- Phaleria macrocarpa reduces glomerular growth factor expression in alloxan-induced diabetic rats
- Antifungal activity of neem leaf ethanol extract on Aspergillus flavus
- Red fruit oil supplementation fails to prevent oxidative stress in rats
- Nicotine supplementation blocks oocyte maturation in Rattus norvegicus
- Andrographis paniculata extract induced apoptosis of adenocarcinoma mammae in C3H mice
- Weekly lifestyle counselling improves glucose level in type 2 diabetes mellitus patients
- Soy-isoflavone supplementation tends to reduce menopausal symptoms in postmenopausal women
- Plasmodium falciparum infection and the risk of anemia in school children
UNIVERSA MEDICINA

Editor-in-Chief
Prof Adi Hidayat, MD, MS, PhD

Manuscripts Editor
Prof Murad Lesmana, MD

Editorial Board
Prof H.A. Prayitno, MD, SpKJ, PhD
Prof Muzief Munir, MD, SpA(K)
Prof Julius E. Surjawidjaja, MD, SpMK
Raditya Wratangka, MD, SpOG(K), PhD
Oktavianus CH. Salim, MD, MS
Elly Herwana, MD, MBIomed, PhD
Pusparini, MD, SpPK, PhD
Maria Regina Rachmawati, MD, SpRM, PhD
Melianti, MD, SpFK
Yenny, MD, SpFK
Richard Tjan, MD, DTMH

Distribution and Marketing
Eddy Kasim, DS
Julius I. Mulla, MD

Secretary
Rita Hemawati

Accreditation: 58/DIKTI/Kep/2013

Correspondence Address:
Medical Faculty, Trisakti University
Jl. Kyai Tapa No.260
Grogol - Jakarta 11440
Phone: 021-5672731 ext. 2611
Fax: 021-5660706
Homepage: www.univmed.org
Email: editors@univmed.org

Published by Faculty of Medicine Trisakti University
Guidelines For Authors

Univera Medcina is a quarterly medical journal and accepts medical and health-related original articles (research papers) in English or Indonesia. The Indonesian papers will be translated and published in English.

SUBMISSION
Submitted manuscripts should not have been published before and must not be under simultaneous consideration by any other journal.

Manuscript preparation
The manuscript should be formatted as follows: A4 (212 x 297 mm), with margins of at least 2.5 cm, use double-spacing in a serif font (e.g., Times), 12-point and limited to approximately 16 pages in length, including references, tables and figures. Do not justify the right margin.
Number pages consecutively in the upper right-hand corner of each page, beginning with the title page. Each manuscript component should begin on a new page in the following sequence: title page, abstract and key words, text, acknowledgments, references, tables, each table on a separate page, complete with title and footnotes, legends for figures. All manuscripts should be accompanied by a cover letter from the author responsible for correspondence.

Title page
This should carry the title of the article, the names and addresses of all authors (the institution to which the work is to be attributed should be listed first), the name, address, fax number and email address of the corresponding author, and a short running title for the headings not more than 40 characters.

Abstract and key words
Original research articles must include a structured abstract that contains no more than 250 words and should consist of background including objective, methods, design, results and conclusions. Below the abstract, provide a list of 3–10 key words.

Text
The text of research papers should be divided into sections with the following headings: introduction, methods, results, discussion, and conclusions.

Acknowledgements
Acknowledge only persons who have made substantive contributions to the study.

References
It is the authors' responsibility to check all references very carefully for accuracy and completeness. Number references consecutively in the order in which they are first mentioned in the text. Identify references in the text. Tables and Lists by Arabic numerals in superscript and parenthesis. "Unpublished observations" and "personal communications" may not be used as references; if cited, a letter from the person quoted granting permission must be submitted. Authors should avoid using abstracts as references. Abbreviate journal names according to the Index Medicus system. (Also see International Committee of Medical Journal Editors Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Sample References http://www.nlm.nih.gov/bmd/ uniform_requirements.html). Examples of correct references are given at the end of these instructions.

Tables and Illustrations (Figures)
Type or print each table with double spacing on a separate sheet of paper. Number tables consecutively in the order of their first citation in the text and supply a brief title for each. Do not use internal horizontal or vertical lines. Give each column a short or abbreviated heading. Authors should place explanatory matter in footnotes, not in the heading. Explain in footnotes all nonstandard abbreviations. For footnotes use the following symbols, in sequence: *, ++, **, ***. Identify statistical measures of variation, such as standard deviation and standard error of the mean. Figures should be either professionally drawn and photographed, or submitted as photographic prints of quality digital prints. For x-ray films, scans, and other diagnostic images, as well as pictures of pathology specimens or photomicrographs, not draw paper, glossy, black-and-white or color photographic prints, usually 127 x 173 mm (5 x 7 inches). Figures should be numbered consecutively according to the order in which they have been cited in the text. If a figure has been published, acknowledge the original source and submit written permission from the copyright holder to reproduce the material. Permission is required irrespective of authorship or publisher except for documents in the public domain.

Units of Measurement
Measurements of length, height, weight, and volume should be reported in metric units (meter, kilogram, or liter) or their decimal multiples. Temperatures should be in degrees Celsius. Blood pressures should be in millimeters of mercury, unless other units are specifically required by the journal.

Sending the Manuscript to the Journal
Manuscripts, written in English, can be submitted electronically as an e-mail attachment to the following address: http://www.univmed.org or alternatively to the following address: Editor's Office, Universa Medcina, Fakultas Kedokteran Universitas Trisakti, Kampus B USAKTI L2, 6 Jl. Kyai Tapa No. 260, Grogol-Jakarta Barat 11440, Phone (62-021) 5672731 (ext. 2504) Fax 62-021-5660706 Email editors@univmed.org.
Examples of correct forms of references

Journals
1. Standard journal
List all authors when 6 or fewer; when 7 or more, list only the first 6 and add "et al." Abbreviate journal titles according to Index Medicus style, which is used in MEDLINE citations.


2. Corporate author

3. Volume with supplement

4. Electronic journal without page numbers
Bawaskar HS, Bawaskar PH. Efficacy and safety of scorpion antivenom plus prazosin compared with prazosin alone for venomous scorpion (Mesobuthus tamanu) sting: randomised open label clinical trial. BMJ 2011;343:d7136. doi:10.1136/bmj.d7136

Books and Other Monographs
1. Editor(s), compiler(s) as author

2. Chapter in a book

3. Conference paper

4. Dissertation

Electronic Material
1. CD-ROM

2. Journal article on the internet

3. Monograph on the internet
Manuscript Checklist

As you prepare to submit your manuscript, please go through this list and check-off each as it is completed. Failure to conform to the format requirements will result in your paper being sent back to you, and a delay in publication.

Include original manuscript plus two copies and a CD containing an exact version of the original hard copy.

Double space manuscripts (text, references and tables) and leave right margins unjustified (ragged).
The title is in upper case letters and centered on the page. The author’s names are centered under the title.

Information about the author’s department, university, university address, and e-mail address is provided. Phone and fax numbers appear only on the cover letter. If information is different for each author, multiple asterisks are used.

Provide a structured abstract that conforms with the required abstract format.

An acknowledgement section can be presented after the conclusion.

References are numbered and listed in the reference section in the order that they appear in the text, not in alphabetical order by author’s names. When referring to a source in the text, only the numeral enclosed in square brackets is used for identification.
Pedoman Untuk Penulis

Universa Medica adalah berkala ilmiah yang terbit setiap
caesar wulan (3 kali dalam 1 tahun). Januari-April, Mei-August
September-Desember dan menerima raihan ilmiah di bidang
kesihatan/keiskerian dalam bentuk laporan perelitian original
article research paper dalam bahasa Indonesia atau inggris. Setiap
makalah akan dipublikasi dalam bahasa inggris.

Penguiran Mahalah
Makalah yang dikirim untuk dimuat dalam Universa
Medica belum pernah dipublikasi dan tidak diberikan ke
perencana lain pada waktu yang bersamaan. Naskah dikirimkan
dalam bentuk print out sebanyak 2 (dua) salinan, dalam file
W.RD, serta diberi menggunakan program komputer
Microsoft Word for Windows baik melalui email
(editor@unvmed.org) maupun ke alamat redaksi.

Persiapan teknis makalah
Naskah ditiket pada kertas berukuran 8,27 x 11,69 " (A4)
dengan batas tepi (margin) 1", (12 point Times New Roman, bebas huruf
text serif) 12 point dan menggunakan spasi rangkap 2 (dua)
double space). Setiap bagian/onslide dan naskah dimulai pada halaman
baru, dengan urutan sebagai berikut: halaman judul, abstrak dan
kata kunci (key words), teks kesebelumnya, ucapan terima kasih,
dafat poslu, tabel dan gambar (setiap tabel dan gambar pada
halaman terpisah). Nomer halaman dicantumkan secara berurutan
mulai dari halaman judul pada sudut sebelah kanan bawah
Makalah Hendaknya ditulis maksimal 16 halaman.

Halaman judul
Halaman judul (halaman pertama) harus mencakup a) judul
makalah yang dibuat sesuai mungkin, spesifik informatif dan
ringkas (j清代 best short running head or foot line) tidak lebih dari
40 karakter (hitung huruf dan spasi) yang dicantumkan di bawah
judul, b) nama dan alamat surat penulis, nama departemen dan
kembaga affiliation; c) nama dan alamat penulis untuk
korespondensi serta nomor telepon, nomor faksimili, dan alamat
e-mail.

Abstrak dan kata kunci
Abstrak adalah menurut abstrak yang tidak lebih dari 250
kata untuk laporan penelitian yang ditulis dalam bahasa Indonesia
dan Inggris. Abstrak laporan penelitian merupakan abstrak
(best short running head or foot line) yang berisikan larat belakang terbaru (tajuran penelitian background), metode (methods), hasil (results) dan kesimpulan (conclusions). Kata kunci dicantumkan di bawah abstrak pada
halaman yang sama sebanyak 3-10 kata. Curahakanlah kata-kata yang
sesuai dengan daftar pada Index Medicus.

Teks
Teks makalah laporan penelitian dibagi dalam beberapa
bagian dengan judul sebagai berikut: Perkenalan (Introduction),
Metode (Method), Hasil (Results), Pembahasan (Discussion),
Kesimpulan (Conclusion), dan Ucapan terima kasih (Acknowledgement).

Ucapan terima kasih
Terutama ditujukan kepada 1) pihak-pihak yang
memberikan bantuan dan dukungan. 2) dukungan dari badan
dan lembaga. 3) para profesional yang memberikan kontribusi
dalam penyusunan makalah.

Daftar Pustaka
Daftar pustaka detail sesuai dengan cara penulisan manual
Vancouver dan hanya mencantumkan keputusan yang dipakai
dan relevan. Rujukan diberi nomor urut sesuai dengan peraturannya
dalam teks menggunakan angka Arab dan dalam teks nomor urut dituliskan dalam tanda kurung. Akan yang digunakan hendaknya > 80% merupakan sumber acuan primer
(jurnal) dan mutakhir (10 tahun terakhir). Tabel dan gambar diberi
nomor sesuai dengan urutan peraturannya dalam teks
menggunakan angka Arab. Simpanlah nama jurnal detail sesuai
dengan daftar dalam Index Medicus yang dapat diperoleh pada
abstrak sebagai rujukan.

Contoh cara menuliskan rujukan adalah sebagai berikut:

Artikel dalam jurnal
1. Artikel jurnal standar
Curumumkan semua penulis bila jumlah penulis 6 (enam) atas
kurang: bila 7 (tujuh) atau lebih cantumkan 6 (enam) penulis
pertama dan diikuti oleh et al.
Prud'homme NR, Hurten K.M. Efficacy and safety of long-acting
glucagon-like peptide-1 receptor: a systematic review and
Lebih dari 6 (enam) penulis:
Campbell NRC, Gilbert RE, Leiter LA, Larochelle P, Tober
2 diabetes: update on pharmacologic management. Can J
Physician 2011;57:997-1002.
2. Artikel jurnal yang disertai DOI
Bhatia TA, Darnstadt GL, Hasan BS, Haws RA.
Community-based interventions for improving perinatal and
neonatal health outcomes in developing countries: a review of
DOI:10.1542/peds.2004-1441.
3. Organisasi sebagai penulis
The National Osteoporosis Foundation of South Africa. Use of
generic zoleconate in the treatment of osteoporosis. S
4. Volume dengan suplemen
Gerald G, Spierings EL, Keywood C. Tolerability and safety of
voiepatin with short- and long-term use for treatment of
migraine and in comparison with sumatriptan. Headache
5. Edisi dengan suplemen
Glasser TA. Integrating clinical trial data into clinical practice.
Neurology 2002;58(12 suppl 7):S6-12.
6. Volume dengan bagian
Abend SM, Kalish N. The psychoanalytic method from an
epistemological viewpoint. Int J Psychoanal 2002:83(2 Pt
2):491-5.
7. Edisi dengan bagian
Ahmar K, Madoff DC, Gupta S, Wallace MD, Price RE, Wright
KC. Development of a large animal model for lung tumors.
8. Edisi tanpa volume
Banting DM, Kastrys H, Hartford J.M. Intraoperative frozen
section analysis in revision total joint arthroplasty. Clin
Bisaka dan monografi lisan  
(Atruran Vancouver sebelumnya salah mercantumkan tanda baca koma dan bukannya tanda baca titik koma antara penerbit dan tahun penerbitan)

1. Penulis perorangan

2. Editor, sebagai penulis

3. Organisasi sebagai penulis dan penerbit


5. Bah dalam buku
   Catatan: Atruran Vancouver sebelumnya mencantumkan tanda baca titik dua, sekarang tanda 'p' untuk halaman.

6. Prosiding konferensi

7. Makalah dalam konferensi

8. Laporan ilmiah atau teknis
   Diterbitkan oleh agen penyandang dana sponsor
   Diterbitkan oleh agen pelaksana

9. Nomor Halaman dalam angka romawi


Disertas


Materi audiovisual


Kamus, Encyclopedi, dan rujukan serupa


Materi Elektronik

1. Artikel jurnal dalam format elektronik

2. Monografi dalam format elektronik

Tabel dan gambar

Catat sejauh tabel pada halaman terpisah dan diketik spasi yang lebih (double space). Nomor urut tabel dan gambar sesuai dengan urutan penampilan mereka dalam teks. Untuk catatan kaki (footnotes) pada tabel gunakan symbol dengan urutan sebagai berikut: *, †, ‡, §, ‖, *, †*, ‡*, §*, ‖*.  

Naskah yang diterima redaksi akan diubah oleh penerbit dan redaksi berhak memperbaiki susunan bahasa tampa mengubah isinya. Penggunaan istilah asing non-medis sedapat mungkin dihindari atau disertai terjemahan perjelasannya. Usian perbaikan naskah (terutama menyentuh substansi) akan disampaikan kepada penulis yang bersangkutan.

Naskah dikirimkan ke alamat Redaksi:

Fakultas Kedokteran Universitas Trisakti
Jl. Kyai Tapa No. 260, Grogot - Jakarta 11440
Telepon: (021) 5672731 (inning ext. 2054. Fax: (021) 5660706
E-mail: editors@anivmed.org

Tiras: 800 eks
# Table of Contents

**Volume 32 — May-August, 2013 — Number 2**

## Editorial

HIV antiretroviral preexposure prophylaxis ......................................................... 69

*Richard Tjan*

## Research Articles

**Phaleria macrocarpa** reduces glomerular growth factor expression in alloxan-induced diabetic rats ............................................................. 71

*Evy Sulistyowinagrun and Setiawati*

Antifungal activity of neem leaf ethanol extract on *Aspergillus flavus* .................. 80

*Aly Margaret, Hanna Yolanda, and Lies K. Wibisono*

Red fruit oil supplementation fails to prevent oxidative stress in rats .................... 86

*Maria Dara Novi Handayani, Parwati Abadi Soekarno, and Septelita Inawati Wanandi*

Nicotine supplementation blocks oocyte maturation in *Rattus norvegicus* .......... 92

*Meitria Syahadatina Noor, H.M. Bakhriamayah, Widjati, and Budi Sutargo*

Andrographis paniculata extract induced apoptosis of adenocarcinoma mammae in C3H mice .......................................................... 99

*Nugrohaningsih, Sarjadi, Edi Dharmana, and Hertanto Wahyu Subagio*

Weekly lifestyle counselling improves glucose level in type 2 diabetes mellitus patients 108

*Amalilia Nuggetsiana Setyawati, Inggar Oca Pushtika, and Kusmiyati Tjahjono DK*

Soy-isoflavone supplementation tends to reduce menopausal symptoms in postmenopausal women ......................................................... 118

*Raditya Wratangka and Ati Cicih Mayasari*

*Plasmodium falciparum* infection and the risk of anemia in school children .......... 128

*Surjani Tan, Taniawati Supali, and Heri Wibowo*
HIV antiretroviral preexposure prophylaxis

Richard Tjan
Editor

According to 3 field trials conducted in Africa, one among African women and two among heterosexual couples, antiretroviral preexposure prophylaxis for prevention of HIV-1 has been shown to be effective.\(^{(1-3)}\) In preexposure prophylaxis, persons without HIV infection are given an oral drug before they have sexual contact with HIV-infected partners.\(^{(4)}\) The drug in question is tenofovir disoproxil fumarate (TDF), a prodrug of tenofovir.\(^{(1)}\) On the basis of the 3 field trials, the Antiretroviral Drugs Advisory Committee of the Food and Drug Administration has recommended a combination of antiretroviral drugs (tenofovir/emtricitabine) for preexposure prophylaxis of HIV.\(^{(5)}\)

TDF is currently indicated for the treatment of HIV in adults over 18 years of age or hepatitis B virus (HBV) infection in adults, or both. The drug is called a nucleotide reverse transcriptase inhibitor (NRTI), preventing the synthesis of viral copies by HIV reverse transcriptase or HBV DNA polymerase.\(^{(6)}\) To retard the emergence of TDF resistance, the drug is usually given in combination with another antiviral, such as emtricitabine (FTC). Nucleic acid testing for HIV virus when starting preexposure prophylaxis, may reduce the risk of resistance, but it is at present not an option in developing countries because of its high costs. Rare but potentially serious adverse reactions to TDF are lactic acidosis and toxic effects on the liver and kidneys.\(^{(6)}\) Because administration of TDF to healthy noninfected persons implies using the drug for a prolonged period of many years, the long-term safety of TDF and the TDF-FTC combination has to be clearly established.\(^{(6)}\)

There is also a real possibility that preexposure prophylaxis may lead to relaxation of the customary precautions on the part of the sexual partners, such as engaging in increased risky sexual behavior or abandoning the use of conventional prophylactic measures (e.g. condoms).\(^{(6)}\) This matter should be a problem for health educators.

From a practical point of view, because of the potential of serious liver and kidney disease caused by TDF, the medical practitioner should prescribe preexposure prophylaxis only in high risk cases, and not for prevention of HIV in otherwise healthy individuals, e.g. blood bank personnel or dental practitioners with a low risk of exposure to HIV, which are currently not indicated. Prescription should be done on an individual basis.

Indeed, the old Hippocratic advice of not too readily prescribing any new modes of treatment, or in plain words - Wait and watch- still holds true. This is presumably one of the reasons for not blindly or overenthusiastically accepting HIV preexposure prophylaxis.
REFERENCES


Nicotine supplementation blocks oocyte maturation in *Rattus norvegicus*

Meitria Syahadatina Noor*, H.M. Bakhriansyah**, Widjiati***,

*Department of Public Health, Faculty of Medicine, University of Lambung Mangkurat, Banjarmasin
**Department of Pharmacology, Faculty of Medicine, University of Lambung Mangkurat, Banjarmasin
***Department of Embryology, Faculty of Veterinary Medicine, University of Airlangga, Surabaya
****Department of Obstetrics and Gynecology, Faculty of Medicine, Dr. Soetomo General Hospital, University of Airlangga, Surabaya

Correspondence dr. Meitria Syahadatina Noor Department of Public Health, Faculty of Medicine, University of Lambung Mangkurat Jl. Veteran, Banjarmasin, Kalimantan Selatan Phone: +6281391739795 Email: drmeitria79@gmail.com

ABSTRACT

BACKGROUND
Indonesia has the third largest tobacco consumption in the world after China and India. Nicotine as the main component of cigarette smoke has negative effects on the reproductive system, such as oocyte maturation, ovulation, and fertilization, and increasing the diploidy of oocytes. The goal of this research was to evaluate the effect of nicotine on oocyte maturation in *Rattus norvegicus*.

METHODS
This was an experimental study with post test only control group design. The subjects were 40 rats selected homogenously and randomly. They were divided into a control group (receiving carboxy-methyl-cellulose sodium and 3 treatment groups (I-III) receiving nicotine subcutaneously for 7 days at dosages of 21 mg/ kgBW, 41 kg/kgBW and 84/kgBW, respectively. The observations comprised oocyte maturation stage, viz. germinal vesicle (GV), germinal vesicle breakdown (GVBD), metaphase I and metaphase II. Data were analyzed by one-way Anova with α=0.05, followed by Tukey’s HSD test.

RESULTS
One-way Anova showed significant differences in oocyte maturation in all groups. Tukey’s HSD test showed that for GV, the differing groups were control and I, control and II, I and III. For GVBD, the differing groups were control and I, I and II, I and III. For metaphase I, the differing groups were control with I, II, and III, I and II, I and III. For metaphase II, the differing groups were control versus I, II, and III, I and II, I and III.

CONCLUSION
Low dose of nicotine is capable of affecting oocyte maturation in *Rattus norvegicus*.

Key words: Nicotine, maturation, oocyte, *Rattus norvegicus*
**INTRODUCTION**

According to a statement by the WHO there were 1.3 billion smokers in the world in 2003 and their numbers will increase to 1.7 billion in 2010. It has been estimated that around one billion people died from smoking in the 21st century. Nicotine is the main component of cigarette smoke and the cause of the smoking habit or addiction. Exposure to cigarette smoke affects both active and passive smokers. The effects of cigarette smoke on the reproductive system are to influence the production and function of gametes, ovulation, the reproductive cycle, fertilization, and embryo transport and implantation. The main component of cigarettes is nicotine (C_{10}H_{14}N_{2}), which constitutes 50% of all components. Nicotine is capable of forming free radicals, thus being a pro-oxidant. Nicotine exerts adverse effects on follicle growth, number of follicles, thickness of the endometrium and uterine glands. It also adversely affects cumulus cells and the organization of microtubules and microfilaments in the oocyte during meiosis. In vitro experiments showed that nicotine induced meiotic blockage in metaphase-I of mouse oocytes, while administration of nicotine to mice in vivo resulted in reduced numbers of ovulated oocytes. The study by Dwirahayu found that nicotine blocks oocyte maturation in Rattus norvegicus at dosages of 35, 52.5, and 70 mg/kgBW.
This is because nicotine reduces the size of ovarian follicles, thus affecting the oocyte maturation process.\(^8\)

In view of the above, it was thought necessary to conduct further studies on the effects of nicotine on oocyte maturation, using lower doses of nicotine, in order to determine the minimal dose capable of affecting fertility in females. The doses used were based on those of Kakisina who used doses of 3, 6, and 12 mg/kgBW for determining developmental abnormalities in mouse embryos. The doses had been validated in preliminary studies and were far below the lethal dose.\(^9\) These doses were then converted into doses of 21, 42, and 84 mg/kgBW for use in the Norway rat (\textit{Rattus norvegicus}).

**Experimental animals**

The study subjects were adult female Norway rats (\textit{Rattus norvegicus}). The rats were randomly assigned to one control group and three treatment groups (I-III). The sample size was 9 rats per group, based on \(\alpha=0.05\), \(\beta=0.2\) and effect size = 0.3.\(^{11}\) To anticipate a reduction in numbers through death, the size of the groups was increased by 20%, so that each group contained 11 rats.

**Preparation of nicotine**

The nicotine doses of 21 mg/kgBW, 42 mg/kgBW and 84 mg/kgBW, were adjusted to the weight of individual rats. Liquid nicotine of 70% purity was diluted with twice-distilled water (\textit{aqua bidestillata}) and the calculated dose for each rat was administered.

**Treatment**

The rats were given a subcutaneous injection of nicotine at 21 mg/kgBW (I), 42 mg/kgBW (II) and 84 mg/kgBW (III) for 7 days. These doses were seven times larger than the corresponding dose for mice (7 x mouse dose).\(^{10}\) The controls were given an injection of carboxy-methyl-cellulose sodium (CMC-Na) using a comparable technique and duration of treatment as used for the treatment groups. The injections were performed by experienced personnel using disposable syringes and needles for each injection.

**Harvesting of oocytes**

The rats were given an injection of 10 IU of pregnant mare serum gonadotropin (PMSG), and 48 hours later an injection of 10 IU human chorionic gonadotropin (HCG). Subsequently the rats were mated with vasectomized male rats to induce ovulation. After 17 hours each female was examined for the presence of a vaginal plug. Rats with a vaginal plug were sacrificed by euthanasia for harvesting of oocytes.

Harvesting of oocytes was done by lifting the uterus under a dissecting microscope and looking for the fertilization pouch. The oocytes were released by rupturing the fertilization pouch. The released oocytes were then transferred by means of a modified pipette into a petri dish containing phosphate buffered saline (PBS) as washing medium.

**Staining of oocytes**

The oocytes were placed on a glass slide that was ringed with vaseline and covered with a cover slip. Subsequently the oocytes were fixed in fixing solution (acid alcohol : absolute methanol = 1:3) for at least 24 hours. The slide was then removed from the fixing solution and air-dried. The oocytes were stained in 1% aceto-orcein for 2-3 seconds and washed in decolorizing solution. They were examined under the microscope for germinal vesicles (GV), germinal vesicle breakdown (GVBD), metaphase I and metaphase II. Examination of
the oocytes was done at the Embryology Laboratory, Faculty of Veterinary Medicine, University of Airlangga, Surabaya.

Data analysis
The data on oocyte maturation were obtained from the stages of GV, GVBD, metaphase I and metaphase II. Statistical analysis was performed by means of one-way Anova, followed by Tukey’s HSD test. The level of significance was set at 0.05.

Ethical clearance
This study was given ethical clearance by the Research Ethics Committee, Faculty of Medicine, University of Lambung Mangkurat, Banjarmasin.

RESULTS
The total number of oocytes collected in each treatment group are presented in Figure 1.

The number of oocytes was inversely proportional to the nicotine dose, i.e. larger doses of nicotine resulted in reduced number of oocytes.

The results of one-way Anova showed significant differences in the numbers of oocytes between all four groups (p=0.000). The control group had a significantly larger number of oocytes in comparison with the three treatment groups (p=0.00). The reduction in the number of oocytes in the group receiving nicotine at a dose of 21 mg/BW (I) was significantly smaller than that in the groups receiving nicotine at doses of 42 mg/kg BW (II) and 84 mg/kg BW (III) (p=0.00). The nicotine dose of 42 mg resulted in a smaller reduction in the number of oocytes than the dose of 84 mg, but the difference was statistically not significant (p=0.74).

The maturation of the oocytes was subsequently observed, with the results shown in Figure 2, depicting oocyte maturation stages in the control group and the groups exposed to nicotine at various doses. Exposure to nicotine at a dose of 21 mg/kgBW significantly reduced the numbers of oocytes in the GV stage as compared with controls (p<0.05). Exposure to doses of 42 and 84 mg/kgBW did not significantly alter the numbers of GV compared with controls (p>0.05). In the GVBD stage, exposure to nicotine at 21 mg/kgBW significantly reduced the number of oocytes in that stage as compared with controls.
with controls (p<0.05). Exposure to doses of 42 and 84 mg/kgBW did not significantly alter the numbers of oocytes in the GVBD stage as compared with controls (p>0.05). In metaphase I, a significant reduction in the number of oocytes was found on exposure to nicotine doses of 42 and 84 mg/kgBW in comparison with controls (p<0.05), which was not found with the dose of 21 mg/kgBW (p>0.05). In metaphase II, a significant reduction in the number of oocytes was found on exposure to nicotine at all doses in comparison with controls (p<0.05).

Tukey’s HSD found significant differences between controls and the three nicotine dosage groups with respect to all observed maturation stages, with p=0.000 for all comparisons.

For the GV stage, there were significant differences between controls and I, controls and II, and between I and II, I and III, II and III. For GVBD, the differing pairs of groups were controls and I, I and II, I and III. For metaphase I, the differences were between controls on the one hand and I, II, and III on the other, and also between I and II, and between I and III. In metaphase II stage, the differing groups were controls versus I, II, and III, I and II, I and III.

**DISCUSSION**

In this study there were differences in the number of oocytes released from the ovaries between controls, treatment groups I (21 mg/kg BW), II (42 mg/kg BW) and III (84 mg/kg BW). Our study results are consistent with the study conducted by Mokhtar et al., where it was shown that nicotine adversely affects the number and quality of oocytes and the fertilization rate in animal models.(12) A recent study using confocal microscopy on oocytes from mice exposed to cigarette smoke revealed that these oocytes had significantly thicker zona pellucida and shorter and wider meiotic spindles. Approximately 25% of these oocytes had errors in chromosomal congression or abnormally shaped spindles.(13)

In the present study, observations on the maturation stages of retrieved oocytes, comprising GV, GVBD, metaphase I and metaphase II, showed significant differences at all maturation stages between the control group and the three treatment groups. This study was an improvement over the study of Dwirahayu, who made similar observations with different doses. The study of Dwirahayu showed significant differences for metaphase II only.(8) In contrast, for GV and GVBD there
were no significant differences, and for metaphase I no statistical tests could be performed because all results were zero.\textsuperscript{(6)} Another study using nontoxic doses of nicotine of 1.0, 2.5, 5.0 and 10.0 mmol/L, respectively, showed nicotine to have no adverse effects on GV breakdown.\textsuperscript{(14)}

Free radical or reactive oxygen species (ROS) production by nicotine is the result of inhibition of anti-oxidant enzyme and subsequent lipid peroxidation. Oxidative stress from free radicals or ROS may damage the cell membrane and also induce DNA fragmentation.\textsuperscript{(15)} Oxidative stress also leads to chromosomal instability and programmed cell death, the latter being the main mechanism of oocyte death.

When fully developed or mature oocytes are released from the follicle for ovulation, the meiotic process is completed as shown by their being in metaphase II. If the meiotic process is not completed, it will stop at any given stage, either GV, GVBD or metaphase. If the developing oocytes have not reached their full size when released from the follicle, they cannot become mature, being in the GV and GVBD stages. Medium-sized oocytes may reach maturity although they have not yet completed the meiotic process and have stopped at metaphase I.\textsuperscript{(10)}

The mechanism of oocyte maturation blockage as a result of exposure to nicotine is by systemic oxidative stress and oxidative stress in the follicular fluid. Intrafollicular oxidative stress may cause apoptosis of the follicular granulosa cells, thus impeding the process of folliculogenesis and reducing follicular diameter.\textsuperscript{(4)}

The size of the follicle affects oocyte development because the capacity of oocytes to complete meiosis for full maturation depends on follicular size. If the follicle becomes smaller in size, the maturation process of the oocytes within the follicle will be blocked. The number of oocytes from small follicles are lower than that of mature oocytes from large follicles.\textsuperscript{(16,17)} Immature oocytes are characterized by the GV stage, GVBD and metaphase I, whereas mature oocytes are characterized by metaphase II.\textsuperscript{(16,17)}

The results of the present study showed that each group differs significantly from the others, signifying that low doses of nicotine is capable of affecting oocyte maturational development. Increasing the dose severely affects the development resulting in fewer mature oocytes. Exposure to nicotine from cigarette smoke at any dose significantly affects oocyte development in metaphase I and II. If there are no oocytes in metaphase II, there are no mature oocytes. Ultimately, this results in female infertility because only mature oocytes can be fertilized by sperm. These findings demonstrate the extreme sensitivity of human oocytes to cigarette smoke, and underline the need for experimental animal data to clarify the causes of meiotic blockage.

One limitation of this study was the inability to perform nicotine exposure by inhalation in order to mimic smoking. The implication of the study is that the dose of 21 mg/kgBW, which was capable of reducing the number of oocytes in \textit{Rattus norvegicus}, can be used to calculate the minimal dose in humans, i.e. 1176 mg. One cigarette contains 0.3-2 mg of nicotine, therefore 588-3920 cigarettes smoked actively or passively, are sufficient to impair fertility in women.

CONCLUSION

Nicotine, the major alkaloid in tobacco, is capable of blocking oocyte maturation in the Norway rat (\textit{Rattus norvegicus}). Immature oocytes cannot be fertilized by sperm, invariably leading to infertility.

ACKNOWLEDGEMENT

We thank the staff at the Embryology Laboratory, Faculty of Veterinary Medicine, University of Airlangga, Surabaya and Bambang Setiawan, S. Ked., M. Biomed for enabling us to complete and publish this study.

REFERENCES

1. Rabinoff M, Caskey N, Rissling A, Park C. Pharmacological and chemical effects of


13. Jennings PC, Merriman JA, Beckett EL, Hansbro PM, Jones KT. Increased zona pellucida thickness and meiotic spindle disruption in oocytes from cigarette smoking mice. Hum Reprod 2011;26:878-84.