EFFECTS OF DEVELOMENTAL EXPOSURE TO A MIXTURE OF HYDRAULIC FRACTURING CHEMICALS ON SPERMATOGENESIS IN MICE

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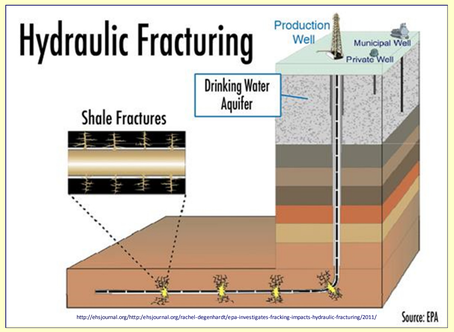
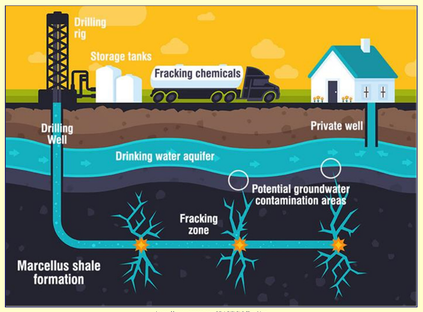
BACKGROUND INFORMATION

- **Hydraulic fracturing or "fracking" is an emerging technology used to optimize the retrieval of previously inaccessible deposits of natural gas thousands of feet below the earth's surface.**



- **Unfortunately, fracking can result in ground and surface water contamination with chemicals that, based on previous research, can interfere with the normal function of a variety of hormone receptors, causing alterations in sperm counts and testis weights, as well as circulating serum hormone concentrations.**



Overall Research Objective

To determine the effects, if any, of exposures to endocrine-disrupting chemicals used in hydraulic fracturing (fracking).

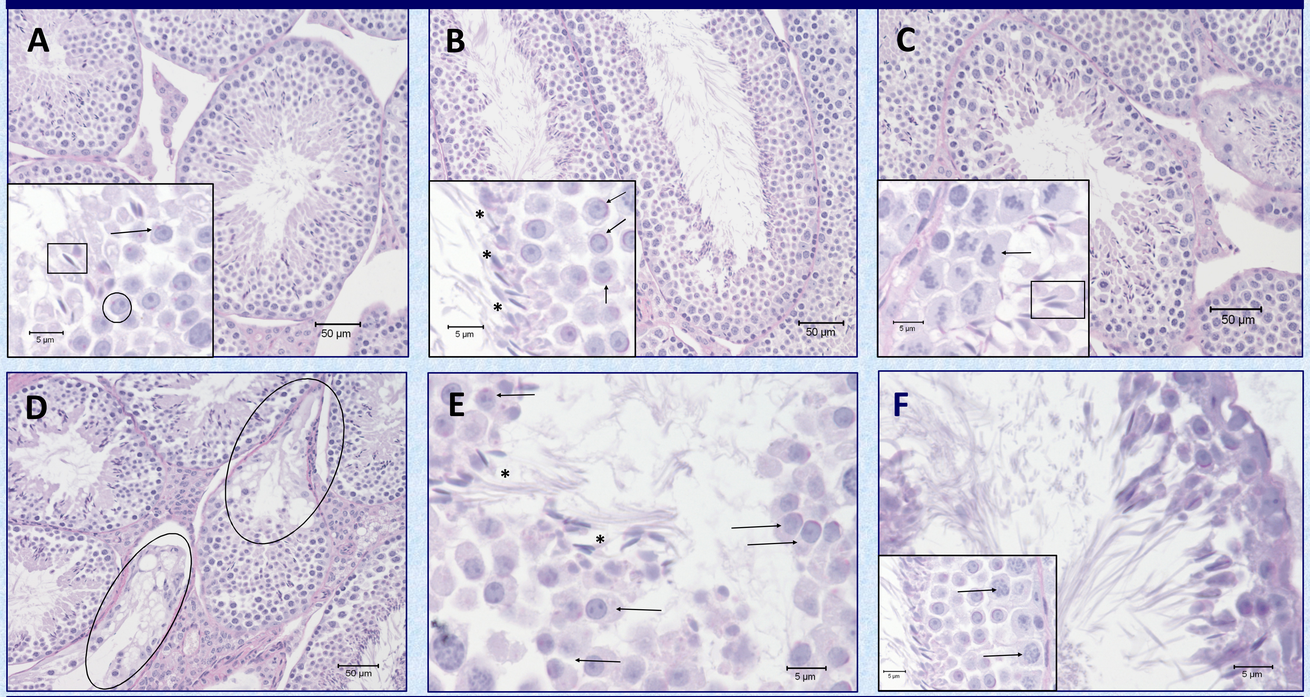
RESEARCH HYPOTHESIS

Developmental exposure to a mixture of selected chemicals used in hydraulic fracturing (fracking) can adversely affect murine spermatogenesis.

Specific Aims

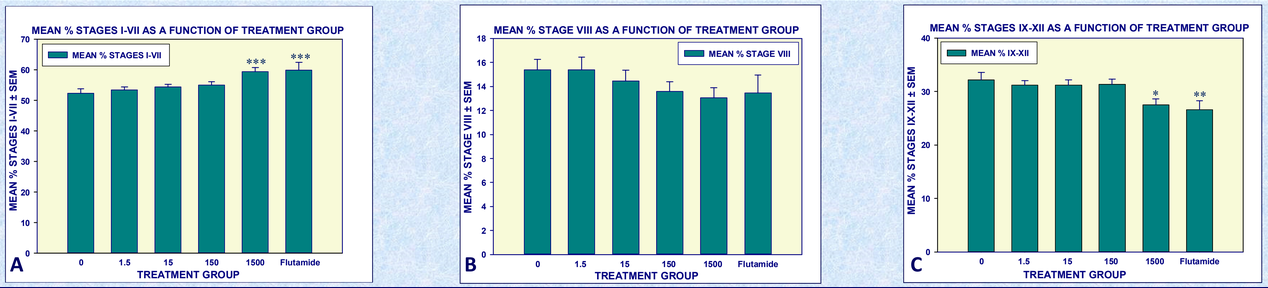
Determine the effects of increasing dosages of a mixture of chemicals used in hydraulic fracturing (fracking) on the percentage of murine seminiferous tubules in different stages of the cycle of seminiferous epithelium.

RESULTS



Staging Classifications (Images A-C): Images A-C show PAS-stained seminiferous tubules representative of Stages I-VII, Stage VIII, and Stages IX-XII, respectively, at 200X magnification, with the insets showing greater detail at 1000X magnification. Stages I-VII (example shown in **A**) are characterized by the presence of both round (*circle*) and elongate (*square*) spermatids. An example of the PAS-positive proacrosomal constituents typical of these stages is also denoted (*arrow*). Stage VIII was assigned to seminiferous tubules undergoing spermiation (**B**) and is characterized by the presence of mature elongate spermatids being released into the tubular lumen (*) and the PAS-positive acrosomal cap (*arrows*). Stages IX-XII, characterized by the presence of only elongate spermatids (*square*), are exemplified in **C**, with the meiotic figures typical of Stage XII denoted (*arrow*).

Histopathologic Effects (Images D-F): Images D-F show pathologic changes in PAS-stained seminiferous tubules. The encircled areas in **D** demonstrate severe tubular degeneration in a mouse from Treatment Group 5 (200X magnification). Staging enabled the identification of an atypical tubule from the same mouse (**E**; 1000X magnification). The stage of the cycle of the seminiferous epithelium cannot be identified for this particular tubule because the round spermatids are representative of multiple stages (*arrows*). Apparently retained elongate spermatids are also denoted in this tubule (*). Staging also facilitated the identification of the tubule shown in **F** (1000X magnification), which was collected from a mouse in Treatment Group 6. This tubule lacks primary spermatocytes, which are denoted (*arrows*) in an example of a normal tubule from this stage (inset).



Distribution of Stages of the Cycle of the Seminiferous Epithelium (Graphs A-C): Treatment group mean percentages of the stages of the cycle of the seminiferous epithelium for are depicted in Graphs A, B, and C. Asterisks are used to represent statistically significant differences between the means for Treatment Groups 2-6 and the mean for Treatment Group 1 (* = P-value > 0.05 and < 0.10; ** = P-value >0.01 and < 0.05; *** = P-value <0.01).

CONCLUSIONS AND FUTURE DIRECTIONS

1. **Developmental exposure to a mixture of chemicals used in hydraulic fracturing can adversely affect seminiferous tubules and the cycle of the seminiferous epithelium.**
2. **Evaluation of smaller subsets of stages of the cycle of the seminiferous epithelium may help further elucidate the specific mechanisms for these antiandrogenic effects.**

MATERIALS AND METHODS

- **Research Animals:** Female C57BL6 mice were orally exposed *ad libitum*, in their drinking water, for 35 days prior to mating and from gestational day (GD) 1 to postnatal day (PND) 21 to either one of five dosages of an equimass mixture of 23 chemicals used in hydraulic fracturing (0, 1.5, 15, 150, and 1500 µg chemical mixture/kg/day, or to the nonsteroidal antiandrogen flutamide. Sixty-nine, 120-day-old, male offspring of these females were assigned to one of six treatment groups, based on their dam's exposure group.

1,2,4-Trimethylbenzene	2-(2-Methoxyethoxy) ethanol
2-Ethylhexanol	2-Methyl-4-isothiazolin-3-one
Acrylamide	Benzene
Bronopol	Cumene
Diethanolamine	Ethoxylated nonylphenol
Ethoxylated octylphenol	Ethylbenzene
Ethylene glycol	Ethylene glycol monobutyl ether
Naphthalene	N,N-Dimethylformamide
Phenol	Propylene glycol
Sodium tetraborate decahydrate	Styrene
Toluene	Triethylene glycol
Xylenes	

- **Treatment Groups:**
  - Treatment Group 1 = 0 µg Chemical Mixture/kg/day
  - Treatment Group 2 = 1.5 µg Chemical Mixture/kg/day
  - Treatment Group 3 = 15 µg Chemical Mixture/kg/day
  - Treatment Group 4 = 150 µg Chemical Mixture/kg/day
  - Treatment Group 5 = 1500 µg Chemical Mixture/kg/day
  - Treatment Group VI = 50 mg Flutamide/kg/day
- **Sample Collection:** On PND 120, selected male mice were euthanized, and their testes were immediately immersion-fixed in 10% neutral-buffered formalin. Sections of each testis were stained with Periodic Acid-Schiff (PAS) to visualize the acrosomal structures.
- **Histopathologic Evaluation (see images in RESULTS):** For each male, 200 to 300 seminiferous tubules were classified based on the presence of both round and elongate spermatids (Stages I-VII); spermiation, or release of mature spermatids into the lumen of the seminiferous tubules, in association with appropriate acrosomal development (Stage VIII); or only elongate spermatids (Stages IX-XII). Seminiferous tubular degeneration and/or unidentifiable germ cell precursor associations were also noted.
- **Statistical Analyses:** Using SigmaPlot (version 13.0), the treatment group means for the relative frequencies of stages of the cycle of the seminiferous epithelium were calculated and compared using one-way ANOVA.

Acknowledgements

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