Absorption of Ultrasound by Normal and Pathological Human Gonadal Tissues *in vitro*

Hiroshi INOUE, Kay I. CARNES and Floyd DUNN

超音波医学 Vol. 20 No. 6 別刷
Jpn J Med Ultrason
1993 p. 349-355

Absorption of Ultrasound by Normal and Pathological Human Gonadal Tissues *in vitro*

Hiroshi INOUE*, Kay I. CARNES and Floyd DUNN

Key Words
Ultrasonic absorption Gonads Cancer

Abstract

The transient thermoelectric method was employed to determine the ultrasonic absorption coefficient α of normal and pathological excised human ovary and testis at 1 and 3.25 MHz. At 1 MHz, α of normal ovary ranged from 0.016 for follicle to 0.046 Np/cm for the tunica albuginea, while the studied pathologies ranged from 0.02 to 0.123 Np/cm. The α increased from 0.018 Np/cm for normal testis from 0.024 to 0.048 Np/cm for the pathological organs studied. The observed changes in the absorption coefficient, of the pathologies from normal, are consistent with the attending changes in water and protein content. The frequency dependence for testis is $f^{1.4}$ and increases with the studied pathologies, while that for ovary is $f^{1.6}$ and decreases with the studied pathologies.

1. Introduction

The use of ultrasound as a therapeutic tool became widespread more than thirty years ago and usage has remained global. The use of ultrasound as a diagnostic tool in human medicine has increased significantly over the past several decades[1] and a huge literature describes the multiplicity of such purposes[2)3)]. There is a belief among radiologists and therapists that exposure to ultrasound is without harmful effects[4]. However, there have been reports describing ultrasonically induced biological alterations in *in vitro* preparations produced by clinical imaging systems[5)–7)], and some have been examined critically and considered to be of sufficient importance to merit further study[8]. A physical mechanism known to be responsible for producing biological effects, under suitable conditions, is associated with the absorption of ultrasound in the body of the specimen with concomitant increase in temperature to possibly damaging levels[9]. Knowledge of the absorption coefficient enables estimates to be made of temperature increases accompanying ultrasound exposure. Such information is necessary for risk determination during ultrasound diagnosis and for dose determination when, for example, ultrasound is employed for hyperthermia treatment of cancer.

Previous *in vitro* measurements of normal ovarian[10] and testicular[11)–13)] tissue specimens

Bioacoustics Research Laboratory, Department of Electrical and Computer Engineering, University of Illinois (1406 W. Green Street, Urbana, Illinois 61801 USA.)
*Department of Electrical and Electronic Engineering, Mining College, Akita University (1-1 Tegatagakuen-cho, Akita 010 Japan.)
原稿受付 1992年10月22日 改訂 1993年2月22日

from a variety of mammalian species showed the absorption coefficient values to depend upon the macromolecular composition of the structure measured, and had little interspecies variation. The present study was undertaken to determine the absorption coefficient values for normal human gonadal tissue and to compare these values with those of previously studied species. Pathological human gonadal tissue was then measured to determine whether differences existed from the nonpathological values. These values were then compared in order to determine whether they were sufficiently different to enable a clinical diagnosis to be made on the basis of absorption coefficient values.

2. Materials and methods

a. The Mammalian ovary

The mammalian ovary is physiologically complex, dynamic and acoustically heterogeneous[10]. It consists of several distinct structres, some of which change as the estrous cycle progresses.

The matrix of the ovary consists of the outer cortex layer and the inner medullary area. The cortex, which occupies one-half to two-thirds of the human ovary[14] is a tough connective tissue layer containing the follicles and corpora lutea and is covered by the germinal epithelium. The medulla is also composed of connective tissue and contains the blood vessels and nerves that support the ovary[15]. The follicle, a principal cortical structure, is the site of growth and development of the oocyte and can be divided into four different categories as it undergoes oogenesis and folliculogenesis. (1) The primordial follicle consists of the oocyte and one layer of flat follicle cells. (2) The primary follicle consists of the growing oocyte and one or more layers of follicle cells that are now cuboidal and known as granulosa cells. (3) The antral follicle also contains the enlarging oocyte and granulosa cells, as well as the antral space and the follicular fluid that occupies it. (4) Just prior to ovulation, the preovulatory, or Graafian follicle, is at its maximum volume of cells and fluid[15].

Following ovulation and expulsion of the oocyte, the granulosa cells undergo hypertrophy, hyperplasia, and biochemical changes to become the luteal cells of the corpus luteum. As the corpus luteum evolves, it becomes highly vascularized and connective tissue develops. The luteal cells accumulate large amounts of lipid as they age. If the animal is not pregnant following ovulation, the corpus luteum involutes and degenerates to become a scar, viz., the corpus albicans. If pregnancy is established, the corpus luteum persists until parturition and then undergoes rapid involution and regression.

b. The Mammalian testis

The mammalian testis is a tubular gland surrounded by a thick, fibrous capsule, the tunica albuginea[15]. The interior is composed of highly convoluted seminiferous tubules surrounded by the interstitium. The human testis is divided into lobules by thin, fibrous septa, each lobule containing one to four seminiferous tubules[15]. The interstitium contains the vasculatue, Leydig cells, fibroblasts, macrophages, and mast cells. The seminiferous tubules are bounded myoid cells and contain support-cells (Sertoli cells), the germinal epithelium, and the luminal area which contains the seminal fluid. The very high water content of mammalian testis, perhaps more than 80%, accounts for its extremely low ultrasonic absorption values[11)12)16].

c. Absorption coefficient measurement methods

Absorption coefficient measurements were made using the transient thermoelectric tech-

nique, which has been described in detail previously[9]. Briefly, chromel-constantan thermocouples were used as the detectors. The 0.003-in. (76.2 μm) wire ends were acid-etched and lap-soldered such that the junction was approximately 13 μm in diameter, in order to reduce the effect of viscous heating at the junction[17]. The tissue to be measured was secured in a holder, which did not interfere with the plane traveling acoustic field. The thermocouple was inserted to a depth of 1-2 mm at the selected site, with the aid of a 30-gauge hypodermic needle which was removed after thermocouple placement. The entire assembly was supported in a Plexiglass tank filled with degassed mammalian Ringer's solution maintained at 37°C. Transducers of resonance frequency 1 or 3.25 MHz, with, respectively, diameters of 5.5 cm and 6.0 cm and forcal lengths of 25 or 20 cm were employed. The transducers, which had been calibrated using the ball radiometric technique[18], were aligned such that the focal point of the sound beam was at the approximate position of the thermocouple junction. The beam profile was then obtained, to determine the position of the junction along the two axes normal to the direction of sound propagation, to within 0.1 mm. Measurements, under computer control, were made at spatial-peak temporal-average (SPTA) acoustic intensities of 1 to 10 W/cm^2 in each specimen (half-power beam width 5 mm). A 1-s exposure was utilized and data points from the straight-line portion of the temperature increase versus time curve were used to derive the absorption coefficient value according to the equation

$$\alpha=\rho C/2I(dT/dt)_0 \qquad (1)$$

where ρC is the heat capacity/unit volume in joules per cubic centimeter per degree Celsius and taken as 0.86 cal/°Cg, I is the SPTA acoustic intensity in Watts per square centimeter and

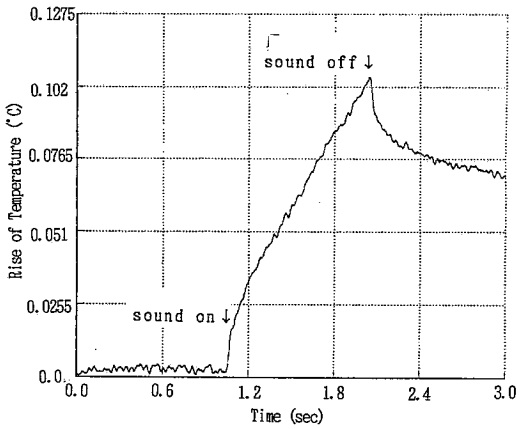

Fig. 1 Representative trace of temperature elevation for an excised mammalian tissue specimen exposed to 1 MHz ultrasound at 5 W/cm^2 for 1 sec.

$(dT/dt)_0$ is the initial time rate of change of temperature in degrees Celsius per second[19]. The computation of the absorption coefficient value includes an iterative procedure that corrects the intensity value for depth of the thermocouple junction in the tissue. A typical trace of temperature elevation in an excised mammalian tissue specimen exposed to 5 W/cm^2, 1 MHz ultrasound is shown in **Fig. 1**. The irradiation period is chosen to be 1 sec. The straight portion of the trace, appearing immediately after the approximetely 0.1 sec viscous rise[9], is used to determine the dT/dt value graphically to evaluate α by eq. (1). The parameters of the apparatus were measured carefully and data, which were determined 24 to 30 times with the SPTA ultrasound intensities of 1 to 10 W/cm^2, were checked for the influence of noise disturbance and averaged to obtain results to three significant figure accuracy. The complete procedure, including discussions of errors, etc., has been published elsewhere[10)11)17)19)22)]. Following the measurement procedure, the entire assembly was removed from the tank. With the tissue and thermocouple still secured in the holder, an incision was made over the junction, and with

the aid of a dissecting microscope, the ovarian structure, in which the junction was located, was identified.

Tissues were obtained from The National Cancer Institute's Cooperative Human Tissue Network, Midwestern Division, Columbus, OH. Specimens were from surgical sources and were frozen in Minimal Essential Media, shipped frozen on dry ice, and stored at −70°C until used. The samples were thawed overnight at 0°C and allowed to warm to 37°C in the tank of Ringer's solution prior to measurement. Detailed pathology reports accompanied each sample which included the age of the patient, the gross description of the sample, and the microscopic diagnosis. Normal ovary specimens ranged from 30 to 56 years while the age of pathological specimens ranged from 31 to 71 years. Normal testis specimens ranged in age from 35 to 77 years, with specific pathological specimens reflecting the age characteristics of each disorder, e. g., seminoma and Leydig cell tumor specimens ranged from 21 to 27 years while atrophic testis specimens ranged from 57 to 80 years.

3. Results

a. Ovary Measurements

The absorption coefficient values determined in this study at 1 MHz are presented in **Table 1**. The entries represent the mean value of n measurements and the standard deviation. The nonpathological, i. e., normal, values are less overall than those previously measured for other species[10], though the relative order is the same. That is, the value for the cortex is greater than that for the medullary tissue and the value for the corpora lutea is near that of the cortex. Due to the limited number of distinct corpora lutea in the specimens, differences due to cycle stage could not be detected. The tunica albuginea, which is a dense connective tissue layer, exhibited the highest value. The fluid filled follicles had the lowest measured value and was similar to that measured previously in the bovine ovary[10].

Table 1 Absorption coefficient values, at 1 MHz, of normal and pathological human ovarian tissues ±SD

Structure	n	Absorption coefficient (Np/cm)	SD
NORMAL			
tunica albuginea	5	0.046	±0.003
corpora lutea	4	0.028	±0.006
cortex	15	0.026	±0.007
medulla	8	0.020	±0.004
follicle	3	0.016	±0.004
PATHOLOGICAL			
papillary serous adenocarcinoma	21	0.020	±0.004
mucosal adenocarcinoma	2	0.039	±0.004
cancerous corpus luteum	2	0.032	±0.001
hemorrhagic areas	6	0.032	±0.003
adenocarcinoma with psammoma bodies	3	0.123	±0.002

n=number of specimens, SD=standard deviation

The largest number of pathological specimens available for measurement were tissues that had papillary serous adenocarcinomas, which constitutes the most common type of ovarian cancer[14]. These tumors secrete serous fluid and it is expected that they would exhibit a relatively low absorption coefficient value (see **Table 1**). As the standard deviation of the measurement for a single structure that had been measured repeatedly in different specimens was less than ±5%, the ±27% SD value for normal cortex must mean that a wide variety of morphological conditions are present.

Hemorrhagic areas were due to ovarian torsion or hemorrhagic corpora lutea. Irrespective of the origin, measurements of hemorrhagic

areas yielded nearly the same values and were lower than those for normal corporal luteal tissues. It is interesting to observe that the absorption value for corpora lutea (CL) which had been invaded by a papillary serous adenocarcinoma was greater than that for a normal CL, or for the value for the papillary serous adenocarcinoma.

A lymphatic blister-like cyst in a mucinous cystadenocarcinoma showed a very low value of absorption at 1 MHz, viz., 0.0052±0.0009 (Np/cm). In the same specimen, but at a different location, the mucinous portion had a very large absorption, viz., 0.041±0.0034 (Np/cm). The connective tissue layer had a nearly normal absorption value. These emphasize the complicated structure of both normal and pathological ovarian tissue.

The psammoma body, which is calcareous in nature, present in a specimen of a papillary serous adenocarcinoma had the very high absorption value of 0.123±0.002 (Np/cm) at 1 MHz; the largest value for any ovary specimen in this study.

b. Testis Measurements

The measured absorption coefficient values for human testicular tissue at 1 MHz are shown in **Table 2**. A statistically significant difference is seen between the value for normal tissue versus that for pathological tissue. The values for the atrophic samples and those with seminomas appear to be the same.

Table 2 Absorption coefficient values, at 1 MHz, of normal and pathological human testicular tissues ±SD

Testis pathology	n	Absorption coefficient (Np/cm)	SD
normal	12	0.018	±0.002
atrophic	10	0.024	±0.004
seminoma	6	0.022	±0.003
Leydig cell tumor	2	0.048	±0.008

n=number of specimens, SD=standard deviation

The value for normal testis is similar to that measured in other species[11)-13)] at 1 MHz, wherein the low value reflects the high fluid content associated with the presence of seminal fluid in the seminiferous tubules and in the interstitial area of the testis[15)]. Additionally, the testis contains minimal amounts of lipid and connective tissue.

The greater absorption ceofficient values for the atrophic and tumorous testicular samples are consistent with the morphological changes attending these conditions, viz., testicular atrophy results in reduced tubular diameter, loss of luminal area, and hyalinization of the tubule basement membrane[20)]. These changes reduce the amount of seminal fluid present, as well as increase the amount of connective tissue.

Seminoma is the most common of testicular cancers[20)]. This tumor fills the testis with a fairly uniform mass of cells which so distort the tubules that the luminal area of the tubule and the seminal fluid may no longer exist, resulting in a greater absorption coefficient value.

The large absorption coefficient value for the Leydig cell tumor also reflects its histopathology. These tumors sometimes cause the stroma of the testis to become fibrous or calcified, the parenchyma is sometimes atrophied due to compression, and crystalline structures are found in about one-half of such tumors.

These results, unlike the results of the ovarian measurements, suggest that noninvasive diagnosis of testicular pathology could be possible by clinical determination of absorption coefficient values.

4. Discussion

Table 3 shows the absorption coefficient values of those tissue samples measured at both 1

Table 3 Frequency dependence of the ultrasonic absorption coefficient of human nomal and pathological gonadal tissues

Tissue	1 MHz (Np/cm)	3.25 MHz (Np/cm)	exponent a
TESTIS			
normal	0.018	0.091	1.38
atrophic	0.024	0.153	1.57
Leydig cell tumor	0.048	0.509	2.00
OVARY			
normal medulla	0.020	0.131	1.59
normal tunica	0.046	0.255	1.45
papillary serous adenocarcinoma	0.020	0.112	1.46
adenocarcinoma with psammoma bodies	0.123	0.363	0.92

and 3.25 MHz and the exponent, a, on frequency, calculated from these data. That is, the absorption coefficient is assumed to follow the power law

$$\alpha_f = \alpha_1 f^a \qquad (2)$$

where α_f is the absorption coefficient at frequency f (in megahertz), α_1, is the absorption coefficient at 1 MHz, and a is the empirically determined exponent on frequency computed from these data.

It is interesting to observe that the testis frequency dependence, of the studied pathological states, decreases from that of the normal specimens while those of the ovary increase.

Though the data are limited, it is possible to discern several results having possible medical application. The very large increase in the absorption coefficient of the Leydig cell tumor over that of the normal testis (nearly 170%) suggests very strongly that hyperthermia treatment could be used to some advantage not requiring radical surgery for the approximately 4% of patients requiring inguinal orchiectomy[21]. Further, as the wave propagation distance for such a procedure conld be limited to a few centimeters, advantage could also be taken of the strong frequency dependence of the absorption coefficient to employ frequencies considerably higher than conventionally used. The data of this study clearly show the absorption coefficient for Leydig cell tumor to be more than 500% greater than that for normal testis tissue, also suggesting an opportunity for more precise diagnosis by ultrasound.

The ovary may not offer such treatment advantage. It is noted that normal ovary can exhibit a very large range of normal tissue absorption properties, e.g., normal medulla and normal tunica show an approximate 2 : 1 absorption coefficient relationship. Further, the papillary serous carcinoma has the same value for the absorption coefficient as normal medulla at 1 MHz, and a lesser value at higher frequencies.

The psammoma body, which exhibits a very high absorption rate at 1 MHz, also had a high absorption loss at 3.25 MHz, viz., 0.363 ± 0.006 (Np/cm). As this material is crystalline in structure, the exponent a, viz., 0.92, is the lowest of the values listed in **Table 3**. This suggests that ovarian cancer may be very difficult to treat using high frequency focused ultrasound hyperthermia.

The a measurement of the normal ovary and testis specimens compared favorably with those reported in the literature for tissues of such specific water, lipid, and protein contents[10)–13)]. Molecular content details of the pathological specimens were not available.

5. Conclusion

The 1 and 3.25 MHz ultrasound absorption coefficient of normal and pathological ovary and testis specimens *in vitro* were determined using the transient thermoelectrical method. The a-values obtained suggest a possible medical application for testis, as discussed in Sec. 4.

Acknowledgements

The authors acknowledge gratefully the partial support of this study by a grant from the United States National Institutes of Health. H. Inoue's stay at BRL was supported by a grant from the Japanese Ministry of Education, Science and Culture.

References

1) Nyborg WL, Ziskin MC(eds) : *Biological Effects of Ultrasound*. p. 1, Churchill Livingstone, New York 1985.
2) White D, Clark G, Carson J, White E(eds) : *Ultrasound in Biomedicine, Cumulative Bibliography of the World Literature in Ultrasound in Medicine and Biology to 1978*. Pergamon, New York 1982.
3) White D, Carson J, White E : Cumulative Bibliography of the World Literature 1979-1982. *Ultrasound Med Biol* **13**(Suppl. 1), 1987.
4) Nyborg WL, Carson PL, Dunn F, et al : Biological Effects of Ultrasound : Mechanisms and Clinical Implications. *NCRP Rep* **74** : 191, 1983.
5) Stewart HD, Stewart HF, Moore RM Jr., Gerry J : Compilation of Reported Biological Effects Data and Ultrasound Exposure Levels. *J Clin Ultrasound* **13** : 167-186, 1985.
6) Taranatal AF, Hendrickx AG : Evaluation of the Bioeffcts of Prenatal Ultrasound Exposure in the Cynomolgus Macaque (Macaca Fascicularis) : I. Neonatal/Infant Observations. *Teratology* **39** : 137-147, 1989.
7) Tarantal AF, Hendrickx AG : 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, 1989.
8) AIUM : Evaluation of Research Reports. Prepared by Bioeffects Committee, *AIUM*, Bethesda, MD 1984.
9) Dunn F, Edmonds PD, Fry WJ : Absorption and Dispersion of Ultrasound in Biological media, In Schwan HP(ed.) : *Biological Engineering*. pp. 205-332, McGraw-Hill, New York 1969.
10) Carnes KI, Dunn F : Absorption of Ultrasound by Mammalian Ovaries. *J Acoust Soc Am* **84** : 434-437, 1988.
11) Goss SA, Frizzell LA, Dunn F : Frequency Dependence of Ultrasonic Absorption in Mammalian Testis. *J Acoust Soc Am* **63** : 1226-1229, 1978.
12) Goss SA, Johnston RL, Dunn F : Comprehensive Compliation of Empirical Ultrasonic Properties of Mammalian Tissues. *J Acoust Soc Am* **64** : 423-457, 1978.
13) Goss SA, Johnston RL, Dunn F : Compilation of Empirical Ultrasonic Properties of Mammalian Tissues II. *J Acoust Soc Am* **68** : 93-108, 1980.
14) Woodruff JD : Pathology : In *Practical Gynecologic Oncology*. pp. 109 - 165, William & Wilkins, Baltimore 1989.
15) Bloom W, Fawcett DW : *A Textbook of Histology*. pp. 858-906, Saunders, Philadelphia 1975.
16) Brady JK, Goss SA, Johnston RL, et al : Ultrasonic Propagation Properties of Mammalian Testes. *J Acoust Soc Am* **60** : 1407-1409, 1976.
17) Goss SA, Cobb JW, Frizzell LA : Effect of Beam Width and Thermocouple Size on the Measurement of Ultrasonic Absorption Using the Thermoelectric Technique. *Proceedings of IEEE Ultrason Symp* **77** : 206-211, 1977.
18) Dunn F, Averbuch AJ, O'Brien WD Jr : A Primary Method for the Determination of Ultrasonic Intensity with the Elastic Sphere Radiometer. *Acustica* **18** : 58-61, 1977.
19) Dunn F : Temperature and Amplitude Dependence of Acoustic Absorption in Tissue. *J Acoust Soc Am* **34** : 1545-1547, 1962.
20) Nistal M, Panigua R : *Testicular and Epididymal Pathology*. Georg Thieme Verlag, New York 1984.
21) Chromette C, Delcourt A, Aieriol M : Nongerminal Cell Tumors of the Testis. In Khoury S, Kiiss R, Murphy GP, Chatelain C, Kar GK, Allan R (eds) : *Testicular Cancer*. pp. 35-45, Liss Inc., New York 1985.
22) Drewniak JL, Frizzell LA and Dunn F : Errors Resulting from Finite Beam Width and Sample Dimensions in the Determination of the Ultrasonic Absorption Coefficient. *J Acoust Soc Am* 88 : 967-977, 1990.