EVALUATION OF A NON-TIMED ARTIFICIAL INSEMINATION PRACTICE APPLIED TO SUFFOLK EWES DURING EARLY BREEDING SEASON

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DEDICATION

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ABSTRACT

The objective of this study was to determine if the AM/PM artificial insemination procedure in cattle works in sheep. Detection of estrus was done using vasectomized rams that were fitted with marking harnesses. Once detection occurred ewes were artificially inseminated 12 to 24 hours after detection. Artificial insemination occurred from August 24 until September 12, 2014 where a total of 49 Suffolk ewe were inseminate. Determination of ewes successful artificially insemination was done by use of a ram fitted with a marking harness and use of ultrasound equipment at completion of the project. It was concluded that there is no difference between artificial insemination at 12 hours or at 24 hours after detection of estrous.

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INTRODUCTION

Artificial insemination (AI) allows producers to introduce genetics from superior sires into their herds or flocks. Historically, AI has had limited use in the sheep industry. Most producers purchase a sire and use the same sire repeatedly in the same flock, thereby reducing the genetic variability. They repeatedly use the same sire because of his success with offspring that produce traits that are wanted by the consumer. This in turn causes certain traits that may have once been important to be weeded out. These genetic traits that are being weeded out by producers are a concern of conservationists. Conservationists are concerned that livestock breeds will become extinct and all animals will begin to look the same due to these breeding practices (Blackburn, 2012). The solution to the genetic diversity problem is by using artificial insemination (AI) in the livestock industry. Producers can purchase semen from genetically proven individuals and still get the outcome they were wanting without losing genetics (Valergakis et al., 2010).

The problem with AI in the sheep industry is lack of practical applications in today's society. In order for AI to become practical there needs to be more research on the many factors affecting AI. Reasons that AI is not practical in sheep are because of all the equipment and experienced personal needed to do the procedure. The process of AI in sheep is more complicated in sheep than in cattle and requires a veterinarian to do the procedure. This can end up possibly cost more money.

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There are possibilities of artificial insemination becoming practical due to the ability of artificial insemination to improve genetics, service more ewes per individual ram than possible in natural breeding, reduce disease risk, and ability to acquire genetics from a ram that the producer does not own or have to transport (Innovis Ltd., 2014). According to Blackburn (2012), in order for their conservation efforts to come into effect there needs to be better insemination methods for sheep and that is the main reason why improvements in sheep AI procedures need to be studied in order to help with conservation of genetics.

Artificial insemination is commonly used in cattle dairies (Hockey et al., 2010). Unfortunately, transvaginal AI is not practiced with sheep due to anatomical differences when compared to cattle (Kershaw et al., 2005). Thus, surgical procedures are required for artificial insemination of sheep. This reduces that likelihood of many sheep producers using the technique. Both sheep and cattle undergo a similar estrous cycle (Senger, 2012). Cattle are typically bred 12 hours after displaying signs of estrus. This is commonly referred to as the AM/PM rule, where cattle showing signs of estrus in the morning (AM) are bred that evening (PM). Due to the similarity in their estrous cycle, practices such as the AM/PM rule of AI used in cows should apply to AI in sheep as well. According to the American Sheep Industry Association (2003), sheep should be artificially inseminated 12 to 18 hours after the sign of estrus. Estrus means standing heat where the female shows signs of receptivity to the ram. Standing heat can be detected in several ways such as behaviors that are expressed or by using a vasectomized buck, a buck that cannot breed the female, with a marking harness.

This study will look at how to improve AI in sheep using the AM/PM procedure used mainly in cattle AI protocol. Also because ewes are multiple ovulators the study is also looking at AI at 24 hours. Typically, hormonal estrous synchronization protocol is used in artificial insemination protocols. This study will rely on the presence of a vasectomized ram to initiate estrus. The main reason of this study is to determine the correct time at which a ewe should be artificially inseminated. If the cycle of the ewe is similar to the cow, then AI at 12 hours will be significantly different than AI at 24 hours.

OBJECTIVES

1. To determine if the AM/PM artificial insemination procedure in cattle works in sheep.
2. To improve efficiency of artificial insemination in sheep.
3. To determine the correct time that ewes should be artificially inseminate to ensure proper insemination will occur.

LITERATURE REVIEW

Estrous Cycle and Estrus

The estrous cycle of the ewe is comparable to that of the cow. The entire cycle occurs in order to produce a valuable egg to be fertilized. Estrous cycle averages 17 days in the ewe, a few days shorter than average cycle of a cow (21d). On day 0 the luteinizing hormone (LH), classified as a protein hormone and produced by the anterior pituitary, will surge. Ovulation occurs when oocytes, are released from the ovary when LH targets the theca interna and luteal cells of the ovary. Ovulation happens 20 to 30 hours after the LH surge. Growth and development of selected follicles and the development of the dominant follicle occur due to the production of LH. Follicle stimulating hormone (FSH) will remain low in the presence of a dominant follicle. While the LH surge occurs, inhibin limits the amount of FSH secreted. Granulosal cells of the ovary produce inhibin and target the anterior pituitary. Due to this the single follicle dominates and becomes the graafian follicle or dominant follicle. The graafian follicle produces the oocyte for ovulation. Granulosa and theca cells of the ovary will become the luteal cells of the corpus luteum at this time (Senger, 2012).

After ovulation, an increase in circulating FSH starts the recruitment of follicles at each follicular wave. Recruitment of follicles means acquiring the follicle that will become the dominant follicle. After recruitment, circulating FSH will decrease. Progesterone, a steroid hormone, will then be secreted by the cells of the corpus luteum. Since the follicle has ruptured estrogen secreted by the ovary will decrease. Progesterone has a negative impact on gonadotropin releasing hormone (GnRH). The hypothalamus produces GnRH and targets the anterior pituitary, which then stimulates either a decrease or increase in LH and FSH.

Decreasing amounts of GnRH being produced causes a decrease in LH and FSH. A functional corpus luteum will last from around day five until day eleven or 12, where peaking amounts of progesterone are produced. The low amounts of LH and FSH being produced prevents follicles from getting to the preovulatory stage. Prostaglandin F2 alpha (PGF2 α) comes into play, when fertilization does not take place (Senger, 2012).

When pregnancy does not occur, the signal of the trophoblastic protein cells of the fetus will not be present to tell the mother that she is pregnant. When there are no trophoblastic protein cells, prostaglandin E2 will not interfere with PGF2α binding sites. PGF2α, produced by the endometrium lining of the uterus, will lyse the CL by stopping the conversion of cholesterol to progesterone. This process is called lutelolysis. The CL must have progesterone in order to continue. Progesterone, digressing, will stop having a negative impact on the hypothalamus thus allowing for GnRH production to increase. Follicle stimulating hormone and LH will also begin to increase as well. FSH will target the granulosal cells of the ovary and allow for follicular growth which causes an increase in estrogen production. Estrogen will reach its highest level right before ovulation (Senger, 2012).

Estrogen, secreted by the follicle in the ovary, is the cause behind estrus or otherwise known as standing heat. Estrus only occurs for about 30 hours (Schoenian, 2012). The female now receptive to the male can become pregnant. Once again LH hits a peak and inhibin will inhibit FSH. This allows only one follicle to become the dominant follicle (Senger, 2012). In sheep, though, this cycle will be laid to rest for a certain period of time which is called anestrous.

Anestrous Period

Anestrous is the period in which the ewe is not able to become pregnant due to the fact that ovulation does not occur i.e. there will be no estrous cycle. This time period starts when daylight increases and goes until the end of summer. Ovulation does not occur because LH secretion decreases (Knights et al. 2014). In order for the current study to work, the ewes need to begin cycling again around the beginning to mid-August to ensure that all females will have a chance to be bred by AI during the time period of the study. Anestrous can be manipulated in many ways; many will use products such as an insert that it placed into the vagina called a CIDR which releases progesterone into the body which then will be taken out after a period of time and then given other hormones to get them into heat or other hormones can be administered on their own as well (Schoenian, 2012). This can be beneficial in many ways but there are other routes that can be used that are more natural. In the current study we will use a natural way to get the ewe out of anestrous, which is called the ram effect.

Ram Effect and Vasectomized Rams

The interval between ovulation and AI is more important than onset of standing heat to AI, therefore it is very important to be able to determine when standing heat is and thus, be able to approximate the time ovulation occurs, in order to increase the probability of fertilization (Hockey et al., 2010). Estrus is hard to determine in sheep because they have practically no outward signs of heat like other animals (Schoenian, 2004). In most studies done with artificial insemination they use timed AI due to the fact that this allows them to breed all the animals at one point and do not have to worry about detecting heat. Studies have noted that some of these hormones given to synchronize the time of AI though can cause more issues within in the animal such as follicle cysts as well as issues with public response to animal cruelty and worries about hormones in food products (Olivera-Muzante, 2011). In order to not have to use hormones to manipulate the estrous cycle there are management practices that can be used.

One of the ways that you can determine if the ewe is in standing heat is by using a vasectomized ram. A vasectomized ram is a ram that has had both ductus deferens cut so that spermatozoa will not be able to get into the ejaculate. Not only can they be used to determine standing heat but they can also help with controlling the estrous cycle of ewes. When the vasectomized ram is presented to a ewe pheromones are produced that can cause the ewe to start cycling. In a study done by Underwood et al. (1944), the "ram effect" was discovered. In the Underwood study they put a ram into a group of ewes, in which were going through a late seasonal anestrous (Perkins and Fitzgerald, 1999). According to Perkins and Fitzgerald (1999), once these females were around the male for a period of time they started to come into estrus and also around the same time.

In order for the "ram effect" to actually work the males and females have to be together or at least near each other for a period of time (Bedos et al., 2010). When first exposed to the ram, the females will only undergo a rapid surge of LH but only for a short amount of time (Bedos et al., 2010). For the actual ram effect to occur, males and females should be exposed to one another for at least three to four days. The first heat the female will go through is a silent heat that a male cannot sense. Some of the ewes will cycle around day 18 while many other will not until a later date around 25 days (Schoenian, 2012). Introducing a vasectomized buck into a herd of females for a time period before the study and during the study will help get the females cycling and determine if they are in heat. Once the ewe is back into heat AI can then occur once ovulation occurs. Although in sheep the anatomy is different than that of cattle so in order to understand how AI works in sheep the anatomy of the sheep must be discussed.

Anatomy of Reproductive System and the Artificial Insemination Protocol

The cervix on any female protects the uterus from the outside and any foreign matter that could potentially damage the uterus. When comparing the cervix of the ewe to the cow, the ewe’s cervix is extremely complicated and does not allow for AI through the cervix. There is a muscular tissue covering the wall of the lumen of the cervix. The muscular tissue constricts the cervix. The cervix is practically a maze in the ewe due to the corkscrew nature of the cervical rings. The opening of the cervix is very narrow and then is followed by another fold that does not line up with the opening thus creating a barrier for any AI instruments to be worked through (Leethongdee, 2009). The anatomy of the ewe’s cervix, as said before, differs from the cow. For one thing the cow, a much larger animal, has larger reproductive anatomy than the sheep. The next part is that the cervix is a lot larger due this fact and not as complicated as the ewe. Since the cow’s reproductive anatomy is larger it is possible through palpation to be able to maneuver the cervix around the AI pipette. The cervix must be bypassed due to this fact, so the use of laparoscopic AI does so (Schoenian, 2012). Although before artificial insemination can occur, heat needs to be detected and in this study the use of the AM/PM rule used in cattle is going to be explored.

AM/PM Rule

Many cattle producers use the AM/PM rule of thumb for AI'ing their cows. This states that if the female shows signs of estrus in the morning, then 12 hours later she will be artificially inseminated or if there are signs of estrus in the evening then 12 hours later in the morning this will occur. This is the optimum time window to inseminate after estrus in order for fertilization to occur (Hockey et al., 2010). This process should work in sheep because the best time to breed sheep is 12 to 18 hours after the onset of estrus (American Sheep Industry Association, Inc., 2003). Also having the ability to check for heat more than once a day, increases the likelihood of inseminating at the correct time. There are a lot of factors that are unknown as to why laparoscopic AI does not work so well in sheep. This study is going to focus on the time at which the sheep are artificial inseminated.

MATERIALS AND METHODS

The experiment was conducted at the Angelo State University Management, Instruction, and Research Center in San Angelo, Texas using 63 Suffolk ewes. All procedures were approved by the Institutional Animal Care and Use Committee prior to project initiating. Ewes that were used in the current study were all from the Angelo State University Management, Instruction, and Research Center flock. All ewes were fed the same nutrition of a mixed ration in the evening, and free choice coastal hay and water during the day. On August 5, 2014, two vasectomized Dorper bucks were placed with the ewes. This verified that the ewes started cycling in order to AI them. On August 19, 2014, the vasectomized bucks were fitted with marking harnesses. Observation of the buck with the ewes began at this point for the duration of the study twice a day, the first being in the morning and the second was 12 hours later. This ensured that identification of estrus was detected close to onset.

The ewes were put into a randomized design. Once the vasectomized buck showed attempts of breeding, designated by color marking on the back of the ewe, the ewes were moved to the barn where the breeding took place. The study started off with one group that was artificially inseminated 12 hours after they were marked but later changed to two groups where one was artificially inseminated at 12 hours and the other was artificially inseminated at 24 hours. This was changed when noticing some of the 12 hour group was not yet ready to AI. So the 24 hour group was added to see which was closer to the perfect timing of AI.

To determine if they were in the 12 hour or 24 hour group depended on if they were in standing heat in the AM or PM. Those that were marked in the AM were in the 12 hour group while those marked in the PM were in the 24 hour group. Artificial insemination occurred every evening unless there was a scheduling conflict. This was to help determine when the proper time was for artificial insemination. Artificial insemination was done from August 21, 2014 until September 12, 2014 when one of the pieces of equipment broke and there were no replacements.

In the current experiment, laparoscopic AI was used to ensure that proper insemination will occur, using frozen semen at the beginning. After noticing that the frozen semen was not as strong as the fresh semen, fresh semen was used for the remainder of the study. Those that were artificially inseminated by frozen semen were taken out of the results due to the fact that freezing semen can have an impact on the fertility of the semen and also did not want to have any more factors in the study. Sheep were withdrawn from food and water prior to this process. Once the procedure was done the ewe was held in a holding pen to ensure no extra stress would occur and the fertilization will take place if she was artificially inseminated in the evening. All ewes were put out to pasture in the am. This occurred until all ewes were inseminated. During the study, on September 5, 2014, the two vasectomized Dorpers were taken out due to the fact that they were borrowed from Texas A&M Extension Center and replaced with two vasectomized Rambouillets from the ASU herd.

Once all of the ewes were bred, excluding five during the study that were not ready to be artificially inseminated and the nine that were not artificially inseminated due to equipment failure, on September 15, 2014 a Suffolk ram fitted with a marking harness were placed with the ewes. Twice a week the ewes were observed for any markings and those that were marked were considered to be bred by the Suffolk rather than by AI. On October 17, 2014 the study concluded with the ultra-sounding of all the ewes that were artificially inseminated.

Data from this study was analyzed using one-way ANOVA procedure in SAS (SAS Institute, Cary, NC). Treatments were considered different at a level of $p \leq 0.05$. Variables to be analyzed were rate of pregnancy of ewes that were bred by laparoscopic AI at 12 hours and at 24 hours.

RESULTS

During the study, some ewes were excluded from the study due to their anatomy not being conducive to AI. Two of the 12 hour group ewes and three of the 24 hour ewes were taken out of the study. A few of the ewes taken out of the study were due to them being too young and their reproductive system was not fully developed. Others had problems with orientation of the reproductive anatomy and the rumen was in the way of being bred. The results from nine of the ewes were taken out of the study. These ewes were artificially inseminated with frozen sperm. All other ewes in the study were artificially inseminated with fresh sperm. The results will focus solely on those that were AI'd by fresh semen. At the end of the study 9 ewes were taken out of the study to due equipment failure.

The study compared AI at 12 hours to AI at 24 hours to determine the best time for AI. Conception was determine by a marking harness and ultra-sounding. There were 11 ewes marked by the clean-up ram that had actually been bred by artificial insemination in the 12 hour group. One of the non-bred ewes was not marked by the ram during the clean-up time in the 12 hour group. There were 7 of the 24 hour group that was marked that had actually been bred by AI. One of the 24 hour group ewes that were not bred by AI had not been marked.

Table 1 shows the conception rates and percent open after ultra-sounding for the 12 hour group and the 24 hour group. The 12 hour group conception rate was 78.57% and the 24 hour group conception rate was 68.75%. There was no difference between the two groups ($p>0.05$).

Table 1. Conception due to artificial insemination 12 and 24 hours after estrus.

	Treatment	
	12-Hour	24-Hour
n	28	16
% Preg	78.57	68.75
% Open	21.43	31.25

DISCUSION

There was no difference between ewes bred at 12 or 24 hours after estrus. This shows that ewes can be bred between those times and still have the same conception rate. Majority of the ewes in the study went into cycle within the first two cycles of estrous, this shows that the "ram effect," demonstrated in a study by Underwood et. al (1944), is conducive for determining estrus before use of AI.

Ewes that are too young had to be taken out of the study due to the fact that their reproductive system was not quite developed enough to be bred by AI. It is easier to find the ovaries after they have already had at least one offspring because they are more fully developed. This was seen during the AI procedure, when it was difficult to find the ovaries due to the size of the ovary and the ewe, thus AI should not be done on young ewes. The other ewes that were taken out of the experiment were ones that had their internal organs positioned in a way that finding the ovaries became very difficult. One of the ewe's rumen was positioned so that they were in the way of the ovaries. Also their rumen may have been too full at the time.

The final ewes that were taken out of the study were due to the use of frozen semen at the beginning. Studies show that fresh semen has a higher conception rate than frozen semen and can cause a significant difference in results. Donovan et al. (2004), suggested the difference was so different that the fresh conception rate was 82% and frozen only being 40%. In addition, frozen sperm once thawed will only have half as much viable sperm as the fresh (Donovan et Al., 2004). To avoid confounding the results of conception, ewes bred with frozen semen was excluded from the analysis.

The AM/PM rule in cows showed to work as well in sheep because they had a 78.57% conception rate. This is shown to be a lot better than some AI procedures done in cow who had a conception rate of 51.8% in a study where Co-Sync-CIDR was used (Kasimanickam et al., 2010). Ewes can actually follow the same protocol as cows because the estrous cycle is comparable to that of the cows. There may have been other factors as to why the conception rate of the cattle was less than ewes because of the use of hormones.

Comparing this protocol to others that use hormones to synchronize estrus, the conception rate of both the 12 hour group and the 24 hour group is a lot higher than a study that used a norgestomet implant with the addition of eCG and/or GnRH. The overall study showed to only have a 55% conception rate (Luther et al., 2006). Adding hormones to a regiment may cause extra outside problems that could cause for a lower conception rate. In another study using different prostalglandin-based protocols for AI and using cervical AI (different from the current study) had results ending from only 24% on up to 59% in conception rate, still showing that the current study had a higher conception rate both at 12 and 24 hour (Olivera-Muzante, 2011). This showed that laparoscopic AI does better than cervical AI as well as the non-use of hormones also helped with conception rates.

Overall in this current study there were some major achievements in learning about AI in sheep. Using hormones to synchronize artificial insemination may cause reasons as to why conception rates in AI may not be as high as natural. This study also shows that AI can be done anywhere from 12 hours to 24 hours after estrus without any harm to the conception rate.

IMPLICATIONS

Based on the results from this study, ewes can be bred anywhere between 12 and 24 hours after they come into heat. For individuals in the sheep industry this is very important because now they do not have to have a lot of workers at one specific time, this process can now be extended over a period of time. In addition, estrus synchronization is not necessary if a vasectomized ran us available for use of the “ram effect” for estrus detection. Further research should be done in order to determine if there is other factors that cause problems in the process of pregnancy during AI such factors could be breed, environmental, or physiological.

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