FULL PAPER *Theriogenology*

The Transfer of Fresh and Frozen Embryos in an Elite Swamp Buffalo Herd

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ABSTRACT. To investigate the development of fresh and frozen swamp buffalo embryos after transfer to synchronized recipients, 14 fresh embryos and 28 frozen embryos, collected from Thai swamp buffalo cows of an elite herd at the Surin Breeding Center, were transferred nonsurgically to 31 synchronized recipients buffalo cows. One fresh embryo was transferred to each of 14 recipients. Twenty eight frozen embryos were transferred to 17 recipients of which 7 cows received 1 embryo, 9 cows received 2 embryos and 1 cow received 3 embryos. Pregnancy was diagnosed by real time B-mode ultrasonography one month after transfer and confirmed by rectal palpation one and two months later. The pregnant cows were kept under observation until calving. The results of fresh embryo transfer showed that 5/14 (35.7%) were pregnant after 30 days, 4/14 (28.6%) remained pregnant until the 3rd month and 2/14 (14.3%) calved. With the frozen embryos, only one cow which received three embryos became pregnant and remained so for 3 months although the embryo did not survive to full term. The overall pregnancy rate using frozen embryos was 5.9% (1/17). The study demonstrated the possibility of performing embryo transfer in elite buffalo herds for genetic improvement, however the use of frozen embryos needed further investigation.

KEY WORDS: embryo transfer, fresh embryo, frozen embryo, swamp buffalo.

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Embryo transfer is an accepted technique for both animal breeding improvement and disease control. It is widely used in beef and dairy cattle. In buffalo only a few successful embryo transfers have been reported since 1983 [6]. In Thailand, the first report on swamp buffalo was done by Chantaraprateep and colleagues in 1989 [5] but until now no further study has been out, due to the low number of embryos produced by the donors. Although swamp buffalo embryos could be frozen by using the program already established in cattle but no development to term has been reported [10]. In murrah buffalo only one calf was born after transfer to 15 recipients, most of them having degenerated or aborted [7, 12]. In Thailand, a genetic elite herd has been established as the National Buffalo Breeding Center. In this center the embryo cryopreservation could be useful for long-term storage of embryos which would enable the optimal distribution of superior genetic resources. The objective of this research was to attempt to perform embryo transfer and embryo cryopreservation in an elite swamp buffalo herd.

MATERIALS AND METHODS

Embryo and cryopreservation: Embryos were collected from the superovulated donors by using FSH or FSH-GnRH in a progesterone-synchronized program as mentioned in previous research [11]. The embryos, at morula to blastocyst stages, described as fresh embryos were either kept for 3 hr after collection in Vigro uterine-base solution®, supplemented with 50X Dulbecco's modified supplement[a] before transferring to synchronized recipients or were frozen in liquid nitrogen (–196°C) for at least one year [1].

Recipient preparation: The recipient cows aged 4 to 5 yrs were selected the basis of their health and normal reproductive tract as judged by rectal palpation. The oestrus cycle of the recipients was controlled by 3 treatments;

a) Intravaginal administration of 1.9 g progestorone in insert silicone elastomer[b] and 10 mg estradiol benzoate[c]

b) Ear implantion of 3 mg Norgestomet[d] with 5 mg, im estradiol-valerate

c) Two injections of prostaglandin F 2[a] (PGF 2α)[e] with 11 days interval (Fig. 1).

Signs of oestrus was looked for twice a day (6–7 a.m. and 16–18 p.m.) after progesterone removal or after the second injection of PGF 2α by using a deviated penile teaser bull. The recipients were rectally palpated on day 6 to 7 after the second oestrus and only those possessing well protruded corpus luteum on the surface of ovary were selected. The embryos were transferred on the 6th or 7th day of the second oestrus cycle.

Embryo transfer technique: The fresh or frozen embryos were transferred nonsurgically into synchronized recipients on day 6 or 7 after oestrus. Epidural anesthesia (5 m*l* of 2% Xylocaine HCl) was used 10 min before transfer to prevent defecation and to minimize straining and uterine contractions during and after transfer. The embryos were stored in a 0.25 m*l* French ministraw between two air pockets and two columns of holding medium (Vigro media supplement with 10% fetal calf serum). The straw was inserted into a

[a] AB Technology Inc., Pullman, WA, U.S.A.
[b] EAZI-BREED CIDR-B®, InterAg, Hamilton, New Zealand.
[c] CIDEROL®, capsule, InterAg, Hamilton, New Zealand.
[d] Crestar®, Intervet International BV, Boxmeer, The Netherlands.
[e] PGF 2α, Prostavet, Gifavet®, Virbac, France.

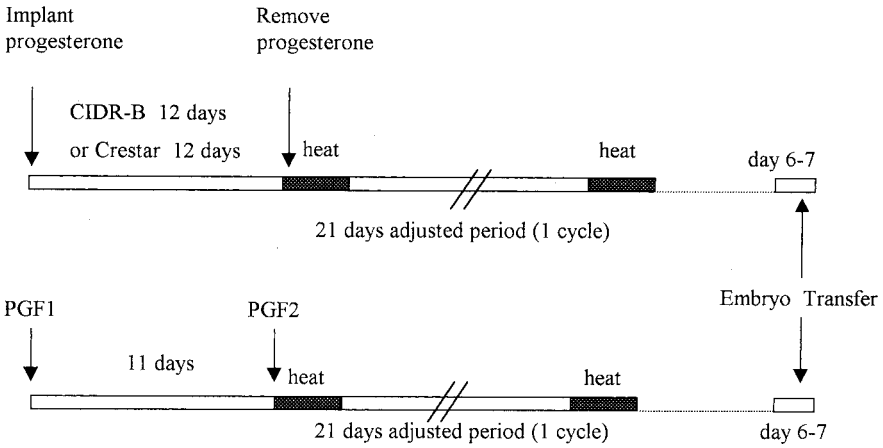

Fig. 1. Program of recipient preparation by using CIDR-B, Crestar® or PGF-PGF treatment.

Table 1. The results of recipient selection after different treatments

Synchronization program	No. of recipients	No. of selected recipients(%)
CIDR-B	20	9(45)
Crestar	20	12(60)
PG-PG	20	10(50)

special artificial insemination gun covered with an embryo transfer sheath fixed at the end. A second external sterile sheath was applied over the first sheath in order to prevent the contamination during vaginal introduction. The tip of the AI gun was placed into the external os of the cervix and pushed through the external sheath before going into the uterus. The embryos were deposited into the ipsilateral uterine horn approximately 5 cm from the uterine bifurcation after which the gun was slowly withdrawn.

Pregnancy evaluation: The recipients were pregnancy diagnosed 1, 2 and 3 mth after the transfer. The first month, by using a realtime-B mode ultrasound rectal[f] with 7.5 mHz[g] and confirmed at the second and the third month by rectal palpation. Pregnant animals were raised in a pasture during the day and fed roughage, concentrate and minerals at night in a barn. Fresh water was provided *ad libitum*.

RESULTS

After the removal of progesterone or the last PGF 2α treatment, it was found that about 90% of buffalo recipients became oestrus with a selection rate of recipients from the three different treatments of 45 to 60% (Table 1). The pregnancy rate using fresh embryos was 35.7% (5/14) at 1–2 months and 28.6% (4/14) at 3 months. Two animals in the latter group aborted after 235 and 243 days. The overall rate of fresh embryo development and the calving rate were the same, i.e., 14.3% (2/14). One male and one female calf was born 300 and 308 days after transfer. Their birth weights were 20 and 30 kg respectively while their weaning weight was 215 kg. They had an average daily weight gain of 762 and 713 gm/day from birth to weaning. For the frozen embryos, the survival rate was only 3.6% (1/28). Only one cow receiving three embryos became pregnant while those receiving one or two embryos did not, then the pregnancy rate at 1–2 and 3 months was 5.9% (1/17) (Table 2) and no frozen embryos survived until full term. An ultrasound picture of pregnant cows 40 days after receiving frozen embryos is shown in Fig. 2.

DISCUSSION

This paper reports the improvements of embryo transfer in a elite swamp buffalo herd using fresh and frozen embryos in Thailand compared to a previous report [5]. In the experiment, the oestrus synchronization of recipient was performed using three different products available in the country. In swamp buffalo, PGF 2α or progesterone in the form of an ear implant or an intravaginal device called "Progesterone Releasing Intraviginal Device (PRID)" had been used to synchronize oestrus successfully. The pregnancy rates varied from 0 to 80% with a fresh embryo calving rate of 30–40% [3, 4, 13]. In this experiment, no difference in the selection rate of recipients was found among the three products; all were around 50%. CIDR-B which has not been used to synchronize swamp buffalo before, was as effective as an ear implant (Crestar®). A mucous or mucopurulant discharge due to vaginal irritation

[f] Aloka, Echo camera, model SSD-500.
[g] UST, 934N3.5.

Table 2. The results of transfer of fresh and frozen embryos in swamp buffalo

Embryo	No. of recipients	No. of embryo transferred per donor	No. of transferred embryos	Survival 1 rate (%)*	Pregnant rate (%) 1–2 mt	3 mt	Calving rate (%)
Fresh	14	1	14	2 (14.3)	5 (35.7)	4 (28.6)	2 (14.3)
Frozen	7	1	7	0	0	0	0
	9	2	18	0	0	0	0
	1	3	3	1	1	1	1
Total	17		28	1 (3.6)	1 (5.9)	1 (5.9)	0 (0)

*calculated on no. of developed embryos to term/no. of transferred embryos

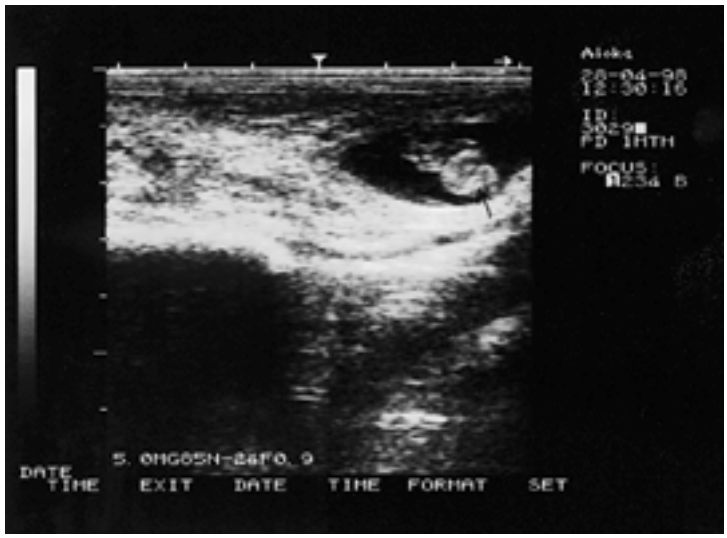

Fig. 2. Pregnant cows 40 days after the transfer of frozen embryos. Arrow indicated foetus.

was observed at the time of insemination as mentioned in a previous observation [4, 13]. It seems that the vaginitis due to the irritation of the intravaginal device does not disturb fertilization and embryo development because normal embryos can be collected from buffalo donors using this technique [11]. The selection of recipients was based on their ovarian structure, assessed by rectal palpation. The selection rate in this experiment seemed underestimated because the corpus luteum of buffaloes is small, more deeply embedded and generally had a less pronounced ovulation papilla than that of *Bos taurus* [6]. The whole structure of ovary is also hidden and this made them difficult to palpate per rectum and to identify the new corpus luteum [9]. It was suggested that the P-level on the day of collection is very useful to confirm multiovulation in the donor. This criteria may be used in recipients as well. In our previous experiment [11], real time-B mode was used to investigate the ovarian structure after superovulation. This might also be applicable for recipient selection.

Our results showed that the success of embryo transfer in this species as judged by this experiment depends largely on the type of embryo. It is obvious that most pregnancies came from fresh embryos transfers i.e., four from fourteen recipients became pregnant and two of them gave birth to calves with normal appearance and normal growth. It can be seen from the literature that the success of embryo transfer in buffalo is poor when compared to cattle. Since the first success of buffalo embryo transfer in Florida, U.S.A. by Drost *et al.* [6], most of the researches has been conducted in murrah buffalo. In swamp buffalo, the first success was reported by our unit [5] at the university farm, and no further study has been conducted since then. This is the first report on the embryo transfer in an elite production buffalo herd, in the country. The pregnancy rate, following non-surgical transfer in buffalo, is generally low around 18% [2, 6]. It was lower than this report, 35% at 1–2 months and 28.6% at 3 months. It was suggested that the low rate is associated with high rate of pregnancy failure, reaching as

much as 40% [8]. In this experiment, the calving rate was 14.3%, two of four pregnant cows aborted during the 8th month of pregnancy (the normal gestation period in buffalo is around 11 months). Swamp buffalo embryo can be successfully frozen by using conventional slow freezing program [1, 7, 10] and the 2-step procedure [12] and it was found that 86.2% of recovered embryos at different stages were normal after being frozen in liquid nitrogen [1]. One hundred percent embryos at the stages of compact morula, early blastocyst and blastocyst can survive, while 83.3% survive at the morula stage and 0% at the expanded blastocyst stage [1]. The results for the frozen embryos was unsatisfactory because the pregnancy rate was very low, only one cow receiving 3 embryos was pregnant at the 3 months and this did not survive to full term. This result was similar to the report on Nili-Ravi buffalo since 0% of slow freezing embryos and 7% (1/15) of two step frozen embryos developed to term [12]. The cryopreservation of embryos will be useful for eliminating the synchronization of recipients and facilitating the utilization of a superior genetic nucleus which has already seen established in Thailand. More investigations on the different factors involving the technique, the freezing medium, the type of cryoprotectants, the degree of synchronization between donor and recipient, are still required in order to improve cryopreservation in this species.

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