REVIEW ARTICLE

# Prenatal Heating Effects of Ultrasound: A Review

Nadzirah Mohamad Radzi, Farah Wahida Ahmad Zaiki

Department of Diagnostic Imaging and Radiotherapy, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia

**ABSTRACT**

The application of ultrasound technology has been widely accepted in clinical settings, particularly in Obstetrics and Gynaecology. This is in light of its ability to detect early foetal malformations apart from enabling foetal monitoring throughout gestation. While ultrasonography is an imaging method that is regularly used in Obstetrics, it is questionable as to whether it is safe for foetuses. The purpose of this paper was to review the evidence regarding the thermal effects of ultrasound exposure on foetal development, particularly. It is hoped that the importance of prudent usage of prenatal ultrasonography will be impressed on clinicians and the public in order to avoid the unnecessary usage of ultrasonography when it is not medically indicated. This is so that the welfare of pregnant women will be looked after, besides contributing to the better health of the next generation by ensuring that the benefits outweigh the known risks or potential harms.

**Keywords:** Ultrasound heating, Thermal effect, Prenatal, Biological effect

**Corresponding Author:**
Farah Wahida Ahmad Zaiki, PhD
Email: farahzaiki@iium.edu.my
Tel: +609-5705336 / +6019-3789263
Fax: +609-5716776

## INTRODUCTION

The principle behind ultrasound technology is the propagation of sound waves from a transducer to the tissues. During their passage through tissues, energy is reflected, refracted, transmitted, and finally received by the transducer as an echo, which results in an ultrasound image that is ready to be interpreted (1–3). Ultrasonography has become one of key investigations for diagnostic purposes in the medical field since its invention in the late 1950s (2). Following the development of the ultrasound machine, ultrasound has become one of commonly-used diagnostic procedures in clinical settings, particularly in Obstetrics and Gynaecology (4). This is due to the fact that it is not only free from radiation; it also provides real-time imaging for lower cost as compared to other imaging modalities (5).

In prenatal studies, early detection of pregnancy is very crucial in order to exclude any foetal malformations or uterine abnormalities (6). Studies have found that ultrasonography is useful in the assessment of gestational ages and diagnosis of many foetal malformations, especially during the early stages of pregnancy (3,4). Apart from Obstetrics, ultrasonography is also widely utilised in various specialties such as vascular studies, Cardiology, Ophthalmology, Gastroenterology, etc. due to its advantage of being an imaging modality that is free from ionising radiation (4).

Despite extensive use in clinical settings, ultrasound produces biological effects which can cause changes to the exposed tissues (4, 7–9). Nevertheless, ultrasound does not bring significant harm to humans since ultrasonography is always aligned with safety measures, especially in the clinical domain. The idea is to have a practice that gives benefits, which outweigh the risks. An understanding of the biological effects of ultrasonography by both practitioners and the public is important so that it can be utilised without adding potential harms.

### Evidence of biological consequences from thermal effects

As an ultrasound beam propagates through different tissues of the body, energy is absorbed and reflected (2,3). A sonographic image is formed as echoes when energy is reflected back to the receiver or also known as transducer. Different tissue compositions absorb different amounts of ultrasound energy, depending on the frequency of the ultrasound beam. The absorption rate rises with an increase in the ultrasound frequency (2). The interaction between the body tissues and the ultrasound beam leads to important mechanisms, which are thermal and non-thermal effects. Both mechanisms are interrelated since cavitations for non-thermal effects

can cause tissue heating, which in turn facilitates non-thermal effects by reducing the threshold for cavitations (2).

Thermal effects that arise from exposure to ultrasound can cause damage to biological tissues (7). These changes can be permanent if the level of exposure is high (7). In fact, any increase in temperature can cause thermal changes to the physiology of tissues (9). Consequently, tissue injuries such as premature cell death, destroyed molecular bonds, altered gene expressions, as well as abnormal cell development and maintenance can take place as a result of heat production (10).

Consistent with this idea, previous researches have revealed that there is an association between the destructive mechanisms of biological cells and the responses of tissues towards heat exposure. The viability of colon cancer cells was found to be diminished when exposed to ablative thermal doses (11). Thermal damage to the biological tissues seemed to be dependent on two factors, which are the amount of heat applied, as well as the duration of exposure (12). A recent study demonstrated that the functionality of breast cancer protein *BRCA2*, which is essential for deoxyribonucleic acid (DNA) repair degraded due to protein denaturation when the cells were subjected to a high dose of heat, which surpassed the minimal thermal dose (13). In an analysis of time-temperature relationship for cell killing, a 1.8-fold increase in heating time was required in order to reduce the temperature of 1°C so that the iso-survival levels can be achieved (14). These findings correspond with the fact that hyperthermia is closely related to the heating time and temperature (15).

In particular, temperature elevation is crucial for cell developmental processes which include cell proliferation, cell migration, and cell differentiation, each of which takes place at different stages of cell growth (16). These indicate that actively growing cells are vulnerable to thermal changes regardless of the state of the cells. Apart from that, other heat-sensitive tissues that are susceptible to an increase in core temperature include the surfaces of bones and the adjacent soft tissues (17–19). The heat sensitivity increases as the bone mineralisation increases (2). Osteocytes have demonstrated rapid hyperthermic cell injury as compared to other cells owing to its low thermal tolerance (20). This thermal effect is strongly associated with the difference in the composition of bone cells (21).

**Effects of ultrasound-induced temperature elevation on foetal growth**

Ultrasound-induced rise in temperature occurs when there are alterations in the physical characteristics of the beam during its transmission through body tissues where heat generated from energy absorption is greater than heat released (22). The characteristics comprise the frequency of ultrasound, focusing beam (which determines the beam width), as well as duration of exposure. Other properties that can influence heat production are acoustic impedance and tissue absorption. Acoustic impedance, or Z, is given by $Z = \rho c$, where $\rho$ is the tissue density and c the speed of sound (23). These ultrasound parameters and body tissue properties play very important roles in the heat production from ultrasonic energy.

Mammals embryos and foetuses are regarded as vulnerable living creatures, especially when they are exposed to agents that can disrupt their developmental processes (24). One of the factors that contribute to their susceptibility is the inability to maintain their core body temperature (16). Most of the time, they depend on the mother's thermoregulatory mechanisms until birth (25). As a matter of fact, the first trimester of pregnancy is the stage that is most vulnerable to heat exposure (26). Thus, any imbalance in foetal homeostasis can potentially affect the foetus, which in turn might result in developmental malformations.

Recently, *in vivo* study has shown that there is considerable decrease in level of rabbit foetal parathyroid hormone at all gestational ages, which indicates hormonal dysfunction due to ultrasound heating (27). In another recent study conducted to investigate the thermal effects on haematological analysis of young rabbit, it has been demonstrated that exposure to prenatal ultrasound results in significant variation on full blood counts constituents (i.e. haemoglobin concentrations, red blood cell, and platelet counts) of newborn rabbit (28,29).

Numerous studies have attempted to highlight the association between exposure to ultrasound and its biological effects to foetuses. As mentioned earlier, any increase in body temperature can result in irreversible effects to biological tissues. As with the rise in temperature following ultrasound absorption by means of interaction with biological tissues, foetal tissues are no exception (30–32). Apparently, tissue heating during exposure to high-intensity ultrasound has altered the biological development of foetuses, subsequently resulting in foetal growth retardation.

The heads and necks of animal foetuses seemed to develop malformations when they were exposed to ultrasound-induced high temperatures for short periods, as well as relatively low temperatures for long periods (33). Following direct exposure to ultrasound, significant temperature elevations were noticed at the animals' blood-brain interfaces which experienced heating through ultrasound absorption and thermal conduction (34). There is possibility of neuronal interruption during prenatal ultrasound exposure since the morphometric brain measurement showed remarkable reduction in volume and surface of brain in rabbit foetus (35).

It was reported that rabbit foetus showed the greatest decrease in foetal weight when exposed to the longest duration of ultrasound exposure (36,37), indicating that disturbances in the biological tissues of foetuses are highly associated with the ultrasound exposure time. Tissue damage aggravates as the duration of exposure increases (38). Meanwhile, another study demonstrated that there was a reduction in body weight of macaque (monkey) offsprings after repeated exposures throughout gestation period (39). The disruptions might in turn act as harmful agents that promote intra-uterine growth restriction (IUGR) in developing foetuses.

There was a 1.3-fold increase in the incidence of IUGR in a group of foetuses that were exposed to higher intensities of ultrasound, as compared to another group which received single-exposure ultrasound throughout gestation (40). As there was a declining trend for the skeletal component than that of soft tissue component, the restriction of foetal growth was more likely to be the effect of reduction in bone growth rather than other factors such as nutritional deficiency (i.e. placental insufficiency) (41). Foetal femur showed a significant increase in temperature as compared to soft tissue when exposed to ultrasound (42).

The increasing concerns regarding teratogenicity effects of heat on foetal cells and tissues have generated a large volume of published studies, which describes how the early embryonic development could possibly be affected by heat exposure. An experimental study emphasising on the effect of heat exposure to the gene expression and cleavage timing in the early embryonic development suggests that reduced oocyte developmental competency was the consequences of the alteration in the GDF9 mRNA expression pattern, which in turn gave rise to delayed embryo cleavage (43). The study indicated that GDF9 protein plays an important role as a potential quality biomarker (44) in the development of ovarian follicular to instantaneously stimulate cell proliferation and prevent premature cell differentiation during the early stage of follicle growth (45). Furthermore, it has been found that early event of embryo cleavage is highly significant as a biological indicator of embryo quality which improves the embryo potential, resulting in higher pregnancy rates (46,47).

Normal development of an embryo is highly dependent on the very early stage of pregnancy; regardless of the technique implemented for fertilisation of an ovum (48). It has conclusively been shown that there is a positive relationship between the quality of sperm and the rate of fertilisation (49). A published article showed that poor sperm quality has significantly lower results in fertilisation rate, which suggests that the sperm parameters in many cases may act as one of the important factors in embryonic development (50). Such finding in sperm reproductive potential is likely to be related to the integrity of the sperm itself. Factors hypothesised to influence the early embryogenesis have been explored in several studies, in which sperm DNA damage is also associated (51–55).

Many scholars hold the view that genetic damage of sperm can be associated with the increase in the core temperature (56,57). In a study which attempts to examine the efficiency of DNA fragmentation assay as a method to analyse the genetic integrity of the sperm, it has been reported that the exposure to heat is one of the agents that accelerates the kinetics of sperm DNA fragmentation (SDF), which may be responsible for genetic sperm damage (58). The incidence of SDF has been addressed in several studies and has been found to be the influencing factor that affects the dynamics of embryonic development (59,60). A study carried out on an animal model confirmed that normal array of active DNA demethylation is disrupted when the oocytes are fertilised with heat-stressed spermatozoa, thereby adversely affecting the subsequent embryo quality and development (61). These occurrences of heat stress-induced sperm defects were likely due to the decreased capability of sperm penetration, resulting in lower fertilisation rates (62,63).

**Other effects of ultrasound exposure**

Other than effects to the foetus, there are also several other known biological consequences of ultrasound exposure, including cellular, genetic, neural, ocular, and pulmonary effects. In a study on the effects of ultrasound at the cellular stage, adenocarcinoma cells were found to undergo apoptosis following exposure (64). Cancer cells also showed reduced proliferation rates before they were completely destroyed (65). Despite this, high-intensity focused ultrasound (HIFU) application on patient with hepatocellular cancer for therapeutic purpose give rise to local damage as well as systemic complications (66). The impact of ultrasound exposure has been recognised not only *in vivo*, i.e. at the cellular level, but also *in vitro* (i.e. connective tissue cells and the tissue regeneration process respectively) (67).

Genetically, an investigation on ultrasound-induced gene transfection showed that cells were successfully transfected using ultrasound of lower frequencies and longer pulse length (68). In the absence of cavitation bubbles which were otherwise created by ultrasound contrast agents, there was no extra advantage on the transfection ratio and survival fraction (69). Besides, cultured Chinese hamster ovary cells showed single-stranded DNA breaks when they were directly exposed to ultrasound (70). The event of DNA strand break is highly due to the cavitation produced by the ultrasound (71), which leads to stresses that contribute to damage mechanism (72).

Neurons are classified as vulnerable cells due to their sensitivity to the adverse effects of ultrasound exposure

(73). In order to block impulse conduction during pain control and anaesthesia, focused ultrasound can be applied since its thermal effects can decrease the amplitude of nerve action potentials (74). Apart from that, a study has found that low-intensity ultrasound exposure is useful in neural stem/progenitor cell induction, as well as neural cell therapy (75). On the other hand, HIFU has been found to be effective in soothing pain as it is able to block the nerve conduction in diabetes-induced *Sprague-Dawley* rat nerves safely (76).

Ultrasonography is also commonly used in Ophthalmology in light of its therapeutic value in the clinical field. Without damaging the apparent tissues, ultrasound can help enhance intracameral fibrinolysis, which is a significant process in controlling blood coagulation (77). Apart from that, the delivery of ocular therapeutic drugs was also found to be accelerated when the cornea, lens, and an area of posterior eye were exposed to ultrasound (78). However, in one case report which used intense focused ultrasound for skin tightening described that the technique seemed to cause epithelial disorganisation and structural changes in corneal stroma (79). Another study also reported that focused ultrasound exposure *in vivo* could potentially cause posterior coats displacement of the rabbit eye (80).

Apparently, lung haemorrhage secondary to ultrasound exposure has been widely studied in relation to the pulmonary effects of ultrasound (81–84). A comparative study, which investigated the potential biological effects of ultrasound to the lung reported that susceptibility to the lung damage was species-dependent. Among mice, rabbits, and pigs, mice were the most sensitive to ultrasound-induced damage while pigs were the least sensitive (85). A broader perspective of the study was carried out to investigate whether ultrasound-induced lung damage was age-dependent. The results of the study demonstrated that adult mice had the most significant suprathreshold haemorrhage as compared to neonatal and juvenile mice (86).

## CONCLUSION

The previous studies have provided important insights into the potential adverse effects of ultrasound when applied to either humans or animals at different stages of cell development. However, too little attention has been paid to the human studies with regards to the prenatal heating effects of ultrasound. This could has been due to the fact that there is a detrimental risk when ultrasound is applied to pregnant mothers without considering their safety. Thus, the awareness of the possible biological effects of ultrasound, along with the factors pertaining to the consequences, is hoped to facilitate safe practices in the clinical field since ultrasonography is widely used in human pregnancies.

## ACKNOWLEDGEMENTS

This work is supported by Research Initiative Grants (IIUM/504/G/14/3/1/1/RIGS 16-300-0464) from International Islamic University Malaysia, Malaysia.

## REFERENCES

1. Aldrich JE. Basic physics of ultrasound imaging. Crit Care Med. 2007;35(5), 131–137.
2. Ter Haar G. Ultrasonic imaging: Safety considerations. Interface Focus. 2011;1(4):686–697.
3. Hassani S. Principles of ultrasonography. J Natl Med Assoc. 1974;66( 3):205–207.
4. Abramowicz JS, Barnett SB, Duck FA, Edmonds PD, Hynynen KH, Ziskin MC. Fetal thermal effects of diagnostic ultrasound. J Ultrasound Med. 2008;27(4):541-559.
5. Edler I, Lindstrom K. The history of echocardiography. Ultrasound Med Biol. 2004;30(12):1565–1644.
6. Saari-Kemppainen A, Karjalainen O, Ylostalo P, Heinonen O. Ultrasound screening and perinatal mortality: Controlled trial of systematic one-stage screening in pregnancy. Lancet. 1990;336(8712):387–391.
7. Izadifar Z, Babyn P, Chapman D. Mechanical and biological effects of ultrasound: A review of present knowledge. Ultrasound Med Biol. 2017;43(6):1085–1104.
8. Sikov MR, Collins DH, Carr DB. Measurement of temperature rise in prenatal rats during exposure of the exteriorized uterus to ultrasound. IEEE Transactions on Sonics and Ultrasonics. 1984;31(5):497 - 503.
9. Shankar H, Pagel PS. Potential adverse ultrasound-related biological effects. Anesthesiology. 2011;115(5):1109–1124.
10. Purschke M, Laubach HJ, Rox Anderson R, Manstein D. Thermal injury causes DNA damage and lethality in unheated surrounding cells: Active thermal bystander effect. J Invest Dermatol. 2010;130(1):86–92.
11. Mouratidis PXE, Rivens I, Ter Haar G. A study of thermal dose-induced autophagy, apoptosis and necroptosis in colon cancer cells. Int J Hyperthermia. 2015;31(5):476–488.
12. Ahmed R. The relation between biological consequences and high temperature in mammals. Int J Zool Res. 2006;2(1):48–59.
13. Tempel N, Laffeber C , Odijk1 H, Cappellen WA, Rhoon GC , Franckena M et al. The effect of thermal dose on hyperthermia-mediated inhibition of DNA repair through homologous recombination. Oncotarget. 2017;8(27):44593–44604.
14. Borrelli MJ, Thompson LL, Cain CA, Dewey WC. Time-temperature analysis of cell killing of BHK

cells heated at temperatures in the range of 43.5°C to 57.0°C. Int J Radiat Oncol. 1990;19(2):389–399.
15. Field SB, Morris CC. The relationship between heating time and temperature: Its relevance to clinical hyperthermia. Radiother Oncol. 1983;1(2):179–186.
16. Ziskin MC, Morrissey J. Thermal thresholds for teratogenicity, reproduction, and development. Int J Hyperthermia. 2011;27(4):374–387.
17. Hynynen K, Deyoung D. Temperature elevation at muscle-bone interface during scanned, focused ultrasound hyperthermia. Int J Hyperthermia. 1988;4(3):267–279.
18. Vyskocil E, Pfaffenberger S, Kollmann C, Gleiss A, Nawratil G, Kastl S et al. Thermal effects of diagnostic ultrasound in an anthropomorphic skull model. Ultraschall Med. 2012;33(7):313–320.
19. Bosward KL, Barnett SB, Wood AKW, Edwards MJ, Kossoff G. Heating of guinea pig fetal brain during exposure to pulsed ultrasound. Ultrasound Med Biol. 1993;19(5):415–424.
20. Kurata K, Takamatsu H. Effect of hyperthermal treatment on the viability of bone-derived cells. J. Biomech Sci Eng. 2011;6(2):101-111.
21. Florencio-Silva R, da Silva Sasso GR, Sasso-Cerri E, Simxes MJ, Cerri PS, Cells B. Biology of bone tissue: Structure , function , and factors that influence bone cells. BioMed Res Int. 2015;2015.
22. O'Brien WD. Ultrasound-biophysics mechanisms. Prog Biophys Mol Biol. 2007;93(1–3):212–255.
23. Nyborg WL, Steele RB. Temperature elevation in a beam of ultrasound. Ultrasound Med Biol. 1983;9(6):611–620.
24. Gluckman PD, Hanson MA, Spencer HG, Bateson P. Environmental influences during development and their later consequences for health and disease: Implications for the interpretation of empirical studies. Proc Biol Sci. 2005;272(1564):671–677.
25. Asakura H. Fetal and neonatal thermoregulation. J Nippon Med Sch. 2004;71(6):360–370.
26. McMurray RG, Katz VL. Thermoregulation in pregnancy. Implications for exercise. Sports Med. 1990;10(3):146–158.
27. Md Dom S, Abdul Razak HR, Ahmad Zaiki FW, Saat NH, Abd Manan K, Che Isa IN et al. Ultrasound exposure during pregnancy affects rabbit foetal parathyroid hormone (PTH) level. Quant Imaging Med Surg. 2013;3(1):49–53.
28. Zaiki FW, Dom SM, Razak HR, Hassan HF. Prenatal ultrasound heating impacts on fluctuations in haematological analysis of oryctolagus cuniculus. Quant Imaging Med Surg. 2013;3(5):262–268.
29. Isa INC, Dom SM. Evaluation of the thermal effects of prenatal ultrasound on hematological analysis of young oryctolagus cuniculus. J Vet Med Sci. 2016;78(9):1399–1403.
30. Barnett SB. Intracranial temperature elevation from diagnostic ultrasound. Ultrasound Med Biol. 2001;27(7):883–888.
31. Barnett SB, Ter Haar GR, Ziskin MC, Nyborg WL, Maeda K, Bang J. Current status of research on biophysical effects of ultrasound. Ultrasound Med Biol. 1994;20(3):205–218.
32. Barnett SB, Rott HD, Ter Haar GR, Ziskin MC, Maeda K. The sensitivity of biological tissue to ultrasound. Ultrasound Med Biol. 1997;23(6):805–812.
33. Maeda K. Diagnostic ultrasound safety: 3. Heating effect on animal fetus in biology. J Health Med Inform. 2014;5(1):1.
34. Lalzad A, Wong F, Schneider M. Neonatal cranial ultrasound: Are current safety guidelines appropriate. Ultrasound Med Biol. 2017;43(3):553–560.
35. Zaiki FWA, Dom SM. Morphometric brain measurement of prenatal ultrasound-induced rabbit fetus using micro-computed tomography (micro-CT). J Teknol. 2016;78(6–7):13–17.
36. Zaiki FWA, Dom SM, Razak HRA, Hassan HF. Prenatal ultrasound heating influences on fetal weight assessment of oryctolagus cuniculus throughout pregnancy. Advance Science and Technology Letters. 2013;33:5–11.
37. Zaiki FWA, Dom SM. The effect of prenatal ultrasound heating throughout gestation on rabbit fetal weight. International Journal of Bio-Science and Bio-Technology. 2014;6(3):71–79.
38. Pellicer B, Herraiz S, T6boas E, Felipo V, Simon C, Pellicer A. Ultrasound bioeffects in rats: Quantification of cellular damage in the fetal liver after pulsed doppler imaging. Ultrasound Obstet Gynecol. 2011;37(6):643–648.
39. Tarantal AF, O'Brien WD, Hendrickx DAG. Evaluation of the bioeffects of prenatal ultrasound exposure in the cynomolgus macaque (macaca fascicularis): 111. developmental and hematologic studies. Teratology. 1993;47(2):159–170.
40. Newnham JP, Evans SF, Michael CA, Stanley FJ, Landau LI. Effects of frequent ultrasound during pregnancy: a randomised controlled trial. Lancet. 1993;342(8876):887–891.
41. Evans S, Newnham J, MacDonald W, Hall C. Characterisation of the possible effect on birthweight following frequent prenatal ultrasound examinations. Early Hum Dev. 1996;45(3):203–214.
42. Drewniak JL, Carnes KI, Dunn F. In vitro ultrasonic heating of fetal bone. J Acoust Soc Am. 1989;86(4):1254–1258.
43. Gendelman M, Aroyo A, Yavin S, Roth Z. Seasonal effects on gene expression, cleavage timing, and developmental competence of bovine preimplantation embryos. Reproduction. 2010;140(1):73–82.
44. Palomino J, Herrera G, Dettleff P, Martнnez V. Growth differentiation factor 9 and bone morphogenetic protein 15 expression in previtellogenic oocytes and during early embryonic

development of Yellow-tail Kingfish Seriola lalandi. Biol Res. 2014;47(1):60.
45. Spicer LJ, Aad PY, Allen DT, Mazerbourg S, Payne AH, Hsueh AJ. Growth differentiation factor 9 (GDF9) stimulates proliferation and inhibits steroidogenesis by bovine theca cells: Influence of follicle size on responses to GDF9. Biol Reprod. 2008;78(2):243–253.
46. Lundin K, Bergh C, Hardarson T. Early embryo cleavage is a strong indicator of embryo quality in human IVF. Hum Reprod. 2001;16(12):2652–2657.
47. Milewski R, Ajduk A. Time-lapse imaging of cleavage divisions in embryo quality assessment. Reproduction.2017;154(2)R37-R53.
48. Goswami G, Singh S, Devi MG. Successful fertilization and embryo development after spermatid injection: A hope for nonobstructive azoospermic patients. J Hum Reprod Sci. 2015;8(3):175-177.
49. Sadeghi MR, Hodjat M, Lakpour N, Arefi F, Amirjannati N, Modarresi T et al. Effects of sperm chromatin integrity on fertilization rate and embryo quality following intracytoplasmic sperm injection. Avicenna J Med Biotechnol. 2009;1(3):173-180.
50. Loutradi KE, Tarlatzis BC, Goulis DG, Zepiridis L, Pagou T, Chatziioannou E et al. The effects of sperm quality on embryo development after intracytoplasmic sperm injection. J Assist Reprod Genet. 2006;23(2):69-74.
51. Tesarik J, Greco E, Mendoza C. Late, but not early, paternal effect on human embryo development is related to sperm DNA fragmentation. Hum Reprod. 2004;19(3)611–615.
52. Ozmen B, Koutlaki N, Youssry M, Diedrich K, Al-Hasani S. DNA damage of human spermatozoa in assisted reproduction: Origins, diagnosis, impacts and safety. Reprod Biomed Online. 2007;14(3): 384–395.
53. Simon L, Castillo J, Oliva R, Lewis SE. Relationships between human sperm protamines, DNA damage and assisted reproduction outcomes. Reprod BioMed Online. 2011;23(6):724 – 734.
54. Carrell DT. Epigenetics of the male gamete. Fertil Steril. 2012;97(2):267-274.
55. Simon L, Murphy K, Shamsi MB, Liu L, Emery B, Aston KI et al. Paternal influence of sperm DNA integrity on early embryonic development. Hum Reprod. 2014;29(11):2402–2412.
56. Pйrez-Crespo M, Pintado B, Gutiйrrez-Adбn A. Scrotal heat stress effects on sperm viability, sperm DNA integrity, and the offspring sex ratio in mice. Mol. Reprod Dev. 2008;75(1):40-47
57. Rao M, Xia W, Yang J, Hu LX, Hu SF, Lei H et al. Transient scrotal hyperthermia affects human sperm DNA integrity, sperm apoptosis, and sperm protein expression. Andrology. 2016;4(6):1054-1063.
58. Santiso R, Tamayo M, Gosбlvez J, Johnston S, Marico A, Fernбndez C et al. DNA fragmentation dynamics allows the assessment of cryptic sperm damage in human: Evaluation of exposure to ionizing radiation, hyperthermia, acidic pH and nitric oxide. Mutat Res. 2012;734(1-2): 41–49.
59. Borini A, Tarozzi N, Bizzaro D, Bonu MA, Fava L, Flamigni C et al. Sperm DNA fragmentation: Paternal effect on early post-implantation embryo development in ART. Hum Reprod. 2006;21(11):2876–2881.
60. Wdowiak A, Bojar I. Relationship between pregnancy, embryo development, and sperm deoxyribonucleic acid fragmentation dynamics. Saudi J Biol Sci. 2016;23(5):598-606.
61. Rahman MB, Kamal MM, Rijsselaere T, Vandaele L, Shamsuddin M, Van Soom A. Altered chromatin condensation of heat-stressed spermatozoa perturbs the dynamics of DNA methylation reprogramming in the paternal genome after in vitro fertilisation in cattle. Reprod Fertil Dev. 2014;26(8):1107–1116.
62. Rahman MB, Vandaele L, Rijsselaere T, El-Deen MS, Maes D, Shamsuddin M et al. Bovine spermatozoa react to in vitro heat stress by activating the mitogen-activated protein kinase 14 signalling pathway. Reprod Fertil Dev. 2014;26(2):245–257.
63. Rahman MB, Schellander K, Luceco NL, Van Soom A. Heat stress responses in spermatozoa: Mechanisms and consequences for cattle fertility. Theriogenology. 2018;113:102-112.
64. Schuster A, Schwab T, Bischof M, Klotz M, Lemor R, Degel C et al. Cell specific ultrasound effects are dose and frequency dependent. Ann Anat. 2013;195(1):57–67.
65. Watanabe A, Nishimura H, Kawashima N, Takeuchi S. Consideration on suppression of cancer cell proliferation by ultrasound exposure using sonochemical and biological measurements. J Phys Conf Ser. 2004;1(1):210–215.
66. Li JJ, Gu MF, Luo GY, Liu LZ, Zhang R, Xu GL. Complications of high intensity focused ultrasound for patients with hepatocellular carcinoma. Technol Cancer Res Treat. 2009;8(3):217–224.
67. Harle J, Salih V, Mayia F, Knowles JC, Olsen I. Effects of ultrasound on the growth and function of bone and periodontal ligament cells in vitro. Ultrasound Med Biol. 2001;27(4):579–586.
68. Cochran M, Wheatley MA. In vitro gene delivery with ultrasound-triggered polymer microbubbles. Ultrasound Med Biol. 2013;39(6):1102–1119.
69. Kodama T, Tomita Y, Koshiyama K, Blomley MJ. Transfection effect of microbubbles on cells in superposed ultrasound waves and behavior of cavitation bubble. Ultrasound Med Biol. 2006;32(6):905–914.
70. Miller DL, Thomas RM, Frazier ME. Single strand breaks in CHO cell DNA induced by ultrasonic cavitation in vitro. Ultrasound Med Biol. 1991;17(4):401–406.
71. Yoshida K, Ogawa N, Kagawa Y, Tabata H,

Watanabe Y, Kenmotsu T et al. Effect of low-frequency ultrasound on double-strand breaks in giant DNA molecules. Appl Phys Lett. 2013;103(6).
72. Warnez M, Vlaisavljevich E, Xu Z, Johnsen E. Damage mechanisms for ultrasound-induced cavitation in tissue. AIP Conf Proc. 2017;1821(1).
73. Ziskin MC, Barnett SB. Ultrasound and the developing central nervous system. Ultrasound Med Biol. 2001;27(7):875–876.
74. Colucci V, Strichartz G, Jolesz F, Vykhodtseva N, Hynynen K. Focused ultrasound effects on nerve action potential in vitro. Ultrasound Med Biol. 2009;35(10):1737–1747.
75. Lee IC, Lo TL, Young TH, Li YC, Chen NG, Chen CH et al. Differentiation of neural stem/progenitor cells using low-intensity ultrasound. Ultrasound Med Biol. 2014;40(9):2195–2206.
76. Lee YF, Lin CC, Cheng JS, and Chen GS. High-intensity focused ultrasound attenuates neural responses of sciatic nerves isolated from normal or neuropathic rats. Ultrasound Med Biol. 2015;41(1):132–142.
77. Yamashita T, Ohtsuka H, Arimura N, Sonoda S, Kato C, Ushimaru K et al. Sonothrombolysis for intraocular fibrin formation in an animal model. Ultrasound Med Biol. 2009;35(11):1845–1853.
78. Nabili M, Geist C, Zderic V. Thermal safety of ultrasound-enhanced ocular drug delivery: A modeling study. Med Phys. 2015;42(10):5604–5615.
79. Kyung Jung S, Yang SW, Soo Kim M, Chul Kim E. Corneal stromal damage through the eyelid after tightening using intense focused ultrasound. Can J Ophthalmol. 2015;50(4):54–57.
80. Silverman RH, Urs R, Lloyd HO. Effect of ultrasound radiation force on the choroid. Invest Opthalmol Vis Sci. 2013;54(1):103.
81. Tarantal AF, Canfield DR. Ultrasound-induced lung hemorrhage in the monkey. Ultrasound Med Biol. 1994;20(1):65–72.
82. Dalecki D, Child SZ, Raeman CH, Penney DP, Carstensen EL. Lung hemorrhage from exposure to ultrasound. J Acoust Soc Am. 1996;99(4):2466–2500.
83. Kramer JM, Waldrop TG, Frizzell LA, Zachary JF, O'Brien WD. Cardiopulmonary function in rats with lung hemorrhage induced by pulsed ultrasound exposure. J Ultrasound Med. 2001;20(11):1197–1206.
84. O'Brien WD, Kramer JM, Waldrop TG, Frizzell LA, Miller RJ, Blue JP et al. Ultrasound-induced lung hemorrhage: Role of acoustic boundary conditions at the pleural surface. J Acoust Soc Am. 2002;111(2):1102–1109.
85. O'Brien WD, Zachary JF. Rabbit and pig lung damage comparison from exposure to continuous wave 30-kHz ultrasound. Ultrasound Med Biol. 1996;22(3):345–353.
86. Dalecki D, Child SZ, Raeman CH, Cox C, Penney DP, Carstensen EL. Age dependence of ultrasonically induced lung hemorrhage in mice. Ultrasound Med Biol. 1997;23(5):767–776.