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EPIDEMIOLOGICAL ASPECT OF DIFFERENT BREEDING STRATEGIES IN DOMESTIC ANIMALS

ЕПИДЕМИОЛОШКИ АСПЕКТИ НА РАЗЛИЧНИТЕ СТРАТЕГИИ НА ОПЛОДУВАЊЕ НА ДОМАШНИТЕ ЖИВОТНИ

EPIDEMIOLOŠKI ASPEKTI RAZLIČITIH UZGOJNIH STRATEGIJA KOD DOMAĆIH ŽIVOTINJA

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Summary: Beside the natural breeding different techniques of technological breeding developed by the man, are applied in animal breeding nowadays. Technological breeding includes techniques like artificial insemination, artificial insemination with flow-cytometrically sex sorted semen, embryo transfer, in vitro fertilization and cloning.

Compareson of the natural breeding with other reproductive techniques showed that natural breeding represents higher risk for the transmission of infection diseases.

Use of artificial insemination and other reproduction techniques depends mainly on positive cost-benefit calculations and differes from one country to unother.

Key words: *animal breeding, reproduction, assisted reproduction, disease control*

Резиме

При оплодувањето на животните во денешно време, покрај природното парење, се користат и различни техники на потпомогната репродукција. Потомогнатата репродукција вклучува техники како што се вештачкото осеменување, вештачко осеменување со сексирана сперма со помош на течна цитометрија, ембрио трансфер, ин витро оплодување и клонирање.

Се покажа дека, во однос на природното парење, примената на методите за потпомогната репродукција го смалуваат ризикот за пренесување на заразните болести.

Употребата на вештачкото осеменување и другите техники на потпомогната репродукција зависат пред се од економските фактори и затоа различно се применуваат во различни земји.

Клучни зборови: *оплодување на домашните животни, репродукција, потпомогната репроодукција, контрола на болестите*

Kratak sadržaj: Pored prirodnog parenja, savremenu proizvodnju domaćih životinja karakteriše i primena različitih tehnika asistirane reprodukcije. Asistirana reprodukcija koristi različite biotehnološke postupke poput: veštačkog osemenjavanja, osemenjavanja semenom sortiranim po polu, embrio transfer, oplođenje in vitro i kloniramnje.

Pokazalo se da tehnike asistirane reprodukcije smanjuju rizik od prenošenja zaraznih bolesti u odnosu na prirodni seksualni kontakt.

Danas je primena metoda asistirane reprodukcije ograničena pre svega ekonomskim faktorima i stoga se one različito primenjuju u raznim zemljama.

Ključne reči: *gajenje domaćih životinja, reprodukcija, asistirana reprodukcija, kontrola bolesti.*

INTRODUCTION

Domestic animals are the animals, which are raised for the benefit of mankind (1). Under this definition they can be divided into two groups. The animals bred for economic purpose (horses, cattle, pigs, sheep, goats, rabbits and poultry) and animals raised for the pleasure (dogs, cats...). Various techniques, which can be used for the breeding in different species of domestic animals, are known today. The suitable technique for breeding is the prerequisite necessary for the successful fusion of male and female gametes and further development of the embryo.

Besides the natural breeding we also have the different variation of technological breeding, which were developed by the man. Technological breeding includes techniques like artificial insemination, artificial insemination with flow-cytometrically sex sorted semen, embryo transfer, in vitro fertilization and cloning. The purpose of each technique is to achieve the specific goal. Economical suitable breeding of large number, healthy animals in wanted selection type are the main goals of all breeders. Expected characteristic of the animals is also an important issue of the breeders raising economic animals. The number or percentage of animals bred with one of techniques described above is fairly different in individual countries and it depends upon to the species, breed and the value of the animals. We should not forget that all technological techniques of the breeding were developed with the specific reason and due to very specific demand.

Since the majority of breeding techniques were in the largest extend developed in the cattle is the purpose of this article to present the goals of different breeding techniques in the light of epidemiological aspect.

Natural breeding (natural mating)

Before the introduction of artificial insemination, the natural breeding was the only form of animal reproduction existing (Ivanov, 1907). In some animal species is this form of animal reproduction still the most widespread. Natural breeding can be divided into mating from a hand and group mating. In the light of epidemiological aspect is this form of animal reproduction the most dangerous for transmission of infection diseases. But, this form can also be relatively safe, as long as we those male and female animals are free from infection diseases. This secure can be achieved by proper identification of the animals and regular control of sex transmissible diseases.

Artificial insemination

Artificial insemination is the first technological manner of animal reproduction. It has started at the end of 19th century (Ivanov, 1912; Dimitrijev, 1934). The first goal of artificial insemination was to introduce the semen into genitals of females in those cases where natural mating was prevented (Kosec, 2002). The most important epidemiological aspect of artificial

insemination was to prevent the transmission of infection diseases i.e. trichomoniasis, campylobacteriosis. Recently these technology gained high develop. Artificial insemination made a real revolution in the selection of animals, after introduction of deep freezing, which enable preservation of spermatozoa for a long or almost unlimited time. The most important precondition in the aspect of epidemiology is that the males, which are used for semen production, are free from infection diseases. With this precondition it is assured that the semen does not present any danger for the transmission of infection diseases.

Artificial insemination is among domestic animals most often used in cattle. As presented in table 1 is the percentage of first inseminations of cows and heifers highly variable between the different countries (2, 3 and 4).

Table 1: The Number and the percentage of first inseminations of cows and heifers in year 2003 (the difference between the countries)

COUNTRY	N – first inseminated cows and heifers*	Insemination (%)
GERMANY	4 769 953	81.2
AUSTRIA *	1 294 924	92.9
IRELAND	app. 1 000 000	37.0
SLOVENIA	193 633	97.5

* - first inseminations of cows and heifers in dairy production

The breeding bulls in insemination centres has to be, according to the guideline 88/407/EGS and from the epidemiological aspect, free from following diseases: tuberculosis, brucellosis, bovine enzootic leucosis, IBR/IPV, bovine genital campylobacteriosis, bovine trichomoniasis and bovine viral diarrhoea (*Diarrhoea viralis bovis*). The guideline mentioned above does not regulate the health status of the bulls intended for natural mating, which is controlled by the regulations prepared in each EU country. In Slovenia the bulls intended for natural breeding needs to fulfil the same criteria as the one in the insemination centres. Since the guideline 88/407/EGS regulates only the health status of bulls in insemination centres, it can be concluded that from epidemiological point of view the artificial insemination is more highly recommended technique, compared to natural mating. It is necessary to interrupt the circle of spreading, if we want to eradicate certain infection disease. The application of artificial insemination is very important to achieve this goal, but since it is also affected with certain expenses, it use depends mainly on positive cost-benefit calculations. As presented in table 1, the use of artificial insemination is in some countries differently developed and this is most likely due to different cost-benefit calculations among countries.

ARTIFICIAL INSEMINATION WITH FLOW-CYTOMETRICALLY SEX SELECTED SPERMATOZOA

Use of flowcytometrically sex selected mammalian spermatozoa has been proven as a powerful tool for increasing the benefits in animal production, for the genetic improvement of farm animals, for the control of sex-linked disease in humans and in wildlife for the re-population of endangered species.

Since the first laboratory tests in the mid eighties the flowcytometrical sexing technology has undergone major improvements up to a level that is now starting to be of interest for commercial application. Nevertheless, the output of sex sorted spermatozoa is still limited compared to the number of a full ejaculate, as each single cell has to be identified and sorted.

First experiments to produce offspring from sex-sorted semen had to overcome the problem of low sort rates employing a normal flowcytometer. Therefore, the first calves were produced

by a combination of sperm sorting and in vitro production of embryos with subsequent embryo transfer (Cran et al., 1993; Cran et al., 1995). Similar experiments were repeated with hormonally synchronized animals recently (Paranace et al., 2003). High speed flowcytometry helped to obtain more sex sorted spermatozoa in a more reasonable time and first calves were born from insemination with fresh (Seidel, Jr. et al., 1997) and frozen-thawed spermatozoa (Seidel, Jr. et al., 1999). Meanwhile the technique is offered commercially for bovine spermatozoa. However, field data indicate that fertility is still very variable and depends on bull effects, which are not necessarily completely due to sorting, but may be related to high dilution effects and reduced compensatory mechanisms among spermatozoa. Especially, on farms with moderate fertility, limited quality of sorted semen may be more extinct.

Meanwhile, the experiments resulted from the cooperation between Federal institute in Mariensee (Germany) and Veterinary faculty of Ljubljana (Slovenia) have shown that the punctual evaluation of motility is insufficient to judge quality of sex-sorted spermatozoa. Better information can be obtained from a 12h thermo resistant test indicating the loss of motility over time. For example these results showed that sorted bull spermatozoa loose more than 90 of their motility under such conditions, whereas unsorted control samples maintain their motility. Similar events can be expected in the female genital tract. However, recent field experiments employing a new set of extenders (Sexcess®) that were developed during this cooperation between Slovenia and Germany showed meanwhile that the fertilizing competence of sex-sorted spermatozoa can be protected. In total 300 heifers on 190 Slovenian farms were inseminated with sex-sorted frozen-thawed spermatozoa or unsorted controls. Pregnancy rates as determined by ultrasonography showed no differences anymore between controls and sex-sorted spermatozoa. Similar data were also obtained with fresh sex-sorted spermatozoa that were used for normal AI in 70 heifers over a three day period after sorting. Also in beef breeds pregnancy rates as determined by ultrasonography between days 30 and 60 were equal between groups (Klinc P, 2005).

The use of embryo transfer technologies in eradication programs of infectous diseases

Embryo transfer and *in vitro* production of embryos are technologies of assisted reproduction, promising quicker and safer advance in genetic quality in domestic animals.

Due to frequently observed infections in highly producing herds, the way of saving the valuable genetically material in the process of sanation of herds and therefore the limiting the risk of infection in uninfected herds is favourable question. If proper procedures are followed, the risk of transmitting infectious disease via embryo transfer is lower than with natural mating or artificial insemination.

The International Embryo Transfer Society (IETS) has encouraged a great deal of experimental work on the risk of transmission of disease from donors to recipients via a 7-day-old blastocyst. There has been much success in demonstrating that with an approved protocol of handling the embryos, to date there is a very little danger in disease transmission with both viruses and bacteria (Philpott, 1993). Possibility of deep freezing of embryos enables preservation of the genome and its reintroduction into healthy herd with very low possibility for transmitting the disease.

Frozen embryos can easily be transported from one location to another, and can be safely maintained in storage while their progenitors are being tested for disease. The distribution of embryos is less hazardous for disease transmission. The number of recipients is small, flushing fluids will dilute pathogens present and, most significantly, embryos can be rinsed (Singh, 1988). The zona pellucida surrounding the bovine embryo has a sponge like surface (Chen and Wrathall, 1989), but appears impermeable to pathogens (Wrathall, 1987). Some pathogens do not easily but some of these can be removed by a trypsin wash (Singh, 1988).

The protocol for an approved system of treatment post flushing and prior to freezing has been adopted by IETS (Stringfellow and Seidel, 1990). These guidelines refer to embryos with intact zona pellucidas.

In Slovenia a study was performed concerning the use of MOET (multiple ovulation and embryo transfer technology) in the IBR/IPV eradication program (Mrkun et al., 2002). The use of embryotransfer in Slovenia is introduced for the years 2001-2003, which is performed by Slovenian ET team according to the national selection program (Table 2). The data from the MOET program in research work are not shown.

Table 2: Comparison between artificial insemination and ET in Germany and Slovenia in years 2001 – 2003

year	Germany		Slovenia	
	first inseminations (%)	embryo transfers (%)	first inseminations (%)	embryo transfers (%)
2001	no data	0.345	97.6	0.016
2002	78.5	0.211	97.5	0.019
2003	81.2	0.209	96.9	0.015

Artificial insemination is established widespread in Slovenia, where 96.9 – 97.6% of all cows and heifers were artificially inseminated in years 2001- 2003. In Germany there is a lover level of artificial inseminations varied around 80%. On the other hand, embryo transfer is more performed in Germany than in Slovenia, but a decrease is observed in Germany from year 2001 to 2003. In Slovenia there is small number of embryo transfers performed, but the number is relatively constant (Table 2).

IN VITRO FERTILIZATION

This procedure usually comprises four separate steps *in vitro*: oocyte maturation, capacitation of sperm, fertilization, and culture of embryos until they can be frozen or transferred to the uterus.

Potential applications of *in vitro* fertilization include supplying embryos from slaughterhouse oocytes, second is to circumvent certain kinds of infertility and a third is as an alternative to harvesting gametes from valuable cows by superovulation. Oocyte maturation could also provide material for cloning by nuclear transplantation and for making transgenic animals (Seidel and Seidel, 1991). *In vitro* fertilization has not been used commercially in cattle in Slovenia. However it is used in the case of circumvent the infertility of valuable cows and in the research work.

CLONING

Cloning is the propagation of genetically exact duplicates (clones) of an organism by means other than sexual reproduction. Cloning of mammals is currently achieved by nuclear transfer (NT), that is replacing the genetic material of an unfertilized ovum (oocyte) with that of an embryonic or somatic cell taken from the animal being cloned, and then transferring the reconstituted embryo to a surrogate mother for rearing.

Globally, all the major livestock species have now been cloned but animal-cloning technology is still only used for scientific and research purposes. The main reasons for limited use is while only 0.1-5 % of cloning attempts yield viable offspring due to complications caused by incomplete reprogramming of the nucleus following transfer, imprinting failure and aberrant