



EMBRYO TRANSFER FOR TRANS CERVICAL IN CATTLE

Ebrahim Babaahmady

Veterinaria Faculty, Ilam University, Ilam, Iran

ABSTRACT

The aim of this study was to analyze the incidence of stage of embryonic development, embryo quality and the time of transfer on pregnancy rate in embryo transfer program timed. We worked a total of 23 Holstein cows as embryo donors, super-ovulated with gonadotropin of pregnant mare serum (PMSG) (2500-3000 UI). 69 cows were used as recipients of Holstein dairy heifers with an age of 20 months and 350 kg average, synchronized with an injection of 500 mg of prostaglandin $F_{2\alpha}$. The result of embryo transfer is influenced by several variables that can be grouped as related to the embryo, with the recipient and the actual transfer. We conclude that non-surgical embryo transfer in cattle provides satisfactory results in terms of production.

Keyword: Embryo transfer, Synchronization, Donors, Recipients, Prostaglandin $F_{2\alpha}$.

INTRODUCTION

The embryo transfer programs have the ability to spread the wealth of superior genetics in livestock and shorten generation intervals in selection programs. The transfer of an embryo from a donor cow and a bull including the uterus of a recipient cow. Presently, this technique is used worldwide, but during the past decade has made little progress, especially in aspects of the response to the super-ovulation treatments and improved pregnancy rates (Wright, 1981). Economic success depends on genetics used, livestock management and marketing and sales skills. The selection of donors, recipient's management, education and technical training of field staff to address handling and superior animal husbandry are the factors that affect the outcome, cost and benefits of embryo transfer programs (Santos et al., 2004). It is useful and encouraging to know that through embryo transfer programs well established and constant can increase the production of superior genetics on a ranch, provided there is a number of factors that are important for good results (Pedroso and Roller, 2004). The period in which the zeal manifested in replacement heifers after joining reproduction and post partum cows are important aspects in order to improve the reproductive efficiency of livestock and are vital to maintain a period of service 85 days of calving interval of 365 days. Females inseminated once the pregnancy diagnosis is performed by rectal palpation (Gil et al. 1999). With the exception of vaginal right diagnosis, other diagnostic methods are not available on all livestock farms (Humblot, 2002). The technology of embryo transfer in cattle requires the selection and management, physical and pharmacological, of the donors and recipients as well as the collection and

transfer of embryos within a short, specified period after estrus (Mapletoft, 2006). The embryo transfer is performed for more than thirty years (Merton et al, 2003). Today it is widely used worldwide (Cutini et al, 2000; Duica et al, 2007) and its main objective is to obtain offspring from genetically superior donor using the recipient's uterus less economic value to bring term gestation (Bóet a., 2006). For the introduction of the technique of embryo transfer in production conditions, the development of non-surgical methods of collection and transfer play an important role (Duica et al, 2007; Peres et al, 2006). Currently the results obtained with the use of non-surgical method are comparable to the surgical procedure, are influenced largely by the skill of the personnel performing the technique (Pedroso, 2003).

Materials and Methods

Therefore a total of 23 Holstein cows as donors of embryos gonadotropin administration of pregnant mare serum (PMSG) (2500-3000 UI) intramuscularly rectal prior diagnosis of a corpus luteum in one cycle their ovaries; past 48 hours is 500 mg supply prostaglandin F₂ alpha (PG). Artificial insemination began at 36 hours applied the (PG) and repeated every 12 hours for up to three services per animal. Two pellets were used in each service semen artificial insemination (AI). The embryo collection was performed on days 7 and 8 after the first AI. A Foley catheter is job No.14 which was introduced trans cervically with the aid of a metal mandrel and rectal control. Uterine horns were washed with buffered saline (PBS) supplemented with fetal bovine serum (FBS) 2%. After the super-ovulation and washing, the embryos collected from a donor are classified according to their morphology and development stage and subsequently frozen or transferred to recipient. At the same time, it must decide which recipients should receive embryos available for the greatest number of pregnancies (Wright, 1981).

The embryos were classified into three categories, according to their morphological characteristics (B, R, M). B: morulae and blastocysts with the zona pellucida intact cell compaction, blastomeres sharpness. R: Embryos with scattered cells and irregular deformed zona pellucida. M: unfertilized ova, embryos and retarded development stages of embryos with signs of cellular degeneration. Only those embryos were transferred classified as B and R. Those remaining after selection in PBS+ 20% FBS: 69 were used as recipients breeding dairy heifers F₂ (Holstein) with an age of 20 months and average weight 350 kg. Achieving synchronization with an injection of 500 mg of PG. Ipsilateral has transferred existing one corpus luteum of the ovary and synchronization ± 1 day with the female donor. Embryonation equipment used consisted of a Teflon catheter (0.5 mm outer diameter) with a metal mandrel which allowed passage of the instrument under control transcervical rectal. And placed in the corresponding horn is retreat mandrel and introduced through a capillary teflon catheter containing the embryo plastic suspended in PBS+ 20% FBS and impelled within the uterine body at the middle portion used a syringe with 2 cm³ of air. Some see the transfer completed, the animals continued in regular operation. The pregnancy diagnosis was performed by rectal between 60 and 80 days to transfer synchronized estrus.

RESULTS

Of the total super-ovulated females responded to treatment for a 78.26% 18. Mean embryos classified as eligible to be transferred was treated three females (Table 1).

Of the 69 embryos equal number of recipients, managed gestation 29, which has a 42.03% success rate. The diagnosis was made between 45 and 80 days rectally.

Table 2 shows that there was obtained as light increase in pregnancy rates (9.09%) when receiving the heat introduced one day before the donor of the embryos (day +1), while forzerodays-1the percentages obtained were not significantly different between groups ($p = .05$).

The highest pregnancy results corresponded to the morulae stage (44.19), while with blasts was achieved 38.46% (Table 3).

Discussion

For greater efficiency in herd reproductive performance, pregnancy diagnosis of cows needs to be done in the shortest possible time after service with the aim of reinseminate those resulting nonpregnant (Thatcher *et al.*, 2002). They give great significance to clinical gynecological examination prior to treatment in order to define ovarian structures present and the clinical characteristics of the uterus as indices to define the state of the estrous cycle in cows found (Bartholomew *et al.*, 2005) before synchronization of estrus.

During the last decade has made remarkable progress in the understanding of the dynamics of growth and development of the ovarian follicle, corpus luteum function and neuroendocrine control (Tenhagen *et al.*, 2004). Parallel studies were conducted aimed at manipulating these functions by pharmacological methods by using Progesterone (Anderson and Day, 1994), Testosterone (Rajamahendran and Manikkam, 1994), Estrogen (Bo *et al.*, 1995), Factors releasing Hormone and Growth Hormone (Twagiramungu, 1995). This has allowed the development of estrus synchronization technologies, superovulation and embryo transfer (Mapletoft *et al.*, 2003) and strategies to improve fertility (Thatcher *et al.*, 2003) and methods to identify and induce the return to service of inseminated females in an unknown state of gestation (Chenault, 2003) or nonpregnant (Chebel *et al.*, 2003) in order to optimize breeding programs using estrus synchronization and artificial insemination, the systems seasonal and delivery services and possibly improve fertility (Lucy, 2005). Thus arose the two procedures re synchronization of oestrus and ovulation in cows led to unknown state of pregnancy or in those who previously made the pregnancy diagnosis.

The embryos were transferred non-surgically to the uterine horn for the ovarian corpus luteum presented under epidural anesthesia. The results of the super ovulations to animals that responded to the application of PMSG (78.26%) and transferable embryos treated females, are slightly lower than those obtained by (Hasleretal, 1987), these results were consistent with published for(Lerneretal, 1986). These poor results may have been influenced by the short period after the part where he was practicing superovulation treatment($x = 50$) days in accordance with (Hasleretal, 1983). In turn for the start of superovulation, was based on the presence rectally.

According to (Nebeletal, 1997), pregnancy rates with the surgical method are high and stable (55 to 65%), whereas via cervical variable results (20 to 60%). Despite this, the fast, easy and economicalitis the latter, it does election to avoid the cumbersome and expensive surgery application in commercial conditions.

With the introduction and widespread use of technology induction and synchronization of estrus Similar phenomena have emerged that are affecting the efficacy of estrus synchronization programs (Santos *et al.*, 2004). These females cause great economic losses due to the impact they have on the lengthening of the interval between births and increased days open especially in dairy cows (Macmillan *et al.*, 2003).

This problem has been the source of many studies and has stimulated the development of various strategies to resynchronize the zeal and induce return to service unknown females in gestation or

nonpregnant (Stevenson and Tiffany, 2004) in order to identify them and reduce the interval between services.

The introduction and improvement of techniques for induction of estrus and intensive program of training of professionals and technicians working in the field of animal reproduction and artificial insemination services in Cuba has contributed to the significant improvement in the efficiency and quality of artificial insemination services in the last 10 years. However, the efficiency shown by these control procedures has been highly variable reproductive (Pedroso and Roller, 2004).

In the development of non-surgical technique many researchers have worked, among which are highlighted (Sugie, 1965; Lawseetal,1975,Hahnetal,1976,Hansen, 1976; Testart, 1977,Brand et al, 1977; Bernardetal, 1978, Wright, 1981,Thompsonetal, 1982), which have different equipment andproceduresdescribed.

The transfer of embryos technique is fast and constantly evolving, varying methods and products used continuously, and the analysis of the ability of each ranch to establish their individual program, considering the needs of production and their ability to work in terms of facilities, location, time of year, as the group of recipients, influencing whether a program works properly. The results of the technique in terms of number of embryos obtained per donor and pregnancy rate, have a direct impact on the economic results of the program. The results depend on the experience and skill of technical, organizational structures in the service of the breeders. Investment in hormones, materials and professional fees have on impact on the cost depending on the results (Duicaetal., 2007). In the process of embryo transfer are included other factors of production, which are not always covered by breeders, because they do not mean cash outlay, but should be recognized as current costs and not deferred or absent. If no pregnancies, if not born then calves, embryos were only an illusion and real economic losses. The most expensive transfers of embryos are not born calves, waste and frustration genetic customers against such expectation (Palma etal., 1993).

Conclusion

However, as the post partum no estrus is one of the main problems affecting reproductive efficiency and effectiveness of artificial insemination programs under this management system deepens during the reduced availability of pastures have been used various biotechnical procedures to induce estrus and ovulation. To date obtained fertility at first service today does not exceed 30% when required at least conception rate of 60% within a period of 70 days.

References

- Anderson, R. H. and M. L. Day. Acute progesterone administration regresses persistent dominant follicles and improvement fertility of cattle in which estrus was synchronized with Melangestrol acetate. *J. Anim. Sci.* 72: 2955-2961, 1994.
- Bartolome, J. A; A Sozzi; J. McHale; K. Swift; D. Kelbert; L. F. Archibald; W. W. Thatcher. Resynchronization of ovulation and timed insemination in lactating dairy cows III: Administration of GnRH 23 day post Ai and ultrasonography for non pregnancy diagnosis on day 30. *Theriogenology.* 63: 1643-1658, 2005

- Bó, G.A., Moreno, D., Cutaia, L., Caccia, M., Tríbulo, R.J., Tríbulo, H.E. (2006). Transferencia de embriones a tiempo fijo: tratamientos y factores que afectan los índices de preñez. Educación Continua. UNCPBA
- Bo, G.A.; G.P. Adams; R.A. Pierson and R.J. Mapletoft. Exogenous control of follicular wave emergence in cattle. *Theriogenology* 43: 31-40, 1995.
- Chebel, R; J. E. P. Santos; S. Junchem; K. N. Galvão; W.W. Thatcher. Effect resynchronization with GnRH on day 21 after insemination on pregnancy rate and pregnancy loss in lactating dairy cows. *Theriogenology* 60: 1389-99, 2003.
- Chenault, J. R; J. F. Boucher; K. J. Dame; J. A. Mayer, and S. L. Wood-Follies. Intravaginal progesterone insert to previous inseminated dairy cows. *J. Dairy Sci.* 86: 2039-2049, 2003.
- Cutini, A., Teruel, M., Cabodevila, J. (2000). Factores que determinan el resultado de la transferencia no quirúrgica de embriones bovinos. *Revista Taurus* N°7, 28-39 y N°8, 35-47.
- Duica, A., Tovio, N., Grajales H. (2007). Factores que afectan la eficiencia reproductiva de la hembra receptora en un programa de trasplante de embriones bovinos. *Revista de Medicina Veterinaria*, julio-diciembre, numero 014. Universidad de La Salle, Bogota, Colombia. Pp 107-124
- Gil, A.; J.L. González; F. Agüero y R. Faure. Diagnóstico precoz de no gestación en bovinos con el Benzoato de Estradiol. *Rev. Cub. Reprod. Anim.* 25: 27-30, 1999.
- Hasler J. F., McCauley A. D., Lathrop W. F., Foote R. H. Effect of donor-recipient interactions on pregnancy rate in a large scale bovine embryo transfer program. *Theriogenology* 1987; 27:139-168.
- Hasler J.F., McCauley A.D., Schermerhorn E.C., Foote R.H. Superovulatory responses of Holstein cows, *Theriogenology* 19 (1983) 83-99.
- Humblot, P. Use pregnancy specific proteins and progesterone assay to monitor pregnancy and determine the timing frequencies and source of embryonic mortality in ruminants. *Theriogenology*. 56: 1417-1433, 2002.
- Lerner S.P., Thayne W.V., Baker R.D., Hensche T., Meredith S., Inskeep E.K., Dailey R.A., Lewis P.E., Butcher R.L., Age, dose of FSH and other factors affecting superovulation in Holstein cows, *J. Anim. Sci.* 63 (1986) 176-183.
- Lucy, M. C. Second Insemination Breeding Strategies for Dairy Cows. *Advances in Dairy Technology* 17: 149-157, 2005.
- Macmillan, K. L; B. V. Segwagwe; C. S. Pino. Association between the manipulation of patterns of follicular development and fertility in cattle. *Anim. Reprod.* 78: 304-307, 2003.
- Mapletoft, R. "Transferencia de embriones bovinos" (2006). *IVIS Reviews in Veterinary Medicine*, I.V.I.S. (Ed). International VeterinaryInformationService, Ithaca NY. Disponible en URL www.ivis.org, Ultima actualización 17-noviembre-2006.
- Mapletoft, R. J. M. F. Martínez; M. G. Colazo; and j. P. Kastelic. The use of controlled internal drug release device for the regulation of bovine reproduction. *J. Anim Sci*81:E, suppl,2: E28-E36,2003.
- Merton, JS. A.P.W. de Roos, E. Mullaart, Ruigh L., Kaal L., P.L.A.M. Vos and S.J. Dieleman, (2003). Factors affecting oocyte quality and quantity in comercial applications of embryo technologies in the cattle breeding industry. *Theriogenology*, 59, 651-674.
- Nebel, R. L. and S. m. Jobst. Evaluation of systemic Breeding Programs for Lactating Dairy cows.A. Review. *J. Dairy. Sci.* 81: 1169-1174,1997.
- Palma, G.A., Brem, G. (1993) "Transferencia de embriones y biotecnología de la reproducción en la especie bovina". Editorial Hemisferio Sur.

- Pedroso, R y Felicia Roller. Impacto de las estrategias farmacológicas para identificar e inducir retorno al servicio de hembras inseminadas en la producción pecuaria. *Rev. Cub. Reprod. Anim.* 30: 13-29, 2004.
- Pedroso, R. Métodos biotécnicos para mejorar la fertilidad del ganado bovino en los programas de inseminación artificial e inducción y sincronización del celo. Tesis, Para la Opción al grado Científico de Dr.Cs. Universidad Agraria de La Habana. La Habana, 17 de julio. 2003
- Peres, L.C., Pincinato, D., Cutaia, L., Bó, G.A. (2006). Simplificación de los programas de Transferencia de Embriones a Tiempo Fijo en Rodeos Comerciales. *Jornadas de Actualización en Biotecnologías de la Reproducción en Bovinos-IRAC.*
- Rajamahendran, R. and M. Manikkam. Effect of exogenous steroids hormones on the dominant follicle maintained by a Norgestomet implant in heifers. *Can. J. Anim. Sci.* 74: 457-464, 1994
- Santos, J. E. P., J. A. Bartolome., R. L. A. Cerri., S. O. Juchem., W. W. Thatcher. O. Hernandez., T. Trigg. Effect of a deslorelin implant in a time artificial insemination protocol on follicle development, luteal function and reproductive performance of lactating dairy cows. *Theriogenology* . 61: 421-435, 2004b.
- Santos, J. E. P., W. W. Thatcher., R. C. Chebel., R. L. A. Cerri., K. N. Galvão. The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. *AnimReprod. Sci.* 82-83: 513-535, 2004.
- Stevenson, J. S and S. M. Tiffany. Resynchronization estrus and Ovulation after not-pregnant diagnosis and various states including cysts. *American Dairy Science Association* , 87: 3658-3664, 2004.
- Tenhagen, M.B.A. and R. Drich., R. T. Surho and W. Heuwieser. Compararison of timed AI after Synchronized Ovulation to AI at estrus: Reproductive and economic Considerations. *J. dairy Sci.* 87: 85-94, 2004.
- Thatcher, W. W; A. Guzeloglu; A. Meikler; S. Kamimura; T. Bilby; A. A. Kowaalski; L. Badingan; R. Pershing; J. Bartolome; and J. E. P. Santos. Regulation of embryo survival in cattle. *Reproduction Supplement*, 61: 253-266, 2003.
- Thatcher, W.W; F. Moreira; S.M. Pancarci; J.A. Bartolome; J. E. P. Santos. Strategies to optimize reproductive efficiency by regulation of ovarian function. *Domestic Animal Endocrinology*. 23: 243-254, 2002.
- Twagiramungu, H.; L.A. Guilbault and J. Dufour. Synchronization of ovarian follicular wave with a gonadotrophinreleasing hormone agonist to increase the precision of estrus in cattle. *A review J. Anim. Sci.* 73: 3141-3151, 1995.
- WRIGHT J. M. Non-surgical embryo transfer in cattle, embryo-recipient interaction. *Theriogenology* 1981; 15:43-56.

Table 1.Results in the super-ovulation.

Treatedfemales	Responded	%	TransferableEmbryos	EmbryosRated(X)
23	18	78.26	69	3

Table 2.Gestation results achieved according to the degree of synchronization between recipients and donors.

Estrous Synchronization (day)	Recipient Females		
	Total	pregnant	
+1	28	14	50.00
0	22	9	40.91
-1	19	6	31.57
Total	69	29	42.03

Table 3.Transfer results according non surgical embryo development.

Estadio Embrionario	Hembras Receptoras		
	Total	Gestantes	%
Morula	43	19	44.19
Blastocisto	26	10	38.46