NOVEL GENES INDUCE UTERINE RECEPTIVITY: THE CHARACTERIZATION OF A SPECIFIC GENE PRODUCT IN THE EWE UTERUS

A Senior Honors Thesis

By

JENNIFER ANN DE GRAAUW

Submitted to the Office of Honors Programs
& Academic Scholarships
Texas A&M University
In partial fulfillment of the requirements of the

UNIVERSITY UNDERGRADUATE
RESEARCH FELLOWS

APRIL 2000

Group: Molecular Genetics

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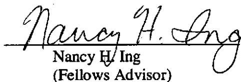
Approved as to style and content by: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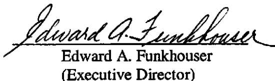
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ABSTRACT

Novel Genes Induce Uterine Receptivity:

The Characterization of a Specific Gene Product

in the Ewe Uterus. (April 2000)

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The normal environment of a sheep uterus is hostile and does not favor embryo growth. Through embryo transfer studies, it has been shown that the steroid hormones estrogen and progesterone allow the uterus to support and nurture developing embryos. However, genes important to uterine receptivity are relatively uncharacterized.

In previous experiments, the technique of Differential Display-Polymerase Chain Reaction was used to identify novel endometrial genes that are expressed in receptive versus non-receptive uteri. Uterine samples from Day 3 and Day 6 of the estrous cycle were examined. Embryo transfer studies indicate that an embryo can be transferred to a uterus that differs at most twenty-four hours. Therefore, a Day 3 embryo cannot survive in a Day 6 uterus and vice-versa. One product, DD5, was found specific to a Day 6 uterus. A partial cDNA fragment of approximately 230 bases was isolated. Northern analysis revealed that DD5 is approximately 900 bases long.

A complementary DNA library was constructed using both oligo-end labeling and random-prime probes involving the original DD5 fragment. A Bacterial Artificial

Chromosome was also used to sequence DD5. Expression of DD5 in the uterus was examined for Day 1, 3, 5, 6, and 7 of the cycle using uterine cross-sections that were radioactively labeled with the partial DD5 sequence. The expression in the glandular epithelium was noted. The pattern of expression of DD5 was compared to that of the estrogen receptor.

A partial sequence of the top strand of DD5 at the 3' end was determined. The position of the poly-A tail was confirmed, indicating that the sequence was at the 3' end. A database search indicated that the 3' region aligned with known ESTs. The complementary DNA library results were inconclusive. Expression of DD5 was high in a Day 5, 6, and 7 uterus, but not evident in a Day 1 or 3 uterus. The glandular epithelium region demonstrated the most significant expression. The glands closest to the lumen had the highest DD5 expression, with the superficial glandular epithelium having the highest.

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INTRODUCTION

With an increasing world population, new strategies for providing food must be developed. The sheep provides an excellent solution to this growing problem. This animal can provide both food and clothing from otherwise unusable forage. Most ewes can produce one lamb per year, but are capable of carrying up to 6 lambs in one pregnancy [1]. Furthermore, they are able to raise up to 4 of these lambs without assistance [1]. Since the gestation time for an ewe is only 140 days, there is much reproductive potential. In order to increase its production, fecundity must be increased. To accomplish this goal, the number of pregnancies per year or the number of lambs per pregnancy must be increased.

The normal environment of a sheep uterus is hostile and does not favor embryo growth. Through embryo transfer studies, however, it has been shown that the female sex steroid hormones, estrogen and progesterone, allow the uterus to nurture and support developing embryos [2]. Steroid hormone receptors transduce the signals from these hormones to form new gene products. Nevertheless, genes important to uterine receptivity are relatively uncharacterized. The long term goal of this project is to understand how these steroid hormones act on the uterus. Consequently, this knowledge can be used to develop techniques for increasing fecundity and thus increasing animal production.

The format of the Biology of Reproduction will be followed.

In previous experiments, the technique of Differential Display-Polymerase Chain Reaction (DD-PCR) was used to identify novel endometrial genes that are preferentially expressed in receptive versus non-receptive uteri. DD-PCR reverse transcribes and systematically amplifies the 3' termini of messenger RNAs with a set of oligo (dT) primers anchored to the beginning of the poly-A tail [3]. Uterine RNA samples from Day 3 and Day 6 of the estrous cycle of the ewe were examined. This cycle is seventeen days long and involves the rising and falling of the sex steroid hormones at different stages (Fig. 1). On Day 3, the sheep embryo first enters the uterus and the levels of both steroid hormones are low. On Day 6, the levels of estrogen and progesterone rise and the embryo begins the development of its tissues. Embryo transfer studies have shown that embryos may be successfully transplanted to a uterus that is at maximum only twenty-four hours out-of-sync with the embryo [2]. As a result, a Day 3 embryo cannot survive in a Day 6 uterus, and a Day 6 embryo cannot survive in a Day 3 uterus. This project's goal involves determining the differences in the endometrial gene expression that prevent such successful embryo transplants.

Thus far, 16 differentially expressed gene products have been identified through the use of DD-PCR. One product, DD5, showed significant expression in the Day 6 uterus as opposed to the Day 3 uterus. Consequently, DD5 is specific to a Day 6 uterus. The cDNA fragment generated is approximately 230 bases in length. This fragment was then PCR-amplified in the presence of an arbitrary decamer. The radioactively labeled PCR products were then separated on a denaturing polyacrylamide gel. By changing primer combinations, products for most of the cellular RNA are represented. Side-by-

side comparisons of samples from Day 3 and Day 6 were made, allowing the identification of this differentially expressed product. Northern analysis revealed the presence of DD5 in all Day 6 samples used and no expression in the Day 3 samples. Furthermore, the Northern analysis revealed that the length of the entire DD5 is approximately 900 bases. DNA sequencing revealed that this product is part of a novel gene sequence and had not been previously discovered. Consequently, its characterization represents the primary focus of this project.

FIG. 1. THE ESTROUS CYCYLE OF THE EWE.

A diagram representing the seventeen day estrous cycle is shown above. The levels of the sex steroid hormones, progesterone and estrogen, rise and fall throughout the seventeen days. On Day 3, the levels of both hormones are low and the embryo first enters the uterus. On Day 6, the embryo begins to develop its tissues and the levels of estrogen and progesterone begin to rise.

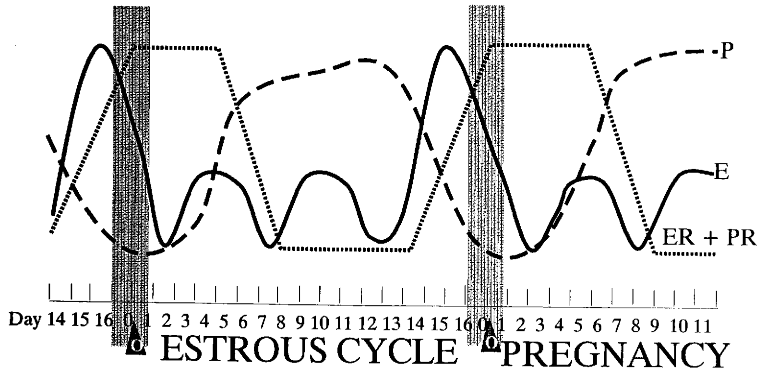

FIG. 1. THE ESTROUS CYCLE OF THE EWE

MATERIALS AND METHODS

Complementary DNA Library

Complementary DNA (cDNA) clones can be used to characterize gene products such as DD5. A set of cDNAs constructed from the messenger RNAs (mRNAs) of a tissue is referred to as a "cDNA library" [4]. The mRNA from a Day 15 cyclic ewe was used to synthesize a cDNA fragment through reverse transcription. DD5 is well-expressed in both Day 15 cyclic and pregnant ewe samples, justifying the use of a Day 15 uterine sample in the cDNA library. The mRNA is first hybridized to an oligodeoxythymidine primer. Reverse transcriptase then binds the primer and synthesizes the first strand of cDNA. Rnase H digestion then nicks the mRNA to leave the RNA primers. DNA Polymerase I binds these primers to synthesize the second cDNA strand. Finally, T4 DNA polymerase and ligase join the fragments of the second strand and polish the ends.

The cDNA fragment was then cloned into the EcoRI site of the vector, lambda bacteriophage. This type of vector infects and lyses *E. coli* cells. This cDNA sample was diluted by a factor of one hundred using SM buffer. Forty different samples containing ten microliters of this dilution and 600 microliters of the *E. coli* cells (XL1 Blue cells) were allowed to incubate for 20 minutes at 37°C. The lambda and the host cells were then grown on forty agarose plates for eight hours at 37°C. Lambda clones were identified as plaques on a lawn of the *E. coli*.

This cDNA library generated approximately 113,760 clones. Since a typical mammalian cell contains 34,000 different mRNAs, this library should contain the full-length DD5. The cDNA fragments embedded on the agarose were transferred to nitrocellulose filters (2 filters per agarose plate). Each filter was rinsed in 0.5 M NaOH and 1.5 M NaCl for two minutes to denature the fragment, 1.5 M NaCl ND 0.5 M Tris (pH 7.4) for five minutes to neutralize the fragment, and 2 x SSC for five minutes to rinse off any residue. Two primers from the original DD5 partial cDNA fragment were radioactively labeled using [^{32}P]CTP to construct oligo end-labeling probes. Each set of filters and a control strip containing increasing amounts of the cDNA and an unrelated plasmid (0.1 ng, 1.0 ng, and 10 ng) were incubated with hybridization buffer for one hour at 42°C. 1.5 milliliters of sodium pyrophosphate was also added to each set of filters. The oligo probes were then added and the filters were allowed to hybridize overnight at 42°C. Positive plaques were then detected using autoradiography and further identified using a secondary screening. The same procedure as above was utilized to determine a positive plaque.

A second cDNA library was also constructed using the same protocol. However, this screening involved random-prime labeling. The partial cDNA fragment (230 bases) was radioactively labeled using [^{32}P]UTP. Forty plates containing a Day 15 pregnant ewe cDNA and *E. coli* were screened. Approximately 40,000 clones resulted. Identified positive plaques were used in a secondary screening.

Screening Genomic Clone

The next method of approach involved the use of a genomic clone. A Bacterial Artificial Chromosome (BAC) was the vector used. Sheep genomic DNA was sheared and inserted into this vector. The partial cDNA fragment was radioactively labeled and used as a probe to find the genomic DD5 clone.

The BAC was first digested with the following combination of restriction enzymes in a total of fifteen different reactions: Eco RI, Bam HI, Hind III, Xba, and NOT I. Each enzyme was used individually and then along with each remaining enzyme to create both single and double digests. All fifteen reactions were then separated on a 1.0% agarose gel. Southern Blotting was then used to transform the fragments to a nitrocellulose filter. The resulting blot was rinsed in 2XSSC to remove any remaining agarose and then baked for two hours at 80°C. A radioactively labeled primer from the original DD5 partial cDNA fragment was used to construct a probe for screening for the BAC fragment that contained DD5. The blot was hybridized at 42°C for one hour. The probe was then added and the blot was allowed to hybridize overnight. The blot was exposed to film overnight. Positive bands on the film indicate that the digested fragment contains the desired DD5.

Subcloning

The DD5 fragment in the BAC is sub-cloned into a plasmid vector designed for expression. One hundred microliters of the BAC was digested with 5 μL of Xba, 30 μL of buffer, and 165 μL of nanopure water. The reaction was then precipitated with 0.3 M

sodium acetate and 3 volumes of ethanol. The vector, pBlueScript (2 μL), was digested with 2 μL of Xba, 4 μL of buffer, and 32 μL of nanopure water. Two microliters of Calf Intestinal Phosphatase (CIP) was then added. Both of these reactions were separated on a 1% low melt agarose gel. Eight bands were cut from the BAC reaction and one band was cut from the pBS reaction. These gel chunks were then melted at 70°C for ten minutes and then transferred to 37°C.

Seventeen ligation reactions were then prepared. The first reaction contained only 1 μL of pBS and 8 μL of nanopure water. The remaining 16 reactions contained each of the eight BAC bands (either 3 or 8 microliters), 1 μL of the vector, and 5 μL of nanopure water in the reactions containing the smaller amount of the BAC. A solution containing the following components was then added to each of the 17 reactions: 2 μL of 10X ligation buffer, 1 μL of T4 DNA ligase, and 8 μL of nanopure water. Each reaction was then placed on ice immediately and incubated at 11°C overnight.

Each reaction was then added to competent cells (JM 83) and grown on LB/ampicillin plates overnight at 37°C. For blue/white colony color selection, 4 μL of IPTG and 40 μL of x-galactose were added. Successful transformations were selected by identifying colonies white in color.

Each single transformed colony was then purified using the Quiagen Plasmid "Miniprep" kit. Twenty-six mini-preps were made. Restriction enzyme digestion using Xba was used to identify colonies that contained the desired clone. All twenty-six digests were separated on a 1% agarose gel.

Sequencing Subclones

Two mini-preps that were successful clones of one of the BAC bands that was positive on the radiography were chosen for sequencing. Each mini-prep was used to construct two reactions containing the following: 2μL of mini-prep, 2 μL of either a universal Forward or Reverse primer, 2 μL of Big Dye (Genetic Technologies Laboratory sequencing dye), and 1 μL of nanopure water. PCR was then used with the following cycles (30 cycles total): 96°C for ten seconds, 42°C for five seconds, and 60°C for four minutes. The resulting samples were then submitted to Genetic Technologies Laboratory for sequencing.

Other Sequencing Techniques

In addition to the subclones, other techniques were used for sequencing. The BAC was sequenced using two universal primers and five different primers synthesized for DD5 from the original partial fragment. In each of these seven reactions, the following components were combined: 16 μL of Big Dye, 2 μL of the primer, 5 μL of the BAC, and 17 μL of nanopure water. In addition to sequencing the BAC, three different plasmid preparations of DD5 were sequenced using the same two universal primers as with the BAC sequencing. Each of these plasmid reactions contained the following components: 2 μL of the plasmid, 2 μL of the primer, 2 μL of the Big Dye, and 1 μL of nanopure water. Bio Rad spin columns were used to further purify all of the BAC and plasmid reactions before they were submitted to Genetic Technologies Laboratory for sequencing.

Database Search

Using the sequence results obtained from Genetic Technologies Laboratory, the databases currently available on the internet were searched. A BLAST search (Genbank website) was performed on the original 230 base fragment and Expressed Sequence Tags (ESTs) were examined. The entire sequence obtained was also submitted to this same database for examining ESTs. Furthermore, the ORF Finder was used to locate possible open reading frames and to determine possible amino acid sequences.

Analysis of Uterine Expression

Slides containing uterine cross sections from various days of the estrous cycle were examined for the expression of DD5. In situ hybridization was used to detect the presence of DD5 in Day One, Day Three, Day Five, Day Six, and Day Seven samples. The same uterine cross sections were examined for the expression of the estrogen receptor. Since the expression of this product has a different pattern than that of DD5, these results serve as a control group for examining DD5 expression. In both the DD5 and the estrogen receptor screenings, the antisense and sense probes were both labeled using [^{35}S] UTP. Sections were counterstained with hematoxylin and mounted with Crystal Mount. Slides were developed after one month of autoradiography. The following ten uterine cell compartments were examined: luminal epithelium (LE), caruncular epithelium (CE), caruncular stroma (CS), stratum compactum (SC), deep glandular epithelium (DGE), middle glandular epithelium (MGE), superficial glandular epithelium (SGE), middle stroma (MS), deep stroma (DS), and myometrium (MY). The

expression of both DD5 and the estrogen receptor were measured in each of the three types of glandular epithelium (DGE, MGE, and SGE) in Day 3, 5, 6, and 7 of the cycle. Qualitative measurements were performed by examining the autoradiographs for each gene product throughout the span of the seven days of the cycle. Comparisons of the expression between the different uterine compartments for each day were made by using the software program ScionImage to measure the density of the labeled dark grains of the tissue sections. The same portion of each section of tissue was examined when comparing the tissue labeled with the sense probe versus the antisense probe.

RESULTS

Complementary DNA Library Inconclusive

The initial cDNA library (oligo-end labeling) resulted in twenty-nine possible positive plaques. All twenty-nine of these positive plaques were rescued from the original agarose plates and used for a secondary screening. From this screening, no positive plaques were identified. The control strips were positive for both screenings, indicating that the hybridization was successful. Consequently, the cDNA library that was screened with oligo-end labeling probes was inconclusive.

The second cDNA library was screened with random-prime labeling probes. It produced one possible positive plaque. This plaque was also rescued from the original agarose plate and used in a secondary screening. No positive plaques were identified in this secondary screening. In both screenings, the control strips were positive, indicating a successful hybridization. Consequently, the random-prime labeling cDNA library was inconclusive.

Location of Genomic Clone

The BAC was digested with different restriction enzymes and then transferred to nitrocellulose using Southern blotting. A radioactively labeled DD5 probe was then allowed to hybridize with the different BAC fragments. The resulting audioradiograph indicates that the BAC contained DD5 (Fig. 2). The fourth lane on the left on the blot was selected for use in the sub-cloning portion of the project. It represents the Xba single

digest of the BAC. Because the signal is extremely high, it contains an adequate amount of DD5.

FIG. 2. SOUTHERN BLOT RESULTS FOR SCREENING THE BAC.

The audioradiograph of the gel used to digest the BAC using restriction enzymes is shown above. A DD5 probe radioactively labeled with [^{32}P] CTP was used to screen the different fragments. From left to right, the lanes represent the following digests: Eco RI, Bam HI, Hind III, Xba, Eco RI and Bam HI, Bam HI and Hind III, Eco RI and Hind III, Eco RI and Xba, Bam HI and Xba, Hind III and Xba, Not I, Not I and Eco RI, Not I and Bam HI, Not I and Hind III, Not I and Xba. The single Xba digest signal was strong so Xba was the restriction enzyme selected to be used for sub-cloning.

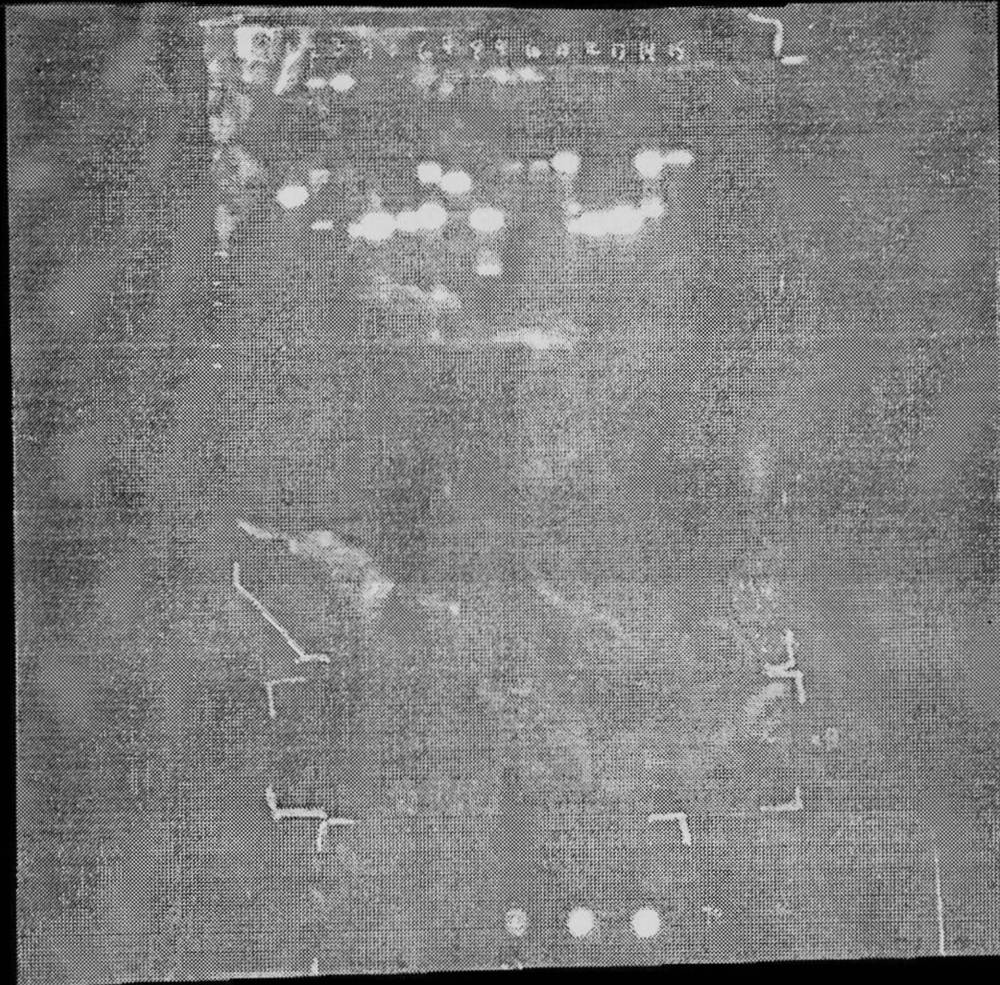

FIG. 2. SOUTHERN BLOT RESULTS FOR SCREENING THE BAC

Sequencing Results

The initial DD5 cDNA fragment isolated from the Differential Display-PCR experiment was known to be close to the poly-A tail. The early sequencing results of the partial cDNA fragment revealed a long string of adenines at the 3' end of the sequence. By sequencing the genomic clone, this adenine stretch was confirmed to be the poly-A tail. Since a poly-A tail is added post-transcriptionally, it will not be present in the sequence of the genomic clone. The results of the BAC sequencing lacked this long stretch of adenines, thus confirming it was in fact a poly-A tail. Furthermore, the sequence of the top strand was determined. Once again, the genomic clone sequence was used to confirm this fact.

The sequence at the point of this publication is 857 bases long (Fig. 4). BLAST searches were performed using Expressed Sequence Tags (ESTs). The 3' region of this sequence had very close alignments to ESTs currently listed in this database. The closest alignment of this region was with the EST numbered AC010210 (human kidney) (Lab on Web). This region near the 3' end represents the original DD5 fragment that was used as a probe.

The Northern analysis indicated that the length of the entire DD5 sequence is approximately 900 bases. The DD5 sequence in Figure 4 is 857 bases. However, the database searches indicate that a major portion of this sequence may be due to the presence of an intron. There were no significant alignments with ESTs along the major portion of this sequence. Only the region near the 3' end aligned with known cDNA's in the databases.

FIG. 3. CURRENT DD5 SEQUENCE.

The DD5 sequence at the time of publication is shown. This sequence is 857 bases in length as opposed to the approximately 900 bases predicted by the Northern results. The presence of the poly-A tail and the top strand orientation is confirmed through the BAC sequencing results. The 3' region represents the original DD5 sequence obtained. It aligns with ESTs found in current databases. The 5' region is suspected to be part of an intron. Sequencing results were obtained from Genetic Technologies Laboratory.

5'cca ctt taa aat ggc aga ctt ggc act tta ggc ctt atg caa aac
ttg cct gat gtt ttt tga aaa ctt tgg cac tta ggc tag ggg aga gag
gga cag att gtg tat gct aat cag ggt cat cta aga agc tca tta
aaa cag tga tta ctg acc cca ctc cca taa ttg ctc tgc tgg gca
ggg gag aaa gac atg aga att tga att ttt caa aag ttc cta aac
agt gct aat gtg gca gct ttg ggt acc aaa att tga gaa act ggg
tgt agc atg cgt ttt gga ggt ggg agc cac taa ggt ggt gtg gga
cgg gtc ggc att tgt gag atg tat agc atg acc tgg gtt att tgt
ctc aaa gac ctg tgc cct cta cgt ccc cag agg tgt gca tgc tca
ggt ctg gtt att ttg aac taa taa ggg gtg aga cct ctg atc tag
aaa gat ctg gct ggg ata agc tcc ctg aat ttc cag att ctc ctg tct
cct tcc ttt ccc agg tgc tgg gcc tgg tgc ggg tgc ccc tgt ata
ccc tgc gag atg gtg tgg gag gcc tgc ctg cct tcc tgg aga ata
cct tta ttg gaa atg cac ggg aac agc tgg tgg aag ccg tcc aga
acc tgg gac tgc tgg aac ctg gct ctt ttg cac acc tta aga ttt
caa ctc ctc cct aga ggc agc cct cca tgg acc cat gaa acc tgc
ctg aga tca gcc ctt tgt act ggg gag gca ggg agg aaa aag
gga atg ttt tct ctt ctg ggc ctg ggc tgc ata gat gat aac aga tta
aaa gag tat gta ata tat tct gag cag agg gac tca tgg cat caa
tgg caa aaa tct gca gct cat ccc ggg ggc ctg aca ttc tca gag
act tcc tct ttt ctt tat att tgt gca cac gct tcc ctg gtg gct cag
aca gta aaa aat cca ctt gta gtg cgg gag acc tgg gtt cta tcc
ttg ggt tgg taa gat ccc ctg gag gag gca tgg caa ccc act cta
gta ttc ttg gct cga gaa tcc cca tgg gca gag gag cct tgc gtg
cta cag tcc atg ggg tca caa agc gnt gga cac anc tga nca
act aag cag aca cat taa gct gat gcc ttt tct **3'**

FIG. 3. CURRENT DD5 SEQUENCE

Furthermore, protein databases were used to determine possible amino acid sequences for this sequence as well as to determine if there were any significant alignments with known proteins. The ORF Finder was used to find possible open reading frames and determine the resulting amino acid sequences. The most significant result involved the 396 base region that spanned from base 460 to base 855. Alignments were found with the LAG-3 protein precursor in humans (Lymphocyte Activation Gene) and with β-galactosidase of Arthrobacter.

DD5 Expression High in Glands

Throughout the estrous cycle, the uterine glands demonstrated pronounced expression of DD5. Although all of ten of the uterine compartments were examined for expression, the three types of glandular epithelium (DGE, SGE, and MGE) showed the most dramatic expression. Consequently, the results of the expression in these three areas were measured for Day 3, 5, 6, and 7. The expression of DD5 was the highest in the superficial glandular epithelium as compared to the other two types of glandular epithelium. On the other hand, the expression of the estrogen receptor increased as the glandular epithelium approached the myometrium (the deep glandular epithelium had higher expression than the superficial glandular epithelium).

DD5 Expression Throughout the Estrous Cycle

DD5 is not expressed in a Day One uterus and a Day Three uterus. Expression of DD5 increases on Day Five. This expression increased through Day Six and Day Seven.

On the other hand, the expression of the estrogen receptor has a different pattern. Expression is significant for A Day One and Day Three uterus, unlike DD5. Also, expression increases in the Day Five, Six, and Seven. The expression of DD5 in a Day One uterus is shown (Fig. 4), as well as the expression of the estrogen receptor on the same day of the cycle (Fig. 5). Furthermore, the expression of DD5 in a Day Six uterus (Fig. 6) differs from the expression of the estrogen receptor on this same day (Fig. 7). On the days that DD5 is expressed, the expression increases as the glands approached the lumen (the superficial glandular epithelium expressed DD5 more than the deep glandular epithelium). The expression pattern of the estrogen receptor has an opposite pattern. Expression is higher in the deep glandular epithelium than in the superficial glandular epithelium.

FIG. 4. EXPRESSION OF DD5 IN THE GLANDULAR EPITHELIUM ON DAY ONE

The expression of DD5 in the glandular epithelium of a uterus on Day One of the estrous cycle is shown. The cross section of the uterus was radioactively labled using [^{35}S] UTP. Hybridization with the DD5 probe is indicated by black grains on the slide. The tissue is stained with hematoxylin. On this day of the estrous cycle, there is no significant expression of DD5.

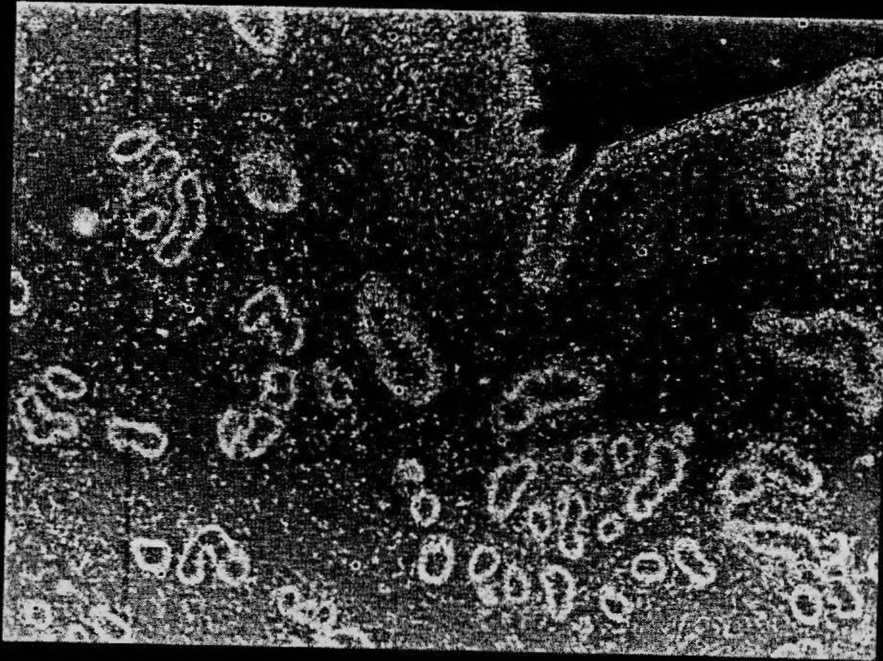

FIG. 4. EXPRESSION OF DD5 IN THE GLANDULAR EPITHELIUM ON DAY ONE

FIG. 5. EXPRESSION OF THE ESTROGEN RECEPTOR IN THE GLANDULAR EPITHELIUM ON DAY ONE

The expression of the estrogen receptor in the glandular epithelium of a ewe uterus on Day 1 of the estrous cycle is shown. The cross section of the uterus was radioactively labled using [^{35}S] UTP. Hybridization with the estrogen receptor probe is indicated by black grains on the slide. The tissue is stained with hematoxylin. Expression of this gene product is high on this day of the cycle. As the glands progress outwards from the lumen, the expression increases. The deep glandular epithelium has greater expression of the estrogen receptor than the superficial glandular epithelium.

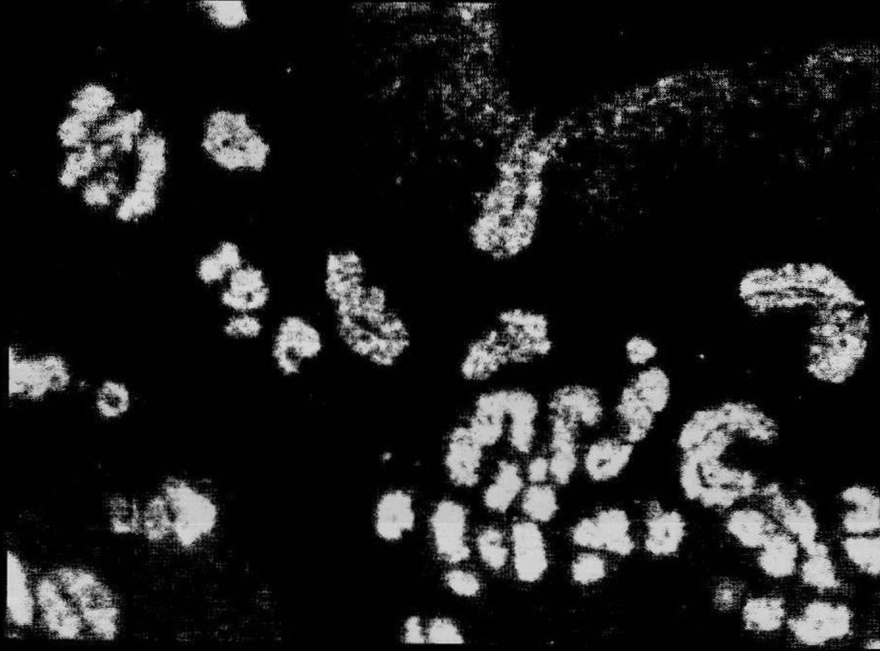

FIG. 5. EXPRESSION OF THE ESTROGEN RECEPTOR IN THE GLANDULAR EPITHELIUM ON DAY ONE

FIG. 6. EXPRESSION OF DD5 IN THE GLANDULAR EPITHELIUM ON DAY SIX

The expression of DD5 in the glandular epithelium of a uterus on Day 6 of the estrous cycle is shown. The cross section of the uterus was radioactively labeled using [^{35}S] UTP. Hybridization with the DD5 probe is indicated by black grains on the slide. The tissue is stained with hematoxylin. Unlike the expression in Day 1, DD5 is highly expressed in the glandular epithelium on this day. As the glands approach the lumen, expression of DD5 increases. The superficial glandular epithelium has a higher expression of DD5 than the deep glandular epithelium.

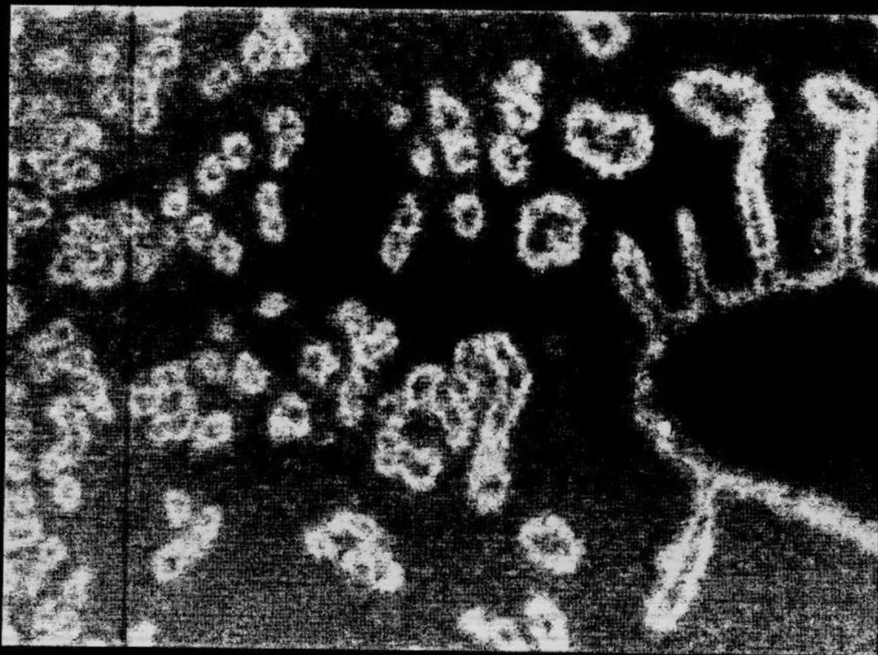

FIG. 6. EXPRESSION OF DD5 IN THE GLANDULAR EPITHELIUM ON DAY SIX

FIG. 7. EXPRESSION OF THE ESTROGEN RECEPTOR IN THE GLANDULAR EPITHELIUM ON DAY SIX

The expression of the estrogen receptor in the glandular epithelium in a Day 6 uterus is shown. The cross section of the uterus was radioactively labled using [^{35}S] UTP. Hybridization with the estrogen receptor probe is indicated by black grains on the slide. The tissue is stained with hematoxylin. Unlike Day 1, expression of the estrogen receptor is low on this day of the estrous cycle.

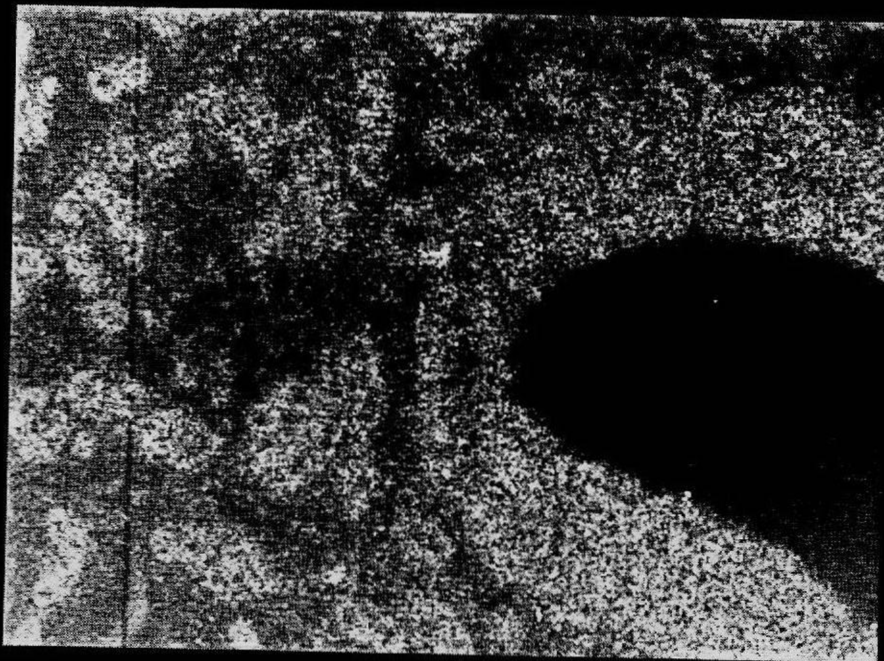

FIG. 7. EXPRESSION OF THE ESTROGEN RECEPTOR IN THE GLANDULAR EPITHELIUM ON DAY SIX

SUMMARY AND CONCLUSIONS

In summary, the role of estrogen and progesterone is critical to the development of embryos in the uterus. The environment of the uterus is normally hostile and does not support growth. However, due to the action of these steroid hormones, the uterine environment changes to that of a nurturing host. Differential Display-PCR was used to identify genes expressed such a receptive uterus as compared to a non-receptive uterus. One of these products, DD5, was found to be specific to a Day 6 uterus. This day represents the point at which the levels of estrogen and progesterone begin to rise and the embryo begins to develop its tissues.

The aim of this project was to characterize this gene product. The partial sequence of it was obtained. Databases currently available reveal that the region near the 3' end of this sequence aligns with ESTs in the databases. Furthermore, the sequencing results indicate that the sequence is near the 3' region of the entire DD5 since the presence of the poly-A tail was confirmed. The top strand orientation of this sequence was also confirmed. Software used to determine open reading frames demonstrated that the same region near the 3' end of the sequence contains an open reading frame that aligns with some found in current databases.

DD5 is expressed primarily in the glandular epithelium of the uterus. Expression increases as the glands approach the lumen. Furthermore, the expression of DD5 was examined in samples from a Day 1, 3, 5, 6, and 7 uterus. No expression was detected in a uterus from Day 1 and Day 3 of the estrous cycle. Expression was significant in a Day 5

uterus and increased in a Day 6 and Day 7. Consequently, the expression of DD5 is found in the glandular epithelium primarily on Day 5, 6, and 7 of the cycle.

This investigation provides many potential applications. By understanding the role steroid hormones play in supporting embryo growth, sheep production can be optimized. Steroid therapy can be used to increase fecundity by increasing the number of lambs per pregnancy. Likewise, steroid therapy can also be used to extend the breeding season and to start younger ewes cycling earlier. Finally, it can be used to reduce embryo mortality in the early stages of pregnancy.

Furthermore, the DD5 gene product may be possibly used as a fertility marker in sheep. Like the LIF protein in the mouse, it could provide and important component of embryo culture so that in vitro manipulations can be more successful for embryos of higher mammals. From those possible applications, it is easy to see the potential in this investigation.

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VITA

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