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Author(s)	OCAMPO, Marlon B.; UENISHI, Rogerio S.; VALDEZ, Conrado A.; PASTOR, Jonathan; CRUZ, Libertado; KANAGAWA, Hiroshi
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BRIEF COMMUNICATION

NON-SURGICAL EMBRYO RECOVERY IN THE WATER BUFFALO

Marlon B. OCAMPO, Rogerio S. UENISHI, Conrado A. VALDEZ,
Jonathan PASTOR¹, Libertado CRUZ¹ and Hiroshi KANAGAWA

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Key words: superovulation, ebb and flow system, water buffalo, embryo recovery

Embryo transfer technology has been successful in cattle, sheep and goats. In water buffalo however, only embryo recovery has been accomplished and it is still plagued by a low recovery rate²⁾. Earlier reports suggested that problems associated with the low non-surgical embryo recovery rate in water buffaloes include overstimulation of the ovaries, which could lead to a failure of the fimbriae to envelop the ovary at the time of ovulation, poor fluid recovery due to the adherence of the uterine wall to the tip of the catheter, which prevents the backflow of fluid, the sharp coiling of the uterine horn and excessive pressure applied on the uterine horn during flushing, which causes the fluid to leak through the utero-tubal junction into the ovarian end of the fallopian tube^{13,14)}.

This study was undertaken to evaluate the applicability of the cattle non-surgical embryo recovery technique, i. e., the ebb and flow system of the uterus in the water buffalo. Other factors relevant to embryo recovery were also studied.

Three river buffaloes (Fig. 1) and one swamp buffalo (Fig. 2) at the Philippine Carabao Research and Development Center were used in the study. Selection of these animals was based on their reproductive cycle.

Each buffalo was first injected with 25 mg of prostaglandin F2 alpha (PGF2 α) followed by 2000 IU of pregnant mare serum gonadotropin (PMSG) ten days later. A second 25 mg of PGF2 α was given 48 hours after the PMSG injection. Seventy two hours later, the buffaloes came into heat and gonadotropin releasing hormone (GnRH) was injected at 250 μ g per animal. Confirmation of estrus was done through physical

Department of Theriogenology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan

¹ College of Veterinary Science and Medicine, Central Luzon State University, Munoz, Nueva Ecija, Philippines

examination. Insemination was carried out using 3 straws of frozen-thawed murreh buffalo semen per buffalo. One straw was deposited in each of the two uterine horns upon detection of estrus and another straw deposited in the body of the uterus 12 hours after.

Prior to uterine flushing, 5–10 ml of 2% lidocaine HCl was administered epidurally between the 2nd and 3rd coccygeal vertebrae. The feces from the rectum were removed and the vulva disinfected using 70% isopropyl alcohol. The cervical canal was then dilated using a cervical expander in order to facilitate the insertion of a two-way Foley catheter (No. 14). After establishing the position of the catheter, the balloon was inflated with 14–16 ml of air until it was fixed in the uterine lumen.

Uterine flushing was carried out by using a 50 ml sterile syringe filled with Dulbecco's phosphate buffered saline (PBS) plus 2 mg of bovine serum albumin per ml concentration through an ebb and flow system (Fig. 3) as described by ELSDEN et al⁵⁾ and ROWE et al¹²⁾. After flushing one uterine horn, the catheter was withdrawn, replaced, and the procedure repeated in the next uterine horn. About 250–400 ml of medium was flushed through each uterine horn and collected in a 1-liter graduated cylinder. The collected medium was allowed to settle for 30 minutes and the supernatant siphoned until about 100 ml was left. This was swirled and decanted into scored plastic petri dishes for examination of embryos under a stereomicroscope. The graduated cylinder was rewashed with a small amount of medium which was also collected and examined.

Table shows the particulars of the non-surgical recovery of embryos in three buffaloes flushed. The number of ovulation was estimated by counting the corpora lutea via rectal palpation. It was noted that the corpus luteum of the water buffalo is smaller, more deeply imbedded and generally has a less pronounced ovulation papilla than that of domestic cattle. This observation coincides with the findings of DROST et al.⁴⁾

Murreh buffalo No. 04 was flushed at approximately 4.5 days (108 hours) after insemination. A degenerated cell (Fig. 4) as manifested by a change in the morphology and presence of lipid droplets in the cytoplasm and an unfertilized oocyte (Fig. 5) were recovered from the right and left uterine horns, respectively. The presence of an unfertilized oocyte could be due to the use of a frozen-thawed semen during insemination. Freezing of semen somehow affects sperm motility and, thereby, its fertilizing capability. However, the reason for the recovery of a degenerated cell is not understood. Buffalo Nos. 54 and 63 were flushed on the 5th day but no embryo was recovered. Buffalo No. 01 was not flushed because it showed signs of heat the day before the collection.

In cattle, the use of the fixed distance circulation system, the variable distance circulation system and the ebb and flow system on non-surgical flushing of embryos had already been established and applied commercially^{1,8,9,10)}. While it is true that

the proximity of the catheter tip to the tip of the uterine horn in the first two systems had a highly significant effect ($P < 0.001$) on the efficiency of recovery^{8,9,10}), there is still a disparity in favor of the ebb and flow system in the non-surgical collection of water buffalo embryo. This latter system was chosen on the basis of its accessibility and practicality in relation to the anatomical structure of the uterine horn of buffalo. The catheter needs only to be placed within the base of the uterine horn, unlike in the other system^{5,12}). Besides, the sharp coiling (Fig. 6) of the uterine horn of the buffalo offered quite a difficulty for the insertion of the Foley catheter beyond its greater curvature as compared to the cattle's uterine horn. Nevertheless, we were able to recover two eggs from one river buffalo using this technique. Furthermore, to overcome the difficulty of inserting the catheter into the uterine horn, it is suggested that the cuff of the catheter be set into the internal os of the cervix, simultaneously flushing both the uterine horns.

PARNPAI et al¹¹). and SHARIFFUDIN and JAINUDEEN^{13,14}) also reported successful recovery of embryos in the water buffalo but had different results. So far, only DROST et al⁴). has succeeded in embryo transfer in the water buffalo, wherein a 35 kg male buffalo was delivered.

Therefore, a thorough and accurate understanding of the factors that affect egg recovery in the water buffalo is essential. These may include the location of the egg in the crevices of the endometrium, where the flushing medium could not reach to dislodge it^{13,14}), and the interval of collection from estrus. A low recovery of eggs after day 4 has been observed not only in cattle but also in buffalo³). This can be explained by the larger volume and surface area of the uterine horn to be flushed making it physically more difficult to recover the egg. However, a more plausible explanation could be that a portion of the egg is expelled shortly after entering the uterus. This agrees with an observation on the abnormal transport and premature entry of the egg into the uterus of a superovulated animal⁶). Moreover, the nutritional status of the donor likewise affects the ovulation response. Usually, the buffaloes are allowed only to graze in a pasture, wherein they are taking in more carbohydrates and fiber than protein. Rations with a higher level of crude protein greatly influence the animal's level of luteinizing hormone and response to GnRH, which eventually affects the ovulation response of the animal concerned⁷).

In this respect, more detailed studies on the factors affecting embryo recovery and transfer, physiology and anatomy of the reproductive tract of the water buffalo are still necessary.

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TABLE. Particulars on the non-surgical recovery of water buffalo embryo.

Type and No. of buffalo	Embryo recovery after mating	No. of ovulations (Corpus luteum)		No. of embryos recovered		Amount of medium used		Remarks
		left ovary	right ovary	left uterus	right uterus	left uterus	right uterus	
MB No. 04	4.5(days)	1	3	1	1	400(ml)	350(ml)	1 deg. cell, 1 unfertilized oocyte
MB No. 54	5.0	1	1	—	—	380	250	good flushing, medium recovery with tissue debris
SB No. 63	5.0	1	2	—	—	300	300	good flushing, medium recovery with tissue debris
MB No. 01	x	x	x	x	x	x	x	"in heat"

MB : ---Murrah buffalo
 SB : ---Swamp buffalo
 — : ---No embryo recovered
 x : ---Not flushed

Embryo recovery in buffalo

EXPLANATION OF PLATE

- Fig. 1 Typical Murrah buffalo with curled horns.
- Fig. 2 Swamp buffalo has sickle-shaped horns curved backward toward the neck region.
- Fig. 3 The ebb and flow system is a method in which the base of a uterine horn is occluded with a cuffed cannula and a to-and-fro cyclic system is used for flushing the uterine horn.
- Fig. 4 A degenerated cell recovered from Murrah buffalo No. 04.
- Fig. 5 An unfertilized oocyte flushed from the same animal.
- Fig. 6 The reproductive organ of the water buffalo is marked by the sharp coiling of its uterine horn.

