TASA DE GESTACION EN HEMBRAS BOVINO DESPUÉS DE LA TRANSFERENCIA DE EMBRIONES FRESCOS O EMBRIONES CONGELADOS PRODUCIDOS IN VIVO O IN VITRO

PREGNANCY RATES IN DAIRY HEIFERS FOLLOWING TRANSFER OF FRESH EMBRYOS, OR *IN VIVO VS. IN VITRO* DERIVED FROZEN EMBRYOS

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RESUMEN

La Transferencia de Embriones o Embryo Transfer (ET) es de gran interés para la cría, debido a sus potenciales aplicaciones en la mejora genética. Nuestra investigación divulga los datos obtenidos de ET del procedimiento para la mejora genética de los bóvidos en el Sur de Italia de 1997 a 2005. El principio del superovulación se hizo entre el día 9 y 11 del ciclo del estro. Se trató a cada donante con las dosis que disminuían de LH-FSH porcino durante 5 días combinados con $PgF_{2\alpha}$ e inseminados artificialmente 2 ó 3 veces. Los embriones tranferibles, recuperados 7 días después del AI, fueron implantados en receptoras sincronizados. El exceso de embriones era congelado y sucesivamente implantado en vacas 7 días después del celo inducido. En un ensayo llevado a cabo en el periodo 2003/2005, se produjo *in vitro* congelado los embriones (OPU-IVP) obtenidos de una fábrica comercial. Los ovocitos fueron aspirados por Ovum Pick para arriba (OPU), fueron fertilizado in vitro y después cultivado por 5 días en los oviductos de las ovejas sincronizadas (cultura *in vivo*). 284 lavados uterinos fueron realizados con un promedio de 5.0±5.1 embriones tranferibles. La tasa de gestación fue de 55% (463/842). Se implantaron los 322 embriones congelados, donde 171 terminaron en gestación, sin diferencia significativa con los obtenidos usando embriones frescos. Mientras que 72 embriones implantados OPU-IVP dieron un bajo numero de gestaciones (20) que dieron lugar al 27.8% de preñez. Los resultados totales obtenidos en los diversos ensayos indicaron que la transferencia del embrión, sean frescos/congelados frescos es una técnica conveniente que puede mejorar el valor económico del grupo de estudio. En nuestra experiencia ET con los embriones OPU-IVP dieron resultados pobres que sugieren un uso limitado en la práctica del campo.
Palabras clave: bóvinos, transferencia de embriones, embriones congelados, tasa de gestación.

ABSTRACT

Embryo transfer (ET) is of great interest for animal breeding, because of its potential applications in genetic improvement. Our research reports the data obtained from ET procedure applications for bovine genetic improvement in south Italy (41°N; 16°E) from 1997 to 2005. Beginning of superovulation was made between day 9 and 11 of the oestrus cycle. Each donor was treated with decreasing doses of porcine LH-FSH for 5 days combined with $PgF_{2\alpha}$, and artificially inseminated for 2 or 3 times. Transferable embryos, recovered 7 days after last AI, were implanted in synchronized recipients. The surplus of embryos were frozen and successively implanted in cows 7 days after induced estrus. In a trial carried on 2003/2005 period, frozen *in vitro produced* embryos (OPU-IVP) obtained from a commercial factory, were used. Oocytes aspirated by Ovum Pick Up (OPU), were *in vitro* fertilized and then cultured for 5 days into the oviducts of synchronized sheep (*in vivo* culture). 284 flushing were totally performed, with a mean of 5.0±5.1 transferable embryos for flushing. The pregnancy rate (PR) was 55% (463/842). As concerns 322 implanted frozen embryos, 171 resulted in pregnancies (PR=53%) with no significant difference with those obtained using fresh embryos. Whereas 72 OPU-IVP implanted embryos gave a low number of pregnancies (20) which result in the 27.8% of PR. The overall results obtained in the different trials indicate the embryo transfer, with fresh as well fresh/frozen embryos, is a suitable technique that can improve the economic value of herd. In our experience the ET with OPU-IVP embryos gave low results that, at moment, suggest a marginal use in the field practice.
Key words: bovine, embryo transfer, frozen embryos, pregnancy rate.

INTRODUCTION

Multiple Ovulation and Embryo Transfer (MOET) is of great interest for animal breeding, because of its potential applications in genetic improvement (Lonergan, 2007) as soon as for bypassing infertility caused by ovulation and fertilization failure and early embryonic death. So, it's possible to choose animals with both high production and environmental adaptability, use their genetic heritage and increase the

breeding productions (Funk, 2006; Abdel-Azim and Schnell, 2007). The introduction of on-farm embryo freezing of in vivo-derived embryos allowed the wide-spread commercial use of frozen embryos. The present study is an attempt to quantitate embryos production after superstimulation in Frisona Italiana dairy cows and subsequent pregnancy rates (PR) following embryo transfer over the past 9 years, using fresh and frozen embryos. Moreover we used frozen embryos obtained from two technologies: the Ovum Pick Up (OPU) and the In Vitro Production (IVP). The OPU/IVP was initially applied to obtain multiple ovulation for embryo production from infertile cows with high genetic values, is now utilized also in cattle industry to enhancing breeding scheme designs. The *in vitro* produced embryos will provide the basis for more advanced technologies such as cloning and transgenic embryo production too.

MATERIALS AND METHODS

Our work reports the data obtained from embryo transfer procedure applications for bovine genetic improvement in south of Italy (41°N; 16°E) between 1997 and 2005. Throughout the trials, between day 9 and 11 of the estrus cycle of donor, the following procedures of superovulation was used. Decreasing doses of *porcine*-FSH-LH (PLUSET®, Serovet; Italy, FSH:LH ratio 1:1) were administered twice a day for 5 consecutive days (IU: 150 - 125 - 100 - 75 - 50). Generally, 800-1000 UI for cow and 500 UI for heifer donors, were used. For estrus synchronization 0,524 mg of PgF_{2a} (Estrumate, Shering-Plough, Italy) was injected in the second day at donors and at the third day at recipients. Synchronized heifers were chosen as recipients on the basis of body weight (>350 kg), reproductive activity (nulliparous and cyclic), body condition score (2.75), and manual/ultrasonographic evidence of a good corpus luteum. In superovulated donors, 2 or 3 artificial inseminations (8 hours apart) were performed after beginning of estrus. Embryos were recovered from the uterus by a nonsurgical flushing technique 7 days after AI (day 0), using modified PBS, with Fetal Calf Serum (1%) and Bovine Serum Albumin, fraction V (0.02%) and evaluated in 100 ml Petri dishes containing PBS. Then the transferable ones were washed 3 times in a holding medium, to remove contaminants from the pellucid zone and packaged in 0.25 ml straws. When transferable embryos were more than recipients, they were frozen in medium containing glycerol (10%) as cryoprotectant. In recent years (2003/2005) *in vitro/in vivo* produced (IVP) frozen embryos distributed by a commercial company (Laboratorio di Tecnologie Riproduttive, Cremona, Italy), were implanted. Oocytes from selected donors were recovered by non-surgical OPU technique. After IVF (2 days), the presumptive zygotes were in vitro cleaved in micro-drops of SOF (Synthetic Oviductal Fluid) and transferred in the oviducts of synchronized sheep for *in vivo* growing (7 days) then re-collected and frozen in one-step glycerol (10%) medium (Galli et al., 2001; Galli et al., 2003). After thawing (10'' at room temperature + 20'' in water at 28°C), embryos were passed (5 minutes each) in 3 consecutive medium with decreasing molarity of sucrose (6.6 M, 3.3 M, 0 M) in order to remove cryprotective agent, and then implanted in recipients 7 day after stimulated estrus. All data are presented on tables as mean per year (±s.d.). Pregnancy rates of recipients were compared using Chi square test (General Linear Models procedures of SAS® software - SAS Institute, 1990).

RESULTS AND DISCUSSION

Between 1997 and 2005, 284 recoveries (Table 1) were totally performed collecting 2536 embryos (9.7±7 for each flushing); 1251 of them (49%), were evaluated of good or high quality, with a mean of 4.9±5.5 transferable embryos *per* flushing. Among these 842 (67.3%) were not surgically transferred soon after collection utilising generally one embryo per recipient. Throughout the observation period, the number of collected embryos varied from 5.6 to 12.8, instead the number of transferable ones fluctuated from 4.0 to 6.3, reflecting the European Embryo Transfer Congress (AETE) published values. The remaining 409 embryos (32.7%), were frozen in glycerol (10%) medium. The pregnancy rate was 55% (463/842) for fresh embryos and, as concerns 322 implanted frozen embryos (Table 2), 171 resulted in pregnancies (53.2%). To compare the PR among frozen embryos we considered only the period between 2003 and 2005 in which there were totally implanted 191 *in vivo*-derived embryos (Table 3, last 3 ys.), and 72 OPU-*in vitro*-produced embryos (Table 3). PR of 55% (105/191) for *in vivo* produced embryos was statistically different (*p=0.05*) from PR (27.8%; 20/72) obtained in OPU-IVP implanted embryos. The better results progressively obtained year by year (Table 1), especially in the last period, is clearly related to the improvement of both the recovery/implant techniques and recipient's management.

Table 1. Results of 9 years of recoveries and fresh embryo transfer in dairy cows.

Year	Recoveries data				Embryo-transfer data	
	Successful recoveries		Embryos collected N° (x±d.s.) Range	Transferable N° (x±d.s.) Range	Implanted** N° (x±d.s.) Range	Pregnancies N° (x±d.s.) Range
	No*	Yes				

1997	8	10	75 (7.5±5.0) 1÷16	63 (6.3±5.4) 0÷16	20 (2.2±2.9) 0÷8	6 (0.6±1.0) 0÷3
1998	12	26	145 (5.6±5.4) 1÷19	121 (4.6±4.8) 0÷19	66 (2.7±2.3) 0÷7	18 (0.7±1.1) 0÷3
1999	4	30	309 (10.3±8.4) 1÷45	149 (4.9±5.0) 0÷16	102 (3.4±3.9) 0÷13	58 (3.2±1.4) 0÷6
2000	2	26	255 (9.8±6.8) 1÷30	134 (5.5±7.1) 0÷29	89 (3.4±3.3) 0÷11	46 (1.7±2.2) 0÷8
2001	1	26	231 (8.9±6.3) 1÷30	103 (3.1±3.2) 0÷12	85 (3.3±2.7) 0÷9	51 (1.1±1.7) 0÷6
2002	0	28	340 (12.1±8.3) 1÷33	127 (4.5±4.7) 0÷15	88 (3.1±3.5) 0÷11	51 (2.4±2.1) 0÷8
2003	4	27	281 (10.4±7.4) 1÷24	144 (5.3±4.9) 0÷16	99 (3.7±3.5) 0÷12	63 (2.3±2.6) 0÷8
2004	0	36	460 (12.8±7.4) 1÷30	196 (5.4±5.1) 0÷15	131 (3.6±3.4) 0÷11	74 (2.0±2.4) 0÷8
2005	2	42	440 (10.5±8.3) 1÷45	214 (5.1±5.6) 0÷26	162 (3.8±3.5) 0÷12	96 (2.3±2.3) 0÷8
TOTAL	33	251	2536 (281.8±125.3) 75÷460	1251 (49%) (139.0±45.5) 63÷214	842 (67.3%) (93.5±39.5) 20÷162	463 **(55%)** (51.3±27.1) 6÷96

**Any embryo collected. **Not transferred embryos were immediately frozen on farm.*

Table 2. Pregnancy rates of 8 years of frozen/thawed in vivo-derived embryos transfer.

Year	Embryos frozen on farm	
	Implanted (n)	Pregnancy (n) (%)
1998	9	2 (22)
1999	36	16 (45)
2000	34	20 (58)
2001	17	9 (33)
2002	35	19 (54)
2003	48	31 (64)
2004	91	50 (54)
2005	52	24 (46)
Total	322	171 (53.2)

Table 3. Pregnancy rate of 3 ys. of frozen/thawed OPU-IVP embryos transfer. The fertilized oocytes were *cultured in vivo* in sheep oviducts (temporary recipients).

Year	Implanted (n)	Pregnancy (n) (%)
2003	31	10 (32)
2004	27	5 (18)
2005	14	5 (35)
TOTAL	72	20 (27.8)

CONCLUSIONS

The overall results obtained in the different trials indicate the embryo transfer utilising fresh, as well frozen in vivo-derived embryos is a suitable technique that can improve the genetic and economic value of the herds, because of its potential use in genetic selection strategies and crossbreeding schemes (Hansen, 2006).

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