NSIIC Grant Intermediate and Final Report

Cervical Insemination: The Method of the Future

June 30, 2022

Membrane Protective Technologies, Inc.

Contents

Executive Summary

This study began development of a system to improve small ruminant insemination. Currently laproscopic artificial insemination is the only commercially efficacious method of artificial insemination (AI). Unfortunately, this method is much more aggressive than is utilized in cattle, swine and horses. Within this grant, we are enabling a commercially viable alternative to laproscopic AI, transcervical insemination, predicated on treatment and relaxation of cervical tissue. The portion of the full system developed in this grant comprises:

1) an agent which softens and relaxes cervical tissue,
2) a preliminary delivery mechanism for said agent, and
3) initial development of a process for positioning and restraining the ewe during insemination.

A total of 8 treatment agents for softening were tested; all agents were naturally derived, tissue-safe extracts which are also sperm safe. All were tested first in abattoir tissue (N = 223). Two different treatments (A and B) were then further tested in live ewes (in estrus, n= 22). Both treatments drastically improved transcervical penetration (P<0.01) over control (no treatment). In fact, the treated tissue allowed passage through the cervix 100% of the time, and into the uterine body in most cases. Such penetration enables pregnancy rates similar to live cover in other ruminants, and swine.

AI rod size had an impact on success of passage into the uterus, but both traditional ½ cc and ¼ cc, were able to be used. Using ¼ cc AI rods, Treatment A enabled 100% success traversing the cervix, while treatment B enabled passage 75% of the time, improving passage depth by 2-3 inches over control (an 40% improvement). With ½ cc rods, both treatments averaged 88% success, compared to the control at 40%, improving depth of penetration by 1 inch over control. **Overall 100% passage into the uterine body was achieved 82% of the time using the treatments developed in this grant. This is unprecedented progress toward a transcervical AI in small ruminants.**

Breeding trials achieved a 40% pregnancy rate in estrus positive, treated ewes but sample size was severely limited due to confounding problems with equipment and seasonality.

The data generated in the past 6 month, meet the grant objectives, demonstrate proof of concept, and importantly highlight areas of focus for phase II. Preliminary experiments for the next phase of development were executed, enabling movement into commercialization of a product that enables the safe, non-traumatic insemination of small ruminants.

Background

Current Technology

Artificial insemination (AI) is a common and extremely valuable method for breeding livestock, allowing for greater control over genetics, decreased expense, greater income per animal, and removing the need to transport animals for live cover. Transcervical AI (TCAI) is a widely used and highly successful for large ruminants such as cattle, swine and horses, but the required laparoscopic AI for small ruminants leaves animals at a high risk for adverse events, injury, death, or failure to fertilize.

During TCAI a slender rod is passed through the cervical rings, into the uterine body or horns, then a straw of semen is injected via the steel rod into the uterus. TCAI is performed with either ½cc-sized or ¼cc-sized rods. In sheep it is difficult to pass the rod (of either size) because cervical rings are convoluted, and no internal guidance is possible {1,2}. Currently TCAI may result in an inflammatory response that decreases the likelihood of fertilization, and repeated damage to cervical tissue can cause scarring and perhaps infertility {2}.

The alternative to TCAI is Laparoscopic AI (LAPAI), in which sperm is deposited into the uterine horns transabdominally. This bypasses all the difficulties posed by the cervix, but has risks including sedation, bodily stress due to head down positioning, is expensive, time consuming, and requires a skilled operator with costly equipment and a veterinarian. Like all surgical procedures, it poses a risk for infection or adverse reaction to the sedative{2}.

Via this grant, MPTI began the development of a new a non-hormonal technology to, temporarily treat the local tissue of the cervix and prepare it for the TCAI process without altering normal, healthy cervical structure and function, and without producing systemic affects which might interfere with ewe health. The long-term goal is to develop a full system that **enables the use of cervical artificial insemination in sheep by the trained lay person**. The development of this technology is multifaceted and includes optimized unique extenders and sperm handling techniques, cryopreservation methods, as well as sperm delivery techniques. In previous work, MPTI developed sperm related cryopreservation technology which were applied toward the second objective, below.

Within this grant proposal **MPTI focused on the key aspect of insemination** and proof of concept, with the objectives of:

(1) developing a cervical ripening agent for ewes; such an agent assists in insertion of AI rods into the reproductive tract by slightly, and temporarily, modifying the physiology of the cervix.

(2) enable the consistent passing of an AI rod into the cervix, which by increasing the depth and ease of penetration, will

(3) enable pregnancies in AI'd ewes.

Success was be determined by an agent that enables the passing of an AI rod through at least 1 of the cervical rings in 80% of the treated ewes. Secondarily, a 35 day pregnancy rate of at least 40% in treated ewes will indicate success of the preliminary system.

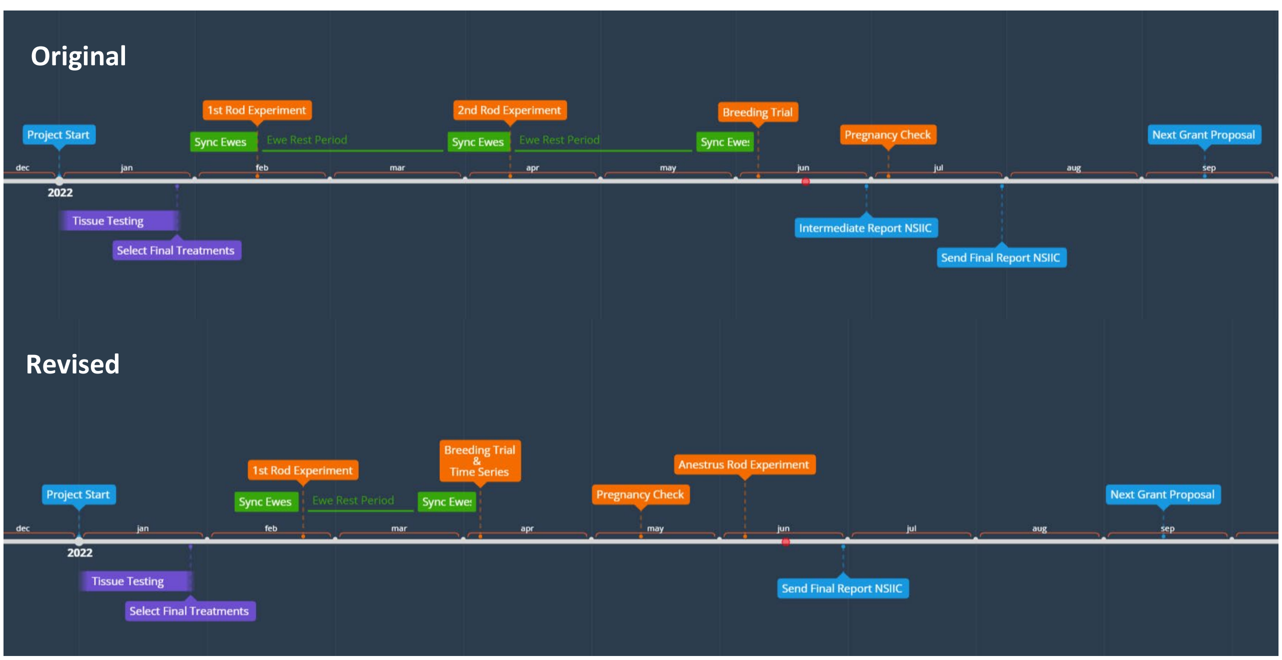


Figure 1: Original timeline and modified timeline for project, in the following segments – tissue testing, two rounds of rod passing without insemination, and a breeding trial with pregnancy checking at 35 days.

Experimental Process and Results

Within this grant, 8 natural products were tested; for proprietary reasons the components will not be disclosed, but they are plant derived extracts that are physiologically compatible with the delicate cervical tissues. As a control, Lidocaine, a product with known efficacy in this application was included with abattoir tissue testing.

Overall there were 8 individual treatments tested on 223 uteri (27 replicates) and 24 ewes in a total of 3 experiments (3 replicates) using the general technique as detailed in Fig 2.

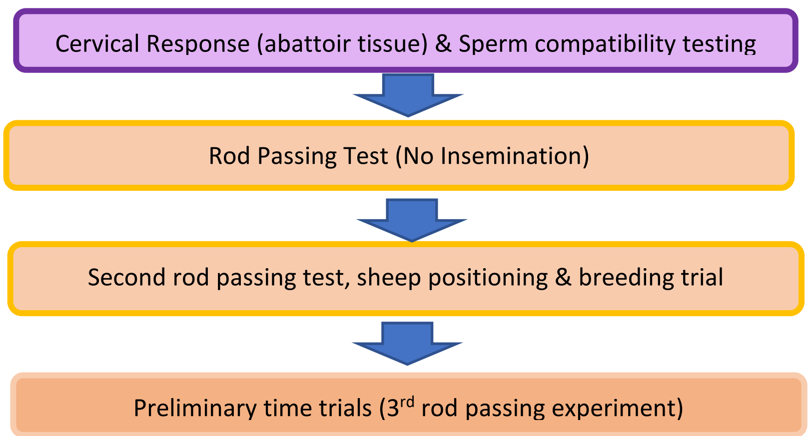


Figure 2: Major steps of the study. Purple indicates in vitro, orange in vivo and in situ testing.

Cervical Response Testing (Objective 1)

Delivery mechanism: **In addition to the softening agents themselves, a preliminary delivery mechanism was also designed and refined using abattoir tracts.** Direct liquid delivery was initially used, but problems with backflow which reduced the contact between the agent and the cervical surface (fornix) limited the effectiveness. Secondarily, an absorbent plug was tried. Said plug, soaked in treatment solution, was inserted into the vagina, held in place by the usual force of the surrounding muscles. It was inserted to the proper depth to ensure surface contact between the plug and the fornix (and, in the case of the abattoir tissue, clipped in place to simulate pressure of living ewe vaginal muscles). This method was successful at delivering treatment to the cervix. We obtained ewe and bison cervical tracts from local abattoirs; such tissue mimics local responses in living tissue for a brief period, modelling *in vitro* response to softening agents. Tracts were treated with the respective softening agent (1 hour treatment time), then ¼ cc AI rods passed through the cervix mimicking TCAI. The estimated number of rings passed was recorded. Post-treatment the tracts were dissected, and length and cervical anatomy recorded then relationships between cervical structure and ease of passage was examined. Figures 3-5 show some example tracts, highlighting the interesting and challenging features of ewe anatomy.

A total of 223 tracts were utilized in experiment, with 99 tracts in a first round of tests, enabling reduction in the number of treatments for experiment 2. Each treatment was tested 11-14 times. **The final treatments selected (referred to hereafter as A and B) were examined in 13 and 12 replicates, respectively.**

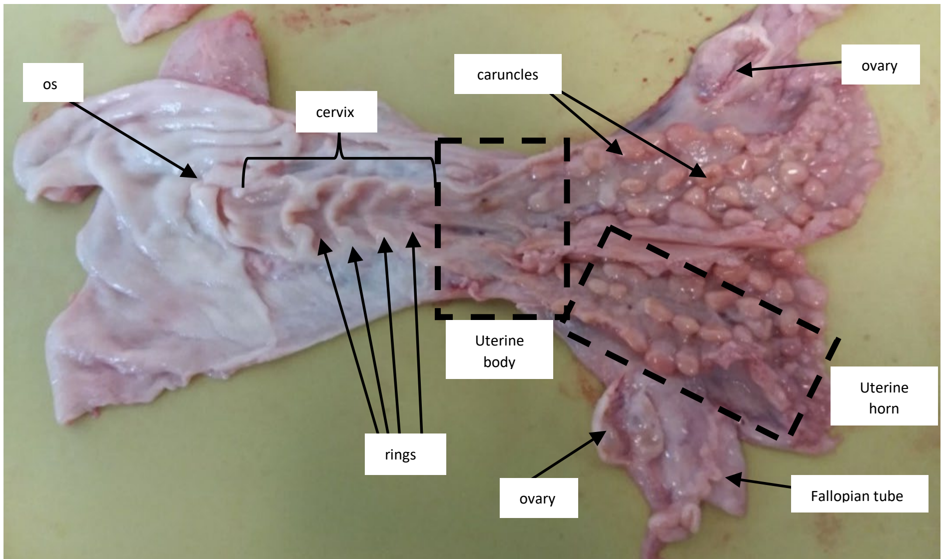


Figure 3: We dissected ewe reproductive tracts, exemplified here with notes on relevant anatomy. The tract has been cut lengthwise and laid flat – normally this tract would be 'rolled up' to make the cervix a long tube. In this case, the 3 rings are relatively well aligned.

Figure 4: Variation in sheep cervical osses (openings) pictured with the vaginal tissue folded back for plain display. Note the variation in structure and orientation relative to the vaginal walls – the os sometimes faces away from the vaginal opening or has multiple layers of flaps blocking entrance into the cervix, which makes entering the cervix unpredictable and difficult during the AI process.

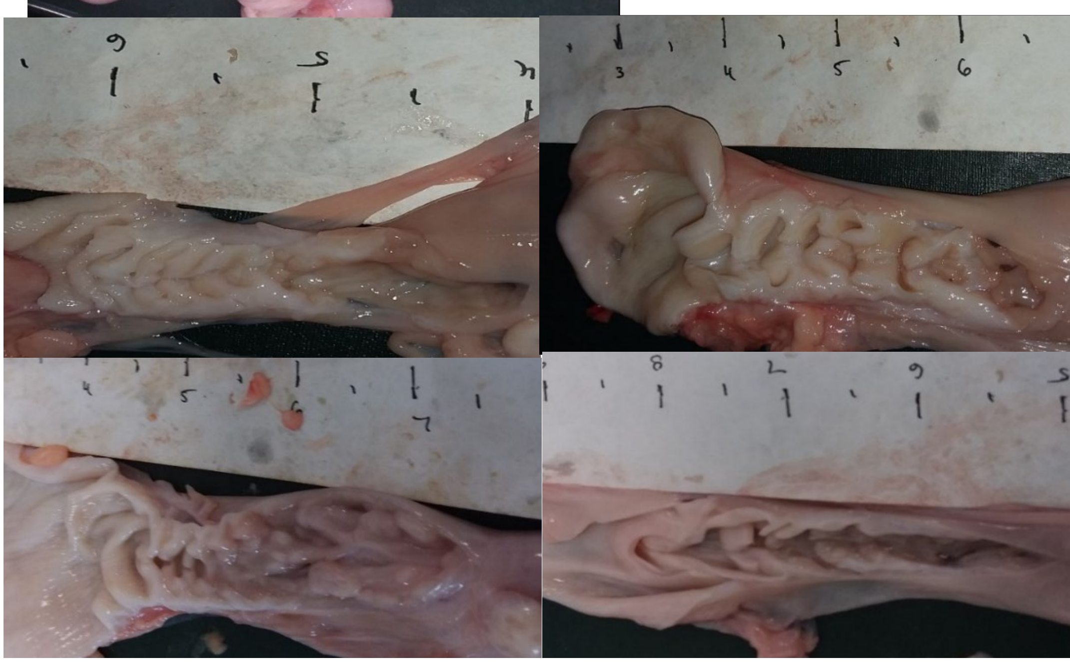
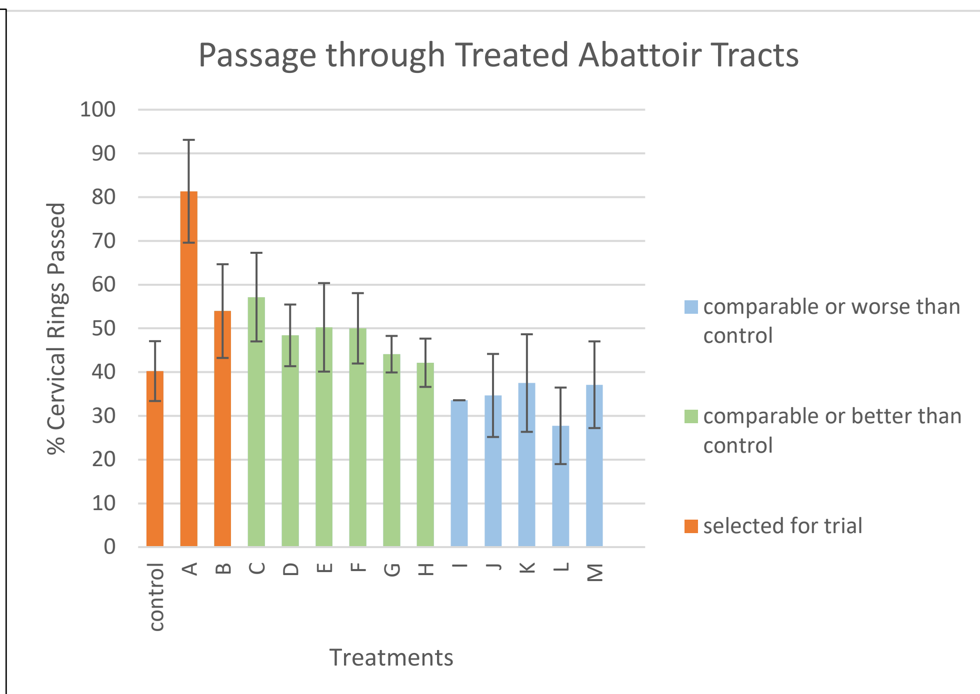


Figure 5: Dissected cervical tracts showing variation in the internal structure of the rings. Note the distinct differences in ring number, shape and orientation; these variations make TCAI a difficult technique to practice, and illustrate the need for selective breeding for ease of AI'ing.

Ring passage data were transformed into a percentage of rings passed to normalize the data and allow comparison across a variety of tissues, breeds, ages, and other etiological differences. A paired T-test showed there were trends in improvement which indicated that certain treatments could be promising if tested *in vivo* (Figure 7; p=0.064). The responses were sufficiently different to narrow the list of potential agents to three, which could then be tested *in vivo* and *in situ* in a breeding trial. One of the potential reasons for a lack of significance is that these tracts cannot mimic systemic effects, lack a blood supply or input from surrounding tissue – enzymatic reactions, hormonal responses, or effects requiring living tissue simply cannot be fully modeled in dead tissue. In addition, and perhaps most importantly, we had no way of classifying data by breed, age, culling reasons, and the like.

Our findings verified the work of others in classifying cervical anatomy{2} and expanded on our understanding of the relationship between anatomy and challenges with the cervical AI process. Interestingly, **there was no statistical correlation between the number of rings in the cervix, and the number of rings able to be passed by the AI rod technician; in other words, 'simpler' cervices are not necessarily easier to pass.** In the tissue tested (ewe age and breed unknown), the average length was 14 cm, containing between 3 and 7 rings (average 5). In some dissected tracts, the cervical rings failed to develop properly and produced a complete blockage of the cervical tract (infertility was likely the reason for culling). In a live cover situation, cervical blockage would result in an inability to conceive, and if overridden by LAPAI, could result in birthing difficulties. The technology developing under this grant, which ameliorates difficulty posed by cervical anatomy, would allow identification of a blocked cervix. This in turn, would allow sheep producers to more quickly carry out selective breeding which, as in the swine and bovine industry, can improve multiple aspects of fertility. These results highlight the <u>need</u> for the technology we are developing.

Figure 7: Results of abattoir tissue testing showing effective treatments as measured by the percentage of passage through cervix by treatment. Treatments in green performed numerically better than the control. Treatments in orange were selected for the trial. This selection was based not only on performance but also commercialization potential, availability and the like. Error bars indicate the SEM.



Sperm Safety Analysis (Objective 3)

A successful cervical softening agent will be safe not only for the ewe's tissues but also for the sperm used to inseminate. We expected the cervical tract and AI rod to retain a residue of the treatment which might combine with the semen inside the cervix or uterus therefore experiments were performed using ejaculate samples from boars to **evaluate the toxicity of each proposed treatment and blends. Sperm samples were incubated with each agent** (0.1 – 1 µL) for up to 3 hours, then the motility characteristics were measured (using computer assisted sperm analyzer). Motility is a useful measure of sperm health. One treatment (Treatment V; Fig 8b) was immediately discarded from further replicates since incubation resulted in almost complete sperm cell death. A total of 4 replicates were executed within this experiment. **All other treatments fared well over the ranges tested (Fig 8a and b).**

In vivo and breeding trials (Objective 2 & 3)

In order to evaluate safety and efficacy of the top 2 softening agents (Treatments A and B), two *in vivo* experiments were designed: live ewe rod-passing and a breeding trial. In both cases the ewes would be synchronized into estrus according to industry standard procedure for TCAI (CIDR insertion for 14 days; CIDR removal and PG600 injection 48 hours prior to insemination at day 12). In the rod passing experiment, no semen was delivered, but in the breeding trial ewes would be inseminated and the pregnancy results used as a measure of the success of the technique.

Fifteen Rambouillet ewes were acquired. One ewe was removed for health reasons, and the remaining **14** were synchronized and the rod passing experiment performed in February, with an IMV Technologies ¼ cc ovine/caprine AI sheaths on a ¼ cc AI gun. **Four ewes were used for each treatment group (control, A, and B).**

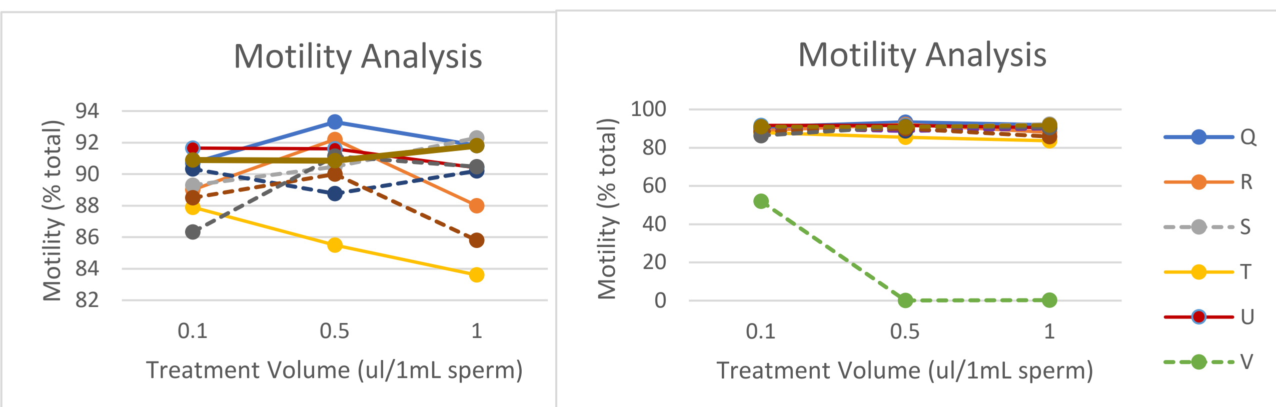


Figure 8 : Results of sperm safety test performed using CASA (computer assisted sperm analysis). Right includes spermicidal treatment V, while left shows details of successful treatments. Control sample average was ~ 90% motile. There is no significant difference between treatments, except V.

Each ewe was treated with the softening agent for 60 min using the developed delivery method, then led to a chute and positioned with their hips at an elevation to assist the AI tech in navigating the vaginal and cervical canals. Plugs were removed, and it was noted that most plugs had a layer of white mucus and discoloration on the end, indicating contact with the cervical surface; such discharge is a common sign of a healthy estrus cycle. Then the AI rod was passed as deeply into cervix as possible without undue pressure. The depth of the os and final depth of penetration, as well as an estimation of the number of cervical rings passed was marked on the rod sheath. The measurement procedure was similar to that outlined by Perry{4}, though we also recorded estimates of the depth of the os and any detectable rings.

Excitingly, treatment A resulted in a 100% rate of rod passage and penetration into the uterus. With ¼cc rods, **Treatment B had 75.15% passage, while the control was 30% success. The control rate is similar to reported success in industry and literature {5,6}.** The average depth of penetration for treatments was also greater than that for control (Fig 10, 11). The average length of a ewe cervix is 5.7cm; though the length of the vaginal cavity can vary between 11.4-17.8cm, with an average of 14cm. **Thus, a consistent increase in depth of passage by 2.5cm (1 inch) represents a ~20-25% increase in cervical penetration, which could drastically improve the chance to fertilize.**

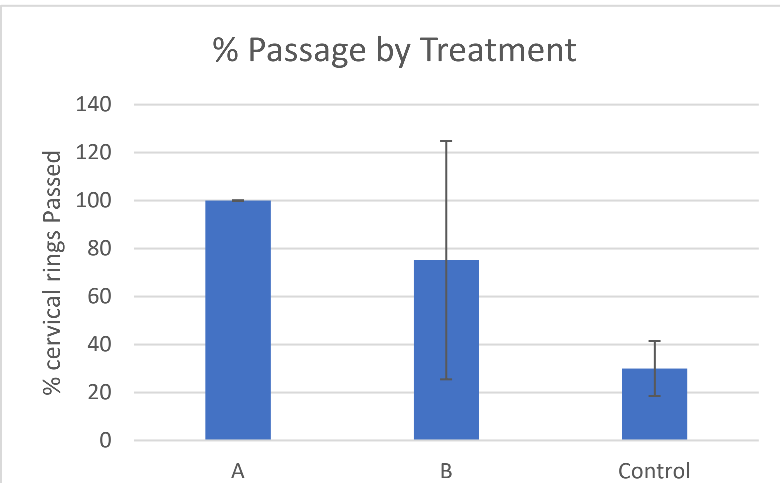
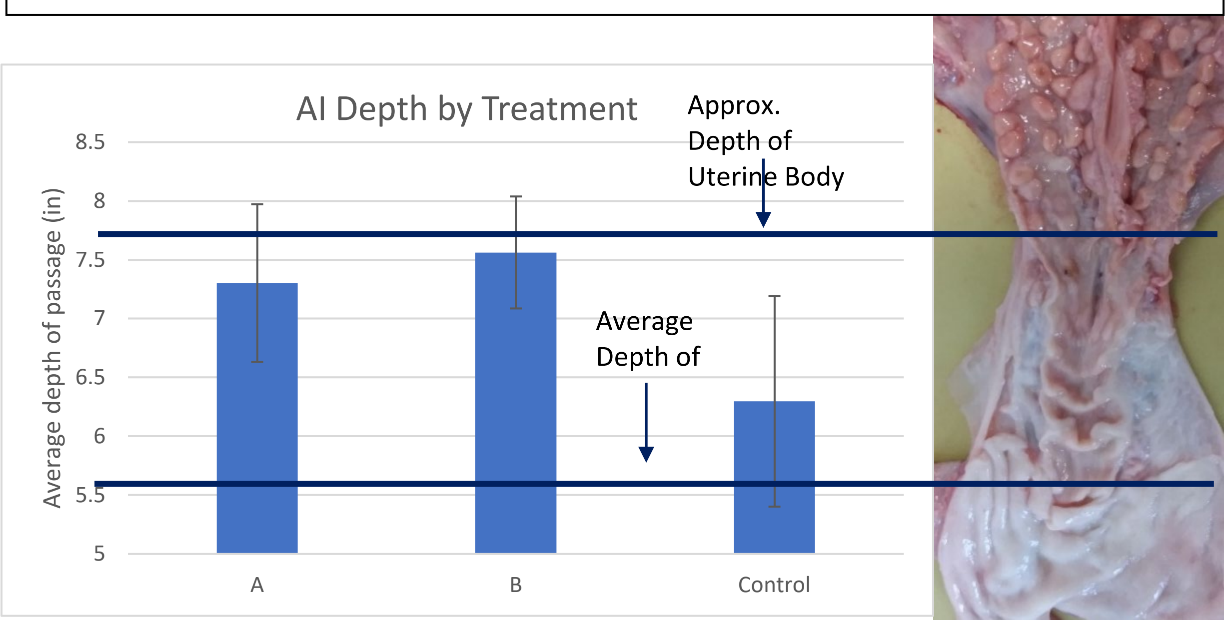


Figure 10: Results of AI rod passage in terms of % of cervical rings passed, based on the average determined from abattoir tissue tests performed earlier in the year. Note that treatment A has error bar (1 std.dev.) height of 0, because passage into the horn was achieved in each ewe.

Figure 11: A visualization of absolute depth of passage by treatment. On the right is an image of a dissected ewe tract, aligned with the measurements on the graph, to demonstrate the significance of a one-inch increase in depth. ***It should be noted that ALL treatment groups had passage beyond the os whereas control passed the os depth < 50% of time.***



The success of the first phase led us to redesign the second and third experimental sets in hopes of gathering more data – not just on the efficacy of the treatments, but the methods by which the ewes were positioned for the rod passing, and the length of time the treatments were effective.

Unfortunately, ewes are short day breeders, fertile in the autumn and winter, and as the seasons progress toward spring fertility drastically decreases. As 4 to 6 week period was required between synchronization cycles to avoid creating ovarian cysts, our timeline for experiments and breeding within the grant period was tight and did not fit a fertile period. Rather than having three synchronization-and-pass cycles as originally designed, we would have to inseminate the ewes during the second phase (April of 2022). Unfortunately, due to fitness issues, several ewes had to be removed from the breeding trial even though they were able to be used in the rod passing trial. A total of 10 ewes were used for the breeding portion and 17 for the rod passing portion. Therefore, experimental groups for breeding were reassigned as control (n=2), Treatment A and B (n=4 each).

The data from the second experiment were further confounded by several factors. Six of the ewes synchronized showed no signs of estrus, indicating they were likely out of season. Moreover, supply issues resulted in an inability to obtain ¼ cc sheaths for AI rods. Previous data from cervical response experiments indicated these are less effective for insemination of ewes. When using the larger rods and lack of estrus, so it was imperative to optimize ewe position. Therefore, the data in the section are further confounded by 3 different techniques to position ewes.

All that said, the experiment yielded significantly positive and useful data.

1) **An optimum angle range for ewe positioning was determined.**
Within the first portion of the breeding, ewes were held in a standing position with a wide canvas belt used to raise their hips slightly, at an angle around 35 degrees. Ewes showed no discomfort at being lifted slightly. However, the inseminator believed the belt was compressing the abdomen and making it difficult to pass the AI through the ewe cervices. Thus, it was contrived to create a platform on which the back feet were positioned. This raised the ewes' hips at an angle between 13 and 24 degrees, which improved passage.

2) **Rod passing confirmed data obtained from first experiment**
Although statistical analysis indicated a statistical tendency (P= 0.0647; difference of means) between treatment groups, in terms of actual practice of AI the results are very distinct: **the control had a mean at 40% cervical passage, while the treated ewes' rods were passed at 88.6%, more than twice the control.**

   In terms of absolute depth of passage of AI rods (in), **Treatment A** (**mean = 7.96**, 95%CI [6.80, 7.96]) **provides a statistically significant (p<0.01) improvement over the control** (**mean = 5.97**, 95%CI [5.08, 6.86]). Interestingly, **Treatment B** (**mean = 6.97**, 95%CI [6.39, 7.55]) is not statistically different from either the control or Treatment A but **Treatment B still had a 1-inch (2.5 cm) improvement in treatment depth over control.**

   Like experimental set 1, **in 100% of the treated ewes the cervical os was passed** indicating efficacy of treatment even in non-estrus ewes, **even when using an ½ cc rod**. ***Moreover, the data reiterate the ability to deposit semen in the uterine body without invasive laparoscopy***.

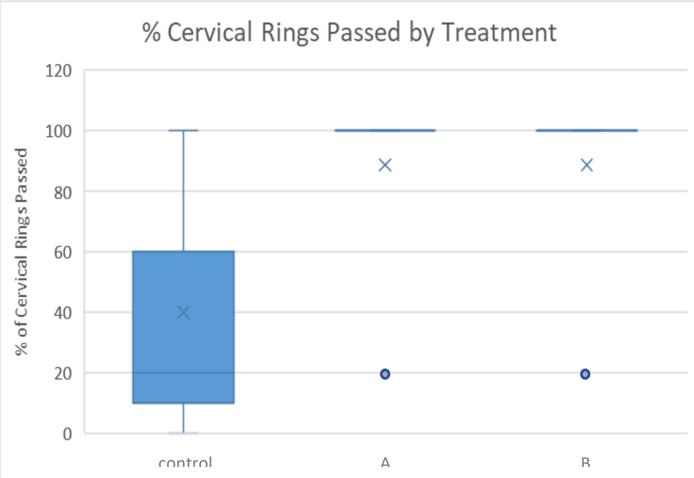


Figure 9: box and whisker plot showing % of cervical rings passed with ½ cc rod and sheaths. The mean is shown as an ×, the median as a horizontal line through the box. Outliers are shown as blue dots. Note that this diagram is unusual, because for the treatments, the median, Q1 and Q3 are all the same (making IQR = 0).

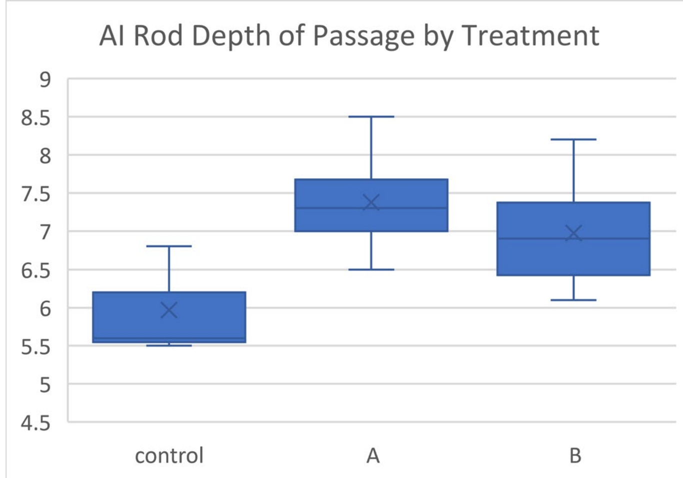


*Figure 10: box and whisker plot showing depth to which ½ cc AI rod was passed. Note that the **difference between means is more than 1.5 inches which is 50-75% of the length of a cervical tract.***

3) Pregnancies were obtained (OBJECTIVE 3)

Ewes fit to perform in the breeding trial were inseminated with frozen ram semen packaged at 200 million sperm/ml extended in GameteGuard®-OF. Sperm had a 3hr post-thaw quality of 48.60% motility and 89.37% live intact.

Of the ewes that showed estrus and were positioned correctly (n=5, 1 control, 2 treatment A and 2 treatment B) two treated ewes were pregnant for a rate of 40%. Although numbers were significantly less than originally proposed, this indicator of success is exciting!

The results of the preliminary breeding trial are encouraging. All the ewes that were pregnant were ewes inseminated when experimental procedure (namely positioning method) had been refined through practice.

Preparation for Phase II

In the aforementioned experiments, a treatment time of 1 hour was used. To determine if this treatment time should be explored as a second phase, two ewes were exposed to either treatment A or B (no untreated/control animals) for 1 hour, and AI rods passed every 30 min. As expected, there was a striking general trend of decline in efficacy over time (Fig 11). **This is a positive response as the treatment is designed to be transient. Moreover, it appears a shorter treatment time is likely efficacious, a commercially beneficial finding.**

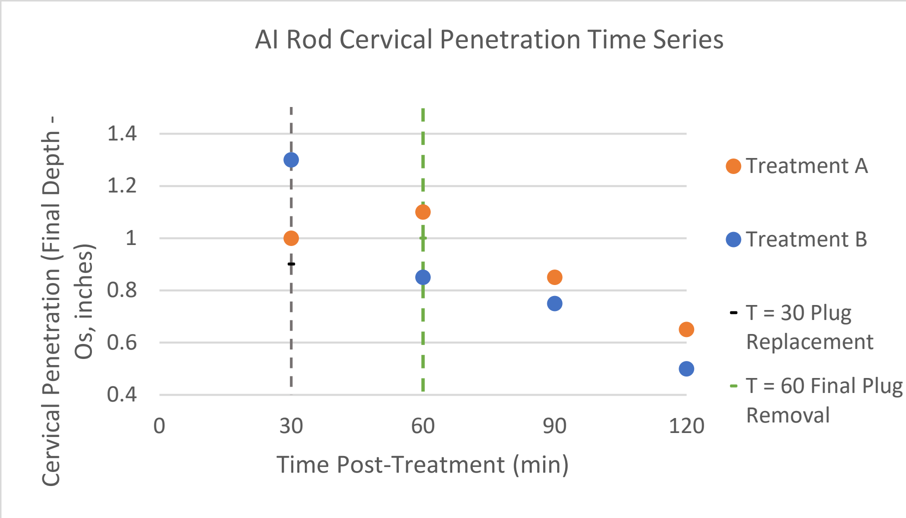


Figure 13: Effect of elevation angle on passage depth. Error bars indicate std. dev. 11: treatments A and B time series showing passage into cervix at 30 min intervals for 2 hours. Plugs were inserted at T = 0, removed at T = 30 min to allow a rod to be passed, and another plug inserted which was removed at T = 60 min and not replaced.

Delivery mechanism: In addition to the softening agents themselves, a preliminary delivery mechanism was also designed and refined using abattoir tracts. This will require some modification in phase II to address commercial-level use.

Anestrus response: a rod passing experiment was performed. Treatment A alone was tested, because it was most successful throughout the study, and the number of available ewes limited (n= 9). While penetration depth was reduced to ~5.5 (past the os, and at least 2 to 3 rings) in rather than the in-estrus depths reaching ~7.5 in, this may be expected as in-estrus animals have a lower vaginal and cervical muscle tone and increased mucus production to ease the ram's penetration and assist semen flow into uterus.

Elevation angle: Finally, to better understand the effect of elevation angle on ease of penetration, the ewes in the anestrus trial were tested at angles of 0, 18 and 27 degrees using the original chute and blocks on which their back feet were set. It was found that an elevation angle of 18 degrees improved the passage depth compared to either 0° or 27° (see figure 13). While this will be further explored in a larger study, it forms the basis for the design of future trials.

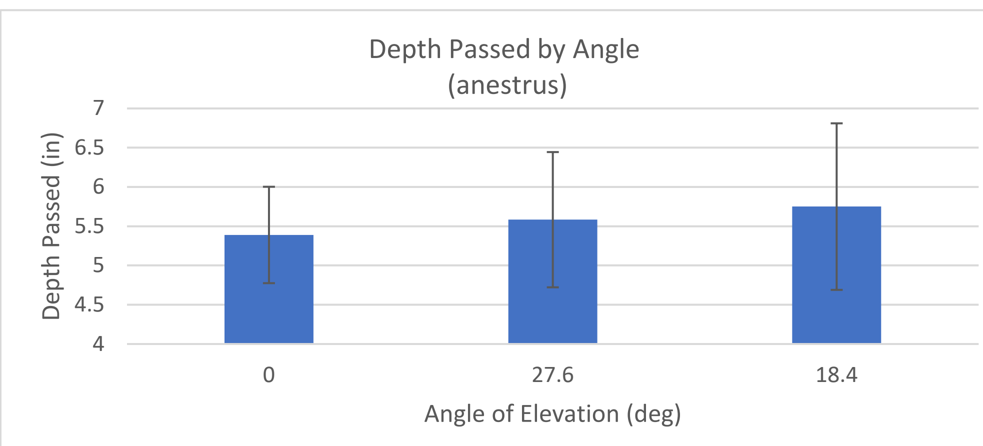


Figure 13: Effect of elevation angle on passage depth. Error bars indicate standard deviation.

Problems, Delays or Adverse Conditions

A brief explanation of non-ideal circumstances can be found below, with notes on how these complications can be avoided in future, and their potential impacts.

1) *Appropriate sheath sizing during the second in situ experiment:* a supply chain issue caused resulted in insufficient ¼ cc sheaths for the insemination. Therefore, ½ cc sheath and AI gun were used which was less than ideal (see pg 10 for details). While the ewes did not show any unusual distress behaviors, the tissue stretching may have triggered a negative inflammatory response thereby preventing pregnancy.
2) *Failure to synchronize:* A lack of response to synchronization drugs due to physiological unresponsive state (not "in season") the effective sample size was 5 animals. In future, breeding trials will be conducted in the autumn to take advantage of natural hormonal and behavioral cycles that improve fertility.
3) *Sample size:* Though the sample size of ewes, and the experimental timeline was limited due to grant monetary constraints, much was done with the resources to hand. Data in small sample sizes of breeding trials can be difficult to interpret due to natural biological variation.

Objectives for Next Grant

MPTI looks forward to pursing the next steps to bring this transformative technology to market. For Phase II of the grant, we will focus in 5 major areas, each focused in areas that will create a reliable, repeatable device to optimize pregnancy using a minimally invasive technique.

- Optimization of a delivery system of Avantaid™ (the commercial name developed for our product). While the plug utilized in this set of experiments was useful, there were some difficulties with positioning and preparation, therefore we will explore alternative methodologies and residence time.
- Sheep positioning to encourage the preferred angle discovered in Phase I. An insert for, or an entirely redesigned, chute will be created. In each case we will consider ease of use, safety, cervical position, ewe comfort, cost and portability.
- Synchronization techniques optimal for intrauterine insemination will be explored to ensure ovulation window, and to address seasonality challenges.
- A large breeding trial is necessary to commercially demonstrate this technology.
- Finally, we will explore the commercial aspects of this technology including pricing elasticity, methods to disseminate, market addressability and facility needs.

Conclusions

Study Outcomes

The objectives of success were achieved. Objective 1: passing of an AI rod through at least 1 of the cervical rings in 80% of the treated ewes. Within this study, the two final treatments produced cervical response which allowed an average of 75-100% passage into the uterine body/horn with ¼ cc *and* ½ cc AI rods. **A 100% passage into the uterine body was achieved 82% of the time.** This is unprecedented success in small ruminants. Objective 2: A successful delivery mechanism was developed.

Objective 3: a 35-day pregnancy rate of at least 40% in treated ewes will indicate success of the preliminary system. Pregnancy rates were 40% when considering ewes in estrus, but out-of-season. Overall the softening agent and method for delivery and ewe positioning are very successful; the knowledge gained about treatment efficacy and delivery, appropriate methods for positioning and restraining ewes for maximum ease during insemination, and the results of the rod passing should be used to design another study refining the system and method, hopefully with a larger population of ewes to be bred in the autumn.

APPENDIX A:

Market Impacts

Although the small ruminant market in the U.S. is underdeveloped compared to European countries, an increasing diversity in population, the need for sustainable livestock coupled with lower capital requirements will enable growth in the marketplace for the show animals, wool production, meat production and lamb producers. The United States has ~5.2 million sheep with ~2.96 million breeding ewes{8}. According to the Food and Agriculture Organization (FAO) of the United Nations, the U.S. sheep meat market was worth $151M-$178M/year, and in Europe $476M- $508M, over 2016-2020; the European goat meat and milk market together was worth in $202M in 2020 {4}.

The laparoscopic AI market is financially lucrative (~$60M annually) for the club lamb market{6}. It is estimated 60-80% of club lambs could be cervically AI'd providing a robust market for related technology; moreover, the ~7 million U.S. meat and milk ewes are candidates for such technology. Currently ewes are bred via LAPAI or live cover, and with this technology not only would we gain most of the ~0.6 million ewes currently LAPAI'ed (0.6 million LAPAI ewes at $100 per ewe = $65M){8} but commercial-type producers as well. Current cost of LAPAI inhibits a significant number of breeders; many smaller operations understand the genetic gain enabled by AI but cannot afford LAPAI. It is estimated a transition to cervical AI would save > $30 million dollars annually by reducing the AI costs{8}. Additionally, the benefits of AI on improving herd genetics and selective breeding for desired markets (club lamb, wool, milk production, etc.) can be brought to the small ruminant process quickly, which will positively impact sheep production globally.

The partnership with Crego Livestock enables a direct to market approach where technology can be effectively and efficiently distributed to the marketplace.

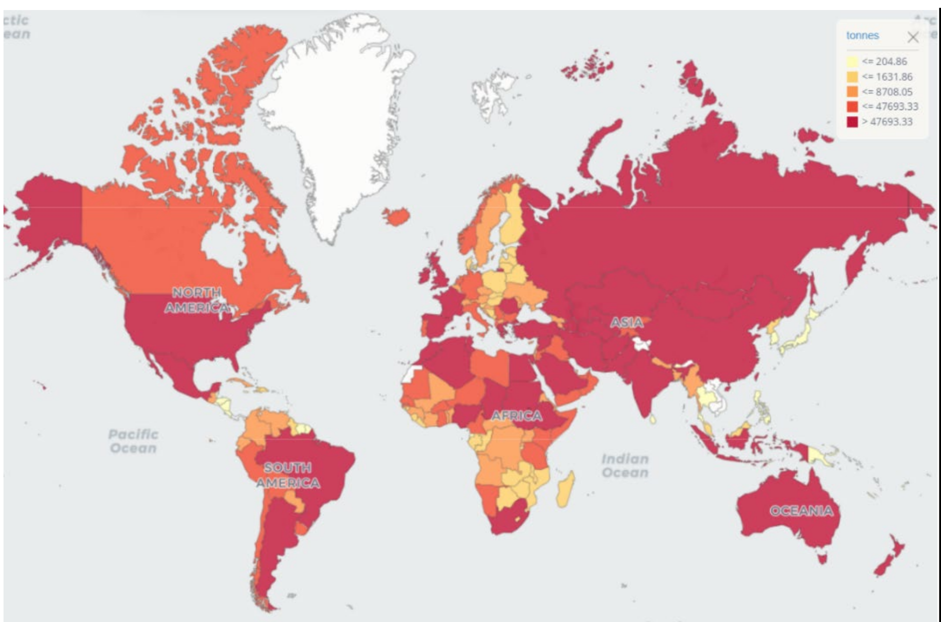


Figure 12: Sheep meat production in tonnes over the years 2000-2020. This map helps visualize the distribution of the market globally, illustrating the importance of this industry and the potential for widespread impact of any technology improving the genetic and breeding process for sheep producers. Source: FAO {8}.

Public Summary

Artificial insemination in small ruminants currently requires surgical intervention. MPTI aims to change this by the development of a method to enable insemination in the method utilized by cattle, swine and horses. The United States has ~5.2 million sheep with ~2.96 million breeding ewes{8}. According to the Food and Agriculture Organization (FAO) of the United Nations, the U.S. sheep meat market was worth $151M-$178M/year, and in Europe $476M- $508M, over 2016-2020; the European goat meat and milk market together was worth in $202M in 2020.

Through this grant, MPTI achieved the first step in cervical insemination, that is, passage of an artificial insemination rod into the uterine body without hormones or artificial chemicals 82% of the time in live animals. This result required development of a method to deliver treatment to the cervix, a protocol to enable biological response, and sperm compatibility. Finally, a small breeding trial was undertaken to demonstrate initial proof of efficacy.

The enablement of a low-cost method of insemination such as transcervical AI will have more than a $30 million impact in the U.S. sheep industry and will improve the reproductive health and wellness of sheep and goats.

Special Conditions

We declare that there were no special conditions on the use of the awarded funds.

Acknowledgements

MPTI is deeply grateful for the partnership with Crego Livestock, who have provided us with vital assistance in selecting and caring for our experimental animal cohort, whose freely-given wisdom helped shape experimental design and practice.

We would like to thank Double J Meat Processing (Ault, CO) Colorado Lamb Processors (Brush, CO) for providing us fresh bison and ewe reproductive tracts for our tissue experiments.

Finally, we thank NSIIC for the funding which made this study possible and for the opportunity to present our findings herein.

Budget

Please see attached spreadsheet for list of expenditures, and PDF for invoices and documentation.

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