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Some factors affect of embryo-flushing in dairy cattle

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ABSTRACT

The authors examined the factors affect of embryo-flushing (n=613) in field conditions. They searched interaction between age of donors (cow vs. heifer) and embryo quality and yield, sought correlation between the milk production of donors and the number of transferable embryos, degenerated embryos and oocytes. The other hand the effect of different kind of superovulation treatments on embryo production was examined. Results of the present study show that the age of donors was influenced for the efficiency of the embryo-flushing. Cow donors product significant (P<0.001) more eggs than heifers, but virgin heifers product better embryos than cows. Significant correlation (r= -0.26; P<0.01) was founded between milk production and embryo-quantity and there wasn't correlation (r= 0.08) between milk production and flushed eggs (unfertilized oocytes and degenerate embryos). The treatment of superovulation determines the successfulness of ET.

(Keywords: cow donors, heifer donors, embryo-flushing, embryo-production, embryo quality, milk production, superovulation)

ÖSSZEFOGLALÁS

Az embrió-kinyerést befolyásoló néhány tényező tejelő tehenészetben

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A szerzők az embrió-átültetést rutinszerűen alkalmazó gazdaságban értékelték az embrió-kinyerések (n=613) eredményességét. Összefüggést kerestek a donorok kora (tehén illetve üsző) illetve a nyerhető embriók mennyisége - minősége illetve a donorok tejtermelési színvonala és az embrió-produkciója illetve az egyéb (petesejt, degenerált) képlet között. Ezen kívül különböző szuperovulációs kezeléseknek (csökkenő vs. fix dózis) ez embrió-produkcióra gyakorolt hatását vizsgálták. A donor korának szignifikáns (P<0.001) hatása van a nyerhető embriók számára, illetve a minőségére. A donorok tejtermelése és az embrió-produkciója között statisztikailag igazolt negatív összefüggést

(r=–0.26; P<0.01) állapítottak meg. A tejtermelés és az egyéb kinyert képletek esetében az összefüggés nem volt számottevő (r=0.08). A szuperovulációs kezelés szintén befolyásolja az ET eredményességét.
(Kulcsszavak: tehén donor, üsző donor, embrió-nyerés, embrió-mennyiség, embrió-minőség, tejtermelés, szuperovuláció)

INTRODUCTION

Over the past 20–25 years the biotechnology in cattle breeding has improved much. The first calf born from embryo transfer (ET) in 1978 in Hungary and the first successfully transfer using frozen embryo had done in 1983 (*Cseh and Dohy*, 2003). Embryo transfer is that when the embryo is removed by flushing from the horn of the uterus before implantation and transferred into the horn of the uterus of synchronized recipient (*Haraszti and Zöldág*, 1993). The methods of the ET were made to possibility to use the potential oocytes of female. Embryo technologies use in cattle breeding has been to increase the number of progeny and top genotypes (*Galli et al.,* 2003). Increase the reproductive rate of the dairy cattle with ET has made it possible to predict accurately the genetic development through higher intensities of selection and shorter generation intervals and more female progenies (*Dohy*, 1999).

The complex process of the embryo transfer stands two parts. One of the embryo flushing, and the other is the embryo transfer. The successfully of the flushing can be measured by the number of embryos and eggs. The effects of the flushing are the seasonality, age of donors, superovulation methods. Cows product more usable embryos than heifers under some conditions. The point of view of donor's condition has an effect on number of transferable embryos (*Hasler et al.*, 1987). Although a number of new technologies have been adopted within the ET industry in the last decade, the basic procedure of superovulation of donor cattle has undergone little improvement over the last 20 years. The greatest problem in the embryo transfer industry is unreliability of superovulatory response. This wide variability creates greatly increase costs. FSH (*Follicle Stimulating Hormone*) and eCG (*Equine Chorionic Gonadotropin*) can generate superovulation (*Lopes da Costa et al.*, 2001). Superovulation can be generated with the help of PMSG also (*Haraszti and Zöldág*, 1993). The most famous treatment uses FSH. *Elsden et al.* (1979) showed that a higher ovulatory response was obtained with the use of FSH than with PMSG. *Goulding et al.* (1996) suggested that day 9 or 10 of the oestrous cycle is more appropriate to start the superovulatory treatment. The generally treatment consist of 8 injections, contain decreased amount FSH. Prostaglandin treatment is accompanied by the 5. FSH injection. This causes the oestrus in the donor with the help of luteolyse of the *corpus luteum* (*Haraszti and Zöldág*, 1993). *Kanitz et al.* (2002) showed increasing amount of FSH, the number of ovulations increases significantly until a plateau was reached for the parameter, but it is not possible to increase ovulation number with further increasing FSH doses. The superovulation treatment produces more embryos than if we use BST (*bovine somatotrophin*) (*Gong et al.*, 1993). As long as mean embryo production remains at less than 6, with a range of (0 to >60), with 20% of donors producing 0 embryos, superovulation will remain an expensive, inefficient procedure (*Hasler*, 2003). The age of donor has an affect on embryo quality. Milking cows produces poor quality embryos (*Leroy et al.*, 2005). The embryo quality has a significant affect on the pregnancy rate (*Szabari et al.,* 2007), but there is no interaction between the development of the embryo and pregnancy rate (*Bényei et al.,*

2006). It can be flushed less embryos from heifers then from cows (*Ax et al.*, 2005). Otherwise the keeping and feeding technology of the donor have great influence on the number and quality of embryos.

The aim of this study were examined the factors affect of embryo-flushing in field conditions. Interaction was searched between age of donors (cow vs. heifer) and embryo quality and yield, sought correlation between the milk production of donors and the number of transferable embryos, degenerated embryos and oocytes and different kind of superovulation treatments on embryo production was examined.

MATERIAL AND METHOD

The donors cows had an outstanding production, and the donor heifers had an excellent pedigree or come from ET. Holstein cows (n=386) and heifers (n=227) were superovulated using FSH (OVAGEN, ICPbio). The superovulation was induced by a total dose of 8.8 mg to 17.6 mg FSH via intramuscular, twice a day, during four days, in the mid-luteal phase of the oestrous cycle (8-11 day), and a dose of PGF_2£ analogous intramuscularly 48 hours later. Three methods of superovulation were applied. Two of these protocols used standard amount FSH (2.5 ml/injection, sum 17.6 mg FSH; 2 ml/injection, sum 14.08 mg FSH) and the other applied falling (-0.5 ml/day, sum 8.8 mg FSH) FSH doses during the 4 days. The donors were artificially inseminated 12 and 24 hours after the onset of oestrus. Embryos were collected non-surgically at day 7 post-insemination. Embryos were classified with stereomicroscope. All embryos were assigned a developmental stage and quality grade according to standards set forth by the International Embryo Transfer Society. Zona pellucida damaged embryos were not used.

Individual embryos were loaded into the centre of 0.25ml straws. Straws were frozen by using a controlled slow freezing method, in ethylene glycol (*Voelkel and Hu*, 1992; *Hasler et al.*, 1997). The straws were transferred to a precooled liquid nitrogen freezer (EUROTHERM). After the straws were cooled to -30 °C at a rate of 0.3 °C per minute, before plunging into liquid nitrogen (*Dochi et al.,* 1998). Frozen straw was thawed in a water bath (37 °C, 12 sec.). The fresh and frozen embryos were transferred directly into synchronised recipients. The recipients were synchronised using with prostaglandin (ESTRUMATE). Heat was detected with rectal control in 3 days after synchronised. Results of embryo-transfer were controlled with rectal palpation in 40–50. days after transfer.

Data were evaluated by T-test and Chi-square test using the *SAS* 9.1 (2004) statistical software package.

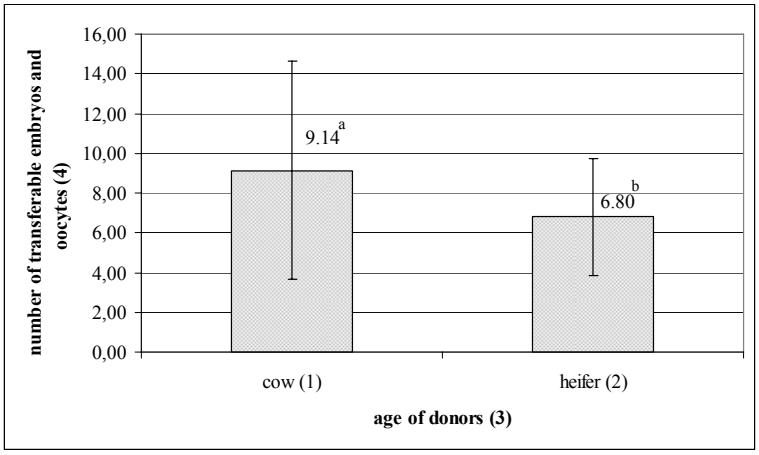
RESULTS AND DISCUSSION

The donors were cows and heifers. The rate of heifer donors from the examined flushing (n=613) was 37.03%. The average number of eggs (transferable embryo + oocyte) per flushing was 8.63. Comparing the effect of the age on the number of flushed eggs (9.14 vs. 6.8) with T-test, the result was significant ($P<0.01$) (*Figure 1*).

More than 2 eggs (2.9) from cows were obtained per flushing than from heifers, which means one more transferable embryo. On the average 7.8 embryos were flushed per donors by *Ax et al*. (2004) which meant 1 more embryo than in our job. The quality of flushed embryo from heifers was significantly better (*Figure 2*).

Figure 1

The effect of age (cow vs. heifer) on number of transferable embryo and oocyte



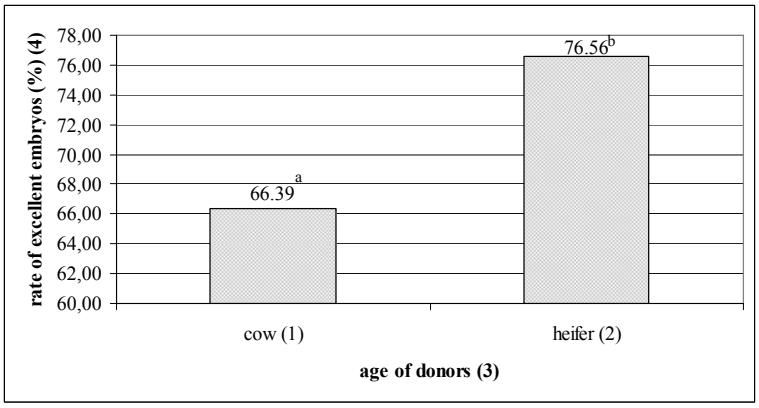
Different letters mean significant differences (P<0.001) (*A különböző betűk a szignifikancia különbségét jelentik P<0,001*)

1. ábra: A kor (tehén vs. üsző) hatása az átültethető embriók és petesejtek számára

Tehén(1), Üsző(2), Donorok kora(3), Átültethető embriók és petesejtek száma(4)

Figure 2.

The rate of excellent embryo quality in various (cow vs. heifer) donors



See *Figure 1* (*Lásd 1. ábra.*)

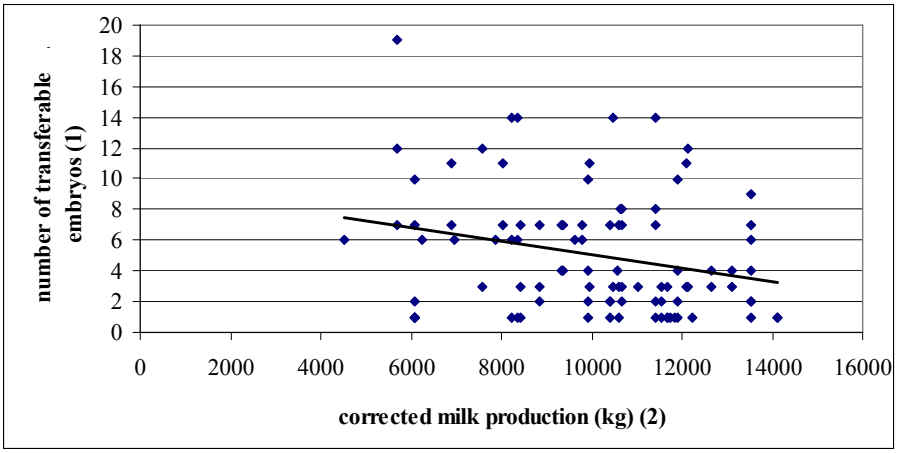
2. ábra: Különböző donorkorcsoportok embrióprodukciójának első osztályú embrióinak aránya

Tehén(1), Üsző(2), Donorok kora(3), Első osztályú embriók aránya(4)

The age of donors and the quality of flushed embryos were compared with Chi-square. The proportion of first class, intact embryos from heifers is statistically higher then from cows. The milk production has an influence on donor selection. In this case the authors examined the corrected milk yield (305d) of donors and the number of embryo (*Figure 3*).

Figure 3.

The correlation between corrected milk production and the number of transferable embryos



r= -0.26, P<0.001; y= -0.0004x+9.5026

3. ábra: A tejtermelés és az ültethető embriók számának az összefüggése

Ültethető embriók száma (1), Tejtermelés (2)

In our experiment we found statistically negative correlation between the two variables (r= -0.26; P<0.01). Cows with higher milk production were able to product less embryo. Because the ET is in a tight interaction with production, so this negative antagonism is not surprising. *Novotny et al.* (2005) found the same correlation (r = -0,35) between the milk and embryo production.

According to our study the establishment of *Seidel and Seidel* (1981) is true and also the general assumption that the high milk production goes with poor fertility because of hormonal affect. And on the other hand, the selection for milk production causes less embryo yield.

There was no correlation (r = 0.08) between 305d corrected milk and the number of unfertilized oocytes and degenerated embryos.

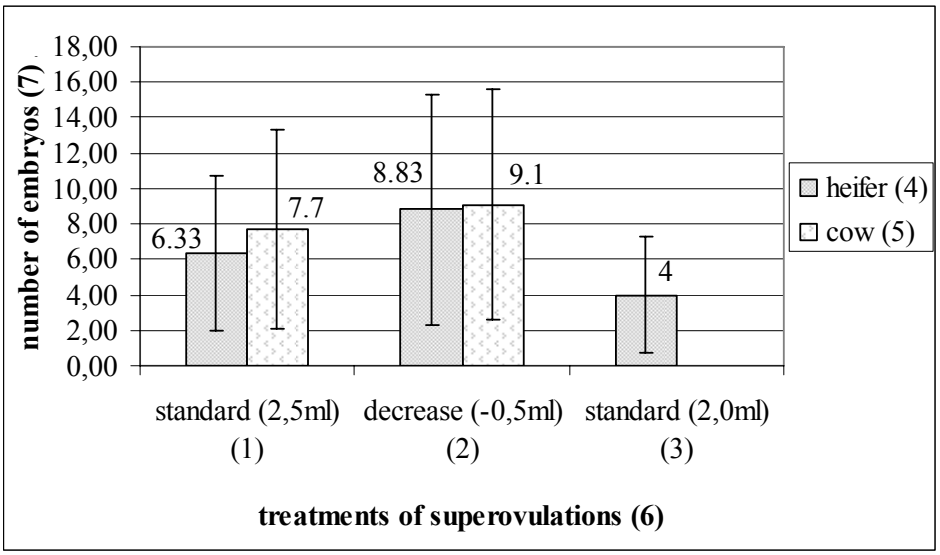
The higher milk production does not cause increasing number of unfertilized oocytes and degenerated embryos, but *Novotny et al.* (2005) determined strong positive correlation (r=0.58) between the two variables. Under these circumstances the knowledge of other factors is very important. With the help of this knowledge the negative effect of milk production could be compensable.

The superovulation of donors has undergone little improvement over the last 20 years. This cause the wide variability the number of transferable embryos. This is why, it

is necessary to know which treatment products more embryos, and which effects should be hindered to reach maximal result, decreasing the great variance. Different kind of treatments causes different numbers of embryos (*Figure 4*).

Figure 4.

The effect on different treatments of superovulation in different age of donors (cow vs. heifer)



4. ábra: Különböző szuperovulációs kezelés hatása tehén és üsző donorok esetében

Standard mennyiség(2,5ml)(1), Csökkenő mennyiség(2), Standard mennyiség (2,0ml)(3), Üsző(4), Tehén(5), Szuperovulációs kezelés módja(6), Embriók száma(7)

The standard injections cause only 7, 5-8 while the decreasing doses produce more than 9 eggs in cows. The differences between the 2 treatments were not significant because the variation. In the case of heifers the decreasing with 0,5 ml doses (total 8,8 mg FSH) gave the best results against the standard doses (17,6 mg and 14,08 mg FSH). Although these differences were not significant. The authors established that the hormonal treatment of superovulation has an affect on the efficacy of ET.

CONCLUSIONS

Applying of the ET in the Hungarian cattle breeding has reached minimal level (<1%) since 1990. This is behind from the countries which have developed cattle breeding and also from our possibilities. The widespread of ET is hindered by financial state of agricultural companies.

The efficiency of ET is influenced by the biological properties of females and environmental factors.

According to our studies more embryos could be obtained from cows donors statistically then heifers. In the case of heifers the rate of excellent quality of embryos was significantly higher.

The appropriate selection of donors is also very important because, the authors find statistically negative correlation between milk production and number of transferable embryos. There was no correlation between milk production and other flushed structures.

The accurately chosen treatment of superovulation determines the successfulness of ET.

Finally we can assess, that the benefits of ET are not applied maximally. The appropriate selection of donors, the higher rate of donors of heifers, more recipients tightly corporation between the participants could be necessary to improve the ET in Hungary.

The ET is not a magic method, because it is not able to compensate the deficiency of management, but it might be an effective tool to increase genetic improvement.

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