# DEVELOPMENTAL AND TERATOGENIC EFFECTS OF ULTRASOUND

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## INTRODUCTION

In obstetrics, the applications of ultrasound cover a broad spectrum. During pregnancy ultrasound is used alone to visualize and monitor fetal development, and it is also used as an adjunct with other procedures, such as amniocentesis, to determine the well-being of the developing fetus. Ultrasound is currently used to assist in chorionic villi sampling, a relatively recent technique designed to obtain genetic information on the fetus during earlier stages of gestation than is possible with amniocentesis. In general, the clinical applications of diagnostic ultrasonography are diverse and its potential use can be justified during virtually all stages of pregnancy.

There are two types of ultrasound used in fetal applications: continuous wave doppler ultrasound (CW) for fetal heart rate monitoring and pulse-echo for visualization. In addition, pulse doppler has found recent use for measuring fetal blood flow. There is an increased concern regarding potential biological consequences that may result from the levels at which these devices operate. Data from a recent survey indicates that some new devices operate at higher exposure levels than those known to have been used in the past (Stewart and Harris, in press). The level of concern is further heightened by the use of pulsed doppler devices to measure blood flow in the fetal heart, placenta, and umbilical vessels. The temporal average intensities of some of these devices approach therapeutic levels.

Possible risks associated with ultrasound exposure on the developing embryo or fetus is an important issue. Embryonic and fetal development is a dynamic process involving rapid growth, functional changes, and complex interaction of various cell populations and developing organ systems. It is essential that development follows its established spatial and temporal program to ensure normal intrauterine maturation. Soon after fertilization, cleavage segregates various portions of the zygote's cytoplasm into specific cell populations. Subsequent development is an intricate system of genetic expression combined with cellular interaction. At specific times during development, cells produce specific gene products. Through cell-to-cell interaction, chemical signals and messenger molecules are

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transmitted and progressively the cells' fate is determined. This process of determining cellular characteristics through genetic expression is referred to as "cellular differentiation." At gastrulation there is a cellular rearrangement which begins giving shape and form to the embryo. Through the mechanisms of selective adhesion and cell migration, the embryonic cells move in very distinct patterns and contact and interact with other specific cell populations. The patterns of gastrulation vary between species, but the end result is the same; the axis of the embryo is determined, the primary germ layers are formed and the process of neurulation is initiated. Subsequent interactions between the germ layers "induce" the formation of embryonic organs and organs systems (organogenesis). The final embryonic pattern, the orderly arrangement of all the organ systems, is obtained by the complex interrelationship of morphogenetic and differentiative events which occur throughout the rest of gestation.

The susceptibility of an embryo or fetus to a physical or chemical agent is strongly determined by its stage of development. From the brief description above, it can be seen that the development from a zygote to a full-term fetus is the dynamic interaction of many systems working in unison. Any agent or insult that disrupts the normal developmental program could potentially jeopardize normal intrauterine development. Because the very young embryo consists of a limited number of cells, the introduction of a physical or chemical agent at this stage of gestation could have a major impact on development. During latter stages of development, if an agent is introduced which has a pronounced effect on cell migration or cellular interactions, it could potentially disrupt the induction of various organ systems and/or limb formation. Recently, German (1984) introduced the "Embryonic Stress Hypothesis of Teratogenesis." German postulates that if an agent alters the production of specific messenger molecules essential for normal development, this functional alteration could have an effect on subsequent cellular interactions. If the resultant developmental error was not lethal to the embryo, the effect could manifest itself as an anatomical malformation. This hypothesis provides a generalized concept by which a single agent can produce a spectrum of developmental alterations; the nature and severity of the defect being determined in part, by the period of gestation when the insult was received.

Rugh's work with x-rays (1968) supports this hypothesis. In the mouse, specific congenital malformations were produced depending on the period of gestation when the insult was given. Here again, a spectrum of developmental alterations was produced by a single agent. In general, x-ray exposures during very early stages of development tended to be embryolethal, whereas exposures latter in development resulted in structural malformations. In the case of maternal hyperthermia, some fetal effects are delayed and are expressed only after a latency period. Therefore, it is not only important to know which endpoint to observe, but also <u>when</u> to look for an alteration in a particular endpoint.

Since fetal ultrasonography is used throughout gestation, there is the risk associated with exposure during critical developmental periods. There is also the possibility that the fetus may receive multiple exposures from sonograms administered over the course of a pregnancy. The mammalian conceptus has little regulatory control over its environment. Factors, such as immature biological barriers, poor immunosurveillance, and relative lack of metabolic detoxification and

thermoregulatory mechanisms, all contribute to the general increase in susceptibility of the fetus to physical and chemical agents (Brix, 1982).

A physical or chemical agent which has an adverse effect on development can act at one or more of three potential sites. The agent can directly affect the fetus or it can elicit its effect on the maternal or the placental system, either of which could indirectly disrupt normal fetal development (Beck, 1981). The possibility of an indirect effect on the fetus is accentuated because normal responses of the maternal system can be altered by other physiologic stresses associated with pregnancy. Potential direct fetal effects from ultrasound are of special concern because the fetus often receives whole body exposure during diagnostic imaging.

In reviewing the work of different investigators careful consideration must be given to various experimental parameters outlined in their studies. Particular attention should be given to actual exposure conditions and dosimetry. Early ultrasound studies provided few details about exposure conditions making it difficult to directly compare the results between different laboratories.

Most reviews on the developmental effects of ultrasound limit themselves to the developmental events between fertilization and birth, although, development does not end at birth. Many fetal systems, such as the neural, visual, endocrine, behavioral and reproductive systems, are immature at birth and development continues well into adolescence. This review considers "development" as a continuum and will be expanded to cover ultrasonic effects on the germ cells, as well as post-natal development. The reported studies are presented in sequence according to the stage of development during which exposure occurred. Because, in a single study, some investigators have exposed subjects at different stages of gestation, the same study may be discussed several times. Emphasis will be placed on experimental design and exposure conditions which may help to explain observed effects. Intensities discussed in the following studies will be stated as spatial average, temporal average (SATA) intensities unless otherwise indicated. Intensities will be stated as specifically as possible from the information supplied by the investigators. Thus, the spatial and temporal intensity characteristics will sometimes be stated, while only "average" or "peak" intensities are given in other instances.

## NONMAMMALIAN SYSTEMS

A wide variety of nonmammalian systems including fish, amphibians, insects, and chickens have been used to investigate the developmental effects of ultrasound. Some of these systems pass through developmental stages that are different or absent in mammalian systems. Variation in yolk content among eggs of different species results in varying cleavage patterns. Polar cell formation and a syncytial developmental stage are unique to Drosophila eggs. Also, placentation and the interaction of maternal compensation are absent in nonmammalian systems. These systems do however, exhibit some basic developmental stages similar to mammals. In general, a great deal of information has been obtained on these systems and their early developmental programs are now well characterized. Therefore, not only do these systems provide useful information on the mechanisms by which ultrasound interacts with organized tissue, but they can also be utilized to obtain information on how ultrasound might potentially affect developmental processes.

In an early study, Selman and Jurand (1964) demonstrated that ultrasound caused intracellular damage in newts. Exposure of Triturus alpestric tadpoles to 1 MHz, CW ultrasound at intensities of 8 - 15 $W/cm^2$ for 5 minutes resulted in destruction of the epidermis and pycnosis of muscle and neural tube cells. Subsequent electron microscopy revealed disruption of nearly all the endoplasmic reticulum in the notochord cells. This effect appeared reversible and within 24 hours more than half the total endoplasmic reticulum had resumed normal configuration.

Andrew (1964) exposed perch spawn to 1.5 $mW/cm^2$ pulsed ultrasound, 48 hours postfertilization for 3 or 5 hours. There were no gross structural defects observed in the eyes or spinal cords of the perch after hatching. Frog eggs which were similarly exposed for 24 hours sometime during their first 11 days of development, showed differences in the rate of development when compared with controls. Unfortunately, information concerning intensity data and pulse parameters were not provided.

Sarvazyan et al. (1982) extensively investigated the effects of ultrasound on amphibian embryonic tissue. Embryos of Rana esculenta, Rana temporaria, and Xenopus laevis at various stages of development and ectomesodermal explants (EME) were exposed to CW and low-intensity pulsed ultrasound. An EME is a small piece of skin ectoderm with its underlying mesoderm, removed from the lateral part of an embryo just after neurulation. With the appropriate conditions, through a process resembling gastrulation, the explant rolls into a sphere resulting in a mesodermal cortex covered by epidermal ectoderm. Both the embryos and the EME's were exposed to 0.88 MHz ultrasound at SATA intensities ranging from 0.025 - 0.1 $W/cm^2$. Pulse repetition frequencies were varied between 10 - 1000 Hz with a constant duty factor of 0.5 and exposure times were between 2 and 30 minutes. The results showed that the sensitivity of the embryo, as well as localization of damage, were dependent on the embryos' stage of development. In general, sensitivity to ultrasound decreased with increased gestational age. In very early stages of development, ultrasonic exposure disrupted the distribution of yolk and pigment. Ultrasonic exposure at the late blastula-early gastrula stage resulted in complete cellular destruction with cells in the animal hemisphere exhibiting the greatest sensitivity. Regions of destruction at this stage were wide and often resulted in death of the embryo. Lesions following exposure at the neurula stage were generally less embryolethal and more localized, usually appearing as a small area of cell necrosis, mainly on the back of the embryo. While there was no observable effect on EME's exposed to CW ultrasound at intensities less than 100 $mW/cm^2$, exposure to pulsed ultrasound below this intensity produced extensive damage. The investigators concluded that pulse repetition frequency was the exposure characteristic that determined the extent of damage to the embryos and EME's. The influence of pulse repetition frequency on EME's also showed species variability with R. temporaria exhibiting maximal sensitivity at lower pulse repetition frequencies (maximum effect at 10 - 20 Hz) than X. laevis (maximum effect at 110 - 130 Hz). The authors concluded that even though results obtained from amphibian embryonic tissue cannot be directly translated to the clinical situation, serious consideration should be given to all exposure parameters (not just intensity) relevant to diagnostic applications of ultrasound.

The fruit fly, Drosophila melanogaster, has been used extensively to investigate the biological consequences of ultrasound. Early studies performed by Fritz-Niggli and Boni (1950) exposed Drosophila during various developmental stages to 0.8 MHz, CW ultrasound at

intensities of 0.7 - 4.0 W/cm$^2$ for 5 seconds to 25 minutes. They found the eggs, larvae and early pupae were all sensitive stages and as metamorphosis progressed, resistance to ultrasound increased rapidly. They also observed that a large number of larvae and prepupae which survived irradiation and continued to develop, later died as late pupae just before emergence. This effect has been termed "delayed lethality" by other investigators.

Selman and Counce also used Drosophila to investigate the effects of ultrasonic treatment on embryonic development (Selman and Counce, 1953; Counce and Selman, 1955). Embryos at various stages of development were exposed for 30 seconds to 1 MHz, CW ultrasound at intensities of 0.3 - 0.5 W/cm$^2$. Direct effects on the embryos were confirmed by microscopic observations made during treatment and subsequent developmental effects were determined by preparations fixed at intervals up to 20 hours posttreatment. Exposure at 0.5 W/cm$^2$ resulted in a slow rotary movement of the central region of cleavage stage eggs. In some cases, death resulted from treatment at preblastoderm stages; in others, there was complete recovery and normal development. The authors proposed that these "vortex-like" disturbances produced by ultrasonic exposure could displace cellular contents resulting in abnormal development. Ultrasonic exposure of late cleavage eggs at intensities of 0.5 - 1.2 W/cm$^2$ produced structural malformations and induced polyploidy. Concurrent microscopic observation of embryos exposed at the syncytial blastoderm stage to intensities of 0.3 - 0.5 W/cm$^2$ showed most of the cytoplasmic disruption localized in the posterior region with the pole cells eliciting the greatest sensitivity. Delayed lethality similar to that observed by Fritz-Niggli and Boni (1950) was reported following ultrasonic treatment.

Child et al. (1980a) suggested that the effect seen by Fritz-Niggli and Boni could be explained by standing wave formation produced by their exposure system. They examined the killing of eggs exposed to 1 MHz traveling and standing wave ultrasound at intensities up to 5 W/cm$^2$ (SPTA). Although they were unable to confirm the delayed lethality effect others reported, they did report the killing of one third of the eggs exposed to traveling waves at 3 W/cm$^2$ and approximately 1 W/cm$^2$ standing wave ultrasound.

Pizzarello et al. (1978) exposed larval and pupal stages of Drosophila to 2.25 MHz, pulsed ultrasound for 2.5 minutes at a temporal average acoustic power of 1.5 mW using a commercial diagnostic unit. Lethality was high in late 3rd instar larvae and pupae under these conditions. Exposure of growing larvae and prepupae resulted in a growth inhibition which expressed itself in the formation of "miniature" adults.

This study suffers from a number of design and reporting flaws. The exposure conditions used in this study certainly guaranteed reflection, therefore, the larvae and pupae were probably exposed to intensities greater than originally planned. Also, meaningful intensity data is missing, "miniature" is not quantitatively defined and there is no statistical analysis of the data. Because of these deficiencies, the validity of the reported effects is questionable.

Due to the potential impact of reporting a biological effect at such a low temporal average intensity, Child et al. (1980b) attempted to repeat Pizzarello's study and an effort was made to replicate the exposure techniques as closely as possible. Child et al. found no effect on survival at 1.5 mW and were unable to confirm the production of miniature flies reported in the Pizzarello et al. study. Although,

when the temporal peak intensity (and the power) was increased tenfold, they observed delayed lethality in one fourth of the exposed larvae.

Pay et al. (1978) demonstrated delayed lethality in pupae exposed to 1 MHz, CW ultrasound. In another study, Pay et al. (1982) showed that although longevity was unaffected, egg laying capacity and egg survivability decreased in female flies exposed as pupae and tested throughout their lifetime. In these studies pupae were exposed to intensities ranging from 0.2 - 4.0 $W/cm^2$ for 10 minutes. Pay's findings are confirmed by the research of Carstensen and Child (1981) who reported that death occurred in 70% of the Drosophila eggs exposed to 1 MHz, CW ultrasound at an intensity of 5 $W/cm^2$ for 30 seconds. To determine if the observed lethality was due to a thermal mechanism, egg sensitivity was tested against heat alone. The findings suggested that the observed effects could not be attributed to heat production and that some other biophysical mechanism of ultrasound must be involved. Subsequent work by Child and Carstensen (1982) exposed Drosophila eggs for 2.5 minutes to 2.25 MHz, pulsed ultrasound at various stages of development. Intensities varied between 20 - 40 $mW/cm^2$ (SATA) or 40 - 100 $W/cm^2$ (SATP), with a pulse repetition frequency of 500 Hz and a pulse duration of 1 μsec. The results from this experiment demonstrated that eggs were very sensitive to the effects of high peak pulsed ultrasound just prior to hatching. This coincides with the stage of development involving tracheal formation and its filling with air. It has been postulated that the interaction of ultrasound with small stabilized gas bodies within the respiratory system may be responsible for the killing observed in Drosophila.

This group has also investigated the killing of Drosophila larvae by microsecond pulses of ultrasound, and the data indicated no obvious relationship between carrier frequency and delayed lethality (Berg et al., 1983). On the other hand, they demonstrated that larval killing and delayed lethality are strongly dependent upon peak intensity (Child et al., 1981). Decreases in survival rates were observed at SATP intensities of 13 $W/cm^2$ (6 $mW/cm^2$ SATA) or greater using 1 μsec bursts and pulse repetition frequencies ranging from 50 - 5000 Hz. Carstensen, Child and coworkers have concluded that temporal average intensity is a poor indicator for these biological effects (Child et al., 1981), and suggests that temporal peak intensities may be the best predictor of nonthermal biological effects (Carstensen, 1983).

Although cavitation produced by the interaction of ultrasound with small gas bodies within the tracheal system may be the postulated mechanism used to explain the effects observed in Child's work, Pay et al. (1985) have recently shown that eggs, 2 hours postfertilization, exposed to pulsed ultrasound (1 MHz, 90 $W/cm^2$ SPPA, 6.5 μsec pulse duration) at SATA intensities greater than 35 $mW/cm^2$, fail to develop. They reported decreased survival at SATA intensities as low as 3 $mW/cm^2$ when eggs were exposed for 10 minutes. The mechanism of ultrasonic action responsible for this high mortality is still unknown. However, it cannot be attributed to ultrasound interacting with air in the tracheal system because ultrasound exposure precedes tracheal development by approximately 11 hours.

Yolk quantity varies greatly between the eggs of birds and mammals, yet their patterns of early embryonic development are quite similar. Both the mammalian and bird blastodisc consist of two layers; the epiblast and the hypoblast. Gastrulation of mammals including humans, resembles that of birds with the formation of a primitive

streak. Cells from the primitive streak subsequently migrate between the hypoblast and the epiblast, giving rise to a trilaminar embryo. Chick embryos have been used extensively in both developmental and teratological studies, because they are relatively inexpensive and easy to maintain. Not only are the developmental stages well documented, but the acoustical properties of both the egg white and yolk have been characterized (Javanaud et al., 1984).

It appears that the chick embryo would be an ideal system for investigating the developmental effects of ultrasound. However, because the embryo is surrounded by a hard shell, investigators have the problem of determining the most appropriate method for delivering ultrasound. Early use of ultrasound in the food industry revealed that ultrasound could be used to evaluate the quality of eggs and to clean the outer shells in preparation for market, but little information was available on what effect ultrasound might have on the eggs' contents. Nikolov (1970) demonstrated that these commercial sonicators could affect the physiochemical properties of eggs. The outer shells appeared quite normal after a 5 - 10 minute sonic exposure but the viscosity and optical density of the inner contents were altered.

Studies centered on development, using lower intensities, found no effect on the embryo if eggs were exposed intact (Vazquex, 1963). The outer shell and air space inside the egg cause significant attenuation and reflection of the ultrasound beam. The outer egg shell itself is impermeable to ultrasound at diagnostic frequencies and it has been estimated that only 1/10,000 of the total acoustic power applied to an intact egg ever reaches the embryo when 2 MHz ultrasound is used (Sofia and Lele, 1975).

There are different techniques used to deliver ultrasound energy directly to the embryo; each has benefits and drawbacks. First, a small window can be cut into the outer shell to expose the embryo. Although this technique minimizes the influence of external factors, it is difficult to define the exposure conditions. In an alternate method the shell is completely removed prior to ultrasound exposure. Eggs can be opened at various stages of incubation and the contents can then be carefully transferred and maintained in a vessel designed for ultrasonic exposure. Although this method may allow more control over the exposure conditions, it removes the embryo from its natural environment and subjects it to various physical factors, which alone may have an adverse effect on development.

Taylor and Dyson (1972) exposed excised chick embryos with 1 MHz, pulsed ultrasound. In this procedure, after the shell was removed, the embryo and its extraembryonic membranes were lifted from the yolk and transferred to an exposure receptacle. The internal contents were discarded and only the embryo and its associated membranes were placed in the ultrasound field. Exposure to "peak intensities" of 40 $W/cm^2$ (pulsed 20 usec on and 180 usec off) during the head process stage (18-20 hours incubation) resulted in an increased incidence of fetal abnormalities. This increase in abnormalities was not observed when the peak intensity was reduced to 10 $W/cm^2$ or when the embryos were exposed during a later stage of gestation (42+ hours incubation). These results support the concept of increased sensitivity of the embryo during early stages of gestation and suggests the importance of peak parameters in determining threshold intensities which might affect development. Barnett (1983) has pointed out that the effects observed by Taylor and Dyson could have been enhanced by their exposure conditions because they exposed chick embryos in glass beakers.

This could result in reflection of the ultrasound beam and possible formation of standing waves which subsequently could intensify the total dose delivered to the embryo.

Barnett (1983) exposed chick embryos through a small window cut into the outer shell. Embryos were exposed to 4.5 $W/cm^2$ (SPTA) pulsed and 100 $W/cm^2$ (SPTA) CW focused ultrasound at 18 - 26 hours incubation. This stage of chick development corresponds to the definitive streak blastoderm stage and is equivalent to approximately 3 weeks gestation in the human fetus. All exposures were for 5 minutes at a frequency of 3 MHz. The pulse exposures used an SPTP intensity of 100 $W/cm^2$ and a 1 usec pulse duration. Although occasional abnormalities were found in both the treated and sham exposed embryos, these were attributed to experimental manipulation of the eggs and not to ultrasound treatment. In general, no lethal or developmental effects were observed during the 72 hours following ultrasonic treatment.

Vazquex (1963) investigated cephalic changes in chick embryos exposed to 0.87 MHz, CW ultrasound. Chick embryos between 29 - 49 hours incubation were exposed through a shell window to intensities ranging from 0.5 - 3.0 $W/cm^2$ for up to 30 minutes. This stage of incubation corresponds to the closure of the neural tube and the initiation of morphogenetic events in auditory development. This ultrasonic exposure resulted in damage to the central nervous system, the auditory organ, oral cavity and facial features. Severity of the malformations was strongly dependent on the stage at which the embryo was exposed, with the earlier stages of gestation showing the greatest sensitivity.

Shpuntoff (1985) used the same exposure method and observed growth inhibition in chick embryos exposed to low frequency ultrasound (29 kHz) on day 5 of incubation. Although this frequency is not used in fetal diagnostic procedures it is used in the field of dentistry and the author cautions that the dental application of low frequency ultrasound may adversely affect rapidly developing oral tissues.

The chick embryo has been used to investigate the effects of ultrasound on a developing system. Positive results have been attributed to experimental manipulations affecting the embryo or exposure conditions which result in phenomena not expected to be major factors in diagnostic situations, e.g., standing wave formation or heat production. The chick embryo model has also been used to study endpoints that are not development oriented, e.g., blood stasis (Dyson et al., 1971, 1974; Dyson and Pond, 1973) and cardiac function (Ruckman et al., 1985). Discussion of these studies have been omitted in this review.

## MAMMALIAN SYSTEMS

There are several distinct advantages to using mammalian systems in the evaluation of developmental effects of ultrasound. Mammals provide a system which more closely approximates the human situation than the nonmammalian systems discussed thus far. The influences of placental interaction and maternal compensation on fetal development can be evaluated. The pregnant mammal undergoes many physiologic changes to provide an environment optimal for fetal development. Ultrasound could affect normal maternal and/or placental functions resulting in a potential compromise on fetal development. The use of a mammalian system thereby increases the chances of detecting an indirect effect on the fetus. Although mammalian systems better approxi-

mate the human situation, extrapolating data beyond the scope of a study must be avoided. Because of variations in reproductive systems and cycles of mammalian species, developmental toxicity data obtained from one species may not be directly applicable to other species.

Developmental testing utilizing mammalian systems are not without its drawbacks. Experimental manipulation of the mother and/or the fetus can introduce stress or other physical factors which may alter the normal embryonic environment resulting in adverse effects on fetal development. In all mammals, including humans, the precise time of fertilization is difficult to determine. Even when timed mating of animals is performed, the method of calculating gestational age may vary among investigators. There is also the problem of accurately determining embryonic and fetal dose.

As mentioned above, there is a great deal of variation in the reproductive systems and cycles of various species. Table 1 compares the approximate gestation periods and developmental times of some important organ systems in humans and other animals compiled by Hoar and Monie (1981). Even within the same species, factors such as strain specificity, experimental manipulations, and varying exposure parameters make comparison of interlaboratory data difficult.

Because organogenesis is considered by many investigators to be a critical period of development, many studies investigating the embryotoxic or embryolethal potential of a drug or physical agent direct fetal exposure to this stage of gestation. Developmental ultrasound studies are no exception, with the bulk of available data coming from fetal exposures during this period. Traditionally, most basic texts on developmental biology begin with a discussion of the germ cells, followed by the processes involved in fertilization and then sequentially cover developmental events as they occur. The following review will summarize the reported effects of ultrasound on developing mammalian systems in a similar fashion; according to the stage of development during which exposure occurred.

## I. The Germ Cells

The first section briefly discusses the effects on testicular and ovarian tissue that have been attributed to ultrasonic exposure. Possible genetic damage produced by ultrasound exposure will not be covered in this review. The endpoints of studies involving exposure of the testes and ovaries have concentrated on two areas, histological damage and functional integrity.

There is little doubt that ultrasound can cause histological damage, but the evidence for functional damage is less clear. Hyperthermia induced by a 5 minute exposure to 3 MHz, CW ultrasound ($1\ W/cm^2$) resulted in a moderate disruption of spermatogenesis and destruction of over half the seminiferous tubules examined in exposed rat testes (Abadir et al., 1979). Ionizing radiation was shown to enhance the ultrasound effect resulting in complete disruption of spermatogenesis. Fry et al. (1978) demonstrated that if the exposure intensity was high enough, testicular exposure to 1.3 MHz ultrasound could produce mortality in adult mice. Mortality was observed in males exposed to $70\ W/cm^2$ (SPTA) for 20 seconds at each matrix location; 36 testicular exposure sites per animal. These investigators found testicular irradiation to CW ultrasound produced a higher mortality rate than pulsed ultrasonic exposure at the same average intensity. Exposed males that survived showed no significant difference in

TABLE 1. Comparative Development of Specific Organ Systems and Gestation Periods*

| | Implantation | Primitive Streak | Neural Plate | Optic Vesicle Formation | S-shaped Heart | Lower Limb Bud | Embryonic Period | Gestation Period |
|---|---|---|---|---|---|---|---|---|
| Man | 7.5 | 17.0 | 19.0 | 24.0 | 25.0 | 32.0 | 57.0 | 267.0 |
| Macaque | 9.0 | 17.0 | 20.0 | 23.0 | 25.0 | 28.0 | 46.0 | 167.0 |
| Guinea Pig | 6.5 | 13.0 | 13.5 | 15.5 | 16.0 | 18.5 | 26.0 | 67.0 |
| Rabbit | 7.5 | 7.5 | 8.0 | 9.0 | 9.5 | 11.0 | 19.5 | 32.0 |
| Rat | 6.0 | 9.0 | 9.5 | 10.5 | 10.0 | 12.0 | 17.0 | 22.0 |
| Mouse | 5.0 | 8.0 | 7.0 | 9.5 | 8.5 | 10.3 | 15.0 | 19.0 |
| Hamster | 5.0 | 7.0 | 7.5 | 8.0 | 8.5 | 9.75 | 12.0 | 16.0 |
| Chicken | - | 0.5 | 1.0 | 1.3 | 2.0 | 3.0 | 5.5 | 21.0 |

* Adapted from Hoar, R. M., and Monie, I. W., 1981, Comparative development of specific organ systems, in: Developmental Toxicology, C. A., Kimmel and J. Buelke-Sam, eds., Raven Press, New York.

fertility when compared to controls. Other investigators have demonstrated that gonadal function in mice is resistant to ultrasound exposure, even when the energies delivered were sufficient to produce burns (Kirsten et al., 1963). Lyon and Simpson (1974) reported similar findings. Male mice were exposed for 15 minutes to either CW or pulsed 1.5 MHz ultrasound. CW exposures were conducted at an intensity of 1.6 $W/cm^2$. Pulsed exposure were conducted at average intensities of 0.9 $W/cm^2$ or 1.6 $W/cm^2$ (45 $W/cm^2$ or 6.4 $W/cm^2$, peak) with pulse durations of 30 usec and 1 msec, respectively. After examination, treated males showed no indication of sterility and no decrease in testicular weight or sperm count when compared with controls.

Histological examination of the testes reveals that the degree of damage at the cellular level resulting from ultrasonic exposure varies with cell type. O'Brien et al. (1979) observed that ultrasound was capable of disrupting testicular tissue by affecting both spermatocytogenesis and spermogenesis. Contrary to the effects following exposure to ionizing radiation, 1 MHz ultrasound (25 $W/cm^2$ SPTA) for 30 seconds showed spermatocytes to be the more sensitive cell type in mice. Similar findings have been observed in rats. Spermatocytes and spermatids in testicular tissue exposed for 5 or 10 minutes to 1.1 MHz, CW ultrasound at an intensity of 1 $W/cm^2$, developed irregular membranes and their intracellular contents were released into the surrounding interstitium (Dumontier et al., 1977). Other cell types, spermatogonia, Sertoli and Leydig cells appear resistant to the effects of ultrasonic exposure (Dumontier et al., 1977; Bailey et al., 1981), and because these stem and support cells maintain their morphologic and functional integrity, only temporary sterility from ultrasonic exposure was observed (Dumontier et al., 1977).

It should be emphasized that the ultrasonic intensities and exposure durations utilized in these studies are well in excess of those used in diagnostic practice and the observed effects have been attributed to tissue heating. In a study which utilized lower intensities, Smyth (1966) exposed the testicular area of mice to 10 $mW/cm^2$, 2.25 MHz, pulsed ultrasound for 10 or 20 minutes on 5 consecutive days. Exposed mice exhibited no differences upon histological examination and no alteration in reproductive competence when compared to controls.

Similar results have been observed in the ovaries of female mice and rats. If ultrasonic intensities are high enough, bilateral sonication of mouse ovaries with 1.3 MHz, pulsed ultrasound can result in death (Fry et al., 1978). Mortality was observed in females exposed to 70 $W/cm^2$ (SPTA) for 20 seconds at each matrix site; each ovary was irradiated at five positions. When exposure intensities were lowered, there was no statistically significant difference in pup weights, number of late resorptions, and runts born to mothers exposed prior to mating. There was, although, a surprising increase in litter size of irradiated females when compared to controls. Lyon and Simpson (1974) found no induction of genetic dominant lethals and no significant alteration in pre-implantation viability in females subjected to ovarian irradiation of the same intensities that were used in the studies on testicular tissue discussed earlier.

Comparable to the observed testicular effects, the alterations observed in ovarian function and cellular architecture are generally attributed to exposure conditions and/or parameters which facilitate tissue heating. Bailey et al. (1983) accessed the ultrasonically induced morphologic damage to mouse ovaries. Mouse ovaries were exposed to 1 MHz, CW ultrasound at spatial peak intensities of 5 to

100 W/cm$^2$ for up to 5 minutes. These intensities are greater than those typically used for clinical therapeutic applications. Observed tissue alterations consisted of pycnosis and general disruption and alteration of cytoplasm of cells and vacuolization of cells and tissue, with the extent of damage varying with cell type. Luteinized cells were found to be the most sensitive, whereas oocytes of all types were the most resistant to ultrasonic insult. In subsequent work, they evaluated the temperature elevation in exteriorized mouse ovaries produced by ultrasonic exposure. Temperature elevations resulting from exposure of 10 W/cm$^2$ (SPTA) or less were not sufficient to produce ovarian tissue damage but 25 W/cm$^2$ (SPTA) or more resulted in damaging thermal levels (Bailey et al., 1984).

Smyth (1966) used the same exposure regime previously described with testicular tissue to investigate effects on the ovary resulting from ultrasonic exposure at diagnostic power levels. Due to apparatus limitations, only the right ovary of each mouse was exposed to ultrasound. Functional and histological results indicated there were no deleterious effects associated with ultrasound.

From the data available, it appears unlikely that ultrasonic exposure to intensities in the diagnostic range produces any effect on the structural or functional integrity of testicular or ovarian tissue.

II. From Fertilization to Organogenesis

There are a few studies which have focused on the effects of fetal exposure during earlier stages of development. The major events in the first seven days of fetal mouse development include cleavage, the concurrent processes of gastrulation and implantation, followed by formation of the primitive streak. With all these developmental events occurring in what corresponds to the first trimester in rodents and reports suggesting increased fetal sensitivity during earlier stages of development, it is surprising more studies have not investigated the potential fetal effects resulting from ultrasonic exposure during these early stages of gestation.

Investigating the effects of pulsed ultrasound on fetal development, Warwick et al. (1970) and Woodward et al. (1970) exposed pregnant mice on the first five days of gestation. The pregnant dams were lightly anesthetized with ether, then semi-submerged over the transducer so that ultrasound could be delivered to their ventral side. The animals were exposed for five consecutive days to either 1, 2 or 3 MHz, pulsed ultrasound, at time averaged intensities of 0.75 - 27.0 W/cm$^2$ (20 - 490 W/cm$^2$, SPTP) for up to 420 seconds. Repeated exposures on each animal on five successive days were performed in an attempt to maximize the chance of producing an effect. Although the intensities used in these experiments are well above those used in clinical practice, the authors found no significant effect on litter size, incidence of resorptions, or abnormalities which could be attributed to ultrasound exposure. This study does suffer from some design flaws. The number of subjects in some exposure groups are small, too small in some cases to detect a low probability event or to generate meaningful statistical data.

Stolzenberg et al. (1980a) exposed mouse embryos in utero to various durations and power intensities of 2 MHz ultrasound. Either CW or burst mode (20% duty factor, 1 or 10 msec bursts) ultrasound was used. Various stages of gestation were chosen for exposure, each representing a critical period of embryonic or fetal development. Exposure of dams on either day 1 (approximately first cleavage), day 2

(2 cell to 4 cell stage), or day 4 (concurrent gastrulation and implantation), were performed to detect any alteration in development which might arise from ultrasonic exposure prior to organogenesis. The other stages of pregnancy on which exposures occurred were organogenesis and organ tissue differentiation, gestational days 8 and 13, respectively. They observed that the mammalian embryo is resistant to insult prior to implantation and that an insult severe enough to affect embryonic development would either kill the embryo outright or produce a growth inhibiting effect resulting in reduced fetal weight with no associated fetal malformation. They also emphasized the potential for an indirect effect on the embryo produced by damaging maternal tissue, such as the uterus, ovaries, or placenta, all of which influence implantation and the maintenance of pregnancy. No adverse effects were observed in the fetuses, placentae, or ovarian tissues of dams exposed to 1 $W/cm^2$ (either burst or CW) for exposures up to 100 seconds. If exposure durations were increased (200 - 400 seconds), deleterious fetal effects were only observed under exposure conditions which produced maternal effects. The authors concluded that the ultrasound produced an indirect effect on the fetus by altering maternal tissue and/or function. Under these exposure conditions, thermistor probes indicated uterine tissue temperature elevations in excess of $40^{o}C$; therefore, the effects were assumed to be thermally mediated.

Akamatsu (1981) used a flush method to remove preimplantation embryos from the oviducts of rats and mice. Embryos in the late morula or early blastula stage were subsequently exposed *in vitro* to ultrasonic or thermal insult. A significant increase in morphological abnormalities and developmental retardation was observed in embryos exposed to 3.0 $W/cm^2$ CW ultrasound at a frequency of 2 MHz for 60 minutes. Similar results were obtained at lower intensities (0.65, 1.0, 1.8 $W/cm^2$) when exposures were extended to 12 hours. Embryos irradiated with 2 MHz, pulsed ultrasound (0.03 - 0.6 $W/cm^2$, SATA; 11 - 200 $W/cm^2$, SPTP) appeared unaffected, whereas embryos exposed to CW ultrasound exhibited similar effects to embryos exposed to elevated temperatures. Again, the results suggest the thermal mechanism of action is responsible for producing the observed embryonic effects.

Stratmeyer et al. (1981) observed a significant weight reduction in mice exposed to ultrasound during early stages of gestation. Mice were exposed *in utero* to 1 MHz, CW ultrasound. Pregnant mice were exposed to either 0, 0.075, or 0.750 $W/cm^2$ for 120 seconds on days 4, 10, or 14 of gestation. The exposure conditions were designed to prevent an increase above normal maternal body temperature (Stratmeyer et al., 1984). Analysis of the data indicated a significant decrease in fetal weights of mice exposed on day 4, but not in the fetuses exposed on days 10 or 14 of gestation.

Pizzarello and coworkers (1978) have reported developmental effects in rat embryos exposed to 2.25 MHz, pulsed ultrasound at low temporal average intensities. Rat embryos were exposed *in utero* on days 3, 5, or 6 of gestation for 5 minutes with a commercial diagnostic unit delivering an average power output of 1.5 mW. After anesthetization, the uterus was exposed by a small medial incision made in the abdominal wall of the pregnant animal. This allowed the transducer to be placed in contact with the uterus and power delivered directly to the uterine wall. Exposure of the embryos produced a decrease in mean fetal dry weight and increased fetal abortion frequency. At an intensity this low, heating due to ultrasonic absorption can be ruled out. When ultrasonic exposure was performed at later stages of development (day 15), these effects were not observed.

The limitations in this study have previously been discussed in the Drosophila work of the nonmammalian section. Because of the clinical implications of Pizzarello's findings, an attempt has been made to replicate this study. Trying to duplicate the exposure conditions and using the same intensities, plus additional exposures at 10 times the peak and average intensity, Child et al. (1984) were unable to reproduce these effects.

Bang (1971) found no developmental effects when fetuses were exposed in utero to 2.25 MHz, CW ultrasound on days 4.5, 5.5 or 6.5. Pregnant dams were exposed to 0.1 - 1.7 $W/cm^2$ for a duration of 60 seconds or up to 4.3 $W/cm^2$ for 15 seconds. When the fetuses were removed and examined on day 18 of gestation, there was no significant difference in either the number of fetuses, the number of gross macroscopic or skeletal malformations between control and treated groups.

III. Organogenesis

Most data on ultrasonic developmental biological effects are from studies which concentrated on potential fetal effects produced from exposure during organogenesis. This is the period of gestation when organs and organ systems are developing. Exposures are performed during organogenesis to maximize the possibility of detecting an observable malformation induced by ultrasound.

Various effects associated with fetal development have been attributed to ultrasound exposure during organogenesis. Shoji et al. (1971, 1972, 1975) reported that maternal exposure to ultrasonic waves produced fetal malformations and an increased incidence of intrauterine death in mice. They experimented with two inbred mouse strains, DHS and A/HeMk. Pregnant mice were exposed on day 8 of gestation to 2.25 MHz, CW ultrasound, at an intensity of 40 $mW/cm^2$ for 5 hours. In these studies the day of plugging was considered gestational day 0, whereas in studies by other investigators the day of plugging was considered day 1 of gestation. In general, fetuses from the treated and sham treated groups had increases in malformations and deaths and decreases in fetal weights when compared with untreated controls. Because sham treatment conditions also induced fetal effects, it was concluded that adverse fetal development resulted from maternal stress produced by the exposure conditions. In subsequent research with rats, fetal effects produced by 100 $mW/cm^2$ elicited strain specificity. Brain hernia and hydrocephalus were among the abnormalities reported in fetuses of Wistar-King A rats exposed in utero. In contrast, Wistar rat fetuses similarly exposed, appeared resistant to the effects of ultrasound (Shoji and Murakami, 1974).

The experiments by Shoji and coworkers have also been criticized because of the exposure procedure used. In these studies the transducer, coupled with glycerine, was placed in direct contact with the ventral side of the pregnant mice as opposed to partially submersing the animal in a water bath. Because of the tissue-air interface on the dorsal side, essentially all the ultrasonic energy is trapped within the animal. Lele (1975) has reported that in mice exposure to 40 $mW/cm^2$ under these exposure conditions could produce a temperature rise in excess of 2.5°C; a temperature rise sufficient to account for any teratogenic effects observed in these experiments.

Garrison et al. (1973) investigated the influence of ovarian sonication on fetal development. These investigators pointed out that the ovaries are essential in maintaining pregnancy, and while most studies concentrate on fetal exposures, the ovaries were the primary target in

this investigation. Rats were exposed on day 8 of gestation to 1.9 MHz, pulsed ultrasound at intensities of 10 or 100 W/cm$^2$ (SATP). Exposures of 10 minutes/ovary were delivered over the lateral abdominal regions transcutaneously or applied directly to surgically exposed ovaries. The investigators found no statistically significant increase in the percent of resorptions and concluded that ultrasound exposure, under these conditions, produced no alteration in ovarian function with regard to fetal development.

External, skeletal and visceral abnormalities have been reported in mouse fetuses exposed on day 8 of gestation to CW ultrasound at a frequency of 2 MHz (Hara, 1980). Fetuses exposed *in utero* to 2 W/cm$^2$ for 5 minutes exhibited a significant increase in the number of brain hernia, anencephaly, cleft palate and skeletal variations when compared with controls.

Fry et al. (1976, 1978) exposed pregnant mice to either CW or pulsed 1 MHz ultrasound. Maternal abdominal surfaces were exposed on days 8 or 9 of gestation for 10 seconds - 10 minutes to CW intensities ranging from 10 mW/cm$^2$ - 10 W/cm$^2$. The pulse exposure regime used the same intensities plus higher intensities up to 150 W/cm$^2$. Relatively long pulse durations, up to 30 μsec, were used. Exposure duration at the highest intensity was limited to 20 seconds at each matrix site; 128 abdominal exposure sites per uterine horn using a beam width of 2 mm. These exposure parameters resulted in an increase in resorption sites in the irradiated dams, but effects to viable fetuses were minimal. When teratogenicity did appear, a higher incidence was associated with CW than pulsed exposure.

In another study, mice were exposed on day 8 of gestation to pulsed ultrasound with average intensities ranging from 0.058 - 0.586 W/cm$^2$ peak intensities; 3 - 10 μsec pulse duration) for 5 minutes (Takabayashi et al., 1981). Fetal anomalies were observed in mouse embryos exposed to 59.4 W/cm$^2$ peak intensity with pulse durations greater than 5 microseconds. If either the peak intensity or the pulse width was reduced, no deleterious effects were observed. These results suggest that specific pulse characteristics, not temporal average intensity, may determine the teratogenic potential of pulsed ultrasound. Unfortunately, this report is brief and important details on methodology are missing. Ultrasonic frequency is not specified and lack of dosimetry information makes replication of this study difficult. Therefore, some caution should be used in the interpretation of these data and results.

O'Brien (1983) induced a significant fetal weight reduction in the offspring of outbred, CF1 mice with 1 MHz, CW ultrasound. On day 8 of gestation, pregnant females were exposed to spatial average intensities up to 5.5 W/cm$^2$ for varying durations. Fetal weight reduction of exposed animals ranged from 5.3% - 17.5% compared to sham treated animals. When the dose parameter was defined as $I^2t$ (where I represents spatial average intensity and t represents exposure time) further analysis of the data revealed that the average fetal weight exhibited a linear dose effect relationship. No significant difference in fetal weight was observed in pregnant hybrid LAF1/J mice irradiated at 2.5 W/cm$^2$ under similar exposure conditions (O'Brien, 1982). O'Brien suggests that the hybrid dams and their progeny elicit greater resistance to the effects of ultrasound compared with outbred strains.

Stolzenberg et al. (1980b) found litter size to be unaffected in mice exposed to 2 MHz, CW ultrasound. A decrease in mean uterine weight was observed in the progeny of dams exposed to 0.5 W/cm$^2$ for

140 seconds or 1 W/cm$^2$ for 60 seconds. Exposure to a spatial average intensity of 1 W/cm$^2$ for 40 - 60 seconds produced a decrease in fetal weight when compared with controls but not when compared with sham-treated fetuses. On the other hand, exposure to 0.5 W/cm$^2$ for 180 seconds produced a significant reduction in neonatal body weight, but only on day 25 postcoitus. Subsequent necropsies revealed that selected organ weights were unaffected by ultrasonic exposure.

Kimmel et al. (1983) exposed mice to 1 MHz, CW ultrasound on day 8 of gestation. Dams were exposed in a 30$^{o}$C water bath to 0.0, 0.05, 0.50, or 1.0 W/cm$^2$ for 120 seconds. Fetuses were removed by laparotomy on day 17 of gestation and were extensively examined for external, visceral and skeletal defects. Although the data indicated a slight increase in the incidence of fetal abnormalities, there were no statistically significant differences between groups which could be attributed to ultrasound.

Edmonds et al. (1979) investigated postpartum survival of mice exposed in utero to ultrasound on day 8 of gestation. Pregnant dams were exposed to 2 MHz, CW ultrasound at a spatial average intensity of 0.44 W/cm$^2$ in a water bath for 60 - 180 seconds. The investigators reported no effect on neonatal mortality and concluded that if the fetuses were affected by ultrasound exposure their viability returned to normal postnatal control values.

Brown et al. (1979, 1981) studied the postnatal behavior and development in mice exposed in utero on days 8 and 9 of gestation. In their earlier report (Brown et al., 1979), altered neurobehavioral development was observed in mouse pups exposed to 1 MHz, CW ultrasound at an intensity of 250 mW/cm$^2$. The abdominal area over the uterine horns were exposed in a 30$^{o}$C water bath for 3 minutes on day 9 of gestation. The ultrasound irradiated pups had accelerated or retarded responses to a variety of reflex developmental tests. In their subsequent work (Brown et al., 1981), dams were exposed on day 8 of gestation, and intensities and exposure durations were slightly modified. The pups exposed in utero to 75 mW/cm$^2$ for 2 minutes exhibited no statistically significant difference in acquisition of conditioned reflexes. Also, pups exposed to 50 or 500 mW/cm$^2$ for 3 minutes exhibited no statistical difference in reflex development assessed by pivoting, walking, forelimb grip, accelerated righting, and a rotorod test.

Sikov and Hildebrand (1976a, 1977) tested the effects of both CW (0.71 and 3.2 MHz; 2.8-32.4 W/cm$^2$, SATA) and pulsed (2.5 MHz; 15 - 410 W/cm$^2$, SATP) ultrasound on development by exposing Wistar rat fetuses at day 9 of gestation. A midline incision was made in the anesthetized mother and the uterine horns were exteriorized. After the uterine horns were exteriorized, two implantation sites in each horn were selected for either ultrasonic or sham exposure. Utilizing this method individual fetuses could be exposed. Exposure times were 5 or 15 minutes and several intensities and pulse durations were used. After exposure the uterine horns were returned to the abdominal cavity and the incision closed. This procedure may produce more stress on the mother and uterine contents, but it allows ultrasonic energy to be delivered directly to the uterine wall. On day 20 of gestation, fetuses were removed, and the effects of sonication were evaluated by detailed teratologic examination. Exposure to sufficiently high levels of ultrasound adversely affected fetal development. The embryotoxic effect was found to be dependent on the mode of ultrasound administered (CW versus pulsed) and the relationship between intensity

and effect was dependent on ultrasonic frequency. From fetal mortality data, an $LD_{50}$ intensity of 17.6 W/cm$^2$ and an "apparent threshold" of 3.0 W/cm$^2$ was calculated. Surviving fetuses had no significant differences in weight or body length although there was an increased incidence in fetal malformations associated with exposure intensities greater than 10.5 W/cm$^2$. The most prominent effect observed from pulsed exposure was the production of fetal cardiac abnormalities. The higher intensities produced increased incidence of septal defects but this effect did not exhibit intensity dependence. In general, fetal abnormalities associated with pulsed ultrasound exposure showed a better correlation with peak intensity than average intensity. In a subsequent investigation (Sikov et al., 1984) they demonstrated that the embryotoxic effect observed in the study above was attributed to heat produced by ultrasound attenuation.

Murai and coworkers (1975a) exposed pregnant rats to 2.3 MHz, CW ultrasound at an intensity of 20 mW/cm$^2$. Exposures of 5 hours were conducted on day 9 of gestation. No significant difference was observed in physical development or orienting behavior of exposed pups. Some delay in reflex development was observed between exposed and untreated control pups; this effect was not observed when exposed pups were compared with sham treated animals. Although no effect in cognitive behavior was observed, the offspring of irradiated rats showed significantly more distinct vocalization when handled and had more distinct escape responses (Murai et al., 1975b). Because much of the observed effects could be attributed to the exposure conditions, it is difficult from this study alone to determine if prenatal ultrasound exposure affects postnatal development.

In an attempt to maximize the chances of producing effects, some investigators perform multiple consecutive exposures on the same animal for several days. McClain et al. (1972) exposed rats to 2.5 MHz, CW ultrasound at an intensity of 10 mW/cm$^2$. Animals were exposed on days 8, 9 and 10 of gestation for 0.5 or 2.0 hours. The ultrasound exposure had no effect on litter size or weight and fetuses exhibited no significant soft tissue or skeletal abnormalities.

These findings are supported by the work of Warwick et al. (1970) and Woodward et al. (1970), previously described. They found no effect on litter size, mean resorption rate, or mean abnormality rate in mice repeatedly exposed to pulsed ultrasound (1, 2 or 3 MHz, 0.75 - 27 W/cm$^2$, time averaged) on days 8 through 12 of gestation. In contrast, Muranaka et al. (1974) reported a significant increase in fetal mortality in mothers exposed to 100 mW/cm$^2$ for 4 minutes daily on days 8 - 14 of gestation. Utilizing 2.3 MHz ultrasound, this exposure regime and 80 mW/cm$^2$ for 10 minutes on the same days of gestation resulted in a 50% reduction in fetal weight. When the intensity was lowered to 20 mW/cm$^2$, there was no significant difference in fetal mortality, growth rate, or external malformations even with daily maternal exposures of 30 minutes.

Stratmeyer et al. (1977), utilizing a 1 MHz, CW source, performed *in utero* fetal exposures in CF1 mice on day 10 of gestation. Anesthetized dams were exposed while in a 30°C water bath to 0.25 or 0.80 W/cm$^2$ for 120 seconds. Fetuses examined on day 18 postcoitus had no significant difference in fetal weight. Examination of the live litters indicated a significant difference in several of the measures of body and selected organ weights at 21, 36, and 51 days postcoitus, although the trend in decreasing weight with increasing exposure was not always consistent. In subsequent work (Stratmeyer et al., 1981, 1984), fetuses born to ICR mice exposed to 0.075 or 0.75 W/cm$^2$ on day

10 of gestation had a significant difference in some of the organ weights at 200 days postcoitus; again, organ weights were generally less in the exposed groups compared to nonexposed groups.

IV. Morphogenesis and the Late Stages of Development

McClain et al. (1972), Warwick et al. (1970), and Woodward et al. (1970) who all utilized experimental procedures previously described, exposed rats and mice repeatedly during later stages of gestations. Results from these studies were similar to their results obtained at earlier gestational exposures. Rats exposed *in utero* on days 11, 12, and 13 of gestation to CW ultrasound (2.5 MHz; 10 $mW/cm^2$; 0.5 or 2.0 hours) had no significant difference in the occurrence of soft tissue or skeletal abnormalities and there was no effect on litter size or weight (McClain et al., 1972). With pulsed ultrasound (1, 2, or 3 MHz; 0.75 - 27.0 $W/cm^2$, time average intensity; 300 - 420 seconds, exposure duration), no effect on litter size, mean resorption rate, or mean abnormality rate could be attributed to *in utero* exposure of mice exposed on days 12 - 16 of gestation (Warwick et al., 1970; Woodward et al., 1970).

Curto (1976) observed early postpartum mortality in mice exposed *in utero* on day 13 of gestation to 1 MHz, CW ultrasound. Mice were exposed in a 30°C water bath to 0.125 - 0.5 $W/cm^2$ for 3 minutes. Curto's results are different than those of Edmonds et al. (1979), who observed no effect on neonatal mortality; there were differences in methodology between the two studies (ultrasound frequency, time of gestational exposure) which make direct comparison of their results difficult.

Stratmeyer et al. (1984) observed no exposure related pattern of fetal weight or body and organ weights in mice exposed *in utero* on day 14 of gestation to 1 MHz, CW ultrasound (0.075 and 0.75 $W/cm^2$ for 120 seconds) and examined on day 200 postcoitus.

Sikov et al. (1976) observed an intensity related prenatal mortality in rats exposed on day 15 of gestation. CW ultrasound (0.95 MHz) up to 1.0 $W/cm^2$ was delivered to exteriorized uterine horns for 5 minutes. Consistent with the results of Edmonds et al. (1979), no postnatal mortality or reduced growth rate was observed; subsequent behavioral testing of the offspring indicated a general delay in neuromuscular development of the exposed animals, although no persistent neuromuscular deficits were observed.

As part of a larger study, Smith (1966) exposed mice to 2.25 MHz at 10 $mW/cm^2$ for 10 minutes per day. In the teratogenic portion of this study, Smith exposed both males and females for 5 consecutive days before mating; during 10 days of mating, and then pregnant females alone throughout gestation until 2 days before delivery. These conditions, multiple CW ultrasound exposures at diagnostic levels, produced no congenital malformations in the offspring born to the exposed group or in the offspring born to the second generation of the exposure group.

CONCLUSIONS

In recent years, as technology has advanced, new medical devices and procedures have evolved with increased capability of monitoring fetal development, even at very early stages of development. Ultrasound is essential in many of these procedures, however some of the newer diagnostic ultrasound instruments operate at higher intensity

levels than earlier instruments. For these reasons, it is more imperative than ever that we understand how, and under what conditions, ultrasound affects biological development.

A review of studies of the effects of ultrasound on developing organisms indicates that the biological data is highly variable and often inconclusive. Most studies reporting deleterious effects have used either intensity levels or exposure conditions which resulted in in situ exposures far in excess of those expected under clinical ultrasound diagnosis and are associated with elevated tissue temperatures. Often studies did not include sham-controls making it impossible to determine if experimental procedures other than ultrasound (maternal manipulation, anesthesia, restraint, etc.) may have produced maternal stress responsible for the observed effects. Few of the endpoints which have been examined involved functional deficits, which are sometimes more sensitive indicators of damage than are anatomic lesions. In general, the biology in the ultrasound bioeffects literature is not contemporary.

Because the current data are inadequate to exclude the existence of immediate effects with low probability of occurrence or delayed developmental effects, future research should concentrate on determining if such effects exist under conditions relevant to diagnostic ultrasound. Ideally, such research efforts would be conducted under a coordinated program designed to utilize the knowledge and methodology of contemporary developmental biology and the established expertise of ultrasound physics and dosimetry. Until more adequate information is available, a prudent approach to using obstetrical ultrasound would be to obtain the necessary diagnostic information using the least possible exposure (a combination of intensity or power and time). Because this approach demands a great deal of judgement on behalf of the clinician, it goes without saying that it also demands a great degree of understanding of the biology and physics of ultrasound exposure.

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