Journal of Animal and Veterinary Advances 6 (4): 496-499, 2007

Some Factors Affecting Embryo Transfer Success in Dairy Cows

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Abstract: The aim of this study was to investigate which characteristics of the animals and of the technique influenced the embryo collection and Conception Rate (CR) in a commercial embryo transfer program. Data from 4 consecutive years (1871 transfers) were statistically analyzed. The day of cycle when the superovulatory treatment was started, the breed and the age of the donor, and the season, had significant effect ($p<0.05$) on the number of collected embryos. The quality of the embryo ($p<0.0001$), the age of the recipient ($p<0.0001$), the depth of the transfer ($p<0.001$) and the absence of trauma, ($p<0.0001$) and the side of the transfer ($p<0.005$), had significant effect on CR.

Key words: Embryo transfer, results, dairy cows, CR, superovulatory treatment

INTRODUCTION

Embryo transfer technology is an effective instrument for genetic improvement in the bovine industry, and breeders may obtain an enhancement of the economic value of their herds as a result of increased genetic value (Merton *et al*., 2003). Over the last 30 years this technique has developed to become a well-established commercial industry (Stroud and Hasler, 2006) although this expansion has been strongly impaired by an inadequate efficiency (Hasler, 2003). In spite of high technical specialization of practitioners working in the embryo transfer field, the results of this technique are still very unpredictable (Durocher *et al*., 2006; Hasler, 2006). The factors that can influence the outcome of this procedure are several and varied, being related to animals, management, environment, operator and other conditions. Aim of this study is to gain a more precise and deep understanding of these factors in order to be able to control them thus improving efficiency and reliability of embryo transfer procedures.

MATERIALS AND METHODS

This retrospective study examined data collected over a 4-year period from a commercial embryo transfer unit operating in the north of Italy.

Data were stored into three different Databases (DB). DB1 contained data regarding superovulation and flushing (n = 204). DB2 contained data regarding embryos (n = 4878) (coming both from flushing and from in vitro production). DB3 contained data regarding the transfers (n = 1871).

Superovulation was performed on cows chosen by the owner, and submitted to three separated clinical evaluations, assessing normal health conditions, occurrence of a normal oestrus and presence of a normal Corpus Luteum (CL), respectively. The superovulatory protocol started 8-14 days post oestrus with the administration of porcine pituitary gonadotropins (Pluset, Laboratorios Calier) and the PGF analogue luprostiol (Prosolvin, Intervet) according to the following dosage schedule (Table 1).

Oestrus was expected 24 h after the last administration of gonadotropins, AI was performed with

Table 1: Dosage schedule

Day	Time	Gonadotropin	PGF
1	8.00 pm	175UI FSH+175 UI LH	
2	8.00 am	175UI FSH+175 UI LH	
2	8.00 pm	150UI FSH+150 UI LH	
3	8.00 am	150UI FSH+150 UI LH	
3	8.00 pm	100UI FSH+100 UI LH	
4	8.00 am	100UI FSH+100 UI LH	18.75 mg lupriostiol
4	8.00 pm	75UI FSH+75 UI LH	11.25 mg lupriostiol
5	8.00 am	75UI FSH+75 UI LH	

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semen of proven fertility for three times, at the onset of oestrus and 12 and 24 h after.

Flushings were performed 7 days after oestrus, using the standard non surgical method (Elsden *et al*., 1976). The embryos collected by uterine flushing were classified according to the rules of the International Embryo Transfer Society (IETS) (Robertson and Nelson, 1998).

ET recipients were selected among cows that were in oestrus 7 days before the transfer. Following a treatment with 18.75 mg of luprostiol at 8.00 pm of the third day of the superovulatory program (Table 1) or 15 mg of luprostiol at 8.00 am of the fourth day if heifers were used. During embryo transfer the recipients were restrained and an intercoccigeal epidural injection of 5-7 mL of lidocaine 2 was administered.

Transfers were considered traumatic when blood was encountered on the catheter after the procedure.

Statistical analysis: In DB1 the effects of the following parameters on the total number of structures and the total number of transferable embryos obtained from flushing were investigated:

- Season of the flushing.
- Group of the donor (heifer, lactating cow, dry cow).
- Breed of the donor (Holstein Friesian, Brown Swiss).
- Days after oestrus at the onset of the superovulatory treatment (8-14 days)
- Size of the CL at the onset of the superovulatory treatment (1-3 cm).
- Different batches of the Porcine Pituitary Gonadotropin (PPG).

In DB2 the effects of the following parameters on the conception rate were investigated:

- Type of embryo (Frozen or Not frozen).
- Type of the cryoprotector (Glycerol Ethylene glycol).
- Source of the embryos (flushing or *in vitro* production).
- Sexing (yes or no).
- Stadium of development of embryos.
- Quality of the embryos (IETS standard).
- Breed of the recipient (Holstein Friesian, Brown Swiss).
- Group of the recipient (heifer, lactating cow dry cow).
- Dimension of the CL of the recipient (1-3 cm).

In DB3 effects of the following parameters on the conception rate were investigated:

- Depth of the transfer (very deep, deep, superficial).
- Atraumatic transfer (yes or no).
- Side of the transfer (Dx-Sx).
- Month of the transfer.

In each DB the variables have been tested looking for significant relations. To look for an effect on the number of embryos obtained Analysis of Variance was used, followed by the Least Square Difference (LSD) test for multiple comparisons; the Chi-Square test was used to study the effect of factors on the Conception Rate (CR). Statistical significance was assumed for $p<0.05$.

RESULTS

Factors concerning superovulation and flushing: The day of the cycle when the superovulatory treatment was started has a significant effect on the number of viable embryos obtained from the flushing ($F = 2.343$; $p = 0.034$). In particular a higher number of embryos was obtained when the superovulatory treatment was started on day 9 when compared to day 11 and 13 (mean value 16.25, 8.75 and 5.0 embryos, respectively).

The breed of the donor had a significant effect on the number of viable embryos ($F = 6.350$; $p = 0.013$). A significantly higher number of embryos was obtained from the Brown Swiss (9.29) than from Holstein Friesian (6.76).

The group of the donor has a significant effect both on the total number of structures ($F = 4.989$; $p = 0.008$) as well as on the number of viable embryos obtained from flushing ($F = 4.384$; $p = 0.014$). The average production in heifers is lower than in cows, being 9.23 vs 15.19 for the total number of structures and 6.0 vs 8.72 for the number of viable embryos.

The season when the flushing was performed had a significant effect on the number of viable embryos ($F = 2.7$; $p = 0.047$). The average number of embryos is lower in summer than in winter (4.85 vs 9.26).

Factors regarding the embryos: The quality of the embryo has a significant effect on the CR ($\chi^2 = 22.119$; $p<0.0001$). The CR obtained transferring embryos of the first class of quality (IETS grading) was 54.51% while the CR obtained using embryos of the third class was 31.88%.

Factors regarding the recipient: The group of the recipient has a significant effect on the CR ($\chi^2=15.005$; $p<0.0001$). The CR in the heifers is higher than in cows (56.36 vs 38.46%).

Factors regarding the transfer: The depth of the transfer has a significant effect on the CR (χ^2=14.751; p<0.001). The CR in the very deep transfers is higher than in the superficial transfers (56.02 vs 14.29%).

A significant difference (χ^2 = 19.948; p<0.0001) in CR exist between atraumatic and traumatic transfers (52.63 vs 17.78%).

The side of the transfer (always omolateral to the CL) has a significant effect on the CR (χ^2=9.404; p<0.005). The CR is higher when the embryo is transferred in the left horn (56.16 vs 48.57%).

All the other investigated effects resulted non significant.

DISCUSSION

Even if the superovulatory treatment was always started at stages in the estrous cycle when a functional dominant follicle was not present (D'Occhio, 1999), the precise day of the cycle in which it was started showed a significant effect on the number of collected viable embryos. This finding in agreement with the results of Lindsell *et al.* (1985) and confirms the idea that the follicle can deeply affect the quality of the oocyte, which in turn is critical to the overall results of the technique (Merton *et al.*, 2003; Sirard *et al.*, 2006).

A significantly higher number of viable embryos was obtained from Brown Swiss cows when compared to Holstein Friesian. Other Authors obtained different results from different breeds (Breuel *et al.*, 1991; Bo, 2006) it is still unknown whether this could depend from physiological status influenced from management and production levels. Also, the finding that heifers produce a lower number of embryos than cows is in agreement with the results of Hasler (2006) and Stroud and Hasler (2006) and in our opinion could be related both to a physiological limit due to an incomplete development of the genital apparatus and to management and feeding, both being often of lower quality for heifers compared with cows.

A variability in the results of superovulation connected with the season has been found by some authors (Hasler *et al.*, 1983) and not by others (Massey and Oden, 1984). Our data show a significant influence of the season in agreement with the well known reduction of fertility that affect dairy cows during the summer.

CR is deeply influenced by the quality of the embryos utilized, (obviously the better is the embryo the higher is the CR). Also, the age of the recipient has marked influence on fertility, with an higher CR in heifers when compared with cows as recently reported from other Authors (Hasler, 2006; Stroud, 2006).

The technical skill in performing the transfer is another source of significant variation of the CR. The depth of the transfer influences the success, with very deep transfers being more successful than superficial ones. An equally significant difference exists between atraumatic and traumatic transfer. So the best transfer should be very deep in the uterus but atraumatic, in a word it should be a skilled transfer.

The last significant difference we found was between right and left transfers. Since the choice of the horn is decided based on the position of the CL, the difference of CR in favour of the left horn might be due to an easier handling of this from a (right-handed) practitioner.

In the list of non significant results it is worth noting the absence of effect of the different batches of the superovulatory drug. Although already demonstrated in the past (Lindsell *et al.*, 1986; Willmot *et al.*, 1990) this finding is still often a current concern for many practitioners.

Likewise our results indicate that CL diameter has no effect on fertility, which is in agreement with the observations of Veronesi *et al.* (2002) that CL diameters is not a good indicator of CL function, although this parameter is often still taken into account in the practice.

CONCLUSION

Between the different factors that have demonstrated an influence on the results of the embryo transfer procedure, some are already well known even if not completely explained and others are tightly linked with the experience of the practitioner. Paying more attention to these aspects should give an improvement of the overall economic success of the technique.

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