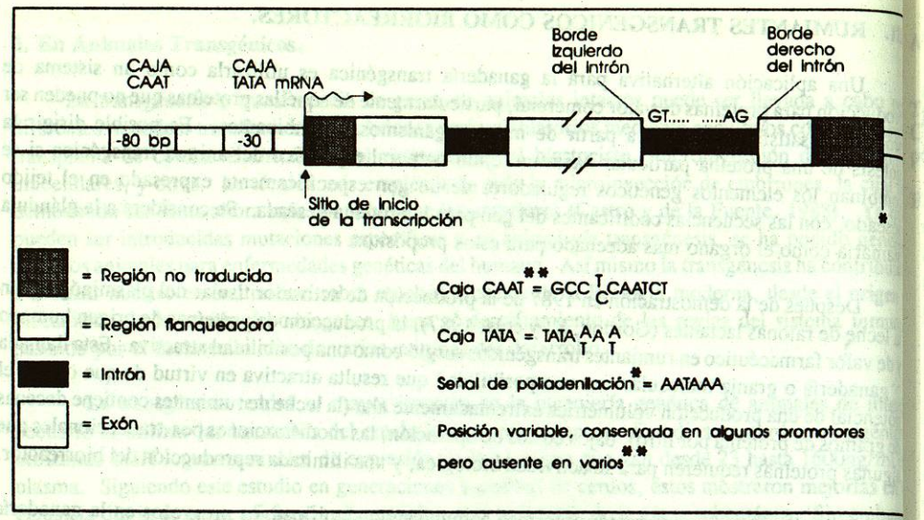

Figura 4. Estructura Molecular de un gen discontinuo modelo. Se esquematizan los principales elemento que componen un gen proteína, típico de mamíferos.

del gen oBLG. El nuevo gen híbrido, empieza con el promotor y elementos de control de la expresió derivados del gen oBLG, a los que le siguen: (1) la región 5' no-traducible de oBLG; (2) la región 5 no-traducible del gen FIX humano; (3) codon de iniciación de la traducción y codones subsecuent requeridas para la síntesis de FIX; (4) codón de terminación de la secuencia codificante para la proteí FIX; (5) secuencias 3' no-traducibles del gen FIX hasta antes de la señal de poliadenilación; (6) regió 5' no-traducible y resto de las secuencias para la proteína oBLG. Para mayor claridad, este complicad arreglo de elementos genéticos se ilustra a continuación:

5'-	Promotor oBLG	5' no-traducible oBLG	5' no-traducible FIX	codones y 3' no-traducible FIX	3' no-traducible oBLG	resto gen oBLG	-3

El gen quimérico se microinyectó en el pronúcleo de cigotos unicelulares de ovinos, lográndo generar cuatro animales transgénicos: dos machos y dos hembras. Se estudiaron a las hembras, l cuales portan alrededor de 10 copias por célula del gen quimérico cada una; éstas fueron apareadas p separado y cada una dió nacimiento a un cordero, los cuales también heredaron el transgen quimér BLG-FIX. Durante la lactancia se colectó leche de cada oveja madre y ésta se analizó para determi la presencia de FIX de origen humano. Este se detectó por radioinmunoanálisis en la leche de amb hembras aunque a muy bajos niveles; aproximadamente 25ng/ml, es decir alrededor de 250 vec menos que la concentración normal del plasma humano. Es de esperarse que el refinamiento de l manipulaciones y construcciones genéticas requeridas para este tipo de sistemas de producció conduzcan a niveles mayores de producción (Clark, A.J. y cols. 1989).

Cuando se logren niveles de eficiencia de expresión comparable para bovinos se estaría hablando de producir, a partir de una sola vaca transgénica productora de 5 galones de leche dirarios, en el orden de 20 g de la proteína recombinante de interés al día.

2. Producción transgénica de activador tisular del plasminógeno humano en la leche de cabras.

Una segunda proteína de origen humano escogida para producirla por un rumiante bioreactor, es el activador tisular del plasminógeno, proteasa de uso clínico, pero cuya producción actual es costosa. Ha sido demostrado que esta proteasa disuelve efectivamente los coagulos de fibrina responsables de oclusiones coronarias.

Para generar cabras transgénicas, los embriones fueron recuperados lavando oviductos asociados con ovarios ovulados, através de una cánula y colectándolos en cajas de petri. Estos fueron microinyectados con el ADN complementario codificante para una variante glicosilada de acción mas prolongada del activador tisular del plasminógeno, bajo el control transcripcional del promotor del gen de la proteína ácida del suero de la leche.

De 29 crías obtenidas de 36 hembras a los que se les transfirieron quirúrgicamente a sus oviductos o úteros los embriones microinyectados, después de cultivarlos en el laboratorio por 72 horas, el transgen se detectó en una cría macho y otra hembra.

La cría transgénica hembra a tenido descendientes en dos ocasiones y de un total de cinco crías, una resultó transgénica. La leche colectada através de su lactación contenía activador tisular del plasminógeno de origen humano con actividad amidolítica comparable al material derivado de sistemas de producción basados en cultivo celular, pero a concentraciones de tan solo unos cuantos miligramos por litro. El diseño de un protocolo simple de purificación les permitió a los investigadores recobrar la proteína recombinante con un grado de pureza electroforética del 98% y un rendimiento total del 25%. Aunque las caracterizaciones de dicha proteína arrojaron diferencias en varios parámetros con respecto a la proteína producida mediante el sistema de producción convencional de cultivo de céluals de ratón, tales como el patrón de glicosilación, los efectos de dichos cambios en la farmacocinética, inmunogenicidad y otros, permanece aun por determinarse. Mas recientemente, se construyó un sistema de expresión usando el promotor del gen de la ß-caseína caprina, así como sucuencias flanqueantes de este gen, para expresar también la región codificante para el activador tisular del plasminógeno de origen humano, obteniéndose muchos mejores niveles de producción (Ebert y cols., 1992).

CONCLUSIONES Y PERSPECTIVAS

La década de los 90's se inicia con renovadas confianza y esperanza en la Biotecnología Anim Después del éxito de la Biotecnología Molecular Industrial, que aporta ya varias proteínas recombinant al mercado, ahora las granjas moleculares ofrecen producir valiosos agentes terapéuticos en grand volúmenes y a bajos costos; sin duda que los rumiantes de granja jugarán un papel central en esta nue era de la Biotecnología Animal.

Muchas son las posibilidades que se desprenden de la manipulación del genoma animal. S contemplan entre otras: 1) Nuevos variedades con mayor velocidad de crecimiento y eficiencia e conversión alimenticia, 2) Animales resistentes a plagas y parásitos. 3) Animales biorreactore produciendo en su sangre o leche grandes cantidades de agentes terapéuticos, 4) Animales co importantes mejoras en sus capacidades metabólicas que les permitan por ejemplo degradar la celulos 5) Animales mejor adaptados al medio, 6) Animales con carne mas magra, etc.

Paralelamente a estas manipulaciones ya en marcha, mucho se beneficiará la nueva Biotecnolog Animal de las mejoras y desarrollos futuros de las tecnologías para generar y cultivar embriones in vit y de las de partición y clonación de embriones. Tambien muy importante serán los trabajos qu actualmente se realizan en el reemplazo de genes por recombinación homóloga en células embrionari pluripotenciales cultivadas in vitro, que luego se reincorporan en blastocistos.

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ETIOPATHOGENESIS OF EPIDIDYMITIS IN RAM LAMBS

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Introduction

Ram lamb epididymitis (LE) occurs primarily in virgin ra from 6 to 18 months of age and is caused by a variety of facult tive anaerobic, gram-negative bacilli in the family Pasteurell eae.[1,3,4,7,9,11,19,20,28,30,33,36,38,39] In the early 1980's, was described in ram-producing flocks in the western Unit States as a distinct disease entity from ram epididymitis (R which occurs primarily in sexually mature rams and is caused Brucella ovis, an obligate aerobic, gram-negative coccobacillu Both LE and RE cause infertility in affected rams, resulting significant economic losses to the sheep-producing industr Although the etiologic agents and pathogenesis of the two di eases are markedly different, both LE and RE induce epididy and testicular lesions, which are indistinguishable by palpati and gross pathology.[7]

The purposes of this report are (1) to provide an overview LE with a historical review of the primary etiologic agents the family Pasteurellaceae; (2) to update the taxonomic status Actinobacillus seminis and the Haemophilus-Histophilus group; a (3) to characterize the phenotypic and biochemical properties three new LE organisms that includes two Actinobacillus-li organisms and a capnophilic gram-negative bacillus, which we identified in our laboratory.

An Overview of Ram Lamb Epididymitis

Historically, five bacterial species and several organis with phenotypic and biochemical characteristics of the fami Pasteurellaceae have been associated with LE.[3,4,9,11,19,28, A. seminis and Histophilus ovis are the two most frequent isolated organisms, while A. actinomycetemcomitans, Haemophil agni, H. somnus, and several facultative anaerobic, gram-negati bacilli, which are frequently referred to as Actinobacillus-li organisms or Haemophilus-like organisms, are occasionally isol ed from either the epididymal lesions or semen of affected ra With the classification and taxonomic relationships of H. agn H. ovis, and H somnus being uncertain, these organisms wh isolated from LE are commonly referred to as the Haemophilu Histophilus group. Other organisms which are occasionally is lated from rams with epididymitis include Actinomyces pyogene Bacteroides species, Corynebacterium pseudotuberculosis, Escher chia coli, Pasteurella species, Staphylococcus species, a Streptococcus species. These organisms, however, are not consi ered specific causative agents of epididymitis in rams.

A. seminis was first reported as a new bacterial species in 1960, while H. ovis, H. agni and H. somnus were first reported in 1957, 1964, and 1969, respectively.[2,3,17,23] The A. seminis isolate was from a ram with epididymitis; the H. agni and H. ovis isolates were from other ovine disease conditions; and the H. somnus isolate was from a bovine infection. Subsequently, H. agni, H. ovis as well as several Actinobacillus-like and Haemophilus-like organisms were isolated from either epididymal lesions or semen from rams with epididymitis.[4,7,9,11,19,20,28,33,39] These gram-negative pleomorphs, which are considered to be either permanent or transient flora on the mucous membranes of the reproductive tract of young rams, cause ascending infections of the epididymes and occasionally the testicles and accessory sex organs.[35] The epididymal and testicular lesions develop most frequently during coldest periods of winter; however, specific factors that predispose the male reproductive organs to these organisms have not been clearly delineated. Regardless of the specific organism associated with LE, acute and chronic epididymitis and orchitis with infertility are the principal clinical manifestations.[7] The effect on fertility may extend to complete sterility and is dependent upon the severity of infection, contamination by inflammatory and bacterial products, testicular involvement and semen production, patency of the genital ducts, and whether the infection is bilateral or unilateral. In yearling rams, the granulomatous lesions induced by A. seminis and H. ovis develop twice as frequently in the tails of the epididymis than as in the heads of the epididymis; however, distribution of epididymal lesions induced by A. actinomycetemcomitans, H. agni, the Actinobacillus-like organisms, and the Haemophilus-like organisms have not been clearly delineated.[30,36,38,39] Some affected animals will have a severe orchitis with purulent exudate, but without any gross apparent involvement of the epididymes.

Today, LE is being reported in the major sheep-producing areas of the world, particularly in ram-producing flocks where mature and young rams are seldom mixed together.[1,9,11,20,33,39] In yearling rams, LE is considered to be an economically important disease due to the adverse effect on ram fertility. This can lead to a decreased lamb crop or require a greater ram-ewe stocking ratio to sustain adequate crop production. Additional losses may be realized in purebred ram raising operations due to unsuitability of the affected ram for sale.

Most clinical diagnoses of LE are based on clinical signs and palpation of the epididymal lesions, while cultures of semen and the epididymal lesions are commonly used to confirm a diagnosis of LE, especially in B. ovis seronegative rams. The etiologic agents of LE are gram-negative pleomorphic bacilli, facultative anaerobic and capnophilic. These organisms exhibit good growth on blood agar when incubated in air with 10 percent carbon dioxide, but generally only fair growth when incubated either in air or under anaerobic conditions. Most organisms will not grow on MacConkey agar. Whenever possible the organisms are identified to species level based on their biochemical characteristics; however, the identification of these organisms has been difficult due to the lack of definitive characteristics. The distinction

between the two Actinobacillus species and the three organisms that comprise the Haemophilus-Histophilus group can be readily appreciated by morphologic appearance and biochemical traits; however, the Actinobacillus-like organisms and the Haemophilus-like organisms cause considerable confusion for the diagnostic bacteriologist.[16,21,28]

Culling of rams with epididymal lesions is the primary method of control.[4-6] Although there are no commercial vaccines available for LE, an experimental A. seminis bacterin has been demonstrated to reduce the incidence of LE under field conditions.[12]

Taxonomic Status of Actinobacillus seminis and the Haemophilus-Histophilus Group

In 1984, the first edition of Bergey's manual of systematic bacteriology classified A. seminis, H. agni, and H. somnus as "species incertae sedis", indicating that these organisms do not qualify for inclusion in the their respective genera, while H. ovis was not described as a distinct taxon.[18,21] Subsequent investigations helped clarify the antigenic, biochemical, cytochemical, and genetic relationships among these organisms.[10,13-16,22,24,27,29,31-32,34,37] Walker et al. determined that the deoxyribonucleic acid relatedness among H. agni, H. ovis, and H. somnus was very small but were genetically distinct from A. seminis.[34] They further stated that organisms comprising the Haemophilus-Histophilus group should be considered a single species. In 1990, Sneath et al. officially recognized A. seminis as a taxon in the genus Actinobacillus.[29] In 1991, Piechulla et al. further investigated the genetic relationships both between and within A. seminis and the Haemophilus-Histophilus group.[22] They found that A. seminis was genetically distinct from the Haemophilus-Histophilus group; determined that the Haemophilus-Histophilus group was a single taxon but did not satisfy the genetic requirements to be classified in the genus Haemophilus; and officially reclassified the organisms of the Haemophilus-Histophilus group as Histophilus ovis, which was the original nomenclature assigned in 1956.[23]

Actinobacillus-like Groups A and B Associated with LE

In 1989, Scanlan et al. described two Actinobacillus-like organisms from the semen of virgin rams with epididymitis as groups A and B.[28] The three strains in group A and the two strains in group B were nonmotile, predominantly gram-negative coccobacilli, and exhibited less pleomorphism than the American Type Culture Collection (ATCC) strain of A. seminis 15768. The colonial morphologies of groups A and B were similar to A. seminis, but were smaller in diameter and had a pale yellow color. Groups A and B, like A. seminis, were facultative anaerobic and capnophilic, did not grow on MacConkey agar, and were catalase-positive and oxidase-positive. Group A, like A. seminis, reduced nitrate but group B did not. Group A, group B, and A. seminis produced acid but no gas from glucose, and the utilization of other carbohydrates varied markedly both between and within

groups A and B. Group A utilized arginine, and A. seminis utilized ornithine; but group B did not utilize either arginine or ornithine. A. seminis produce acid phosphatase, alkaline phosphatase and beta-glucuronidase, but was negative for esterase (C4); group B produced acid phosphatase, alkaline phosphatase and esterase (C4); but group A was negative for these four enzymes.

In 1991, Scanlan and Healey evaluated the antimicrobial susceptibility characteristics of A. seminis ATCC 15678, fifteen A. seminis LE strains, and the three group B strains.[25] All strains were susceptible to amikacin, ampicillin, carbenicillin, cephalothin, chloramphenicol, erythromycin, gentamicin, kanamycin, neomycin, nitrofurantoin, novobiocin, penicillin, polymyxin B and tetracycline, but resistant to clindamycin. The A. seminis strains were susceptible to methicillin but resistant to bacitracin and sulfadiazine, whereas the group B strains were susceptible to bacitracin and sulfadiazine but resistant to methacillin. The antimicrobial susceptibility differences between A. seminis and group B, particularly to bacitracin which interferes with peptidoglycan synthesis and sulfadiazine which interferes with folic acid synthesis, provides a criterion to help distinguish these organisms.

Groups A and B had phenotypic and constituent biochemical properties of the family Pasteurellaceae, particularly those of currently defined species in the genus Actinobacillus; however, neither group A nor group B could be identified as species in the genera Actinobacillus, Haemophilus, or Pasteurella based on the taxonomic criteria in the first edition of Bergey's manual of systematic bacteriology.[8,15,21]

Capnophilic Gram-negative Bacterium Associated with LE

In 1991, Scanlan and Healey described a capnophilic gram-negative bacillus (strain A2) from the semen of a virgin ram with epididymitis that had phenotypic and biochemical properties markedly different from those described for the facultative anaerobic, gram-negative bacilli in the family Pasteurellaceae, which have been traditionally associated with LE.[26]

Strain A2 exhibited good growth on blood agar when incubated in air with 10 percent carbon dioxide, but failed to produce visible growth on MacConkey agar.[26] The bacterium also failed to produce visible growth on blood agar when incubated in air or in an anaerobic atmosphere. After 2 days incubation on blood agar in air with 10 percent carbon dioxide at 37°C, the colonies were raised, translucent, and nonhemolytic. Strain A2 was catalase-positive, oxidase-positive, reduced nitrite, and was asaccharolytic when tested for aerobic acid production from various monosaccharides, disaccharides, trisaccharides, alcohols, or glucosides. The bacterium also failed to utilize arginine, lysine, ornithine, or tryptophane.

In 1991, Healey et al. compared the outer membrane proteins (OMPs) of strain A2 with A. seminis ATCC strain 15768, A. seminis LE field strain As8C, and Actinobacillus-like group B strain D107, using sodium dodecyl sulfate polyacrylamide gel electrophoresis and two dimensional gel electrophoresis.[16] The A. seminis