

REASSESSMENT OF TERATOGENIC RISK FROM ANTENATAL ULTRASOUND

Abstract

Science has shown that risk of cavitation and hyperthermia following prenatal ultrasound exposure is relatively negligible provided intensity, frequency, duration of exposure, and total numbers of exposures are safely limited. However, noncavitational mechanisms have been poorly studied and occur within what are currently considered "safe" levels of exposure. To date, the teratogenic capacity of noncavitational effectors are largely unknown, although studies have shown that different forms of ultrasound-induced hydraulic forces and pressures can alter membrane fluidity, proliferation, and expression of inflammatory and repair markers. Loose regulations, poor end user training, and unreliable ultrasound equipment may also increase the likelihood of cavitation and hyperthermia during prenatal exposure with prolonged durations and increased intensities. The literature suggests a need for tighter regulations on the use of ultrasound and further studies into its teratogenicity.

Keywords

• Cavitation • Microstreaming • Sonoporation • Teratogens • Ultrasonography • Prenatal

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Introduction

"There are undoubtedly several mechanisms by which ultrasonic radiation may affect animal tissue. Moreover, experience in X-radiology has shown that it is unwise to assume that absence of immediate effects always implies that no damage has occurred" [1].

Science lies in a state of continual fluctuation and progression. As technologies and paradigms are amended, accepted theories are reviewed and tested against new understanding. Often these well-accepted theories stand the test of time; but sometimes they do not. In the case of antenatal ultrasound the progression of scientific theory and the application of this tool in other arenas has given us a greater understanding of how ultrasound behaves at the cellular level, an intimate understanding that was not available several decades ago. Concern based on this fresh understanding dictates that we take a second look at the safety of this otherwise extraordinarily useful tool.

The study of ultrasound is not new to science although the number of its current uses is

bourgeoning. (For a listing of some of its uses in medicine, manufacturing, and research, see Table 1.) As early as 1826, the Swiss physicist, Jean-Daniel Colladon, partnered with the engineer, Charles-Francois Sturm, to measure the speed of sonic waveforms underwater against the speed of light estimating the former at 1435 meters per second. Later, ultrasound was studied and utilized for submarine navigation as early as World War I and by the 1930s it saw uses in radar and metal flaw detection of military crafts. Within the medical field high-intensity ultrasound was exapted as a neurosurgical tool for creating lesions within brain tissue, similar to the use of laser. By the 1940s, however, enthusiasm for this new tool rapidly outweighed caution leading to numerous unwarranted therapies for conditions such as arthritis, gastric ulcers, eczema, asthma, elephantiasis, hemorrhoids, and angina pectoris (see [2] for review).

Nevertheless, a growing skepticism closely shadowed its rise in popularity and the realization that sound force could produce tissue damage lead to both a reduction in

its widespread use and further research into its safety. This initial caution was especially apparent in obstetrics: various cell culture, animal, and human studies were performed to determine whether prenatal exposure to ultrasonic radiation could adversely affect early development. While numerous studies were performed, overall the results appeared to support the supposition that at lower intensities, shorter durations, and limited number of exposures ultrasound was not a considerable danger (for review, see [3]).

Scientists began recognizing the potency of ultrasonic cavitation and cavitationally-induced hyperthermia as early as the 1950s; however, knowledge then is not what it is today [4,5]. While there is still much we don't know about the interaction of ultrasonic waves with biologic tissue at varying intensities and frequencies, we do know that noncavitational and potentially deleterious mechanisms are active below safety cutoffs [6]. Whether they are actively teratogenic remains unaddressed. Unfortunately, each decade since the application of ultrasound in obstetric medicine

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its popularity has continued to skyrocket, increasing the risk of adverse side effects. In modern obstetrics, it is standard practice to utilize ultrasound to diagnose and date the pregnancy as well as to continue to monitor the growth of the fetus, even though studies have suggested that risks may outweigh the benefits in such circumstances [7,8]. Even women experiencing non-at-risk pregnancies generally receive multiple unwarranted ultrasounds during a given pregnancy [9]. And yet thorough safety studies have not been performed despite the growing evidence that ultrasound is a potentially dangerous tool that requires the utmost delicacy and caution in its application. Respected researchers in the past have questioned ultrasound safety, despite that the typical range of prenatal exposure does not seem to cause obvious malformations. As Holland and Apfel report [10], Frizzell [11], Kremkau [12], the National Council on Radiation Protection and Measurement [13], and the National Institutes of Health Consensus Committee [14] all reviewed safety studies on ultrasound and each respectively concluded that "diagnostic ultrasound may not be totally innocuous and recommend[ed] that more research be aimed specifically at test systems that would provide a better database for developing reasonable estimates of bioeffects and of risk" (p. 2059 from [14]). Ziskin and Petitti [15] also point out most poignantly that:

"[...] the inability to finding convincing proof of an effect, either from epidemiology or from physicians' experience, does not preclude the possibility of [adverse effects from ultrasound] happening. Statistical reasoning shows that even with large population studies, it is difficult to identify a small increase in the rate of a commonly occurring event. Subtle effects, long-term delayed effects, and certain genetic effects, could easily escape detection" (p. 91).

Just as Ziskin and Petitti have suggested, such assumptions are still present today which continue to falsely impress upon the science that safety studies have been adequate and thorough (for example, see [16]). Research into transient cavitation and thermal effectors have been considerable, and provided A.L.A.R.A. (As Low As Reasonably Achievable) guidelines are followed, risk from these effectors during

Table 1. Uses of ultrasound in medicine, manufacturing, and research.

1. Diagnostic sonography providing structural imaging, including prenatal ultrasound.
2. The ablation of target tissue, such as during neurosurgery or tumor removal, and the breakdown of calculi such as kidney stones or gallstones.
3. Transcranial ultrasonic stimulation, similar to transcranial magnetic stimulation (TMS).
4. Vasodilation, providing better visualization of the vasculature during cardiovascular procedures.
5. Targeted drug delivery, utilizing focused ultrasound to make the target tissue more permeable, e.g., the blood-brain barrier, skin, etc.
6. Wound healing, e.g., bone fractures and ulcers.
7. Bactericidal properties when synergized with antibiotics.
8. Elastography, in which ultrasound is used to determine the elasticity of a given organ which can help discern the overall health of that organ.
9. Transmembrane delivery of products into target cells, e.g., nonviral genes or nutrients.
10. Acoustophoresis: the use of ultrasound on an ionic medium to create an electric charge.
11. The purification of agricultural products.
12. Heat transfer in liquids for production of substances such as ethanol.
13. The purification of metals.
14. Manipulation and characterization of particles in the bio- and physical sciences.
15. The testing of metals, plastics, aerospace composites, wood, concrete, cement, etc. in manufacturing in order to measure thickness and locate flaws within the material.

prenatal ultrasound should be relatively negligible; however, work on the bioeffects of noncavitation mechanisms remains sparse and current ultrasound machines do not calculate risk related to stable cavitation and microstreaming. Therefore it is imperative that our science and our medicine reflect the growing understanding of this complexity.

Biophysical mechanisms of prenatal ultrasound and its teratogenic potency

Even though ultrasonic waveforms are capable of creating considerable damage through mechanisms of cavitation and extreme hyperthermia, the causal factors of teratogenicity arising from prenatal ultrasound are probably noncavitation in nature, except in instances of end users' nonadherence to safety guidelines. Convention within the field of physics, contrary to fields of study within medicine, utilizes the terms *cavitation* and *noncavitation* to describe the effects of ultrasound on a given medium. When force is applied to a fluid medium in the form of compression/expansion waveforms, gaseous bubbles arise at a given atmosphere of negative pressure during the expansion half-cycle. For pure water, bubble formation requires more than 1,000 atmospheres of pressure in order to occur, a level of pressure unheard of even in today's most intense ultrasounds. However,

when a liquid medium contains solids, such as cellular material, gases become trapped in crevices within these solids. The presence of already-formed gaseous cavities thereby lowers the threshold for cavitation because bubble formation is already present. The gaseous cavities during the expansion half-cycle expand with the liquid medium while in the compression half-cycle likewise compress. At lower pressures, these cavities either reabsorb into the medium or remain relatively stable and oscillate with the sonic waveforms; this is referred to as *stable cavitation*. However, during *transient cavitation* such as occurs with higher intensity ultrasounds or lower frequencies, the cavities rapidly increase in size until at which point pressure becomes too great in the surrounding medium and the bubble collapses [17]. The implosion creates water jets of extreme pressure that can damage cellular membranes and disturb intracellular contents [18,19]. The implosion also produces an extraordinary rise in temperature of approximately 5,500 °C due to the intense compression of gases by the liquid (for a summary of information, see [19]). For some perspective, the heat generated from cavitation is only slightly less than the estimated temperature of the surface of the sun. While it is amazing that such extreme temperature exposure doesn't destroy a tissue outright (which is mainly due to the rapid cooling rates in the surrounding medium estimated at over 10° °Cs⁻¹), a build-up of temperature from

multiple gaseous implosions in a local area can subsequently trigger the denaturation of proteins, changes in lipid membrane fluidity, alterations in intracellular signaling, and even cell death [19-22]. The compression of gases by liquid following cavity implosion also leads to the production of free radicals, which can wreak havoc on tissues [23]. The phospholipid membranes are particularly vulnerable due to their chemical composition such that they are easily scavenged by free radicals, but carbohydrates, proteins, RNA, and DNA may also be targets of oxidation [24-26]. Previous work has in fact suggested that ultrasound has mutagenic capacity and this mutagenesis may largely be due to the reactive oxygen species (e.g., hydroxyl radicals) produced during cavitation [27,28].

Higher intensities and lower frequencies, however, are not the only factors for concern: long exposure even in what appears to be "safe" ranges of intensity and frequency can trigger the slow growth of cavities because the bubble with each acoustic cycle generally shrinks less than it grows. This can ultimately lead to cavitation and for this reason it is imperative that duration of exposure for all forms of diagnostic and therapeutic ultrasound, but especially for prenatal ultrasound, is minimized to that which is absolutely necessary. In addition, even though extreme hyperthermia is mainly produced by cavitation, the force of sound as an energy source can nevertheless transfer that energy to the medium in the form of heat. Therefore, shorter durations should also minimize risks due to the noncavitationary transfer of heat energy to exposed tissues.

Cavitation seems to occur at particular thresholds of intensity, frequency, and duration, and provided that the prenatal ultrasound scan remains within accepted guidelines risk for cavitation and extreme hyperthermia are relatively low. (Risks due to poor regulations over the use of medical ultrasound will be discussed later.) However, stable gaseous cavities do still form and oscillate within biologic tissues during normal exposure; microstreaming is a potential problem at almost any range of intensity, frequency, and duration; and the transfer of low-grade heat

energy to the local tissue may also disrupt the cell's biochemistry [29].

Noncavitationary mechanisms include radiation pressure, force, torque, shear stress, and microstreaming. Each of these places various pressures and forces on the cell directly, parallel, and tangentially. Stable cavities remain intact for numerous acoustic cycles and can create transient pores in the cell membrane as well as disrupt the organization of organelles and other intracellular materials through acoustic streaming [1,30]. These forces together increase membrane porosity by "poking holes" into the phospholipid bilayer which subsequently triggers the influx and efflux of important cell signaling molecules [30]. This flux in cell signaling alters activity of numerous intracellular pathways and can ultimately lead to changes in gene expression [31]. For instance, due to the extreme ratio in levels of intracellular-to-extracellular calcium this ion rushes into the cell upon ultrasound exposure triggering numerous calcium-dependent pathways [32,33]. Calcium is also a necessary ion for the resealing of the broken membrane by triggering fusion of lysosomes to the outer membrane in a LAMP-1-dependent manner thereby repairing the pores created [34,35]. As per example, Al-Karmi et al. [36] have shown how ultrasound-induced calcium signaling affects conductance of the cell, finding that in calcium-laden medium, frog skin exhibits a significantly larger level of conductance. In fact due to ultrasound's conductive capacity it is currently being used for transcranial stimulation in humans [37,38]. Ultimately, more and more noncavitationary bioeffects are being reported in the literature, citing the modulation of membrane fluidity, cell proliferation, and presentation of inflammatory and repair markers [6].

As mentioned, cellular disorganization is a potential problem of microstreaming. As early as 1967, Connolly and Pond [1] suggested that the intracellular disorganization noted in Selman and Counce and Selman's [18] *Drosophila* experiments could be explained by vortices arising from ultrasound-induced microstreaming within the cell, disrupting organelles and various other cellular components. Likewise, the oscillation of stable

cavities in the liquid within the cell could feasibly create a similar disruption, while oscillation of bubbles outside the cell surface disrupt the membrane by creating transient pores within the phospholipid bilayer. Radiative forces have been shown to increase membrane porosity and Koshiyama et al. [30] have found that these noncavitationally-induced pores can be as large as 1.4 nm, a similar size as the diameter of gap junctions that allow the direct passage of larger signaling molecules between connected cells [39].

While safety studies have traditionally defined "harm" based on the level of tissue damage produced *in vitro*, *ex vivo*, and *in vitro*, it is apparent today that deleterious effects need not be relegated to necrosis but may instead be biochemical in nature, as Connolly and Pond [1] had so astutely noted:

"The ability of muscles to pump Na^+ ions from their interiors depended on metabolic reactions to yield the necessary energy. Obviously some biochemical lesion had been caused [by ultrasound exposure]. Histological changes were not seen when the muscle was stained and sectioned. In this case a biochemical lesion has certainly preceded any possible histological one" (p. 114 in [1]).

Total number of exposures is also an important variable in terms of outcome. In a 1989 study, Tarantal and Hendrickx [8] exposed prenatal macaques to ultrasound five times per week on gestational days (GD) 21-35, three times per week on GD 36-60, and once a week on GD 61-150. Each exam lasted 10 minutes and spatial-peak temporal-average (i.e., acoustic output) was well within current obstetric ranges. The authors reported altered birth weight and crown-rump length, generally reduced levels of physical activity as compared to control monkeys, and lower white blood cell counts comprising reductions in segmented neutrophils and monocytes. All effects had normalized by age 5-6 months. It is uncertain whether these neonatal phenotypes resulted solely from noncavitationary mechanisms or cavitationary thresholds had been reached even at the low intensity of $12 \text{ mW}\cdot\text{cm}^{-2}$. However, mirroring this simian study, frequent ultrasounds in humans have been closely correlated with neonatal birth weight.

Newnham et al. [7] subjected 1415 women with single pregnancies to ultrasound examinations at 18, 24, 28, 34, and 38 weeks of gestation, while a control group of 1419 women received a single ultrasound at week 18. Intrauterine growth restriction was significantly higher in the experimental group as measured by birth weight, such that a significant number of infants fell below the tenth percentile and even the third percentile mark. Because a considerable duration occurred between pregnancies within the Newnham et al. study [7] in which the stimulus (ultrasound) was removed, the differences in outcome suggest that multiple ultrasounds can have additive or exponential effects on development.

Much research has been done attempting to ensure that negligible damage occurs from cavitation and cavitation-induced hyperthermia during routine ultrasound exposure. As will be subsequently reviewed, this reliability may be vulnerable to such things as variability in end user application (e.g., lax adherence to recommended guidelines for exposure) and even doubtful reliability of the machines themselves. However, we know relatively little about noncavitational effectors and yet these likely pose the greatest threat in current application. Due to radiative forces and pressures, significant biochemical and physical pathologies occur at lower thresholds than are seen with cavitation-related phenomena. As Koshiyama et al. [30] have reported, pore formation in the phospholipid bilayer need not be stimulated by any mechanical or electrical force but simply requires the insertion of enough water molecules into the inner hydrophobic region of the bilayer to exceed a critical value. This suggests that very subtle effectors can have extreme chemical effects on cell structure and signaling.

Increased risk due to deregulation

As a general review for the reader, the American Institute of Ultrasound in Medicine (AIUM) 2010 guidelines [40] recommend the use of ultrasonography during the first trimester in normal non-risk pregnancies for the following purposes: 1) to confirm of pregnancy; 2)

to estimate gestational age; 3) to diagnose multiple pregnancies; and 4) to confirm cardiac activity. For the second and third trimesters, they likewise recommend the use of ultrasound in normal pregnancies in order to 1) estimate gestational age; 2) evaluate fetal growth; 3) determine fetal presentation; 4) evaluate fetal well-being; and 5) as a basic screening for fetal anomalies. We will discuss further the potential risks to some of these recommendations based upon early and multiple exposures.

While the science's greatest forthcoming challenge lies in the study of noncavitational effectors of ultrasound *in vivo*, the governing bodies that oversee and regulate medical ultrasound face considerable challenges of their own. Prior to 1992, the levels of absolute intensity in ultrasonography were determined by these governing bodies; however, since 1993 it is the end user who determines the levels of ultrasound appropriate for a given examination. The American Institute of Ultrasound in Medicine (AIUM) recommends intensities no higher than $94 \text{ mW}\cdot\text{cm}^{-2}$ for obstetric purposes, although the machines are generally capable of reaching intensities up to $720 \text{ mW}\cdot\text{cm}^{-2}$. While this flexibility has distinct benefits in medical application, it is also vulnerable to variations in judgment by the end user. In fact, in a 2007 study [41] Sheiner et al. have shown that in a survey of 130 end users approximately 82% lacked adequate understanding of the thermal index and 96% failed to demonstrate appropriate understanding of the mechanical index. Most alarmingly, only 20% of the end users knew where these safety indices were located on the machines; ironic that these indices should be helping to inform the end user of whether the patient is in danger of overexposure. Placing the onus of safety on undereducated end users is a considerable risk to patients receiving ultrasound.

The first eight weeks of pregnancy referred to as the embryonic period are the time of an infant's greatest vulnerability to teratogenic exposures. Founder cells are actively dividing and expanding their populations. Agents which target these founder populations can have some of the furthest reaching effects such that irreparable insults can be passed on to all subsequent progeny. It is therefore

extraordinarily surprising that the AIUM stipulates in their practice guidelines that ultrasounds utilized within the first trimester are acceptable for diagnosing and dating pregnancy and that "[diagnostic] ultrasound studies of the fetus are generally considered safe . . ." (p. 8 in [40]). The American College of Obstetricians and Gynecologists (ACOG) and the American College of Radiologists (ACR) likewise advocate the use of ultrasound for dating and fetal monitoring (for review see [42]). Guidelines however by other organizations outside the United States such as the Alberta Medical Association of Canada explicitly state that routine ultrasounds should not be performed solely for these purposes. Ironically even ACOG concludes that there is insufficient evidence that ultrasound reduces infant morbidity, raising the question why these examinations are being performed in the first place (Alberta CPG Working Group for Prenatal Ultrasound [43]; for review see [42]). The AIUM goes on to state that the "diagnostic procedure should be performed only when there is a valid medical indication"; however, it indicates that diagnosis and dating of pregnancy are acceptable indications. The AIUM recommends the use of the A.L.A.R.A. principle, which stipulates that intensity and duration of exposure be "As Low As Reasonably Achievable", i.e., the end user should utilize the lowest intensity and length of exposure necessary in order to acquire the desired image. However, length of exposures varies considerably by end user judgment and as we've discussed earlier the longer the exposure time the more likely the threshold for cavitation will be reached. Therefore unwarranted exposures, especially those occurring in the first trimester, and multiple exposures partnered with poor regulations and end user training pose considerable risks to patient safety.

Probably the greatest concern in ultrasound safety has recently been unearthed in a series of studies by Mårtensson et al. [44,45]. Mårtensson's group studied ultrasound transducer error rates across equipment from seven different major manufacturers, totaling 676 transducers. On average, 40% of those transducers were defective; the company with the lowest rate totaled 20% while the company

with the highest was an astonishing 67%. They went on to study transducer reliability in all ultrasound machines within a single hospital setting, finding that 81 of the 299 actively used transducers were faulty. Fault with the transducer can degrade image quality, which may subsequently prompt the end user to increase intensity in order to capture a useful image. As A.L.A.R.A. instructs, end users should use the lowest intensity and shortest duration possible in order to acquire the necessary image. In the case of faulty transducers that intensity could be higher on average and therefore this is an area of study that requires much more attention both by scientists and regulatory bodies.

Ultrasound as compared to other imaging techniques is exceptionally cost effective for practitioners and hence there is a preference for its use within medicine. In fact, ultrasound is so cost effective that numerous private companies have begun offering additional ultrasound services to expectant parents, referred to as "keepsake images". Parents can have additional images or even full-length videos taken of their unborn children, promoted as a way to "start the family photo album early". Private companies have also begun offering "ultrasound parties" in which an entire family can gather around at home while a 3D or 4D ultrasound is performed. In 2004 the FDA issued a statement warning against the unmedicalized use of ultrasound which such companies have subsequently ignored [46]. In addition, fetal heart rate monitors which utilize a form of doppler ultrasound to provide an audio of the fetal heart have been available on websites such as Amazon and eBay for some time. Not uncommon in reviews of these products, parents describe the frequency with which they use the monitors in order to listen to their babies' heartbeats:

"Goodness I love my fetal Doppler. [...] I have not had a day where I could not hear the heartbeat from week 8 of pregnancy when I got it! The heart rate isn't exact but I can hear my little one at any time of the day and as clear now (16 weeks) as when I got it! [...] My doctor thinks I got an amazing deal! (reviewed in [47]).

It is alarming to consider that some mothers are daily subjecting their unborn children to

unregulated use of ultrasound. In such an instance there is no oversight as per length of exposure of a given focal area nor of overall total exposure. Under these circumstances, the risk of cavitation damage may grow exponentially. Thankfully, some governmental bodies are beginning to regulate unmedicalized use of ultrasound, such as the Connecticut House Bill 5635, *An Act Concerning Ultrasound Procedures for Medical and Diagnostic Purposes*, passed by Governor Rell in 2009.

And finally, doctors seem particularly eager to prescribe the use of early and multiple ultrasounds partly due to fear of legal reprisal should a developmental abnormality be missed:

"Of particular concern to all parents is the risk of an abnormality in their baby. Consumer demand for reassurance in this regard is becoming overwhelming and the birth of an undetected abnormal child may often be followed by attempts at litigation. Failure to perform an ultrasound, cardiotocograph or other medical tests at an appropriate time are commonly cited in writs against doctors, midwives and hospitals" (Senate Community Affairs References Committee, 1999).

While at first glance some individuals may be tempted to place blame on physicians for succumbing to patient and legal intimidation or on the fervent insistence of parents to have ultrasounds, the situation is far more complex than this. First and foremost the thorough science behind our understanding of potential risk is lacking. And it is that science which would ultimately inform both regulatory bodies and practitioners as to how this tool need be safely applied. Renewed caution is therefore necessary from those who regulate and apply ultrasound while safety research takes the time to catch up.

Discussion

Ultrasonic forces affect tissues through cavitation and noncavitation effectors which differ according to combinations of intensity, frequency, and length of exposure. Threshold of cavitation also varies by tissue type such that bone requires a much lower threshold than soft tissue [48]. And it is also

currently unknown whether multiple prenatal ultrasonic exposures could have additive or exponential results on phenotype, as illustrated by the earlier studies of Newnham et al. [7] and Tarantal and Hendrickx [8]. In short, understanding the potential for ultrasonic teratogenicity is an extraordinarily complex scientific undertaking, one which is still ongoing today.

As is hopefully apparent, further research is needed to clarify how noncavitation mechanisms may affect prenatal development within normal clinical range. It is also important to gauge whether cavitation and hyperthermia are occurring in everyday obstetric practice due to end user variability and transducer malfunction. Lastly, because the use of ultrasound in medicine has now advanced beyond the science itself, it is also prudent to draw the reins and once more apply greater caution to our use of this tool within obstetrics such as unwarranted, early, and multiple ultrasounds. In 1967, Connolly and Pond [1], on reviewing early ultrasound studies on *Drosophila* and avian embryos, noted, "a full research programme into the precise effects of diagnostic ultrasound is strongly indicated especially in regard to reproductive cells" (p. 114 in [1]). Since that time scientists have reassured physicians that there is negligible risk of teratogenicity from cavitation and extreme hyperthermia provided intensity, frequency, and duration are safely maintained. However, we currently have no means of estimating or measuring physical and chemical damage due to noncavitation mechanisms nor their cumulative effects from multiple exposures.

While the science continues to progress, we strongly recommend to physicians that they use greater caution in the application of obstetric ultrasound (Table 2): routine scans are not recommended for diagnosing, dating, or monitoring of an embryo or fetus without indication of potential pathology. While parents may be eager to date their pregnancies and determine the sex of their unborn children, counting all arms, legs, fingers, and toes, it is important for the physician to communicate the potential risks to their patients so they may understand that ultrasound is not just a picture but an

invasive tool. We also recommend that the governing regulatory bodies communicate greater caution to the larger community; that they advocate stricter use within clinical practice; that they support the development of better educational programs for doctors and end users; and most importantly that they require hospitals and clinical practices have their ultrasound equipment frequently and thoroughly tested for reliability. Ultrasound is not only a clinical tool, it's a vital part of medical business, and for parents it is a paramount milestone in the early lives of their children; therefore it may be difficult to convince people that its application should be limited without incontrovertible proof that teratogenicity occurs within clinical ranges. However, in the case of prenatal ultrasound, when we are discussing the safety of an unborn child caution should be our first and foremost priority. In addition to (and sometimes in

Table 2. Recommendations to obstetricians and end users in the use of prenatal ultrasound.

1. Avoid the use of unwarranted ultrasound examinations, including for the purposes of diagnosing and dating a normal pregnancy.
2. Avoid the use of ultrasounds within the 1st trimester if possible.
3. Avoid the use of multiple unwarranted ultrasounds if possible.
4. Utilize the lowest intensity, highest gain, and lowest duration of exposure possible to get the image necessary (A.L.A.R.A.).
5. Make a point to keep the wand moving and do not hover over any single focal area for any considerable length of time.
6. Perform a maintenance on actively-used ultrasound machines multiple times throughout the year to ensure peak performance.
7. Inform your patients of the potential risks to the baby from ultrasound exposure.

conflict with) the safety standards outlined by the AIUM, we are recommending a reduction in the number of medically unnecessary ultrasounds including for the purposes of diagnosing, dating, and monitoring of a normal pregnancy; a reaffirmation of the A.L.A.R.A principle; improved maintenance schedules of actively-used ultrasound machines; and the communication and dissemination of risk-

related information to the larger community so that people may make better informed decisions about their personal health care.

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References

- [1] Connolly C., Pond J., The possibility of harmful effects in using ultrasound for medical diagnosis, *Biomed. Eng.*, 1967, 2, 112–115
- [2] Woo J., A short history of the development of ultrasound in obstetrics and gynecology. *History of ultrasound in obstetrics and gynecology*, Part 1, 2008, retrieved on 10/12/2009 from <http://www.ob-ultrasound.net/history1.html>
- [3] Dewhurst C.J., The safety of ultrasound, *Proc. R. Soc. Med.*, 1971, 64, 996–997
- [4] Dognon A., Simonot Y., 1951. Cavitation et hémolyse par ultrasons de fréquences différentes, *C. R. Hebd. Séances Acad. Sci.*, 1951, 232, 2411–2413
- [5] Křížek V., Kolominsky J., Tepelné účinky ultrazvuku ve tkáních, *Čas. Lék. Čes.*, 1951, 90, 482–486
- [6] Johns L.D., Nonthermal effects of therapeutic ultrasound: the frequency resonance hypothesis, *J. Athl. Train.*, 2002, 37, 293–299
- [7] Newnham J.P., Evans S.F., Michael C.A., Stanley F.J., Landau L.I., Effects of frequent ultrasound during pregnancy: a randomised controlled trial, *Lancet*, 1993, 342, 887–891
- [8] Tarantal A.F., Hendrickx A.G., Evaluation of the bioeffects of prenatal ultrasound exposure in the cynomolgus macaque (*Macaca fascicularis*): I. neonatal/infant observations, *Teratology*, 1989, 39, 137–147
- [9] You J.J., Alter D.A., Stukel T.A., McDonald S.D., Laupacis A., Liu Y., et al., Proliferation of prenatal ultrasonography, *Can. Med. Assoc. J.*, 2010, 182, 143–151
- [10] Holland C.K., Apfel R.E., Thresholds for transient cavitation produced by pulsed ultrasound in a controlled nuclei environment, *J. Acoust. Soc. Am.*, 1990, 88, 2059–2069
- [11] Frizzell L.A., Biological effects of acoustic cavitation, In: Suslick K.S. (Ed.), *Ultrasound: its chemical, physical and biological effects*, VCH, New York, 1988, 287–303
- [12] Kremkau F.W., Bioeffects and safety, In: *Diagnostic ultrasound: principles, instrumentation and exercises*, 2nd ed., Grune and Stratton, New York, 1984, 166–277
- [13] Nyborg W.L., Carson P.L., Miller D.L., Miller M.W., Ziskin M.C., Carstensen E.L., et al., *Biological effects of ultrasound: mechanisms and clinical Implications*, National Council on Radiation Protection and Measurement, Bethesda, 1983
- [14] National Institutes of Health Consensus Committee, *Diagnostic ultrasound imaging in pregnancy*, 1984, NIH Pub. No. 84-667
- [15] Ziskin M.S., Petitti D.B., Epidemiology of human exposure to ultrasound: a critical review, *Ultrasound Med. Biol.*, 1988, 14, 91–96
- [16] Grether J.K., Li S.X., Yoshida C.K., Croen L.A., Antenatal ultrasound and risk of autism spectrum disorders, *J. Autism Dev. Disord.*, 2010, 40, 238–245
- [17] Tezel A., Sens A., Mitrugoti S., Investigations of the role of cavitation in low-frequency sonophoresis using acoustic spectroscopy, *J. Pharm. Sci.*, 2002, 91, 444–453
- [18] Counce S.J., Selman G.G., The effects of ultrasonic treatment on embryonic development of *Drosophila melanogaster*, *J. Embryol. Exp. Morphol.*, 1955, 3, 121–141

[19] Suslick K.S., The chemical effects of ultrasound., *Sci. Am.*, 1989, 260, 80–86

[20] Basile A., Biziato D., Sherbet G.V., Comi P., Cajone F., Hyperthermia inhibits cell proliferation and induces apoptosis: relative signaling status of P53, S100A4, and Notch in heat sensitive and resistant cell lines, *J. Cell. Biochem.*, 2008, 103, 212–220

[21] Kampinga H.H., Thermotolerance in mammalian cells: protein denaturation and aggregation, and stress proteins, *J. Cell Sci.*, 1993, 104, 11–17

[22] Yatvin M.B., The influence of membrane lipid composition and procaine on hyperthermic death of cells, *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.*, 1977, 32, 513–521

[23] Riesz P., Kondo T., Free radical formation induced by ultrasound and its biological implications, *Free Radic. Biol. Med.*, 1992, 13, 247–270

[24] Davies K.J., Protein damage and degradation by oxygen radicals, I. General aspects, *J. Biol. Chem.*, 1987, 262, 9895–9901

[25] Quinlan G.J., Gutteridge J.M., Hydroxyl radical generation by the tetracycline antibiotics with free radical damage to DNA, lipids and carbohydrate in the presence of iron and copper salts, *Free Radic. Biol. Med.*, 1988, 5, 341–348

[26] Stadtman E.R., Levine R.L., Free radical-mediated oxidation of free amino acids and amino acid residues in proteins, *Amino Acids*, 2003, 25, 207–218

[27] Macintosh I.J., Davey D.A., Relationship between intensity of ultrasound and induction of chromosome aberrations, *Br. J. Radiol.*, 1972, 45, 320–327

[28] Newcomer E.H., Wallace R.H., Chromosomal and nuclear aberrations induced by ultrasonic vibrations, *Am. J. Bot.*, 1949, 36, 230–236

[29] Krasovitski B., Kimmel E., Shear stress induced by a gas bubble pulsating in an ultrasonic field near a wall, *IEEE Trans. Ultrason. Ferroelectr. Freq. Control*, 2004, 51, 973–979

[30] Koshiyama K., Yano T., Kodama T., Self-organization of a stable pore structure in a phospholipid bilayer, *Phys. Rev. Lett.*, 2010, 105, 018105

[31] Deng C.X., Sieling F., Pan H., Cui J., Ultrasound-induced cell membrane porosity, *Ultrasound Med. Biol.*, 2004, 30, 519–526

[32] Carafoli E., Calcium signaling: a tale for all seasons, *Proc. Natl. Acad. Sci. USA*, 2002, 99, 1115–1122

[33] Zhou Y., Shi J., Cui J., Deng C.X., Effects of extracellular calcium on cell membrane resealing in sonoporation, *J. Control. Release*, 2008, 126, 34–43

[34] Reddy A., Caler E.V., Andrews N.W., Plasma membrane repair is mediated by Ca²⁺-regulated exocytosis of lysosomes, *Cell*, 2001, 106, 157–169

[35] Yang F., Gu N., Chen D., Xi X., Zhang D., Li Y., et al., Experimental study on cell self-sealing during sonoporation, *J. Control. Release*, 2008, 131, 205–210

[36] Al-Karmi A.M., Dinno M.A., Stoltz D.A., Crum L.A., Matthews J.C., Calcium and the effects of ultrasound on frog skin, *Ultrasound Med. Biol.*, 1994, 20, 73–81

[37] Mihran R.T., Barnes F.S., Wachtel H., Temporally-specific modification of myelinated axon excitability in vitro following a single ultrasound pulse, *Ultrasound Med. Biol.*, 1990, 16, 297–309

[38] Tufail Y., Yoshihiro A., Pati S., Li M.M., Tyler W.J., Ultrasonic neuromodulation by brain stimulation with transcranial ultrasound, *Nat. Protoc.*, 2011, 6, 1453–1470

[39] Pébay A., Peshavariya H., Wong R.C.B., Dusting G.J., Non-classical signalling mechanisms in stem cells, In: Atwood C.S. (Ed.), *Embryonic stem cells: the hormonal regulation of pluripotency and embryogenesis*, Intech, Rijeka, 2011, 317–336

[40] American Institute of Ultrasound in Medicine, AIUM practice guideline for the performance of obstetric ultrasound examinations, *J. Ultrasound Med.*, 2010, 29, 157–166

[41] Sheiner E., Shoham-Vardi I., Abramowicz J.S., What do clinical end users know regarding safety of ultrasound during pregnancy?, *J. Ultrasound Med.*, 2007, 26, 319–325

[42] Washington State Health Care Authority, Ultrasonography (ultrasound) in pregnancy: health technology assessment, 2010, taken on 08/28/2012 from http://www.hta.hca.wa.gov/documents/final_report_ultrasound.pdf

[43] Alberta Clinical Practice Guidelines Working Group for Prenatal Ultrasound, Guideline for the use of prenatal ultrasound: First trimester, Alberta Medical Association, Edmonton, 1998

[44] Mårtensson M., Olsson M., Brodin, L.-Å. Ultrasound transducer function: annual testing is not sufficient, *Eur. J. Echocardiogr.*, 2010, 11, 801–805

[45] Mårtensson M., Olsson M., Segall B., Fraser A.G., Winter R., Brodin L.-Å. High incidence of defective ultrasound transducers in use in routine clinical practice, *Eur. J. Echocardiogr.*, 2009, 10, 389–394

[46] Rados C., FDA cautions against ultrasound ‘keepsake’ images, *FDA Consum.*, 2004, 38, 12–16

[47] Williams E.L., Casanova M.F., Prenatal ultrasound: it’s not just a photograph, *Autism Sci. Dig.*, 2011, 1, 58–60

[48] Abbott J.G., Rationale and derivation of MI and TI - a review, *Ultrasound Med. Biol.*, 1999, 25, 431–441