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Multiple Ovulation and Non-Surgical Embryo Transfer in Cattle by Using Intravaginal Controlled Internal Drug Release (CIDR) Progesterone Inserts

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Abstract

This paper has been presented to share the protocol and techniques of multiple ovulation and non-surgical embryo transfer in cattle among Nepalese animal scientists. Three elite Holstein cows kept for research and study purpose in the animal farm of the University of Queensland, Gatton, Australia were selected as donors of the embryo for this multiple ovulation and embryo transfer program. Similarly, 11 Holstein heifers of age 14 to 20 months from the same farm were selected as recipients for the program. Synchronization of estrus in both donor and recipients and superovulation in donor has been done by using intravaginal controlled internal drug release (CIDR) progesterone inserts, GnRH, FSH and $PGF_{2á}$ as per a set protocol. Oestrus was detected by heat mount detector and artificial insemination in donors was done immediately after detecting heat and repeated every 12 hours for 2 to 3 times with semen of proven bulls. Embryos from the donors were recovered by flushing using Foley catheter. The embryos were then evaluated microscopically according to the stage of development and quality of them. The embryos were non-surgically transferred into recipients by using embryo transfer gun. Unrecovered embryos were terminated by injecting $PGF_{2á}$ after 6 days. The pregnancies in recipients were diagnosed on 45th dayof transfer by real time ultrasonography (USG). All of the recipients (100%) came in estrus within 72 hours of removal of CIDR while it took 96 hours after removal of CIDR to donors to show the sign of estrus. Sixteen embryos were recovered from three donors out of which 11 (68.75 %) were transferable. Number of transferable embryos could be collected from one donor which could affect the average result. Five cows (62.5%) were pregnant when diagnosed by ultrasonography (USG) after 7 weeks.

Key words: oestrus synchronization, superovulation, embryo evaluation

Introduction

The most obvious and widely used purpose of multiple ovulation and embryo transfer (MOET) in domestic animals including cattle is genetic improvement of the animals. MOET helps to increase genetic superiority in animals by increasing selection intensity in dams, reducing generation interval, increasing accuracy of selection and increasing variation of the traits (Noakes *et al.* 2001).

Other advantages of multiple ovulation and embryo transfer are as follows:

1. MOET can be used to transfer superior genetic material from one country to another which is far more efficient than transfer of large animals (Noakes *et.al.* 2001, Senger 2003).
2. The MOET program can be used for biodiversity conservation by using the technology in reproduction of endangered animals (Senger 2003).
3. The calving time of animals can be matched with availability of feed (e.g. pasture) and

marketing opportunity to increase the profit from the enterprise (Bearden & Fuquay 2000) and to make the management easy.

4. MOET can be used in research studies about female reproduction, endocrinology, embryology, genetics and animal diseases (Bogh & Greve 2009, Noakes *et al.* 2001).
5. There is less possibility of disease transfer from parent to offspring born by embryo transfer technology (Noakes *et al.* 2001).
6. Animals with infertility can be treated with MOET technology and twinning can also be induced by using MOET (Seidel & Seidel 1991).

Although transfer of embryos imported from abroad has been done in Nepal in the past, complete process from multiple ovulation up to the transfer has not been practiced till now. This paper is presented to share the protocols and techniques for multiple ovulation and embryo transfer in cattle to Nepalese scientists.

Methodology

Animal used

Three high producing Holstein cows of the age from 73 months to 133 months from The University of Queensland, Gatton dairy were selected as donors for this study. Durations from last calving for three donors were 3 months, 10 months and 14 months respectively. Similarly, eleven Holstein heifers of age 14 months to 20 months were selected as recipients for the study.

Husbandry

All of the animals are kept for research and study purpose the University of Queensland, Gatton. Animals are fed with total mix ration (TMR) with milking twice daily. Oestrus is detected by heat mount detector (Kamar) and artificial insemination is used to breed the animal.

Oestrus synchronisation and superovulation

Oestrus synchronization in donors

Delicate and complexly balanced level of gonadotropin releasing hormone (GnRH), gonadotropins (FSH and LH), ovarian steroids (estrogen and progesterone) and prostaglandin $F_{2á}$ by endocrine and neuro-endocrine systems are responsible for overall cyclic activity during oestrus cycle of animals (Cunningham & Klein 2007, Noakes *et al*. 2001, Kojima 2003). The oestrus in donors is synchronized for two purposes. First, by synchronizing we can collect embryos from several donors at the same time, and the second, it helps to bring both the donors and recipient animals in same stage of cycle which is a must for physiological adaption of embryo from donor in the uterus of recipients (Senger 2003).

Major organ manipulated is the CL and either lifespan of CL is shortened or prolonged for oestrus synchronization by using prostaglandin or progestagen respectively (Bearden & Fuquay 2000, Niasari-Naslaji 1996). Administration of prostaglandin results in low blood progesterone level by regression of CL which in turn causes elevated level of endogenous gonadotropins (FSH and LH) due to higher GnRH secretion (Senger 2003, Bearden & Fuquay 2000) which in turn causes all of the animals with functional CL of 5 to 17 days come into oestrus within 4 days (Bearden & Fuquay 2000).To cover animals with immature CL (age of less than 5 days), two injections of $PGF_{2á}$ in 11-14 days apart is used (Bearden & Fuquay 2000).

Progestagen (or progestin) method was used to synchronize oestrus in both donors and recipients in this study. In progestagen method, progesterone is used for approximately 7 days which inhibits release of GnRH and gonadotropin with halted follicular growth and ovulation (Bearden & Fuquay 2000, Niasari-Naslaji 1996) in animals with functional CLs which regress within progesterone supply period (Senger 2003, Bearden & Fuquay 2000). Removal of progesterone causes spontaneous oestrus in these animals while the other animals with functional CL till the end of progesterone supply are brought to oestrus in 3 to 5 days by injecting $PGF_{2á}$ (Senger 2003, Bearden & Fuquay 2000, Jainudeen, Wahid & Hafez 2000). Intravaginal controlled internal drug release (CIDR) inserts were used as the source of progesterone.

Superovulation in donors

To acquire the benefit from embryo transfer program, it is essential to produce more number. of oocytes in single cycle from an elite dam to increase genetic gain by increasing selection intensity in female. This multiple ovulation was achieved by injecting follicle stimulating hormone (FSH) which either recruit new follicles for ovulation by inhibiting the function of inhibin (Cunningham & Klein 2007) or prevent

already growing follicle to become atretic (Niasari-Naslaji 1996). Superovulation protocols also comprised of $PGF_{2\alpha}$ to regress pre-existing CLs. Superovulation protocol in donors has been initiated by making sure that the reproductive cycle of donors and recipients coincide (Bearden & Fuquay 2000). Detail protocol of oestrus synchronization and superovulation program in donors is as in Table 1 and figure 1 below.

Table 1. Detail protocol for synchronization of oestrus and superovulation in donors

Day	Hormone/Device	No./Dose
0	CIDR	One
2	GnRH	2 ml I/M
6	FSH 1 & 2 / Heat detector (kamar)	4 ml am and pm I/M
7	FSH 3 &4	3 ml am and pm I/M
8	FSH 5 & 6 / Remove CIDR	2 ml am and pm I/M
9	FSH 7 & 8 / $PGF_{2\alpha}$	FSH - 1 ml am and pm I/M $PGF_{2\alpha}$ – 5 ml am and pm I/M

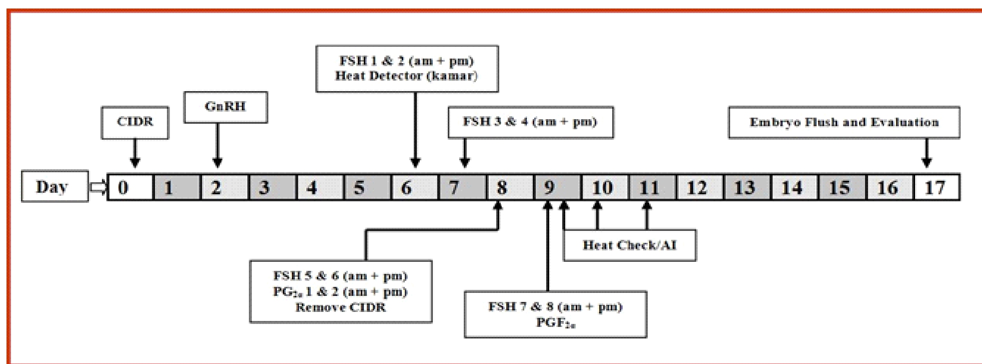

Fig. 1. Detail protocol for oestrus synchronization and superovulation in donors

Synchronization of oestrus in recipients:

Oestrus in the recipients were synchronized in two way i.e. synchronization of oestrus among recipients to transfer the embryo to all of the recipients at the same time and synchronization of oestrus of recipients with donors for physiological adaptation of embryo from donors in uterus of recipients. Detail protocol of oestrus synchronization in recipients is depicted in table 2 and figure 2 below.

Table 2. Detail protocol for oestrus synchronization in recipients

Day	Hormone/device	No./Dose
0	CIDR	One
7	$PGF_{2\alpha}$ / Apply heat detector (kamar)	5 ml I/M
8	Remove CIDR	

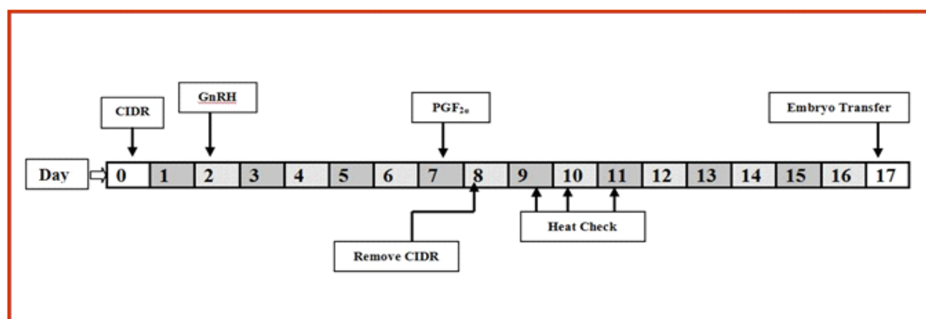

Fig. 2. Detail protocol for oestrus synchronization in recipients

Artificial insemination

Artificial insemination was done immediately after detecting heat and repeated after every 12 hours for two or three times with semen from proven bulls.

Embryo collection

The embryos were recovered from the uterus by non-surgical technique as described below.

- Epidural anaesthesia was applied through epidural space above tail head.
- Numbers of corpura lutia (CL) were estimated by palpating per rectum.
- The recovery of embryo was done by using Foley catheter through cervix with manipulating per rectum.
- The catheter was inserted through the cervix into the uterine body and the balloon of the catheter was inflated.
- Uterus was filled and emptied with continuous flow of medium from disposable plastic infusion bag through the Foley catheter and embryos were trapped by filtering the fluid on a small disc.
- Embryos from filter were transferred into searching disc by rinsing the filter.

Embryo evaluation

After rinsing the embryos from filter to searching disc, the dick was systematically examined under microscope (first in low power and then high power) to find the embryo and transferred to another disc with fine micropipette. After locating and transferring all embryos they were evaluated microscopically according to stage of development (Table 3) and quality of embryo (Table 4).

Table 3. Criteria used to define the stage of development of embryos

No.	Stage
3	Morula
4	Compact Morula
5	Early Blastocyst
6	Blastocyst
7	Expanded Blastocyst
8	Hatched Blastocyst

Table 4. Criteria used to define the quality of embryos

No.	Stage	Features
1	Excellent	Perfect shape, size and structure for its age
2	Good	Slightly asymmetrical with some imperfections on non-significant characters like shape of zona pellucida
3	Fair	Small in size, moderate number of excluded cells, a few degeneration, precise but not severe anomalies
4	Poor	Very small, failure of compaction, significant degeneration, varying cell size
5	Dead or degenerating	Severely degenerated

Cryopreservation of embryos

Excess of the embryos to the number of recipients were cryopreserved. Embryos were washed, evaluated and placed into freezing medium for 10 minutes. Straw was filled with freezing medium half way after rinsing with the medium, and approx 5 mm air column and another column of freezing medium with embryo and

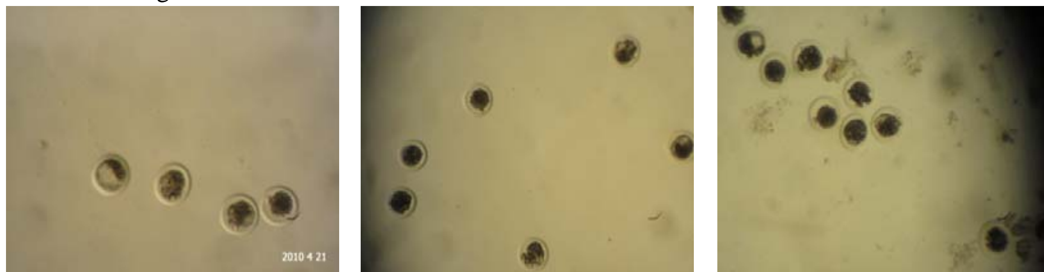

Fig. 3. Embryos recovered from the program under X1000 magnification

finally sealed with heat. The straw was freezed by placing into the freezing machine. The cooling rate was average 0.5°C/minute. When straws reached -30°C, it was plunged into liquid nitrogen and stored in liquid nitrogen.

Transfer of embryos

The embryos were non-surgically transferred into recipients as follows.

- At first, ovaries of all recipients were palpated to find out the quality of CL and side of ovulation.Epidural anaesthesia was applied through epidural space above tail head.
- Two recipient cattle were removed after palpation due to the lack of quality CL.
- Straws with embryos were loaded into a embryo transfer gun.
- Embryo transfer device was passed through the cervix and passed up to the horn ipsilateral to the corpus luteum and embryo was deposited into the horn.

Termination of unrecovered embryos

Five ml $PGF_{2\alpha}$ is injected to each animals after 6 days to terminate unrecovered embryos.

Pregnancy diagnosis

The pregnancies in recipients were diagnosed on day 45 of transfer by real time ultrasound or ultrasonography (USG). The probe or transducer was inserted into the rectum and the images were analysed on the screen. Cattle with foetal fluid and mass of conceptus in uterus were declared as positive.

Results and Discussion

Response of oestrus synchronization in donors and recipients

Eight (73%) recipients cow out of eleven came in heat within 48 hours of removal of CIDRs. Rest of the cattle (27%) recipients showed oestrus after 72 hours of removal of CIDRs. Similarly, Two donors (50%) came in oestrus within 48 hours of removal of CIDRs. One (25%) came in heat after 96 hours of removal of CIDR.

Thus, 50% of donors and 73% of recipients came in heat on day 10th of the program and 25% of donors and 27% of recipients showed symptoms of oestrus within 2 days after that.

Superovulation response in donors and quality of embryos

Numbers of embryo collected from donors are as below.

Table 5. Number of embryos recovered from each donor

No.	Total no of embryo collected	No. of transferable embryos
D1	12	9
D2	3	2
D3	1	0

Quality of embryo recovered from the program is as below.

Table 6. Quality of embryo recovered in the program

Donor	Number of Embryos	Stage of Development	Quality	Code	Total Transferable
D1	3	Unfertilized			9
	1	Blastocyst	Excellent	6-1	
	3	Early blastocyst	Good	5-2	
	2	Compact morula	Excellent	4-1	
	2	Compact morula	Good	4-2	
	1	Compact morula	Fair	4-3	
D2	1	Unfertilized			2
	2	Compact morula	Good	4-2	
D3	1	Unfertilized			0
Total					**11**

Pregnancy rate

Five cows (62.5%) were found pregnant when diagnosed by ultrasonography (USG) after 7 weeks. Although final result of multiple ovulation and embryo transfer (MOET) program is pregnant cattle, the overall success of the program depends upon many factors like superovulation response in donors, degree of synchronization of oestrus among donors and recipients, number and quality of embryo recovered etc. The number of embryo received per donor cow varies greatly ranging from 0 to 40

(Betteridge and Smith 1988; cited in Niasari-Naslaji 1996) and this is probably the most unpredictable part of MOET success.

In MOET program run in Gatton dairy of the University of Queensland, 4 cows were initially selected as donor but 1 cow was removed from the program before complete hormonal treatment. Therefore, all comparisons here will be done assuming only three donors. Total 16 embryos were obtained in the program and 11 were transferable out of them. Thus, 5.3 total embryos per donor and 3.7 transferable embryos per donor was the result of this MOET program.

Total number of embryos per donor (5.3) and transferable embryos per donor (3.7) in this experiment is little less than those found by Mikkola (2005). Mikkola (2005) recovered 8.6 total and 5.4 transferable embryos per donor. Similarly, it is also less than the response found by Hasler *et. al.* (1983). In an extensive study on Superovulation in Holstein cattle, Hasler *et. al.* (1983) obtained 5.1 total embryos per donor. Our result is comparable with this result. Ax *et al.* (2005) has tested Superovulation response in prepubertal Holstein heifers and found 7.7 total and 4.7 transferable embryos per donor in commercial setting.

Thus, total and transferable number of embryos obtained in this program is less than those reported by other scientists and also less than average commercial expectation. No transferable embryos could be collected from one donor (No. 871) which affected our average result. This donor cow actually had not shown good oestrus and also came in heat after two days of other donors. In addition, this cow had only one artificial insemination (AI) in comparison to 3 or 4 AI to other donors.

Regarding pregnancy rate, 5 out of 8 (62.5%) embryo transferred recipients were pregnant when checked with USG on 45th day of transfer. This result will be lower if we consider all of the recipients initially selected for the program (11). One cow was removed from the program before completing hormonal treatment and other two were removed from the program during embryo transfer due to lack of good quality CL on them. This pregnancy rate (62.5) is within the average rate of pregnancy reported in ET (McMillan 1998). Thiber and Nibar (1992) has reported 60-70% pregnancy rate while transferring embryos from prepubertal heifers. Hasler *et al.* (1983) reported 67% pregnancy rate in embryo transfer in large number of Holstein cattle. Purcella *et al.* (2005) have found that concept rate depends upon quality of embryo. They have reported 71.6% conception rate for embryo of grade 1 and 64.7% conception rate for grade 2. Thus, pregnancy rate on this program can be regarded as average industry standard.

All of the procedures during oestrus synchronization, superovulation, embryo collection and transfer have been done hygienically and with caution. Due to the objectives of the program and inability of preplanning in advance, donor and recipients cattle were selected randomly. This seems to be only factor affecting the overall result of the program. Therefore, proper selection of donor and recipients could improve the result of the program. As this program is designed to teach university students about MOET, handling by many (which is unavoidable) might have some influence to the result of the program.

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References

Ax, R. L., S. Armbrust, R. Tappan, G. Gilbert, J. N. Oyarzo, M. E. Bellin, D. Selner and T. C. McCauley. 2005. Superovulation and embryo recovery from peripubertal Holstein heifers *Animal Reproduction Science* **85:**71-80.

Bearden, H. J. and J. W. Fuquay. 2000. *Applied animal reproduction*. Prentice-Hall, Inc., NJ, USA.

Bogh, I. B. and T. Greve. 2009. Assisted reproductive technologies. In: *Veterinary reproduction and obstetrics,*[Eds. D. E. Noakes, T. J. Parkinson and G. C. W. England] Saunders Elsevier pp. 855-893.

Cunningham, J. G. and B. G. Klein. 2007. *Textbook of veterinary physiology*. Saunders Elsevier, St. Louis, Missouri.

Hasler, J. F., A. D. McCauley, E. C. Schermerhorn and R. H. Foote. 1983. Superovulatory responses of Holstein cows. *Theriogenology* **19:**83-99.

Jainudeen, M. R., H. Wahid and E. S. E. Hafez. 2000. Ovulation induction, embryo production and transfer. In *Reproduction in farm animals* [Eds. E. S. E. Hafez and B. Hafez). Lippincott Williams and Wilkins. pp405-430.

Kojima, F. N. 2003. The estrous cycle in cattle: Physiology, endocrinology, and follicular waves. *The Professional Animal Scientist* **19:**83-95.

McMillan, W. H. 1998. Statistical models predicting embryo survival to term in cattle after embryo transfer. *Theriogenology* **50:**1053-1070.

Mikkola, M., P. Mäntysaari, N. Tammiranta, J. Peippo and J. Taponen. 2005. Effect of dietary protein on embryo recovery rate and quality in superovulated heifers. *Animal Reproduction Science* **87:**193-202.

Niasari-Naslaji, A. 1996. Studies on oestrus synchronisation and superovulation following controlled ovarian follicular development in dairy and beef cattle. In: *Department of farm animal medicine and production*, 199. Brisbane, Australia: The University of Queensland.

Noakes, D. E., T. J. Parkinson and G. C. W. England. 2001. Embryo transfer in large domestic animals. In *Arthur's veterinary reproduction and obstetrics* (Eighth edition), Oxford: W.B. Saunders. pp 819-836.

Purcella, S. H., W. E. Beala and K. R. Gray. 2005. Effect of a CIDR insert and flunixin meglumine, administered at the time of embryo transfer, on pregnancy rate and resynchronization of estrus in beef cattle. *Theriogenology* **64:**867–878.

Seidel, G. E. and S. M. Seidel. 1991. *Training manual for embryo transfer in cattle*. Food amd Agriculture Organization of the United Nations, Rome, Italy.

Senger, P. L. 2003. *Pathways to pregnancy and parturition*. Current Conceptions Inc., Washington, USA.

Thibier, M. and M. Nibar. 1992. Clinical aspects of embryo transfer in some domestic farm animals. *Animal Reproduction Science* **28:**139-148.