)
Post-collection	3	75 %[1]	6.70^{a}	0.14^{y}
A Trt	3	23 %[2]	53.2^{b}	3.66^{z}
B Trt	3	22 %[2]	57.2^{b}	3.79^{z}
Control	3	14 %[2]	58.3^{b}	3.72^{z}

Different number superscripts indicate significant differences between motility percents ($P < 0.001$).
Different letter superscripts (a,b) indicate significant differences between lipid peroxidation levels ($P < 0.001$).
Different letter superscripts (y, z) indicate significant differences between lipid free radical amounts ($P < 0.001$).
Negative correlations were found between motility percentages and lipid peroxidation ($r = -0.913$) amounts and between motility percentages and lipid free radical ($r = -0.940$) amounts.

Centrifugation is a common step to concentrate the specimen in the cryopreservation preparation process. Yet it has been suggested that centrifugation may cause undue stress.[14] It has been observed in dog semen, that lower centrifugal speeds resulted in a higher amount of sperm lost in the supernate while viability losses were higher at increased centrifugational velocities. Yet, membrane integrity was maintained at the various speeds. [15] Our evaluation of the seminal fluid extracted before and after centrifugation, show very little difference in lipid stability (refer to Table III and IV). Neild and colleagues reported a capacitation-like phenomenon after centrifugation of stallion sperm.[16] Thus, permeability alterations may be due to other sources than lipid peroxidation.

Protein degradation due to peroxidation has been observed. [8] Baumber et al. correlated high peroxidation levels in post-thawed semen with DNA fragmentation. [17] This may actually be the cause for the reduced fertilization capability recognized in sperm after cryopreservation . [18]

As observed in bovine semen, our results showed higher levels of peroxidation in frozen-thawed semen versus fresh or cooled (refer to Tables III and IV). However, due to the trends at various points in the procedural analysis, our results do not demonstrate the stress in relation to cooling and thawing as suggested by Chatterjee and Gagnon.[19] Instead, the largest accumulation of both lipid peroxides and lipid radicals was observed during the addition of the freezing egg yolk extender (refer to Tables III and IV). Egg yolk contains phosphatidylcholine, similar to that already present in the membrane of sperm. Though studies have demonstrated the protective effects of egg yolk, [20] our results indicate a negative side. Misik and colleagues [21] demonstrated the amplification in MDA levels in egg yolk held at 22 °C compared to 50 °C. The introduction of a diluent with ongoing lipid peroxidation or the addition of a product, specifically lipid peroxides (LOOH), will enhance lipid peroxidation.[22] Thus, alternative extenders need to be investigated. Braun and colleagues observed an increase in the membrane integrity and motility of frozen stallion semen with the exclusion of egg-yolk. [23] Dacaranhe and Terao observed reduced iron-peroxidation levels with the presence of phosphatidylserine. [24] A trial on frozen stallion semen has already verified an improvement in motility with the addition of phosphatidylserine . [25]

The addition of antioxidants, such as Vitamin E, may need to be incorporated into breeding schemes to reduce lipid peroxidation rates. Increases in semen quality have been observed in [26] turkey, boar, [12, 27] ram,[28] and horse samples [17]with antioxidant supple-mentation. Dietary antioxidant supplementation has also increased semen quality in boars [27] and roosters .[29]

In the swine industry, semen cryopreservation is not frequently used due to the low number of piglets commonly produced. Previous studies have suggested that the structure of the sperm cells is damaged by the mechanical stress of freezing, thus reducing function One example is the difference observed in the membrane lipid stability before and after freezing. In the present study, we attempted to find when these changes occur by comparing semen samples from various procedural steps in the freezing process. The most significant lipid stability change was observed after an egg-yolk extender was added to the sample. Our results suggest the lipid alterations may not be due to the mechanical stress of freezing but rather by the extenders used. Though many freezing protocols use egg-yolk as a protectant and nutrition source for sperm, new extenders that stabilize lipids may need to be investigated. Also, dietary vitamin supplements may also improve outcomes. Further research is needed.

Literature Cited

1. Engel S., Schreiner T. and Petzoldt R. Lipid peroxidation in human spermatozoa and maintenance of progressive sperm motility. Androl. 1999; 31: 17-22.
2. Verma A and Kanwar KC. Human spermatozoal motility and lipid peroxidation. Ind. J. Exp. Biol. 1999; 37: 83-85.
3. Huang, YL, Tseng WC and Lin TH. In vitro effects of metal ions (Fe, Mn, Pb) on sperm motility and lipid peroxidation in human sperm. J. Toxic. Environ. Health.. 2001; 62, A: 259-267.
4. Zabludovsky, N, Elites F, Geva, E, Berkovitz, E, Amit A, Barak Y, Har-Even D and Bartoov B. Relationship between human sperm lipid peroxidation, comprehensive quality parameters and IVF outcome. Androl. 1999; 31: 91-98.
5. Mennella, MR and Jones R. Properties of spermatozoal superoxide dismutase and lack of involvement of superoxides in metal-ion catalyzed lipid peroxidation reactions in semen. Biochem. J. 1980;191: 289-297.
6. Alvarez, JG and Storey BT. Lipid peroxidation and the reactions of superoxide and hydrogen peroxide in mouse spermatozoa. Biol. Repro. 1984; 30: 833-841.
7. Duru NK, Morshedi M, Schuffner A, Oehninger S. Cryopreservation-thawing of fractionated human spermatozoa and plasma membrane translocation of phosphatidylserine. Fertil Steril. 2001 ;75: 263-268.
8. Medeiros, CMO, Forell F, Oliveira AT and Rodriquez JL Current status of sperm cryopreservation: why isn't it better? Therio. 2002; 57: 327-344.
9. Comaschi, V, Linder L, Farruggin G, Gesmudo N, Colombi L and Masotti L. An investigation on lipoperoxidation mechanisms in boar spermatozoa. Biochem. Biophys. Res. Commun. 1989; 158: 769-775.
10. Awda BJ, Mackenzie-Bell M, Buhr MM. Reactive oxygen species and boar sperm function. Biol Reprod. 2009; 81 :553-561.
11. Kim S, Lee YJ, Kim YJ. Changes in sperm membrane and ROS following cryopreservation of liquid boar semen stored at 15 °C. Anim Reprod Sci. 2011;124:118-124.
12. Cerolini S, Maldjian A, Surai P, and Noble R. Viability, susceptibility to peroxidation and fatty acid composition of boar semen during liquid storage. Ani Repro Sci. 2000; 58: 99-111.
13. Cerolini S, Maldjian A, Pizzi F and Gliozzi TM. Changes in sperm quality and lipid composition during cryopreservation of boar semen. Reprod. 2001; 121: 395-401.
14. Gil J, Soderquist L and Rodriquez-Martinez H. Influence of centrifugation and different extenders on post-thaw sperm quality of ram semen. Therio. 1999; 54: 93-108.
15. Rijsselaere T, Van Soom A, Maes D and de Kruif A. Effect of centrifugation on in vitro survival of fresh

diluted canine spermatozoa. Therio. 2002; 57: 1669-1681.
16. Neild, DM, Gadella BM, Colenbrander B, Aguero A and Brouwers JFHM. Lipid peroxidation in stallion spermatozoa. Therio. 2002; 58: 295-298
17. Baumber, J, Ball BA, Linfor JJ and Meyers SA. Reactive oxygen species and cryopreservation promote deoxyribonucleic acid (DNA) damage in equine sperm. Therio. 2002; 58: 301-302.
18. Dobrinski I, Thomas PG, and Ball BA. Cryopreservation reduces the ability of equine spermatozoa to attach to the oviductal epithelial cells and zonae pellucida in vitro. J Androl. 1995; 16: 536-542.
19. Chatterjee S and Gagnon C. Production of reactive oxygen species by spermatozoa undergoing cooling, freezing and thawing. Molec Repro Develop. 2001; 59: 451-458.
20. Vishwanath R and Shannon P. Storage of bovine semen in liquid and frozen state. Ani. Repro. Sci. 2000; 63: 23-53.
21. Misik V, Gergel D, Alov D, and Ondrias K. An unusual temperature dependence of malondialdehyde formation in $Fe^{2+}/H_2O_2^-$ Initiated Lipid Peroxidation of Phosphatidylcholine Liposomes. Physiol. Res. 1994 ;43: 163-167.
22. Tang L, Zhang Y, Oian Z and Shen X. The mechanism of Fe initiated lipid peroxidation in liposomes: the dual function of ferrous ions, the roles of the pr-existing lipid peroxides and the lipid peroxyl radicals. Biochem. J. 2000; 352: 27-36.
23. Braun, J, Hochi S, Oguri N, Sato K, and Torres-Boggino F. Effect of different protein supplements on motility and plasma membrane integrity of frozen-thawed stallion spermatozoa. Cryobio. 1995; 32:487-492.
24. Dacaranhe CD and Terao J. A unique antioxidant activity of phosphatidylserine on iron induced lipid peroxidation of phospholipid bilayers. Lipids. 2001; 36: 1105-1110.
25. Wilhelm KM, Graham JK, and Squires EL. Comparison of the fertility of cryopreserved stallion spermatozoa with sperm motion analyses, flow cytometric evaluation and zona free hamster oocyte penetration. Therio. 1996; 46: 559-578.
26. Donoghue AM and Donoghue DJ. Effects of water and lipid soluble antioxidants on turkey sperm viability, membrane integrity and motility during liquid storage. Poul. Sci. 1997; 76: 1440-1445.
27. Brzeezinska-Slebodzinska E, Slebodzinski AB, Pietras B and Wieczorek G. Antioxidant effect of vitamin E and gluthathione on lipid peroxidation in boar semen plasma. Biol. Trace. Elem. Res. 1995; 47: 69-74.
28. Câmara DR, Silva SV, Almeida FC, Nunes JF, Guerra MM. Effects of antioxidants and duration of pre-freezing equilibration on frozen-thawed ram semen. Therio. 2011 ;76:342-350.
29. Neuman, SL, Lin TL, and Hester PY. The effect of dietary carnitine on semen traits of white leghorn roosters. Poul. Sci. 2002; 81: 495-503.

Global Journals Inc. (US) Guidelines Handbook 2014

www.GlobalJournals.org

FELLOWS

FELLOW OF ASSOCIATION OF RESEARCH SOCIETY IN MEDICAL (FARSM)

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

- The "FARSM" 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., FARSM.

FARSM accrediting is an honor. It authenticates your research activities. After recognition as FARSM, you can add 'FARSM' 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:

FARSM 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%.

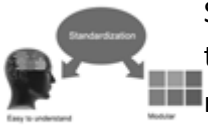
Once FARSM title is accorded, the Fellow is authorized to organize a symposium/seminar/conference on behalf of Global Journal Incorporation (USA). The Fellow can also participate in conference/seminar/symposium organized by another institution as representative of Global Journal. In both the cases, it 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 FARSM 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 FARSM, you will be given a renowned, secure and free professional email addres 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 FARSM 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 FARSM 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 FARSM, you may send us a scanned copy of all of you 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 FARSM 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 FARSM 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 FARSM 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 FARSM member can decide its price and we can help in making the right decision.

The FARSM 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 MEDICAL (MARSM)

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

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

MARSM accrediting is an honor. It authenticates your research activities. Afterbecoming MARSM,you can add 'MARSM' 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.

MARSM 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 MARSM, you willbe 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 MARSM 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 MARSM, 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 Open Association of Research Society (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.

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

Global Journal of Medical Research

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

ISSN 9755896