FRAKSI HEKSAN EKSTRAK BIJI PEPAYA MUDA DAPAT MENGHAMBAT PROSES SPERMATOGENESIS MENCIT JANTAN LEBIH BESAR DARIPADA FRAKSI METANOL EKSTRAK BIJI PAPAYA MUDA

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ABSTRAK

Fraksi ekstrak heksan mengandung dua golongan zat aktif yang bersifat antifertilitas yaitu golongan steroid dan golongan triterpenoid yang diperkirakan bersifat antifertilitas, walupun mekanisme kerjanya belum jelas. Rancangan penelitian yang digunakan ialah '' *Pre-test post-test control group design''*. Penelitian ini memakai 30 ekor mencit jantan strain balb C , umur sekitar 12 minggu dengan berat 20-22 gram, kemudian dikelompokkan secara random menjadi 3 kelompok yang masing-masing terdiri dari 10 ekor. Satu kelompok kontrol (P0 = yang diberikan aquabides), dan dua kelompok perlakuan (P1 = kelompok perlakuan yang diberikan fraksi heksan ekstrak 20 mg/20 gram/hari, P2 = kelompok perlakuan yang diberikan fraksi ekstrak metanol 20 mg/20 gram/hari). Setelah 36 hari perlakuan lalu dilakukan pemeriksaan testis dan darah mencit. Data yang diperoleh dianalisis secara statistik dengan menggunakan uji normalitas Kolmogorov Smirnov Goodnees of Fit test, uji homogenitas, dan uji anova. Didapatkan hasil bahwa fraksi heksan ekstrak maupun metanol dapat menurunkan jumlah sel spermatogonia A, sel spermatosit primer pakhiten, sel spermatid, dan sel Sertoli secara sangat bermakna ($p < 0,01$), sedangkan jumlah sel Leydig dan kadar hormon testosteron menurun tidak bermakna ($p > 0,05$). Dari hasil penelitian ini dapat disimpulkan bahwa fraksi heksan ekstrak biji pepaya dapat menurunkan jumlah rata-rata sel spermatogonia A, spermatosit primer pakiten, spermatid, sel Sertoli, sel Leydig dan kadar hormon testosteron lebih besar dari pada fraksi metanol ekstrak biji pepaya muda.

Kata kunci : spermatogenesis, testosteron, fraksi heksan ekstrak biji pepaya muda dan fraksi metanol ekstrak biji pepaya muda, mencit jantan

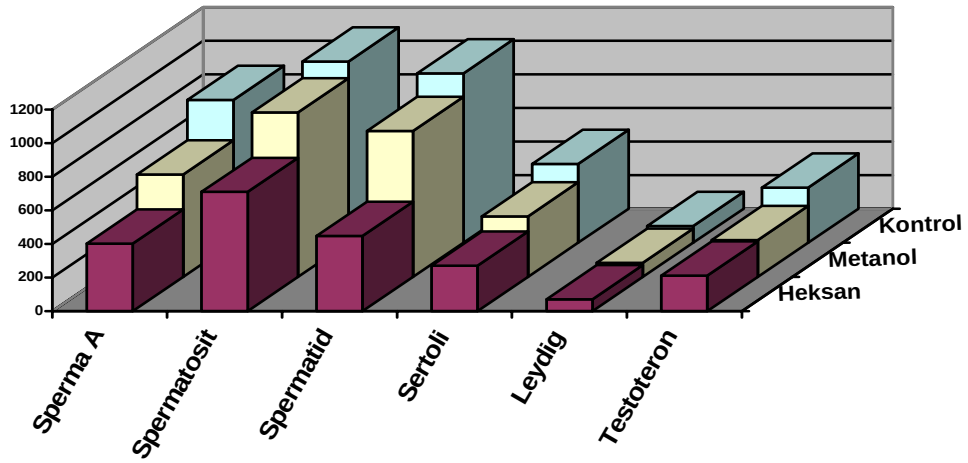
Pendahuluan

Salah satu alasan rendahnya partisipasi pria dalam keluarga berencana karena kontrasepsi pria yang tersedia sangat terbatas jenisnya (Arsyad, 1990; Hartono, 1996). Masalah tersebutlah yang menjadi landasan mengapa perkembangan teknologi kontrasepsi perlu lebih mengarah pada pria (Sumaryati, 2004; Wilopo, 2006). Biji pepaya muda merupakan salah satu bahan alam yang mempunyai khasiat antifertilitas (Chinoy, 1994; Ucha, et al. 2001). Penelitian ini dilakukan untuk membuktikan bahwa fraksi heksan ekstrak biji pepaya muda mempunyai efek menghambat proses spermatogenesis lebih kuat daripada fraksi metanol ekstrak biji pepaya muda. Biji pepaya yang dipakai dalam penelitian ini adalah biji pepaya muda (*Carica papaya, Linn*) lokal Bali.

Metode Penelitian

Penelitian ini dengan rancangan *Pre-test Post-test Control Group Design* (Campbell and Stanley, 1968). Sebanyak 30 ekor mencit jantan Balb-C, dikelompokkan secara *simple random sampling* menjadi tiga kelompok: kelompok kontrol (P0) yang diberikan akuabides; kelompok perlakuan 1 (P1) yang diberikan fraksi heksan ekstrak biji pepaya; kelompok perlakuan 2 (P2) yang diberikan fraksi metanol ekstrak biji pepaya. Data kuantitatif: dihitung jumlah sel-sel spermatogonia A, sel-sel spermatosit primer pakhiten, sel-sel spermatid, sel-sel Sertoli, sel-sel Leydig dan kadar hormon testosteron. Derajat kemaknaan ditetapkan dengan $\alpha \leq 0{,}05$.

Hasil Penelitian



Gambar 1. Histogram jumlah sel spermatogonia A, sel spermatosit primer pakhiten,

spermatid, Sertoli, Leydig dan testosteron mencit strain Balb-C

Dari hasil analisis *oneway* Anova didapatkan jumlah rata-rata sel spermatogonia A kelompok kontrol 852, kelompok perlakuan pertama 402, kelompok perlakuan kedua 610. Ketiga kelompok mempunyai perbedaan yang sangat bermakna (p=0,000). Hasil analisis LSD didapatkan jumlah sel spermatogonia A kelompok kontrol berbeda sangat bermakna dengan kelompok 1 (p=0,000) dan dengan kelompok 2 (p=0,002). Kelompok perlakuan 1 berbeda sangat bermakna dengan kelompok perlakuan 2 (p=0,006).

Jumlah sel spermatosit primer pakhiten kelompok kontrol 1080, kelompok perlakuan pertama 710, kelompok perlakuan kedua 980. Ketiga kelompok mempunyai perbedaan yang sangat bermakna (p=0,000). Hasil analisis LSD mendapatkan jumlah sel spermatosit primer pakhiten kelompok kontrol berbeda sangat bermakna dengan kelompok 1 (p=0,000) dan kelompok 2 (p=0,002). Kelompok 1 dengan kelompok 2 terdapat perbedaan yang sangat bermakna (p=0,000).

Jumlah sel spermatid pada kelompok kontrol adalah 1010, pada kelompok perlakuan pertama adalah 448, pada kelompok perlakuan kedua adalah 870. Ketiga kelompok mempunyai perbedaan yang sangat bermakna (p=0,000). Hasil analisis LSD mendapatkan kelompok kontrol berbeda sangat bermakna dengan kelompok 1 (p=0,000) dan kelompok perlakuan 2 (p=0,037). Antara kelompok 1 dengan 2 terdapat perbedaan yang sangat bermakna (p=0,000).

Jumlah sel Sertoli pada kelompok kontrol adalah 470, pada kelompok perlakuan pertama adalah 270, pada kelompok perlakuan kedua adalah 360. Ketiga kelompok mempunyai perbedaan yang sangat bermakna (p=0,000). Hasil analisis LSD mendapatkan jumlah sel Sertoli kelompok kontrol berbeda sangat bermakna dengan kelompok 1 (p=0,000) dan kelompok 2 (p=0,007). Kelompok 1 terdapat perbedaan yang bermakna dengan kelompok 2 (p=0,022).

Jumlah sel Leydig pada kelompok kontrol adalah 100, pada kelompok perlakuan pertama adalah 70, pada kelompok perlakuan kedua adalah 84. Ketiga kelompok tidak mempunyai perbedaan yang bermakna (p=0,476). Kadar hormon testosteron pada kelompok kontrol adalah 329 ng/dl, pada kelompok perlakuan pertama adalah 211 ng/dl, pada kelompok perlakuan kedua adalah 220 ng/dl. Ketiga kelompok tidak mempunyai perbedaan yang bermakna (p=0,640).

Pembahasan

Penurunan jumlah sel spermatogonia A disebabkan oleh zat aktif yang terkandung dalam fraksi heksan ekstrak biji papaya (steriod dan triterpenoid) maupun yang terkandung dalam fraksi metanol ekstrak biji pepaya muda lokal Bali (alkaloid), zat tersebut diduga bersifat antifertilitas. Penurunan jumlah spermatogonia A ini diduga juga karena hormon estradiol (E2) maupun hormon progesteron (P4) yang terdapat dalam fraksi heksan. Kedua hormon tersebut akan menyebabkan terganggunya sekresi FSH dan LH (Fora, 2006). Estradiol menyebabkan penekanan hipotalamus dan hipofisis anterior sehingga menyebabkan GnRH dan hormon gonadotropin (FSH dan LH) terhambat (Golub *et al.* 2004; Turek, 2005). Terhambatnya FSH ini akan menyebabkan terganggunya pula proses mitosis dan proliferasi spermatogonia A.

Penurunan sel spermatosit karena terganggunya fungsi dari sel Sertoli sehingga menyebabkan suplai laktat dan piruvat menurun, laktat dan piruvat merupakan sumber energi (Jutte *et al*. 1981). Bila jumlah sel spermatosit mengalami kerusakan dan mengalami degenerasi maka sel spermatosit ini akan difagositosis oleh sel Sertoli sehingga jumlah sel spermatosit berkurang (Nakamura and Hall, 1997)

Penurunan spermatid karena terganggunya fungsi dari sel Sertoli yang menyebabkan suplai laktat dan piruvat akan menurun. Laktat dan piruvat merupakan sumber energi dari spermatid (Jutte *et al*. 1978). Penurunan sel spermatid ini kemungkinan melalui beberapa mekanisme seperti adanya gangguan dalam proses meiosis, gangguan dalam proses spermiogenesis awal, lepasnya spermatid ke lumen tubulus seminiferus dan apoptosis spermatid. (Donnel *el al.* 1996).

Jumlah sel Sertol ini membentuk *blood testis barrier*. Adanya *blood testis barrier* ini akan menyebabkan terbentuk *microenvironment* yang optimal untuk berlangsungnya proses spermatogenesis (Johnson and Eviritt, 1990). Jika fungsinya terganggu maka sekresi *androgen binding protein* (ABP), suplai nutrient, *growth factors,* laktat, tranferin juga terganggu, yang mengakibatkan proses spermatogenesis akan terganggu, karena zat tersebut sangat dibutuhkan dalam proses spermatogenesis (Lohiya, et al. 2002; Niederberger, et al. 2004).

Fraksi heksan ekstrak biji pepaya lokal Bali yang muda tidak dapat menurunkan secara bermakna sel Leydig maupun kadar hormon testosteron, kemungkinan disebabkan karena sel-sel leydig tersebut paling kuat terhadap pengaruh dari luar dibandingkan sel-sel spermatogenik, hal ini sesuai dengan pernyataan Johnson and Everitt (1990) yang melaporkan bahwa sel-sel dalam tubulus seminiferus mempunyai sensintivitas yang berbeda-beda terhadap pengaruh dari luar.

7. Simpulan dan Saran

7.1 Simpulan

Berdasarkan hasil penelitian serta pembahasan dapat disimpulkan hal-hal sebagai berikut:

1. Fraksi heksan ekstrak biji pepaya muda menurunkan jumlah sel spermatogonia, sel spermatosit primer pakhiten, sel spermatid dan sel Sertoli dengan sangat bermakna dan penurunannya lebih besar daripada fraksi metanol ekstrak biji pepaya muda.
2 . Fraksi heksan ekstrak biji pepaya tidak dapat menurunkan jumlah sel Leydig dan kadar hormon testosteron

7.1 Saran

1 Perlu dilakukan penelitian lebih lanjut tentang efek samping dan efek reversibilitas dari pemberian fraksi heksan ekstrak biji pepaya muda.
2. Perlu dilakukan penelitian pemberian fraksi heksan ekstrak biji pepaya muda terhadap manusia, apabila penelitian tarhadap binatang percobaan sudah dianggap cukup

HEXANE FRACTION OF UNRIPE PAPAYA SEED EXTRACT (*CARICA PAPAYA, LINN*) INHIBITS SPERMATOGENESIS OF MALE MICE (MUS MUSCULUS) STRONGER THAN METHANOL FRACTION OF UNRIPE PAPAYA SEED EXTRACT

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Fraction of hexane extract contains glycosides and triterpenoids, which is assumed to have an anti fertility ingredient, so it can be used as a male contraceptive, although the mechanism of action is not yet clear. This study used the pre-test and post-test control group design, using 30 male mice of balb C strain, aged 12 weeks, weight 20-22 gram, subsequently grouped by random into 3 groups each consisting of 10 male mice. One control group (P0 = control group) was given double distilled water, and two treatment groups (P1 = treatment group) was given fraction of the hexane extract of young Carica papaya seed 20 mg/20gram/day, P2 = treatment group) was given fraction of the methanol extract of young Carica papaya seed 20 mg/20 gram/day). After 36 days of treatment, evaluation of the testis and blood, of the male mice was conducted. Data were analysed by normality test of Kolmogorov Smirnov Goodness of Fit, homogeneity test, and Anova test. This study showed that cells of spermatogonia A, primary pakhiten spermatocyte, spermatid, and Sertoli cells, decreased significantly ($p < 0,05$) but Leydig cells and testosterone did not decrease significantly ($p > 0,05$). It is concluded that fraction of hexane extract of carica papaya seeds can decrease the mean number of cells spermatogonia A, spermatocyte of primary pakhiten, spermatid, Sertoli, and Leydig cells and the level of testosterone hormone better than fraction of methanol extract of young Carica papaya seeds.

Keyword : Spermatogenesis, testosterone, fraction hexane extract of young Carica papaya seeds and fraction methanol extract of young Carica papaya seeds, male mice.

Introduction

The low participation of men in Family Planning Program is partly due to the fact that only a limited number of male contraceptive devices are currently in available (Arsyad, 1990; Hartono, 1996). Therefore, it is extremely important that appropriate technology be developed to produce contraceptives that can be used specifically by men (Sumaryati, 2004; Wilopo, 2006). Unripe papaya seeds are known to be one of the natural substances that have anti-fertility effect (Chinoy, 1994; Ucha, et al. 2001).The purpose of this study is to investigate whether hexane fraction of unripe papaya seed extract has a stronger effect to inhibit the process of spermatogenesis than the methanol fraction. In this study, unripe seeds were collected from local Balinese papaya fruits.

Research Method

This study was an experimental research using *pre-test post-test control group design* (Campbell and Stanley, 1968). The study used 30 male mice strain Balb-C which were determined by simple random sampling into three groups: control group (P0); treatment Group 1 (P1); treatment group 2 (P2). Quantitative data: counted of cells of spermatogonia A, cells of spermatocyte primary of pakhiten, cells of spermatid, cells of Sertoli, cells of Leydig and testosteronee hormone level. Significance level was determined with $\alpha \leq 0.05$

Result

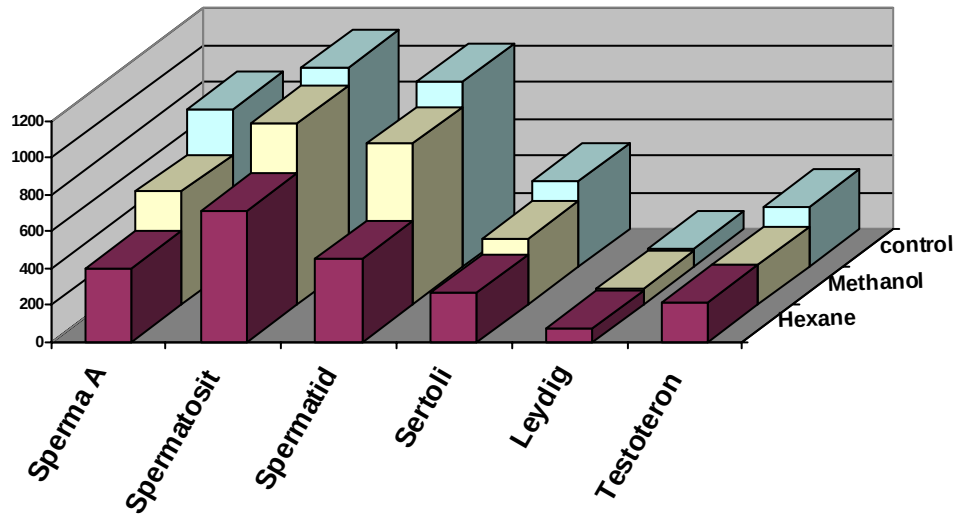

Figure 4.1 Histogram showing the level of spermatogonia A, pachiten primary spermatocyte, spermatid, Sertoli and Leydig cells and testosterone in mice strain Balb-C.

The analysis of *one-way Anova* showed that the average number of Spermatogonia A cells in the control group was 852, treatment group 1 was 402, and treatment group 2 was 610. The analysis result by LSD showed the number of spermatogonia A cells in the control group differed significantly from that of group 1 (p=0.000) and group 2 (0.002). Treatment group 1 was significantly different from treatment group 2 (p=0.006).

The number of primary pachiten spermatocyte cells in the control group was 1080, in treatment group 1 was 710 and in treatment group 2 was 980. The three groups showed significant differences (p=0.000). The result of LSD analysis showed the number of primary pachiten spermatocyte cells in the control group differed significantly from that in the treatment group 1 (p=0.000) and group 2 (p=0.002). The difference was significant between both treatment groups (p=0.000).

The average number of spermatid cells in the control group was 1010, in the treatment group 1 was 448, and in treatment group 2 was 870. It clearly showed that the three groups had significant differences (0,000). The result of LSD analysis showed significant difference between the control group and treatment group 1 (p=0.000) and between control group and treatment group 2 (p=0.037). The result of comparison between treatment group 1 and 2 was significant (p=0.000).

The number of Sertoli cells in the control group was 470, in the treatment group 1 was 270 and in treatment group 2 was 360. The three groups had significant differences (p=0.000). The result of LSD analysis showed that number of Sertoli cells in control group differed significantly from that in treatment group 1 (p=0.000) and group 2 (p=0.007) respectively. The difference was significant between both treatment groups (p=0.022).

The number of Leydig cells in the control group was 100, in treatment group 1 was 70 and in treatment group 2 was 84. The three groups had no significant differences (p=0.476). The level of testosterone in control group was 329 ng/dl, in treatment group 1 was 211 ng/dl and in treatment group 2 was 220 ng/dl. The three groups had no significant differences (0.640).

Discussion

Reduction of spermatogonia A cells might be due to the active substances contained in the hexane fraction of unripe papaya seed extract (steroid and triterpenoid) and in the methanol fraction of the same unripe papaya seed extract. These substances are thought to have anti-fertility features. Reduction of the number of spermatogonia A cells was probably due to the effect of estradiol (E2) as well as progesteron (P4) contained in the hexane extract. Both hormones are responsible in the inhibition of FSH and LH secretion. Estradiol supresses the hypothalamus and anterior hypophysis and inhibits GnRH and gonadotropin (FSH and LH) (Golub et al. 2004; Turek, 2005). Inhibition of FSH further disturbs the process of mytosis and poliferation of spermatogonia A cells.

Spermatocyte cells may decrease because of the disturbance in Sertoli cells function, which initiate the decrease of lactate and piruvate supplies, which are energy sources (Jutte *et al*. 1981). If spermatocytes are disrupted and degenerated, the cells will be phagocitized by Sertoli cells and thus reduce the number of spermatocyte cells (Nakamura and Hall, 1997)

The decrease of spermatid cells was caused by the inhibited function of Sertoli cells, which resulted in reduction of lactate and piruvate supplies. Lactate and piruvate were energy sources of spermatid cells (Jutte et al, 1978). Spermatid cells are decreased by several mechanisms such as disturbance in meiosis process, disturbance in primary process of spermiogenesis, the escape of spermatid into lumen of *seminiferous tubules* and apoptosis of spermatid cells (Donnel et al, 1996).

The number of Sertoli cells also forms blood testis barrier. Blood testis barrier generates optimum microenvironment, which takes place during the process of spermatogenesis (Johnson and Eviritt, 1990). If the number of Sertoli cells decreases and its function is disturbed, it can disturb secretion of androgen binding protein (ABP), nutrient supply, growth factor, lactate, and transferin, which then disturbs the process of spermatogenesis (Lohiya, et al. 2002; Niederberger, et al. 2004).

So if Sertoli cells are disturbed, it will also disturb the process of spermatogenesis.

The hexane fraction of local ripe papaya seed extract cannot decrease the number of Leydig cells and level of testosterone significantly and it may be caused by the Leydig cell has the strongest deficiency to external factors among the spermatogenic cells. This

result is similar to that of Johnson and Everitt (1990), who reported that the cells within tubulus seminiferus had different sensitivity to external factors.

VII. Conclusions and Recommendations

7.1 Conclusions

Based on the results and discussions of this study, it can be concluded as follows:

1. Hexane fraction of ripe papaya seed extract reduces spermatogonia A cells, primary pachiten spermatocyte cells, spermatid cells, Sertoli cells significantly and the reduction is greater than that caused by methanol fraction of ripe papaya seed extract.
2. Hexane fraction of ripe papaya seed extract does not reduce Leydig cells and testosterone level

7.2 Recommendations

1. A further study on the side effects of the administration of hexane fraction of unripe papaya seed extract needs to be carried out
2. A further study on reversibility effect of the administration of hexane fraction of unripe papaya seed extract needs to be carried out
3. A study on the administration of hexane fraction of unripe papaya seed extract to humans needs to be carried out after the study on animals has been considered sufficient.

REFERENCES

Arsyad, K.M. 1990. “Kemungkinan Pengembangan Kontrasepsi Pria”. Medika 12. (4). 42-51

Campbell, D.T., and Stanley, J. D. 1968. “Experimental and Experiment quasi Experimental Design for Reasarch”. Chicago.

Chinoy, N. J., D’Souza, J. M., Padman, P. 1994. “Effects of Crude Aqueous Extract of *Carica papaya* seeds in Male Albino Mice.” Reprod Toxicol. 8 (1) : 75 – 9.

Donnell, L.O., McLachlan, R.L., Wreford, N.G., de Kretser, D.M., and Robertson, D.M. 1996. “Testosterone Withhdrawal Promotes Stage-Spesific Detacment of Round Spermatids from the Rat Seminiferous Ephithelium”. Biol. Reprod. (55): 895-900.

Fora. M.A. 2006. “Interrelationship Beetwen The Hypothalamus, Pituitary and Testis”. Endrocrinilogy of Male Reproductive Sytem.

Golub, M.S., Kaufman, F.L., Campbell, M.A., Hong Li. 2004. “Progesterone Hazard Identification”. In: Evidence on the Developmental and Reproductive Toxicity of Progesterone.

Hartono, H. 1996. “Keluarga Berencana dan Kontrasepsi.” Pustaka Sinar Harapan. Jakarta.

Johnson, M. and Eviritt. 1990. “Essensial Reproduction.” 3 nd Edition. Blackwell Sci. Pub. Oxford London, Edinburg.

Jutte, N.H.P.M., Grootegoedja, Rommerts, F.F.G, Van Der Mollen, H.J. 1981.”Exogenous Lactate is Essential for Metabolic Activities in Isolated Spermatocytes and Spermatogenesis. J. Reprod. Fert. 62.: 399-405.

Lohiya, N.K., Manivannan, B., Mishra, P.K. Pathak, N., Sriram, S., Bhande, S.S., Pannerdoss, S. 2002. “Chloroform Extrac of Carica Papaya Seeds Induces Long-Term Reversible Azoospermia In Langur Monkey.” Asian J of Androl. 4 (1): 17-26.

Lohiya, N.K., Pathak, N., Mishra, P.K., Manivannan, B. 1999. “Reversible Contraception with Chloroform Extract of *Carica papaya*, Linn. Seeds in Male Rabbits.” Reprod Toxicol. 13 (1) : 59 – 66.

Nakamura, M. and Hall, P.F. 1997. Efects 5-Thio-D-Glucose on Proteins Synhesis Invitro by Various Types of Cells from the Testis. J. Reprod. 49: 95-97.

Niederberger, C.S., Shubhada, S. Kim, S.J. and Lamb, D.J. 2004. “Paracrin Factors and Regulation of Spermatogenesis”. Word Journal of Urology. 11. (2) : 120-128.

Sumaryati, A. 2004. “Tahun Ini KB Pria Mulai Digalakkan”. Badan Koordinator Keluarga Berencana Nasional. Available from: http://www.bkkbn.go.id/article_detail.php. Accesed, 26, Oct. 2005.

Turek, P.J.. “Hypothalamic-Pituitary-Gonadal (HPG) Axis and Control of Spermatogenesis”. In: Endocrine evaluation. Male Reproductive Laboratory Departement of Uroligy Universitas of California at San Francisco. San Francisco, California.

Ucha – Nwachi, E.O., Ezeokoli, D.C., Adogwa, A.O., Offiah, V.N., 2001. "Effect of Water Extract of *Carica papaya* Seed on the Germinal Epitelium of the Seminiferous Tubules of Sprague Dewley Rats. " Kaibogaku Zasahi. 76 (6) : 517-21.

Wilopo, S.A. 2006. "Perkembangan Teknologi Kontrasepsi Pria Terkini". Gema Pria. Available from: http://pikas.bkkbn.go.id/gemapria/article-detail php. Accesed, Jun 18, 2006.