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Ultrasound from Conception to 10^{+0} Weeks of Gestation

Scientific Impact Paper No. 49
March 2015

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1. Introduction

Ultrasound scanning machines are designed and built to conform to strict international regulations. In the hands of well-trained, competent operators this equipment has proved an invaluable resource, especially since, in contrast to imaging techniques based on ionising radiation, there is no evidence that repeated exposure has cumulative and/or detrimental effects. Ultrasound has become central in the management of the problems of early pregnancy, particularly but not exclusively in the management of women after assisted conception and those in whom miscarriage is feared or ectopic pregnancy is suspected.

Much effort has been devoted to the issue of the safety of diagnostic ultrasound devices, with particular attention having been paid to the unavoidable heating of tissue resulting from the output of acoustic power. In day-to-day use, operators are trained to limit ultrasound output using the displayed 'safety indices'. This ensures that the temperature increase in tissues caused by ultrasound equipment should be below 1.5°C. This is a widely adopted safety threshold that is below what is found to be teratogenic over long periods in laboratory studies in mammals. Ultrasound also has mechanical effects independent of its ability to heat tissue and can produce acoustic cavitation and generate biologically active free radicals. For example, ultrasound may impart momentum to tissues and cause amniotic fluid to flow in the direction of its beam: a phenomenon termed 'acoustic streaming'. Cells that are bound together will tend to resist this force and thus, on exposure to ultrasound, experience some displacement from their position of equilibrium and lateral forces can produce shear within tissue. While the force is estimated to be very small, and very unlikely to cause harm, the effect on a developing conceptus remains uncertain.

This paper will address the issues of ultrasound in the embryonic period in the light of the most current evidence and guidance from national and international ultrasound safety committees and organisations. In particular, it will consider both medical and nonmedical use of the commonly used ultrasound modes in the embryonic stage of human development (up to 10 weeks of gestation).

2. Diagnostic ultrasound

2.1 Safety indices

Antenatal ultrasound examination results in the exposure of the developing embryo/fetus to thermal and mechanical stress, and the potential effects of these are represented by the thermal index (TI) and the mechanical index (MI) respectively.

The following empirically derived indices are designed to represent in real time 'reasonable worst case' conditions:

i) TIS is the relevant index when soft tissue is being examined,
ii) TIB when bone lies at the focus of the imaging beam, and
iii) TIC when bone lies close to the transducer face.

The TI is the ratio of the machine power output to the power required to raise the temperature of soft tissue (TIS) or bone (TIB) by 1°C. In the context of obstetric imaging, the heating effect is of importance.[1] The 'as low as reasonably achievable' (ALARA) principle should guide the operator and so it is recommended that the higher the TI, the shorter the ultrasound exposure should be. It is recommended that TIS is monitored during scanning in the first 10 weeks of pregnancy and TIB thereafter.[1]

The MI is a parameter whose value is proportional to the peak negative pressure in the imaging pulse and inversely proportional to ultrasound frequency. An MI of less than 1.0 indicates that effects arising from acoustic cavitation are very unlikely. The MI relates to imaging ultrasound modes and the prerequisites

for obtaining good images during pregnancy are usually favourable as there is no air in the fetal lungs, no gas in the intestines and the fetus is surrounded by amniotic fluid. Thus, there is usually no need to increase the energy output levels and the MI can be kept below 1.0.

2.2 Scanning mode

Acoustic output of ultrasound machines is dependent, in part, on the scanning mode and is thus under the control of the person performing the examination. As far as is known, the most commonly used 'B-mode' ultrasound is safe when applied using standard obstetric presets encountered in the clinical setting, given that generated intensities are low.[2] Most clinical obstetric and early pregnancy ultrasound examinations typically last 20 minutes and rarely exceed 40 minutes. Recent years have seen the increased use of pulsed Doppler ultrasound in early gestation: for example, for screening for chromosomal abnormalities by using Doppler assessment of the ductus venosus velocity waveform or tricuspid valve insufficiency at 11–13^{+6} weeks.[3] Colour Doppler and pulsed wave Doppler involve greater average intensity and power outputs than B-mode as expressed by higher displayed TIs; hence the potential risk to the fetus from heating is increased. It is recommended that parameters should be adjusted by the operator so that the TI is maintained less than or equal to 1.0 when performing Doppler ultrasound, particularly in gestations under 10 weeks, and to keep the exposure time limited.[4]

The use of 3D ultrasound does not necessarily imply higher ultrasound exposure than 2D ultrasound and indeed scanning time may be reduced. After acquiring a dataset for 3D ultrasound, the image is often analysed offline. However, 4D ultrasound (meaning real-time moving 3D) is associated with higher energy output than 2D as documented by changes in TI and MI.[5]

2.3 Ultrasound training and knowledge of safety indices by operators

More than 15 years ago, the US Food and Drug Administration (FDA) and then the International Electrotechnical Commission adopted the output display standard (ODS) and issued regulations requiring that the information on the TI and MI be provided by the manufacturers. The ODS information should facilitate the safe use of the ultrasound equipment; it is the responsibility of the operator to control the output energy and to follow the ALARA principle. A basic knowledge of the ODS and of the equipment settings is necessary to fulfil this.

Teaching of the safety principles is part of the training of sonographers and medical practitioners undertaking ultrasound diagnosis. Very detailed but relevant information is provided in the manuals of modern ultrasound machines; however, these are rarely read. Knowledge of the ODS among users is poor, as demonstrated in studies using anonymous questionnaires distributed to European[6] and American[7] experts in obstetric ultrasound. In the two studies, comprising 199 and 130 users respectively, only 30% of participants were familiar with the terms TI and MI. Moreover, only 20% knew how to adjust the energy output on their own ultrasound machine. The knowledge of the safety principles was worse in another survey among residents and fellows in obstetrics and gynaecology in the USA[8] and knowledge of sonographers (technicians) did not vary according to years of experience or credentialed status.[9]

The usefulness of the ODS indices has been criticised and the system not found to be particularly user-friendly. Nevertheless, at present, it is the only information on the output energy available to the user and therefore it is necessary to improve the level of knowledge among ultrasound operators by including the safety aspects of ultrasound in all educational activities concerning ultrasound diagnosis.

3. Ultrasound exposure of gametes and the embryo

3.1 Ultrasound prior to fertilisation

A study from 1982 suggested that ovulation may be premature in women who underwent ultrasound

examination of the ovaries (B-mode) in the late follicular phase.[10] Patients in induced ovulation cycles were followed and timing of follicle rupture after the onset of luteinising hormone (LH) surge or administration of human chorionic gonadotrophin was recorded. Rupture never occurred before the 37th hour in control patients (those who received no ultrasound in the follicular phase). However, premature ovulation was observed at 26–36 hours in about 50% of cases in the study group (ultrasound during the previous 3 days or in the 36 hours immediately following the ovulatory stimulus). These findings were concerning but the study has never been repeated. Other researchers have reported deleterious effects of ultrasound on the menstrual cycle, particularly a decrease in ovulation rates in mice[11] and reduced cumulative pregnancy rates in mice[12] and humans.[13] However, no effects on the quality of the pre-implantation embryo, including DNA and RNA synthesis, were shown,[14] nor on fertilisation rate and embryonic development following in vitro fertilisation (IVF) and embryo transfer.[15] The clinically available data on ultrasound exposure of oocytes during meiosis are confusing. Some researchers reported a deleterious effect on the fertility of patients undergoing artificial insemination, with a reduction in the cumulative rate of pregnancy.[16] A study of ultrasound exposure of meiotically active, preovulatory oocytes showed no differences between rats exposed to ultrasound after the LH surge and controls in terms of pregnancy rate, number of corpora lutea, implantations, pups, and mean pup and placental weights at autopsy on day 22 of pregnancy.[16] Others have claimed an increase in the pregnancy success rate following ultrasound monitoring of follicular growth,[17] although evidently this is not a direct effect of ultrasound but of improved timing. An attempt to clarify this phenomenon was described by Mahadevan and colleagues[18] who suggested that exposure of human oocytes to ultrasound (using a 3.5 MHz probe) during the different phases of meiosis does not significantly influence the developmental potential of the in vitro fertilised embryos. Unfortunately, the relevant exposure parameters were not described, as discussed earlier, except for ultrasound frequency. This makes the interpretation of the findings very difficult.

3.2 Ultrasound exposure of the embryo

The embryonic phase of development (up to 10^{+0} weeks of gestation) is a time of potential vulnerability to any theoretical risk of ultrasound for three reasons:

1. The embryo is very small, measuring only 6 mm in length at 6 weeks to 35 mm by 10 weeks.
2. Cell division is most rapid.
3. Fetal blood flow is limited, the fetal–placental circulation being established only after 11 weeks, making this a time of heightened vulnerability to thermal stress, as heat will be less likely to dissipate than later in gestation.[19]

The size of the embryo and the speed of cell division mean that if there are any harmful effects of ultrasound they are most likely to arise in the embryonic period as opposed to later in pregnancy. Furthermore, the ability of the embryo to dissipate heat is limited, as this would occur through a process of passive diffusion along a temperature gradient through adjacent structures: the coelomic and amniotic cavities. By 11 weeks[20] the fetal–placental circulation is established with fetal blood passing through the umbilical arteries towards the placenta and passing through the villi; these finger-like projections are bathed in maternal blood. In contrast to the embryonic period, after 11^{+0} weeks it is via the placenta that oxygen, waste products and nutrients are exchanged with maternal blood, with oxygenated blood returning to the fetus through the umbilical vein.

3.3 Modelling thermal and other effects and assumptions from in vitro and phantom studies: bioeffects

Thermal phantoms (studies in tissue-like models) that mimic particular tissue paths can be constructed and may be useful in evaluating any potential hazard arising from ultrasound-induced heating. For example, a phantom has been designed to mimic the neonatal head to estimate the temperature rise at several locations in the head during scanning through the fontanelle at typical clinical settings.[21] The

most important source of heating during diagnostic scanning arises from self-heating of the transducer itself since there is imperfect conversion of electrical to acoustic energy at the transducer front face. A phantom appropriate for assessing thermal effects during first trimester scanning has now been constructed. This allows simulation of the anatomy and exposure conditions both of the embryo (where there is a very small 'target' and no flow) and of the early fetus (after 11 weeks) where the 'target' is larger and there is limited flow. Initial results indicate that, in general, the TI underestimates the temperature measured at the probe surface and there is a relatively small heating effect at the focus point of the ultrasound transducer.[22]

3.4 Animal studies: embryonic period of pregnancy

Several researchers have described the influence of maternal ultrasound exposure in the period before implantation. Takeuchi et al.[23] exposed pregnant rats to a 2.5 MHz continuous wave ultrasound field on the second and third day of gestation at spatial average intensities of 150 mW/cm^2. They found no increase in fetal death or malformations after 20-minute exposures. Stolzenberg and associates[24] exposed pregnant mice to 2 MHz of continuous wave ultrasound at a spatial average intensity of 1 W/cm^2 in the first 3 days of gestation. A decreased pregnancy rate was noted after exposure for 200 seconds on day zero and 300 seconds on day three. Decreased birthweight was observed after exposure for 100 and 200 seconds on day zero and 200 and 400 seconds on day one. In another series of studies, ultrasound exposure led to damage of maternal tissue, which was reflected in increased mortality, decreased weight gain and paralysis of the pups.[25] McClain et al.[19] exposed rats to 10 mW/cm^2 continuous wave Doppler ultrasound for up to 2 hours at frequencies of 2.25 and 2.5 MHz. The fetuses were examined on day 20 and no consistent increase in mortality was observed, nor did the authors detect any other abnormalities. When additional experiments suggested the possibility of damage during organogenesis, intensity levels much higher than those generally employed in clinical obstetric practice were used.[26,27]

These animal experiments demonstrate that in vivo exposure to ultrasound at spatial average intensities below 1 W/cm^2 does not affect embryos at the early stage of gestation but limited data suggest that levels of ultrasound power significantly greater than 1 W/cm^2 may lead to changes in maternal tissue with harmful fetal effects.

3.5 Animal studies: later pregnancy

Although studies on mammalian, preferably upper-order primates, are more clinically relevant,[28] the interaction between ultrasound and biological tissues (bioeffects) has been mainly examined using simple in vitro models, and in vivo studies in non-mammalian species.[29] Fetal cerebral neocortical neuronal generation in the brain proliferative zones and their migration to their final destinations following an inside-to-outside sequence occurs in most mammals, including humans. In rodents,[30] ultrasound exposure for 30 minutes or longer during the fetal neuronal migration affected normal migration to the correct destination in the cerebral cortex. What remains unclear is whether a relatively small misplacement, in a relatively small number of cells that retain their origin cell class, is of any clinical significance. Furthermore, there are several major differences between the experimental set-up of Ang et al.[30] and clinical use of ultrasound in humans[31] in that the exposure duration was up to 7 hours in the aforementioned experiment and scans were performed over a period of several days. In addition, given their size, embryos received 'whole-brain' exposure to the beam; the entire brain was within the path of the beam. These types of exposure and duration are not performed in humans. Moreover, the brains of mice are much smaller than those in humans and develop in a few days.

In a further study performed in chicks,[32] pulsed Doppler ultrasound exposure in ovo for more than 4 minutes resulted in significant short- and medium-term memory impairment.[1] However, B-mode exposure did not affect memory or learning function in chicks. Pulsed Doppler ultrasound exposure for more than 15 seconds at the level of the ductus venosus has been associated with liver damage in rodents, with a positive correlation between increased apoptotic activity in the liver and ultrasound

exposure time.[33] At a molecular level, a rise in tissue temperature can result in disruption of mitochondrial metabolism and generate reactive oxygen species in embryonic tissue.[34]

3.6 Human epidemiological evidence

Most epidemiological ultrasound safety studies consider the use of diagnostic ultrasound at around 18 weeks of gestation or later and there are no epidemiological data specifically addressing exposure of human embryos to diagnostic ultrasound. In addition, most epidemiological evidence derives from B-mode scanners in commercial use 20–25 years ago, whereas the acoustic outputs from modern devices have increased 10- to 15-fold over the last decades.[35] If there are adverse effects of ultrasound during pregnancy which are dose- and time-dependent during embryonic or fetal development, then the available epidemiological data are so limited as to preclude any meaningful conclusions to be drawn.

Epidemiological studies of ultrasound during the second trimester have been unable to confirm an association between prenatal ultrasound and adverse perinatal outcomes including childhood malignancies, neurological development, dyslexia, speech development, school performance, intellectual performance and adult mental disease.

3.6.1 Handedness and prenatal ultrasound

The data available for association between prenatal ultrasound and left-handedness remain controversial, with no evidence shown in a Cochrane review but a more recent meta-analysis showing a significant association.[36–38] The current biological understanding of handedness is limited and partly contradicts the epidemiological evidence.[39] In addition, since the clinical significance of the handedness is unclear, a statistical association should not lead to the conclusion that ultrasound is causal or causes harm to the developing brain.

4. Ethical and social policy aspects of nonmedical ultrasound in early pregnancy

Making the heartbeat of the first trimester fetus audible to prospective parents by switching on the Doppler mode of the ultrasound machine has become commonplace in medical early pregnancy scanning, with the main purpose of reassuring them that their embryo/fetus is alive and well. In most cases, parents are not able to discern fetal heart motion of an embryo since there are only a few millimetres of crown–rump length on a 2D image, but can be convinced by the reassuring thumping sound of a Doppler turned to full volume. Doppler-registered heartbeat at 6/7 weeks is also an established method of quality control of IVF/intracytoplasmic sperm injection (ICSI); in Austria, reimbursement of IVF by insurance is dependent on documented Doppler-proven heartbeat in early pregnancy. In the USA, former congresswoman Michele Bachmann sponsored the 'Heartbeat Informed Consent Act' that would specifically require doctors to 'make the baby's heartbeat audible' in a mandatory ultrasound before a planned abortion.[40] If Doppler in the first trimester is a central part of proposed abortion legislation, it must be assumed that 'making the baby's heartbeat audible' is already well established in standard pregnancy care.

Until recently, 3D and 4D ultrasound examinations were carried out late in the second and early third trimester,[41] usually for the purpose of obtaining a 3D profile of the future family member's face for 'keepsake', a procedure called 'baby facing' in ultrasound jargon. Considering the low energy impact of B-mode ultrasound (the basis of both 2D and 3D), it is unlikely that fetuses subjected to a 3D ultrasound session outside a medically indicated context in the second half of pregnancy are at risk of being exposed to a critical impact of ultrasound energy. However, there is a move to perform such baby viewing sessions ever earlier in pregnancy.

Almost 15 years ago, the editors of an obstetric ultrasound journal voiced their concern about more and more papers submitted on the subject of Doppler ultrasound applied to ever smaller and younger embryos and even the yolk sac.[42] This caution has had little effect in the intervening years; the firm

belief that ultrasound, even Doppler ultrasound, is an inherently safe technology has become embedded among medical and nonmedical ultrasound operators. Some prenatal photo organisations offer services from vaginal ultrasound as early as at 6 weeks. With such ultrasound being performed early in pregnancy, the main safety issue is therefore the use of pulsed wave and colour Doppler.[43]

As discussed in section 3, the biophysical effects of Doppler ultrasound are dependent on exposure time. Whereas it is unlikely that the registering of 5–8 heartbeats in an embryo using Doppler ultrasound will have any effect, a longer period of 'listening to the heart' – of a minute or more – might theoretically lead to a measureable temperature rise in the insonated thoracic area of the embryo. Embryonic structures prior to 7–8 weeks are static and thus would be continuously exposed to the ultrasound beam. Unfortunately, there are few unequivocal warnings to keep Doppler ultrasound of the embryonic heart in early pregnancy as short as possible. The alternatives to pulsed wave Doppler are to use M-mode ultrasound, from which exactly the same information can be gleaned and which imparts much less energy, or simply to demonstrate the heart pulsations on 2D ultrasound.

A recent FDA consumer update has advised that ultrasound devices (including Doppler heartbeat monitors) should only be operated by trained healthcare professionals for medical reasons and it 'strongly discourages' their use solely for creation of 'keepsake' images or videos.[44]

5. Existing guidelines for first trimester ultrasound

National and international ultrasound societies regularly issue statements on the safe use of ultrasound in pregnancy. Prudency and following the ALARA principle are recommended when using ultrasound in pregnancy in general.[45] No specific recommendations have been issued regarding the use of imaging ultrasound in the first trimester (except for Doppler, see below). The use of ultrasound for the purpose of providing souvenir images or video recordings of the fetus is discouraged regardless of the gestational age.[46]

Recent reports on the increasing use of Doppler ultrasound in early pregnancy led to recommendations on its use during the first trimester because of its potentially higher energy output. The World Federation for Ultrasound in Medicine and Biology and the International Society of Ultrasound in Obstetrics and Gynecology issued a joint statement that has been adopted by several other international organisations.[45] It states that Doppler (i.e. pulsed wave [spectral], power and colour Doppler) should not be used routinely in all early pregnancies. The statement gives recommendations as to the levels of TI (less than or equal to 1.0) and time limits (5–10 minutes, maximum 60 minutes) when pulsed wave Doppler is used for a medical indication in early pregnancy. However, these guidelines refer most specifically to ultrasound carried out at 11–14 weeks (early fetal) rather than the embryonic period.

6. Conclusion

Ultrasound scanning in the embryonic period is particularly valuable in several important clinical scenarios, where the usefulness of ultrasound in guiding clinical management exceeds any theoretical, unquantified but almost certainly very small, risk to the embryo of the ultrasound itself. While there are presently no grounds for questioning the safety of diagnostic ultrasound in this context, ultrasound imaging is increasingly being used without obvious medical justification and the possibility of subtle long-term adverse effects should be kept in mind.[20] Patients who welcome early confirmation of pregnancy by ultrasound may not be aware that this period, up to 10^{+0} weeks of gestation, is the period during which prenatal development would be expected to be most susceptible to perturbation.

B-mode ultrasound is central to many clinical decisions and is highly unlikely to be associated with immediate or long-term harm to the embryo or fetus when applied using standard obstetric presets encountered in the normal clinical setting.[24] On a precautionary principle, however, we still recommend

that ultrasound, including B-mode, in the embryonic period is performed only where clinically indicated, or within the aegis of a research study whose design considers the potential for biological effects arising from the use of ultrasound. In particular, souvenir ('keepsake') ultrasound is not recommended since the benefits cannot outweigh any potential risks.

Colour and pulsed wave Doppler involve greater average intensity and power outputs than B-mode and higher TIs are easily achievable.[21] Animal studies suggest that prolonged, high intensity ultrasound exposure, particularly to pulsed wave Doppler, in the embryonic and early fetal periods can be associated with permanent harmful biological effects. Although the animal studies are not analogous to the clinical use of ultrasound in human pregnancy, the use of colour and pulsed wave Doppler for all but brief insonation is not recommended during the embryonic period of development. In animal studies the probe is typically focused at a specific point, whereas in clinical ultrasound usage the 'dwell time' (the time the ultrasound focus is fixed at a specific point) is typically very short – of the order of seconds. It is therefore unlikely in clinical usage that significant energy would be dissipated into a very small area of tissue.

When performing Doppler ultrasound before 11^{+0} weeks, the machine parameters should be adjusted by the operator so that the TI is maintained less than or equal to 1.0 and the exposure time should be limited to that necessary to obtain the clinically required information. Although no specific guidance pertains to ultrasound in the embryonic period, operators should be familiar with the relevant international guidance for ultrasound at 11–13^{+6} weeks.[45] These matters pertaining to ultrasound safety are now contained in the new RCOG ultrasound curriculum.

There is no information on repeat ultrasound exposure in the embryonic period, nor is there any epidemiological information on the cumulative effects of ultrasound in relation to future effects on human development. Therefore, such use should be avoided, for example, in conceptions after fertility treatment. This topic could be investigated if the duration of each ultrasound scan and the mode and average TI were recorded, allowing data to be collected for epidemiological research in this important, neglected area.[47]

7. Opinion

- Ultrasound in the embryonic/early fetal period is important and justified for many clinically indicated conditions.
- B-mode ultrasound used in standard obstetric presets for clinical reasons from conception to 10^{+0} weeks of gestation is safe and the benefits outweigh any theoretical risks.
- The routine use of colour and pulsed wave Doppler is associated with higher power output. Although there is no evidence that this is harmful, it is for this reason that all but brief insonation is not recommended during the embryonic period.
- Where the embryonic heart is insonated, low power output energy should be used initially with an increase in gain if necessary; M-mode is a lower energy alternative to pulsed wave Doppler.
- The TI and exposure time should be limited to those necessary to obtain the clinically required information.
- Repeated ultrasound exposure in the embryonic period should be avoided unless clinically indicated. This is based on a precautionary principle, not because there is evidence of any harm.
- Knowledge of the safety principles by professionals using ultrasound is important.

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Appendix I: Glossary

3D ultrasound	An image is produced using B-mode ultrasound scanning in several different planes to give a 'volume' rather than a 'slice' view
4D ultrasound	Real-time 3D ultrasound
ALARA	As low as reasonably achievable
Apoptosis	Programmed cell death
B-mode	The most commonly used ultrasound mode which allows cross-sectional visualisation of tissue, usually in black, white and grey
Colour Doppler	An ultrasound mode that allows visualisation of, for example, blood vessels
FDA	US Food and Drug Administration
IVF	In vitro fertilisation
MI	Mechanical index
ODS	Output display standard
Pulsed wave Doppler	A mode of Doppler in which a representation of, for example, an arterial or venous waveform is visualised. This allows measurement of the resistance or velocity in the vessel.
TI	Thermal index

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Conflicts of interest:
Mr Lees: ultrasound companies (GE, Siemens, Samsung, Toshiba) have sponsored study evenings, lent ultrasound equipment for research and/or contributed to research and training funds. He is Editor-in-Chief for *Fetal and Maternal Medicine Review*. Mr Lees is supported by the National Institute for Health Research (NIHR) Biomedical Research Centre based at Imperial College Healthcare NHS Trust and Imperial College London. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

Professor Abramowicz receives royalties as a contributing author to UpToDate.

Mr Axell is funded by an NIHR CSO Healthcare Scientist Doctoral Fellowship Award.

Professor Brezinka, Professor Salvesen, Dr ter Haar, Professor Marsal and Miss Smith: none declared.

The final version is the responsibility of the Scientific Advisory Committee of the RCOG.

The review process will commence in 2018, unless otherwise indicated.

