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43

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Title : Physical, Morphological and Biochemical Effects of Prenatal Ultrasound Exposure on Rabbit Fetus

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Ultrasound is extensively used in various clinical specialties throughout the developed world since it was perceived to lack in bioeffects. Somehow, through the advances in the capability of ultrasound equipment that triggered greater image processing power, the revision of potential bioeffects is needed to be corroborated. Hence, this study is designed to determine the physical, morphological and biochemical effects on fetus development. This *in-vivo* experimental study involved twelve pregnant rabbits, exposed to ultrasound exposure durations of 30, 60 and 90 minutes in the middle of the stipulated gestational stages (1st stage; gestational day (GD) 6-7, 2nd stage; GD 17-18, 3rd stage; GD 28-29). Acoustic output parameters were kept constant (frequency = 7.09 MHz, intensity (ISPTA) = 49.4 W/cm2, power = 56.0 W, thermal index (TI) = 0.2 and mechanical index (MI) = 1.0). The rabbits were euthanized and data were analyzed using SPSS 21. Total 136 fetuses (1st stage, n = 34 (25%); 2nd stage, n = 28 (21%); 3rd stage, n = 74 (54%)) were analyzed for physical, structural and ultrastructural morphological, biochemical and haematological analyses. Physical analysis found to have significant differences in fetal weight between exposed and control groups at all stages ($P < 0.001$, $P = 0.01$, $P < 0.001$, respectively) with a negative correlation between different exposure durations and fetal weight at 1st and 3rd stages ($P = 0.02$, $r = -0.40$, $P = 0.04$, $r = -0.23$, respectively). There were also significant differences in brain volume and surface at all stages of gestation ($P < 0.05$). Ultrastructural morphological analysis showed statistically significant in apoptotic neurons and glial cells (AC) count at 1st and 2nd stages ($P < 0.05$) with positive good and fair correlation ($P < 0.001$, $r = 0.53$; $P < 0.001$, $r = 0.47$, respectively). While, biochemical investigation reported that significant differences in AC at all stages ($P < 0.001$) with mean AC depicted lowest in control groups, in congruent to structural morphological analysis where neuronal cell death (NCD) count were significant at all stages ($P < 0.001$) and mean NCD least in control groups. Haematological analysis reported that significant differences in red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin (Hb) concentration, platelet (PLT) count and lymphocytes (LYM) count ($P < 0.001$, $P = 0.04$, $P < 0.001$, $P < 0.001$, $P = 0.01$ respectively). There were negative correlation of exposure with RBC, Hb and PLT ($P = 0.01$, $r = -0.38$; $P = 0.04$, $r = -0.27$; $P = 0.02$, $r = -0.32$, respectively), yet a positive correlation with LYM ($P = 0.03$, $r = 0.30$). Results suggested that ultrasound might interfere with the sensitive stages of developing fetus by both thermal and mechanical effects that probably induced hyperthermia and heat stress to the fetus *in-utero* hence, plausibly interrupted the biological cells. Further empirical research is needed to endeavor for being sufficient to draw a conclusive safety statement of prenatal ultrasound and contributing to the current body of knowledge.