LIBERTY UNIVERSITY

Anti-Müllerian Hormone Gene Expression in Mouse Testes Elucidated via Reverse Transcription Polymerase Chain Reaction

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Abstract and/or Background

Background: Mice are effective embryological model organisms owing to their anatomical, physiological, and genotypic human analogy. AMH is a peptide hormone regulating sex development. In adult male mammals, AMH supports sperm production and causes regression of the Müllerian duct while in females the Wolffian duct regresses without testosterone. Thus, there is a dual nature dynamic at play in bipotential gonadal development: testosterone maintains Wolffian ducts and virilizes the urogenital sinus via androgen receptor while AMH represses the female reproductive tract anlagen. A housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was also examined as it encodes a moonlighting protein capable of performing mechanistically distinct functions and exuded band presentation in ovary and testes samples. **Methods**: Anti-müllerian hormone (AMH) production in mouse testes was elucidated by Reverse Transcription Polymerase Chain Reaction (RT-PCR), the most sensitive technique commonly used to characterize gene expression in cells and tissues. While a standard PCR requires hours to complete, a modified "fast" PCR takes ~30 minutes which could allow for advanced integration into undergraduate laboratory courses. Therefore, studies were performed to flashlight putative "fast" PCR efficacy by detecting anti-Müllerian hormone (AMH). Total RNA was isolated from fresh frozen mouse testes using a rapid spin column-based method and analyzed by spectrophotometry. RT was performed using a commercial kit to generate cDNA templates. PCR was employed at "standard" and "fast" pace to amplify AMH and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; housekeeping gene). **Results:** Rapid isolation delivered RNA of quality >2.10 (OD260/280) and quantity 491.4 ug/ml. Both AMH and GAPDH were detected by standard PCR. Importantly, GAPDH was successfully detected by "fast" PCR although "fast" results for AMH were inconclusive. **Conclusions:** Bridging the undergraduate-graduate dichotomy, an RT-"fast" PCR methodology proved conducive to GAPDH presentation. The newfound efficiency gain could afford Liberty students pedagogy often suspended until post-graduate endeavors, inculcating competitive advantage generative of downstream academic and industrial success.

Introduction and/or Research Question

Reverse transcription polymerase chain reaction (RT-PCR) is the most sensitive technique commonly used to characterize gene expression in cells and tissues. Reverse pivoting the central dogma of cell biology, RT-PCR retrotranscribes RNA to DNA (contextualized complementary DNA (cDNA)) to mRNA, and can thus accentuate mouse reproductive organ AMH mRNA presence or absence. Single-stranded RNAs can use host DNTPs and capsid reverse transcriptase enzymes to synthesize a DNA strand and the RNA can be degraded while the newly synthesized DNA strand functions as the template for another new DNA strand. This paradigm is the converse logicality upon which RT-PCR is built and concomitant mRNA amplification is indicative of AMH mRNA in mouse reproductive organs. While a standard RT-PCR requires hours to complete, a modified RT-"fast" PCR takes ~30 minutes, which could allow for advanced integration into undergraduate laboratory courses. It was hypothesized that AMH gene expression could be detected by RT-"fast" PCR, an accelerated protocol, to meet teaching-lab timeframes conducive to biomedical curricula integration. Therefore, studies were performed to flashlight putative RT-"fast" PCR efficacy by detecting anti-Müllerian hormone (AMH) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Methods

Total RNA was isolated from fresh frozen mouse testes using a rapid spin column-based method and analyzed by spectrophotometry. Spin column-based RNA isolation is a non-toxic and rapid method to isolate total RNA from the tissue samples. The silica-based membrane will selectively bind to the RNA and DNA due to the silica's highly positive charge that has a strong affinity to the RNA and DNA's negative charge. Rapid isolation delivered RNA of quality >2.10 (OD260/280) and quantity 491.4 ug/ml. RT was performed using a commercial kit to generate cDNA templates. PCR was employed at "standard" (95°C for 3 minutes; 40 cycles of 95°C for 30 seconds, 55°C for 40 seconds, 72°C for 30 seconds; 72°C for 5 minutes) and "fast" (98°C for 40 seconds; 30 cycles of 92°C for 2 seconds, 60°C for 2 seconds; 72°C for 5 minutes) pace to amplify AMH and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; housekeeping gene). PCR products were analyzed by standard silica gel electrophoresis. Experiments were replicated 7 times.

"Standard" RT-PCR	RT- "fast" PCR
95°C for 3 minutes	98°C for 40 seconds
40 cycles of 95°C for 30 seconds	30 cycles of 92°C for 2 seconds
55°C for 40 seconds	60°C for 2 seconds
72°C for 30 seconds	72°C for 5 minutes
72°C for 5 minutes	no temporal component
Total Time (minutes/set): 29.167 min/set	Total Time (minutes/set): 6.6999 min/set

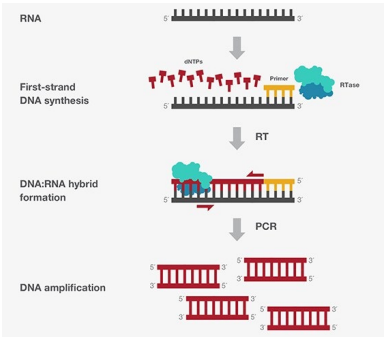

Figure 1. RT-PCR Mechanism Delineation. Reverse transcription polymerase chain reaction (RT-PCR) is the most sensitive technique commonly used to characterize gene expression in cells and tissues. Reverse pivoting the central dogma of cell biology, RT-PCR retrotranscribes RNA to DNA (contextualized complementary DNA (cDNA)) to mRNA, and can thus accentuate mouse reproductive organ AMH mRNA presence or absence. Single-stranded RNAs can use host DNTPs and capsid reverse transcriptase enzymes to synthesize a DNA strand and the RNA can be degraded while the newly synthesized DNA strand functions as the template for another new DNA strand. This paradigm is the converse logicality upon which RT-PCR is built and concomitant mRNA amplification is indicative of AMH mRNA in mouse reproductive organs.

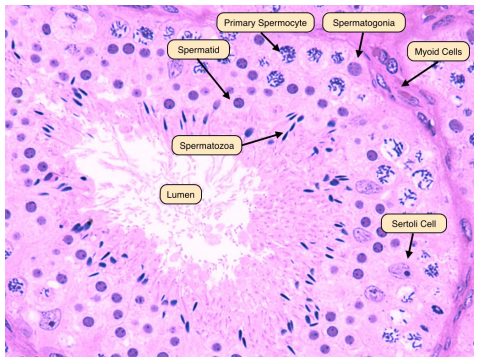

Figure 2. Cytology of Male Testis. Male testicular components and AMH generative loci were elucidated via hematoxylin and eosins (H&E) stain. In the mouse testis, AMH is produced by the Sertoli cells beginning at 12.5 days post-coitum. Stained material constituents thoroughly articulate ploidy-commensurate spermatozoa morphology characterized by the congealing, condensing, and narrowing/elongating of spermatids, primary spermatocytes, and spermatogonia, respectively. Tail formation is emblematic of spermatozoa maturity.

Figure 3. Gross Testicular Anatomy. Caudal aspect of partially dissected male mouse testis.

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#	Sample ID	User name	Date and Time	Nucleic Acid	Unit	A260 (Abs)	A280 (Abs)	260/280	260/230
24		Xu, Jing	11/1/2023 4:53 PM	491.4	ng/µl	12.284	5.831	2.11	1.18

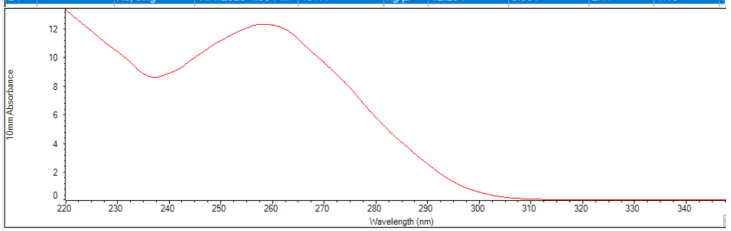

Figure 4. Rapid Spin-column Derived RNA Integrity. Total RNA was isolated from fresh frozen mouse testes using a rapid spin column-based method and analyzed by spectrophotometry. Spin column-based RNA isolation is a non-toxic and rapid method to isolate total RNA from the tissue samples. The silica-based membrane will selectively bind to the RNA and DNA due to the silica's highly positive charge that has a strong affinity to the RNA and DNA's negative charge. Rapid isolation delivered RNA of quality 2.11(>2.10) (OD260/280) and quantity 491.4 ug/ml.

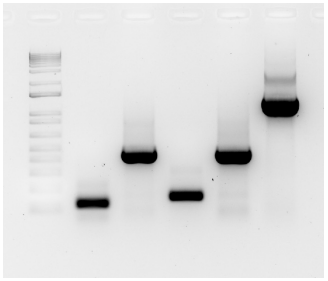

Figure 5. Primer Assessment. Primers were amplification-capable across all experimental samples (AMH, LEU, and GAPDH). Band migration distance through agarose matrices was inversely proportional to molecular weight in kilodaltons. Migration patterns careened from negative to positive resulting from sugar-phosphate backbone inculcated nucleic acid negativity.

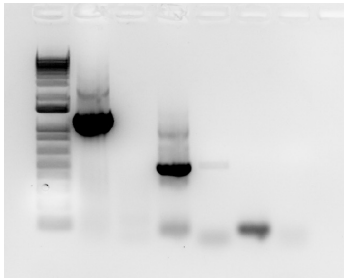

Figure 6. RT- "fast" PCR Comparison. Both AMH and GAPDH were detected by standard PCR. Importantly, GAPDH was successfully detected by "fast" PCR although "fast" results for AMH were inconclusive.

Results and/or Conclusion

Results
Rapid spin-column based RNA isolation delivered RNA of quality >2.10 (OD260/280) and quantity 491.4 ug/ml. Both AMH and GAPDH were detected by standard RT-PCR. Importantly, GAPDH was successfully detected by "fast" PCR although "fast" results for AMH were inconclusive. These data suggest that RT-"fast" PCR could become inextricably ingrown into undergraduate curricula across biomedical disciplines at every university level while scaffolding into manageable teaching lab time constraints.

Conclusions
Bridging the undergraduate-graduate dichotomy, an RT-"fast" PCR methodology proved conducive to GAPDH presentation. The newfound efficiency gain could afford Liberty students pedagogy often suspended until post-graduate endeavors, inculcating competitive advantage generative of downstream academic and industrial success.

Future Work

The novel RT-"fast" PCR technique here employed could be further modified so that AMH detection could become increasingly successful, reproducible, and conclusive pursuant likewise of increased GAPDH housekeeping gene band density. To improve upon the current method, we suggest increasing cDNA concentration. attenuating annealing/extension temperature, and increasing cycle number, with a goal of integrating this technique into undergraduate curricula.

References and/or Acknowledgments

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