Evaluation of the thermal effects of prenatal ultrasound on hematological analysis of young *Oryctolagus Cuniculus*

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ABSTRACT. Elevated temperatures can induce changes in red blood cell (RBC), white blood cell (WBC) and platelet (PLT) counts. Ultrasound heating during obstetric scans has the potential to increase body temperature owing to the phenomenon of absorption. We conducted a study to determine the thermal effects of prenatal ultrasound on RBCs, hemoglobin concentration (Hb), WBCs and PLTs in young rabbits. We selected 69 rabbits that were 1 month of age and 73 that were 5 months of age, and allocated them to four groups. The control group consisted of four pregnant does that were allowed to have a full term delivery without any ultrasound exposure. The experimental groups were subjected to one-time ultrasound exposure for 30, 60 and 90 min in the middle of each gestational stage accordingly. RBCs and Hb showed significant reductions in the experimental groups of 1- and 5-month-old rabbits ($P<0.05$). In addition, WBCs and PLTs yielded significant differences in the 1-month group that were not observed in the 5-month group ($P>0.05$). The highest values recorded were those of the WBCs of 1-month-old subjects that received 90 min of exposure at the second stage of gestation. The PLTs were the lowest values recorded in 1-month-old subjects following 90 min of ultrasound exposure at the third stage of gestation. These findings suggest that hematological fluctuations during the early stages of postnatal life persisted until 1 month of age and recovered thereafter, as the subjects progressed into adulthood. Therefore, ultrasound heating can cause significant, yet reversible effects on the hematological parameters of rabbits.
KEY WORDS: platelet, prenatal ultrasound, red blood cell, thermal effects, white blood cell

Ultrasound scanning, from a medical viewpoint, is generally considered safe when used prudently [2]. Ultrasound has become a valuable tool for assessment of the cardiovascular system, reproductive organs, internal abdominal organs, and in ophthalmology. The use of this non-ionizing modality is widespread, particularly in obstetrics and gynecology. Some of the advantages of prenatal ultrasound include: obtaining an accurate due date, diagnosing missed miscarriages and any uterine abnormalities, and monitoring fetal development [4, 8, 28]. Prenatal ultrasound could significantly reduce fetal mortality, because it facilitates early detection of fetal malformations [22].

Despite its medical benefits, prenatal ultrasound has also been utilized for social and business purposes. Most pregnant women worldwide routinely have prenatal ultrasound scans, as they believe it can enhance parental-fetal bonding. The private sector promotes the use of ultrasound imaging for keepsake videos with captivating names that lure parents-to-be to have scans without knowing the side effects. The US Food and Drug Administration (FDA) has warned about the potential hazards of creating these keepsake videos. In fact, several animal and human studies have been conducted to explore the effects of prenatal ultrasound on the developing fetus. Animal studies have suggested that exposure to prenatal ultrasound could lead to low birth weight [7, 18, 23], changes in bone mineral density [17] and an increase in temperature in the fetal brain [3, 10]. Energy absorption of the ultrasound beam [2, 19] can have thermal effects, which is the main potentially adverse biological effect. Bone is the most likely tissue to be affected by the absorption of ultrasonic energy, because of its very high absorption coefficient. However, the absorption rate differs depending on the gestational age and degree of ossification [5].

To the best of our knowledge, the effects of prenatal ultrasound on the hematological parameters of young subjects have rarely been examined. Thus, the purpose of the present study was to examine these effects on red blood cell counts (RBCs), hemoglobin (Hb), white blood cell counts (WBCs) and platelet counts (PLTs) in young rabbits exposed prenatally to ultrasound.

MATERIALS AND METHODS

Animal preparation: A total of 22 female (does) Malaysian bred New Zealand White rabbits (*Oryctolagus cuniculus*) were time-mated. Of these, four rabbits were selected for the control group, and 18 rabbits were assigned to separate experimental groups. The gestational period for a doe ranges from 30–33 days [11] and is divided into three stages, each consisting of 11 days. Following delivery, the offspring were used for data analyses at the ages of 1 and 5 months, based on the group to which they were assigned. All procedures were approved by the Universiti Teknologi MARA Committee on Animal Research and Ethics (UiTM CARE). All rabbits were provided with an ad lib supply of water. However,

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to keep external factors (that might affect the validity of the results) constant, the pelleted feed was measured and fed once daily as 5% of body weight [27]. The body weight was measured fortnightly. The rabbits were kept in an individual cage in an animal house with a 16:8 hr light:dark cycle, and temperature range of 14–28°C [15]. Proper ventilation was provided by using a BioGS air purifier to rid the environments of harmful gasses, such as carbon dioxide and ammonia, released by the rabbits [14].

Prenatal ultrasound exposure: A Phillips HD3 system (Philips Electronics E.V., Herrsching, Germany) fitted with a 5–9 MHz linear-array transducer (L9-5, Philips Electronics E.V.) was used to provide B-mode ultrasound exposure. The transducer operated with a focal depth of approximately 5.5 cm that generally corresponded to the position of the fetus in pregnant does. For each exposure, the maximum frequency used was 9 MHz, and the mechanical (MI) and thermal indices (TI) recorded were 1.0 and 0.2, respectively. The spatial peak temporal average intensity (I_{SPTA}) and the output power varied from 0.13–0.19 W/cm^2 and 0.4–0.7 W, respectively, based on previous characterization of the transducer [1]. These values remained constant for all exposures. During the scans, several transducer maneuvers, such as fanning, sliding and rotating, were done to maximize exposure on as many fetuses as possible. The abdominal region of pregnant does was shaved to facilitate transducer application. Each pregnant doe received a single exposure to ultrasound during the applicable gestational stage of the group to which it was assigned (first stage group, second stage group and third stage group). The exposure lasted 30 min, 60 min and 90 min at first (day 6), second (day 17) and third stages (day 28), accordingly. The control group was allowed to have a full term delivery without exposure to ultrasound. The rabbit restrainer, MyRabbitBurrow, designed by Dom [17] was used to keep the doe calm and cooperative during the scanning.

Full blood count analysis: Blood samples from 1-month-old (0.5–1 m*l*) and 5-month-old (1–3 m*l*) rabbits were collected from the central artery of the ear. The rabbits were restrained in a custom-made rabbit restrainer to facilitate the procedure of blood sampling. The blood samples were placed directly into blood tubes with ethylene diamine tetraacetic acid (EDTA) (Bectin, Dickinson, and Co., Rutherford, NJ, U.S.A.) and were sent to the Department of Veterinary Laboratory Diagnostics, Universiti Putra Malaysia (UPM) for full blood count analysis. The following parameters were assessed and reported for the study: RBCs, Hb, WBCs and PLTs.

Statistical analysis: Group differences were evaluated using the analysis of variance (ANOVA) test of the Statistical Package for the Social Sciences, SPSS version 21.0. Scheffe's post-hoc comparisons were carried out to determine which groups differed significantly from the others. All differences were assumed statistically significant at $P<0.05$. The reported readings represent mean ± SD. Pearson's correlation was used to evaluate correlations among the duration of exposure, stage of gestation and blood analysis.

Table 1. In-house normal ranges for RBCs, Hb, WBCs and PLTs based on the control group that was not exposed to ultrasound

Age in months	Measurement	($\bar{x}$)	SD	Standard error
1	RBCs (×10^{12}/*l*)	4.4	0.26	0.08
(n=10)	Hb (g/*l*)	88.5	5.87	1.86
	WBCs (×10^9/*l*)	5.3	1.68	0.53
	PLTs (×10^9/*l*)	623.8	51.39	16.25
5	RBCs (×10^{12}/*l*)	5.77	0.17	0.05
(n=10)	Hb (g/*l*)	113.4	2.41	0.76
	WBCs (×10^9/*l*)	10.88	1.69	0.53
	PLTs (×10^9/*l*)	368.5	8.62	2.73

n, number of samples; $\bar{x}$, mean; SD, standard deviation; RBCs, red blood cell counts; Hb, hemoglobin concentration; WBCs, white blood cell counts, PLT; platelet counts.

Table 2. Number of young rabbits in each group

Age (months)	Ultrasound exposure		n
	Gestational stage	Duration of exposure (min)	
1	First stage	30	7
		60	7
		90	6
	Second stage	30	6
		60	6
		90	7
	Third stage	30	6
		60	6
		90	8
5	First stage	30	6
		60	8
		90	9
	Second stage	30	7
		60	6
		90	7
	Third stage	30	6
		60	8
		90	6

n, number of samples.

RESULTS

Since there was a lack of reference values for RBCs, Hb, WBCs and PLTs in 1- and 5-month-old rabbits, an in-house normal reference range of both sets of parameters was developed using the outcome of the control groups. In addition, unpredictable oscillations in the hematological parameters of rabbits have resulted in reports of different reference values by several authors. Table 1 summarizes the reference range to which the results of the experimental group was compared. The Q-Q plots for all parameters showed normal distribution, in which the data points fell approximately along a straight line. Table 2 shows the number of young rabbits in each group.

Full blood count analysis of 1-month-old rabbits: In comparison to the control group, RBCs were significantly
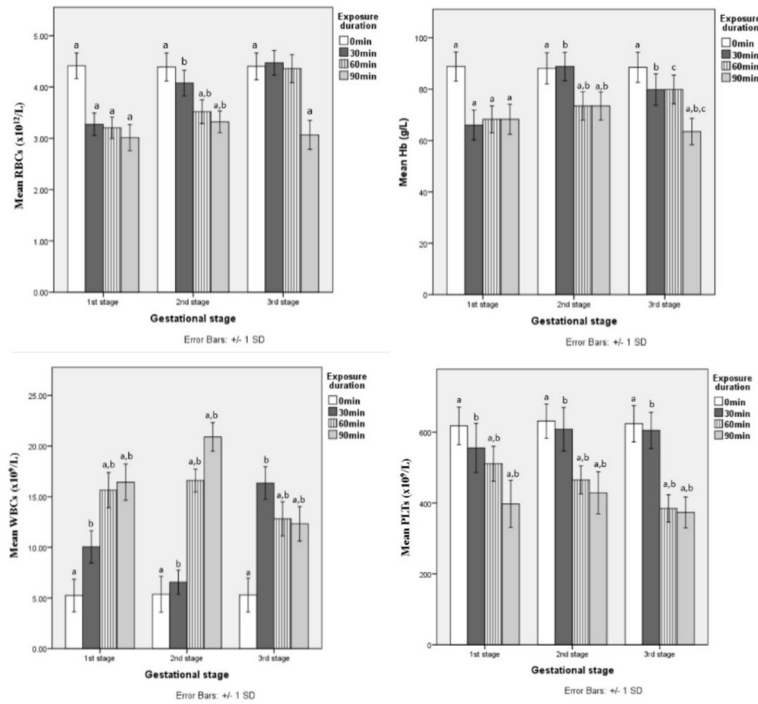
Fig. 1. Hematological analysis of 1-month-old rabbits: RBCs, Hb and PLTs showed significant reductions, particularly after 90 min of exposure at the third stage. On the other hand, the number of WBCs was significantly increased following ultrasound exposure, with the highest reading recorded after 90 min at the second stage. Different superscript letters represent significant differences. Error bars represent ± standard deviation.

reduced in the first stage group (30 min: 3.27 ± 0.22, 60 min: 3.12 ± 0.21 and 90 min: 3.02 ± 0.26); second stage group (60 min: 3.52 ± 0.23 and 90 min: 3.32 ± 0.21); and third stage group (90 min: 3.07 ± 0.28). Similar findings were noted in Hb concentration. WBCs were significantly increased in the first stage group (60 min: 15.64 ± 1.73 and 90 min: 16.43 ± 1.79); second stage group (60 min: 16.60 ± 1.14 and 90 min: 20.90 ± 1.41); and third stage group (60 min: 12.82 ± 1.69 and 90 min: 12.32 ± 1.70). A significant reduction was found in PLTs in the first stage group (60 min: 510.86 ± 49.27 and 90 min: 397.5 ± 66.53); second stage group (60 min: 465.17 ± 39.8 and 90 min: 428.57 ± 59.78); and third stage group (60 min: 384.67 ± 38.64 and 90 min: 373.63 ± 43.65). Figure 1 presents the trends in the hematological parameters of 1-month-old subjects. Both PLTs ($r=-0.44$) and WBCs ($r=0.41$) were correlated ($P<0.05$) with gestational stage; however, neither RBCs ($r=-0.01$) nor Hb ($r=-0.2$) showed any correlation ($P>0.05$) with gestational stage. Correlations ($P<0.05$) were noted between the duration of exposure and RBCs ($r=-0.69$), Hb ($r=-0.62$), WBCs ($r=0.72$), and PLTs ($r=-0.81$), respectively.

Full blood count analysis at 5 months of age: No significant differences ($P>0.05$) were observed among any parameters for any exposure time in the first and third stage groups. However, in the second stage group, only RBCs (90 min: 4.23 ± 0.32) and Hb (90 min: 92.91 ± 6.43) were found to have been significantly reduced, when compared to the control group. WBCs and PLTs yielded no significant differences. Figure 2 presents the trends in the hematological parameters of 5-month-old subjects. The gestational stage was correlated with RBCs ($r=-0.27$) and Hb ($r=-0.29$), and the duration of exposure was correlated with RBCs ($r=-0.44$) and Hb ($r=-0.33$).

DISCUSSION

The present study examined the effects of prenatal ultrasound on full blood count analysis of 1- and 5-month-old rabbits. A complete blood count was performed, as this is considered a conventional laboratory test in medical practice to assess general health. Stress, various disorders and a rise in body temperature can all affect hematological parameters [9, 13]. Ultrasound heating during obstetric scans has the potential to trigger heat stress in pregnant does.

The results of the present study indicate that a single exposure to ultrasound scanning during the gestation period can cause fluctuations in hematological parameters. RBCs of 1- and 5-month-old subjects were significantly reduced in the exposed group. A reduction in RBCs in the present study is consistent with the findings of previous reports on newborns subjected to prenatal ultrasound exposure [1, 16]. These findings could be attributed to the hemolysis that might occur even with a relatively small increase in temperature [6, 12]. Developmental hematopoiesis in mammals
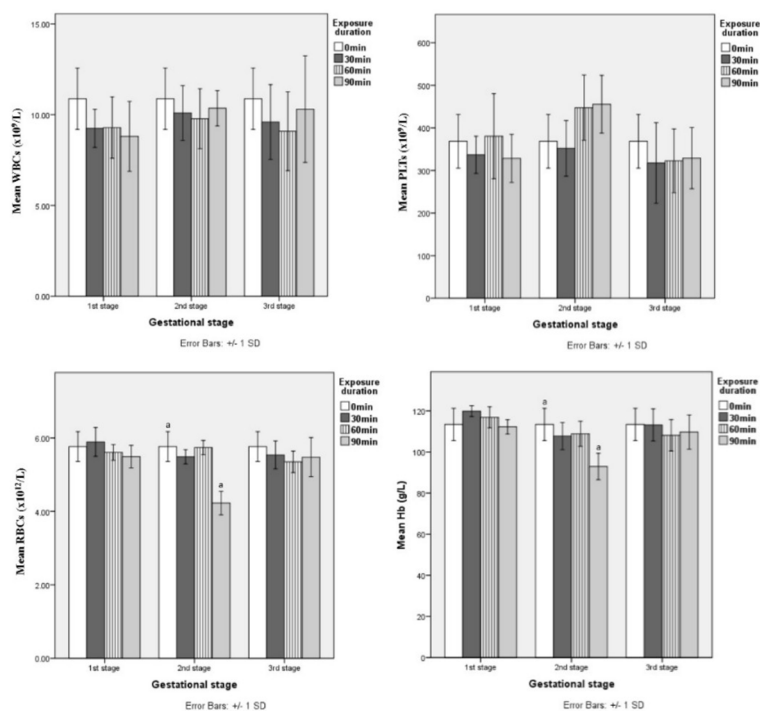
Fig. 2.　Hematological analysis of 5-month-old rabbits: RBCs and Hb showed significant reductions, with the lowest value being recorded after 90 min of exposure at the second stage. WBCs and PLTs yielded no significant differences; however, slight changes were noted between readings. Different superscript letters represent significant differences. Error bars represent ± standard deviation.

is initiated within the yolk sac of the embryo and is taken over by the bone marrow at a later stage of gestation [26]. An arrest in maturation of the myeloid lineage of blood cells might occur owing to heat absorption by the bone as a result of ultrasound exposure at crucial developmental stages [24]. When the body temperature rises, regions of the RBC surface that are devoid of lipids become exposed and leaky, thus resulting in hemolysis [6].

Hb is an iron-containing protein in RBCs that is responsible for oxygen transport around the body. Hyperthermia can cause significant changes in blood properties, especially Hb [29]. When RBCs are destroyed, Hb escapes into the plasma and causes iron depletion [21]. The differences observed in Hb after birth was closely correlated with those in RBC, a finding that is consistent with those of Ahmad Zaiki et al. [1].

Prenatal ultrasound exposure had a short-term effect on WBCs as evidenced by the fact that differences in WBC were only observed in the 1-month-old group. The reasons for the significant leukocytosis observed in the present study remain unclear. However, Tarantal, O'Brien and Hendrickx [26] reported similar findings of a greater number of WBCs in 1-month-old cynomolgus macaques of the ultrasound-exposed group than in concurrent controls, and no significant differences at later stages. A possible explanation for the changes observed in WBCs and PLTs is bone marrow failure. This failure could include an inability of the progenitor population in fetal bone marrow to mature, the delayed

formation of stromal supporting cells or both [25]. In a study by Payton et al. [20], bone marrow was damaged following ultrasound exposure above the diagnostic level.

Even though the effects of prenatal ultrasound in 5-month-old rabbits were less pronounced, the effects were still notable. These findings suggest that hematological fluctuations during the early stages of postnatal life persisted for 1 month and had the ability to recover as the animals grew into adults. Thus, exposure to prenatal ultrasound can induce significant, yet reversible effects.

REFERENCES

1.　1983. Proceedings of the 28th annual meeting of the American Institute of Ultrasound in Medicine and the 12th annual meeting of the Society of Diagnostic Medical Sonographers. October 18-21, 1983 New York, New York. Abstracts. *J. Ultrasound Med.* **2** Suppl: 1–269 [Medline]
2.　Ahmad Zaiki, F. W., Md Dom, S., Abdul Razak, H. R. and Hassan, H. F. 2013. Prenatal ultrasound heating impacts on fluctuations in haematological analysis of Oryctolagus cuniculus. *Quant. Imaging Med. Surg.* **3**: 262–268. [Medline]
3.　Andreassi, M. G., Venneri, L. and Picano, E. 2007. Cardiac

imaging: the biological effects of diagnostic cardiac ultrasound. *Prog. Biophys. Mol. Biol.* **93**: 399–410. [Medline] [CrossRef]

4. Duggan, P. M., Liggins, G. C. and Barnett, S. B. 1995. Ultrasonic heating of the brain of the fetal sheep in utero. *Ultrasound Med. Biol.* **21**: 553–560. [Medline] [CrossRef]

5. Geerts, L. T. G. M., Brand, E. J. and Theron, G. B. 1996. Routine obstetric ultrasound examinations in South Africa: cost and effect on perinatal outcome—a prospective randomised controlled trial. *Br. J. Obstet. Gynaecol.* **103**: 501–507. [Medline] [CrossRef]

6. Gent, R. 1997. Applied Physics and Technology of Diagnostic Ultrasound. Milner Publishing, Adelaide.

7. Gershfeld, N. L. and Murayama, M. 1988. Thermal instability of red blood cell membrane bilayers: temperature dependence of hemolysis. *J. Membr. Biol.* **101**: 67–72. [Medline] [CrossRef]

8. Hande, M. P. and Devi, P. U. 1992. Effect of prenatal exposure to diagnostic ultrasound on the development of mice. *Radiat. Res.* **130**: 125–128. [Medline] [CrossRef]

9. Hill, L. M. and Breckle, R. 1983. Current uses of ultrasound in obstetrics. *Prim. Care* **10**: 205–223. [Medline]

10. Hinton, M., Jones, D. R. and Festing, M. F. 1982. Haematological findings in healthy and diseased rabbits, a multivariate analysis. *Lab. Anim.* **16**: 123–129. [Medline] [CrossRef]

11. Horder, M. M., Barnett, S. B., Vella, G. J., Edwards, M. J. and Wood, A. K. 1998. Ultrasound-induced temperature increase in guinea-pig fetal brain in utero: third-trimester gestation. *Ultrasound Med. Biol.* **24**: 1501–1510. [Medline] [CrossRef]

12. Kaplan, H. M. and Timmons, E. H. 1979. The rabbit: a model for the principles of mammalian physiology and surgery. Academic Press, New York.

13. Karle, H. 1968. Elevated body temperature and the survival of red blood cells. A study on experimental pyrexia in rabbits. *Acta Med. Scand.* **183**: 587–592. [Medline] [CrossRef]

14. Lawrence, C. and Atac, B. 1992. Hematologic changes in massive burn injury. *Crit. Care Med.* **20**: 1284–1288. [Medline] [CrossRef]

15. Lebas, F. 1997. The Rabbit: husbandry, health, and production. Food and Agriculture Organization of the United Nations, Rome.

16. Matics, Z., Gerencsér, Z., Radnai, I., Zotte, A. D., Palumbo, M., Mikó, A., Kasza, R. and Szendrő, Z. 2013. Effect of different lighting schedules (16L:8D or 12L:6D) on reproductive performance and nursing behaviour of rabbit does. *Livest. Sci.* **157**: 545–551. [CrossRef]

17. Md. Dom, S. 2013. Teratogenic effects of diagnostic ultrasound exposure. LAP LAMBERT Academic Publishing, Deutschland.

18. Md. Dom, S., Salikin, M. S., Hassan, H. F. and Mohd Yusoff, N. 2012. The effect of B-mode diagnostic ultrasound exposure on rabbit foetal bone mineral density (BMD). *Radiography* **18**: 197–200. [CrossRef]

19. O'Brien, W. D. Jr. 2007. Ultrasound-biophysics mechanisms. *Prog. Biophys. Mol. Biol.* **93**: 212–255. [Medline] [CrossRef]

20. Payton, O. D., Lamb, R. L. and Kasey, M. E. 1975. Effects of therapeutic ultrasound on bone marrow in dogs. *Phys. Ther.* **55**: 20–27. [Medline]

21. Rother, R. P., Bell, L., Hillmen, P. and Gladwin, M. T. 2005. The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: a novel mechanism of human disease. *JAMA* **293**: 1653–1662. [Medline] [CrossRef]

22. Saari-Kemppainen, A., Karjalainen, O., Ylöstalo, P. and Heinonen, O. P. 1990. Ultrasound screening and perinatal mortality: controlled trial of systematic one-stage screening in pregnancy. The Helsinki Ultrasound Trial. *Lancet* **336**: 387–391. [Medline] [CrossRef]

23. Stolzenberg, S. J., Torbit, C. A., Pryor, G. T. and Edmonds, P. D. 1980. Toxicity of ultrasound in mice: neonatal studies. *Radiat. Environ. Biophys.* **18**: 37–44. [Medline] [CrossRef]

24. Tarantal, A. F. and Hendrickx, A. G. 1989. Evaluation of the bioeffects of prenatal ultrasound exposure in the cynomolgus macaque (Macaca fascicularis): II. Growth and behavior during the first year. *Teratology* **39**: 149–162. [Medline] [CrossRef]

25. Tarantal, A. F. and Hendrickx, A. G. 1989. Evaluation of the bioeffects of prenatal ultrasound exposure in the cynomolgus macaque (Macaca fascicularis): I. Neonatal/infant observations. *Teratology* **39**: 137–147. [Medline] [CrossRef]

26. Tarantal, A. F., O'Brien, W. D. and Hendrickx, A. G. 1993. Evaluation of the bioeffects of prenatal ultrasound exposure in the cynomolgus macaque (Macaca fascicularis): III. Developmental and hematologic studies. *Teratology* **47**: 159–170. [Medline] [CrossRef]

27. Yanni, A. E. 2004. The laboratory rabbit: an animal model of atherosclerosis research. *Lab. Anim.* **38**: 246–256. [Medline] [CrossRef]

28. Zelop, C. C., Bromley, B., Frigoletto, F. D. Jr. and Benacerraf, B. R. 1994. Second trimester sonographically diagnosed placenta previa: prediction of persistent previa at birth. *Int. J. Gynaecol. Obstet.* **44**: 207–210. [Medline] [CrossRef]

29. Zinchuk, V. V. and Borisiuk, M. V. 1992. [The effect of sodium cyanate-modified hemoglobin oxygen affinity on the heat resistance of rats]. *Biull. Eksp. Biol. Med.* **114**: 600–603. [Medline] [CrossRef]