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Standardization of Ultraviolet Irradiation of Channel Catfish Sperm

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Gynogenesis is a naturally occurring phenomenon in lower vertebrates in which offspring receive two sets of chromosomes from the female (Dawley 1989). This occurs when development is activated by sperm, but genetic material from the male is not incorporated into the embryo. Normally, haploid offspring result that do not survive to hatching, although in certain instances suppression of meiosis II or first mitosis can result in diploid gynogenetic offspring (Purdom 1993). Artificially induced gynogenesis has been used in the breeding and genetic study of several fishes, including salmonids (Chourrout 1982; Allendorf et al. 1986), tilapia (Don and Avtalion 1988), and channel catfish (Liu et al. 1992). Gynogenesis has been applied to production of monosex populations, isogenic populations, and inbred lines of fish (Ihssen et al. 1990; Tave 1993). Gynogenesis can be induced artificially by irradiating sperm with ultraviolet (UV) radiation to inactivate genetic material. Eggs are fertilized with the irradiated sperm and shocked by temperature or pressure change to restore diploidy.

Irradiation is usually accomplished by exposing sperm to a UV radiation source providing specified power per unit area ($\mu W/cm^2$). In general, radiation dosages are estimated by trial and error and reported as time of exposure or distance of sperm from the radiation source (Chen et al. 1986; Hussain et al. 1993). Success of genetic inactivation of sperm is typically estimated by the production of haploid or gynogenetic embryos (Varadaj 1993). Success of irradiation, although reported less often, is estimated by the reduction of sperm motility (percentage of actively swimming sperm upon activation with water) (Peruzzi et al. 1993).

The purpose of this study was to establish a general relationship between UV irradiation and motility of channel catfish sperm. The authors used a commercially available UV source, a DNA crosslinker, equipped with a photo-diode that enabled direct monitoring of total irradiation and delivery of specified UV exposures ($\mu W/cm^2 \times s = Joules/cm^2$). The objectives were to: 1) measure the effect of sample concentration on motility of irradiated channel catfish sperm; 2) measure the effect of sample depth on the motility of irradiated channel catfish sperm, and 3) develop a species-independent formula to estimate exposure time or distance from the UV source when metering is not available. The motility values for irradiated sperm observed in this study encompassed the range of values reported for artificially-induced gynogenetic production of channel catfish and other fishes.

Materials and Methods

A Stratagene Stratalinker (Model 2400) DNA crosslinker was used to deliver accurate, meter-controlled levels of UV radiation at 254 nm. Instruments of this type are used commonly in molecular biology laboratories for binding of DNA to nylon membranes. This model contains five 15-W germicidal bulbs which distribute a power per unit area of ~4,000 $\mu W/cm^2$ of 254 nm radiation at 20 cm. Bulb age and other factors can affect power per unit area ($\mu W/cm^2$), but because the crosslinker is controlled by direct metering of total energy per

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unit area (Joules/cm^2), bulb age does not affect the total UV exposure.

Sperm cells were collected from anterior testes (Sneed and Clemens 1963) of healthy, mature channel catfish *Ictalurus punctatus* (1–3 kg and 50–80 cm) and suspended in Hanks' balanced salt solution (HBSS) (Tiersch et al. 1994) at three concentrations (1 g of testis per 10 mL, 20 mL, or 40 mL HBSS). Hemacytometer counts of these samples yielded concentrations ranging from 35,000 to 150,000 sperm cells/mm^3. Because catfish sperm are usually prepared as a suspension based on weight of testis rather than sperm counts, the authors chose to report sperm concentration in g testis per mL HBSS. Percent motility was estimated by placing ~5 μl of sperm onto a microscope slide, activating with ~50 μl of water, and viewing under darkfield illumination at 100×. Only high-quality samples (motility ≥ 70%) were chosen for each experiment.

The effect of sample concentration was determined on 1-mL aliquots of sperm of each concentration placed into petri dishes (35 × 10 mm) and irradiated from 0.00 to 0.50 Joules/cm^2 in increments of 0.05 Joules/cm^2. The effect of sample depth was determined on 7-mL aliquots of sperm (1 g testis/20 mL HBSS) placed into petri dishes of three sizes (35 × 10 mm, 60 × 15 mm, and 100 × 15 mm), resulting in depths of 10 mm, 3 mm, and 1.5 mm. Sperm were irradiated from 0.00 to 1.40 Joules/cm^2 in increments of 0.10 Joules/cm^2. In each experiment, motility was estimated immediately before and after irradiation.

The following formula was derived from the inverse square law based on use of one or more long cylindrical radiation sources. This formula was developed to assist in estimation of total exposure (Joules/cm^2) when direct metering is not available:

$$(n \times w \times t)/(d^2 \times \pi^2) = \mathrm{E}$$

where n is the number of bulbs, w is bulb output at 254 nm (watts), t is the duration of exposure (s), d is the sample distance from the bulbs (cm), and E is the total UV exposure (Joules/cm^2). Bulb output (w) is at 254 nm only and does not refer to the full power rating listed on the bulb (e.g., a typical 15-W bulb has 254 nm output of ~3.3 W). This information is available from the manufacturer. Using this formula, total UV exposure can be estimated, even when metering is not available, given knowledge of the output (at 254 nm) of a UV source.

The formula can be rearranged algebraically to allow calculation of exposure time (t) or distance from UV source (d) when a specified total exposure is desired. For example, the formula was rearranged in the following manner to test for calculation of exposure times:

$$(E \times d^2 \times \pi^2)/(n \times w) = t$$

Three exposures (0.1 Joules/cm^2, 0.2 Joules/cm^2, and 0.3 Joules/cm^2) were chosen and corresponding exposure times (24 sec, 48 sec, and 72 sec) calculated from the formula using the following values: $d = 20$ cm; $n = 5$; $w = 3.3$ W. Another researcher in the laboratory placed 1-ml aliquots of sperm (1-g testis/20 mL HBSS) from each of five fish into petri dishes and irradiated at the three exposure levels chosen, as delivered by metered control within the Stratalinker. Aliquots of sperm, identical to those above, were placed in petri dishes and irradiated at the three exposure times calculated from the formula. Exposure times were measured by stopwatch. Motility was estimated immediately before and after irradiation and compared to motility of sperm irradiated by metered control.

Statistical analyses were performed using Stat-view 512+ for Macintosh®. Motility values (percentages) were arcsine-square root transformed prior to analysis. Data from all studies were analyzed by analysis of variance with the level of significance set at $P \leq 0.05$. Separation of means was determined by Scheffe-F test.

Results

The motility of irradiated sperm samples was reduced from an initial value of 80%

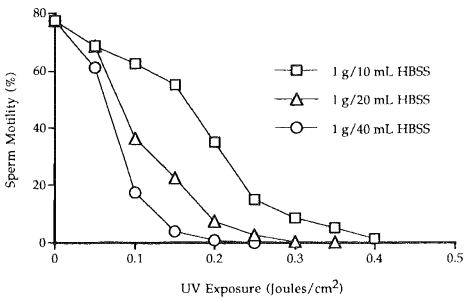

FIGURE 1. *Motility of channel catfish sperm suspended at three concentrations in Hanks' balanced salt solution and irradiated with ultraviolet radiation. Each point represents the mean value of four fish. Motility was significantly different (P ≤ 0.001) at each concentration at all exposure levels except the lowest (0.05 Joules/cm²) and highest (≥0.40 Joules/cm²).*

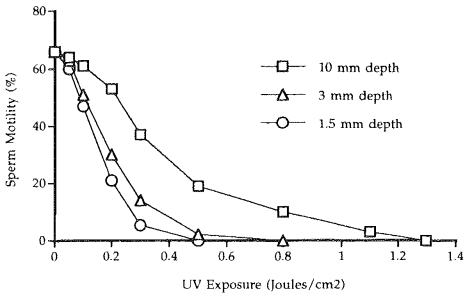

FIGURE 2. *Motility of channel catfish sperm suspended at 1 g testis/20 mL Hanks' balanced salt solution and irradiated at three sample depths. Each point represents the mean value of five fish. At exposure levels of 0.20 to 0.80 Joules/cm², sperm samples of greatest depth (10 mm) displayed significantly higher motility (P ≤ 0.001) than that observed at other depths (1.5 mm and 3 mm).*

to as low as 0% within an exposure range of 0.2 to 0.4 Joules/cm², dependent upon the concentration and depth of samples (Figs. 1, 2). After irradiation, samples with higher sperm concentration had significantly higher motility ($P \leq 0.001$) than samples of lower concentration (Fig. 1). Motility was significantly different ($P \leq 0.001$) at each concentration at all exposure levels except the lowest (0.05 Joules/cm²) and highest (≥0.40 Joules/cm²) (Fig. 1).

After irradiation, sperm samples of 10 mm in depth had significantly higher motility ($P \leq 0.001$) than samples of less depth (1.5 mm and 3 mm) at exposure levels between 0.20 and 0.80 Joules/cm² (Fig. 2).

Motility of samples irradiated for 24 sec (0.1 Joules/cm²), 48 sec (0.2 Joules/cm²), or 72 sec (0.3 Joules/cm²) were not significantly different from the motility of samples irradiated using direct metering (Table 1).

Discussion

The DNA crosslinker proved to be a useful tool for quantification of UV radiation dosages for channel catfish sperm. The unit was easy to operate, and provided accurate, sensor-monitored levels of UV exposure. Crosslinkers are standard equipment in molecular biology laboratories and are, therefore, becoming increasingly available. Although DNA crosslinkers are relatively expensive (~$1,200), UV meters that measure total exposure (Joules/cm²) are typically as expensive. Less expensive UV meters (~$400) that measure power per unit area (μW/cm²) can be used to calculate total exposure (Joules/cm²), but provide less accuracy.

In sperm samples with an initial motility of ≥70%, a concentration of 1 g testis/20 mL HBSS, and a depth of 1.5–3 mm, a radiation level of 0.20–0.30 Joules/cm² consistently reduced motility to ~20%. Chan-

TABLE 1. *Comparison of motility of channel catfish sperm irradiated at metered energy levels, or exposure times calculated from the irradiation formula. Motility scores represent the mean value for five fish. There was no significant difference (P > 0.05) in motility produced by the two irradiation procedures (one-way analysis of variance).*

Metered irradiation		Timed irradiation	
Energy level (Joules/cm²)	% motility mean ± SD	Time (sec)	% motility mean ± SD
0.0	80 ± 9	0	80 ± 9
0.1	66 ± 8	24	77 ± 6
0.2	50 ± 7	48	64 ± 8
0.3	21 ± 6	72	27 ± 9

nel catfish have been produced by gynogenesis using UV-irradiated sperm reduced in motility from ~80% to ~20% (Liu et al. 1992; Goudie et al. 1991); therefore, the values provided above would seem appropriate for the genetic inactivation of catfish sperm and artificial induction of gynogenesis. If increases in sample depth or concentration are necessary, higher levels of radiation would be required to achieve a specified level of motility. For example, a doubling of sperm concentration to 1 g testis/10 mL HBSS would increase the exposure requirement by ~50%.

Irradiation levels observed in this study agree with those observed in other studies. For example, Don and Avtalion (1988) reported gynogenetic production of tilapia by use of sperm irradiated at 3,070 $\mu W/cm^2$ for 90 sec although motility values were not reported. These values convert to a UV exposure of 0.27 Joules/cm^2, a value within the range (0.20–0.30 Joules/cm^2) observed in the present study for reduction of sperm motility in channel catfish to 20%. However, because the relationship between UV exposure and sperm motility is not completely understood, irradiation values should be developed on a species-by-species basis.

In contrast, the formula derived in this study is species-independent and can be applied to irradiation of sperm from other species. Total exposure (Joules/cm^2) can be selected from a general relationship between UV exposure and sperm motility, and exposure time (t) calculated from the formula. Sample distance (d) from the radiation source can be calculated given a specified exposure time and algebraic rearrangement of the formula. Only one of these variables (exposure time or sample distance) can be derived in a single calculation; the other must be assigned in advance. Values calculated from the formula can be affected by factors such as electrical fluctuation, reflectance, or bulb age when direct monitoring of total UV exposure is not available. Also, because the value for distance is squared, small errors in measurement can affect results. For example, a variation of ± 1 cm can change the exposure time (t) by $\pm 10\%$. By use of this formula, the trial and error associated with UV-induced genetic inactivation of sperm could be reduced, and total exposure could be estimated in the absence of a UV meter.

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