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Conference paper

CLONING AND THE LIVESTOCK BREEDING BUSINESS OF THE 21ST CENTURY

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In the past year, cloning has received a great deal of attention because of two cloned animals introduced to the world. In February 1997, a group of Scottish scientists at the Roslin Institute announced the existence of a sheep named "Dolly" which was cloned from adult mammary epithelial cells. Later in August 1997, scientists from ABS Global, Inc., DeForest, Wisconsin announced the existence of a then 6-month old Holstein bull calf they named "Gene" which had been cloned from cells (embryonic germ cell; EGC) originally derived from a fetus. These two events stirred the imagination of people worldwide and set off an ethical debate about who should control and use cloning technology. For farm animal agriculture, there are many interesting lucrative production based business applications for cloning technology. Combining use of cloning with other biotechnologies (i.e. population genetics, molecular biology (recombinant genetics), Marker-Assisted-Selection, in vitro fertilization and embryo transfer), provides producers with tools for managing risk in their livestock operations as well as opportunities for new nontraditional product(s) to meet agricultural demand in the 21st century.

Cloning in cattle: how did we get here?

Research into mass production of cloned farm animals (principally farm animals such as cattle, pigs, sheep and goats) has been ongoing for many years. Actually, Dolly and Gene were not the first cloned farm animals in the world. In the early 1980s clones from split bovine embryos were produced by American Breeders Service (now ABS Global, Inc.), Colorado State University, Granada and University of Wisconsin. An example of two famous split embryo clones are ABS' Duplicate and Divide. They were the first high

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genetic merit Holstein clone bull calves. By the mid 1980s nuclear transfer of disaggregated embryo cells from mid- to late-blastocyst stage were being used to make multiple clones from a single embryo. Individual embryonic cells were isolated and transferred into enucleated oocytes and, subsequently, multiple copies of the founder embryo were produced and calves born. It was also during this period of time that in vitro maturation and fertilization (IVM/IVF) techniques were being perfected for making bovine embryos entirely in the laboratory. Bos taurus and bos indicus cattle are the only farm animal species where IVF technology has been developed and used on a commercial basis.

Soon after nuclear transfer technology was developed for producing cloned bovine embryos it was realized that using a single embryo as a source of clonable cells was limiting for mass production because the founder embryo cells could not be cultured indefinitely. Thus the founder embryo was a temporary source of genetic material for cloning purposes. In the very late 1980s and very early 1990s the search began for a source of genetic material that could provide unlimited nuclear (cellular) material for cloning animals which also could be cryopreserved. Researchers began to look at other embryo cells as a source of genetic material for starting the cloning process. The first cells considered were from the inner-cell-mass (ICM) of the late developing embryo. The first cloned calves using cells from the ICM were produced using nuclear transfer procedures in the early 1990s. However, cells from the ICM could not be preserved and were not culturable for long periods of time. The second cells considered were embryonic stem-cells derived from an early developing embryo following descriptions and procedures first demonstrated in the mouse. Although several labs have reported established culture of ES cells and several attempts have been made to produce cloned cattle using them no live calves as yet have been produced. ABS Global researchers had been using this type of cell for cloning purposes since 1992 with some limited success but with no live calf production. Presumably, the reason for failure of the cells to support development of a functional live healthy calf is incomplete placental development which leads to abortion near the end of the first trimester of pregnancy. This problem was first reported by ABS researchers in the mid- 1990s. It became apparent that to complete the developmental process and get a 'live calf on the ground' either another cell type had to be found which had full developmental capabilities (totipotency) or that some form of chimeric embryo (using bovine ESC-like cells mixed with normal developing embryonic cells) could be used to support development and production of a live calf. Several attempts were made to produce a live chimeric calf with mixed genomes. In 1995, a new approach was formed using primordial germ

cells (PGCs) as the starting cloning material derived from a developing fetus. These cells provide the founding genetic material for a new cloning process which meets the criteria for mass production of cloned cattle. This technology was successfully demonstrated by the production of 'Gene'.

Cloning in cattle: where are we going?

In the fifteen minute talk we will discuss some of the opportunities that lie ahead for the uses of farm animal cloning and how its commercial applications can benefit traditional and non-traditional livestock breeding enterprises in the 21st century.

KLONIRANJE I POSAO UZGOJA STOKE U 21. STOLJEĆU

Prošle godine kloniranje je privuklo mnogo pozornosti zbog dvije klonirane životinje predstavljene svijetu. U veljači 1997. skupina škotskih znanstvenika u Institutu Roslin objavila je postojanje ovce po imenu "Dolly" koja je klonirana iz odraslih epitelnih stanica dojke. Kasnije, u kolovozu 1997. znanstvenici iz ABS Global, Inc., u DeForestu, Wisconsin, objavili su postojanje onda 6 mjeseci starog teleta mužjaka pasmine Holstein, nazvano "Gene", kloniranog iz stanica (embrijska zametna stanica; EGC) koje su prvobitno nastale iz fetusa. Ta su dva događaja uzbudila maštu ljudi širom svijeta i pokrenula etičku raspravu o tome tko bi morao nadzirati i upotrebljavati tehnologiju za kloniranje. Za uzgoj farmskih životinja postoje mnoge isplative primjene tehnologije kloniranja koje se temelje na unosnoj proizvodnji. Povezivanjem primjene kloniranja s drugim biotehnologijama (tj. populacijska genetika, molekularna biologija (rekombinirana genetika), selekcija uz pomoć markera, oplodnja in vitro i presađivanje embrija (transfer) pruža proizvođačima alat za svladavanje rizika u radu sa stokom kao i mogućnosti za nove netradicionalne proizvode, kako bi se udovoljilo poljoprivrednoj potražnji u 21. stoljeću.

Kloniranje goveda: kako smo došli ovamo?

Istraživanje masovne proizvodnje kloniranih domaćih životinja (uglavnom domaćih životinja kao što su goveda, svinje, ovce i koze) provodi se već mnogo godina. Zapravo Dolly i Gene nisu prve klonirane domaće životinje u

svijetu. Početkom 1980-ih klone iz razdvojenih goveđih embrija proizveo je Service američkih uzgajača (American Breeding Service) (sada ABS Global, Inc.), Državnog sveučilišta Colorada u Granadi i Sveučilište u Wisconsinu. Primjer dvaju glasovitih klona razdvojenog embrija su Duplicate i Divide ABS-a. Oni su prvi Holstein telići mužjaci visoke genetske vrijednosti. Do sredine 1980-ih upotrebljavano je prenošenje jezgre rastavljenih (disagregiranih) stanica embrija od srednjeg do kasnog stadija stanica blastocista za stvaranje mnogostrukih klona iz jednog embrija. Izolirane su pojedine stanice embrija i prenesene u jajnik stanica bez jezgre, a zatim proizvedene mnogostruke kopije embrija osnivača, te rođena telad. U tom istom razdoblju usavršene su in vitro tehnike dozrijevanja i oplodnje (IVM/OVF) za stvaranje embrija goveda isključivo u laboratoriju. Bos taurus i bos indicus goveda jedine su vrste domaćih životinja na kojima je razvijena tehnika IVF i upotrijebljena na komercijalnoj osnovi.

Ubrzo nakon što je razvijena tehnologija prenošenja jezgre (nucleus transfer) za proizvodnju kloniranih embrija goveda shvatilo se da upotreba jednog embrija kao izvora stanica koje se mogu klonirati ograničava masovnu proizvodnju, jer se stanice osnivači embrija ne mogu beskonačno uzgajati. Tako je embrij osnivač bio privremeni izvor genetskog materijala za kloniranje. Na samom kraju 1980-ih i u početku 1990-ih počelo je traganje za genetskim materijalom što će moći dati jezgrovni (stanični) materijal za kloniranje životinja koji će se moći očuvati zamrzavanjem. Istraživači su počeli promatrati i druge embrionalne stanice kao izvor genetskog materijala za početak procesa kloniranja. Prve stanice uzete u obzir bile su iz unutarnje mase stanice (ICM) kasno razvijenog embrija. Prva telad klonirana upotrebom stanica iz ICM-a proizvedena je primjenom postupaka nuklearnog transfera (jezgrovnog prenošenja) početkom 1990. Međutim, stanice iz ICM-a nisu se mogle sačuvati i nisu se mogle uzgajati za dugo vrijeme. Druge razmatrane stanice bile su embrionalne stanice-stapke (stem-cells) nastale iz ranog razvoja embrija nakon opisa i postupka najprije prikazanih na miševima. Iako je nekoliko laboratorija izvijestilo o postizanju kultura ES stanica i bilo nekoliko pokušaja da se pomoću njih klonira govedo do danas još nije proizvedeno živo govedo. Istraživači u ABC Global upotrebljavaju ovaj tip stanice za kloniranje od 1992. s ograničenim uspjehom, ali bez proizvedenog teleta. Po svoj prilici razlog za neuspjeh stanice da podrži razvoj funkcionalnog života zdravog teleta je nepotpuni razvoj placente, što dovodi do abortusa pred kraj prvog tromjesečja trudnoće. O ovom su problemu prvi izvijestili istraživači ABS-a sredinom 1990-ih. Postalo je jasno, da bi se završio razvojni proces i dobilo "živo tele na zemlji" treba naći drugi tip stanice koji će imati potpune razvojne sposobnosti (totipotenciju) ili se može upotrijebiti neki oblik himeričnog

embrija (upotrebom goveđih stanica poput ESC pomiješanih s molarnim embrijskim stanicama u razvoju) kao podrška razvoju i proizvodnji živog teleta. Bilo je nekoliko pokušaja da se proizvede živo tele s miješanim genomima. Načinjen je novi pristup 1995. godine upotrebom prvobitnih stanica zametka (PGC) kao početnog klonskog materijala nastalog iz razvoja fetusa. Ove stanice pružaju temeljni genetski materijal za novi postupak kloniranja koji zadovoljava kriterije za masovnu proizvodnju kloniranog goveda. Ta je tehnologija uspješno prikazana proizvodnjom "Gene"-a.

Kloniranje u goveda: kamo idemo?

U 15 minuta govorit ćemo o nekim mogućnostima u kloniranju domaćih životinja te kako njihova komercijalna primjena može biti od koristi tradicionalnim i netradicionalnim uzgajačima stoke u 21. stoljeću.

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