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Report of the Short-Term Consultancy
from Dec 7 to Dec 21, 1988
on the
Embryo Transfer Sub-project
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Consultancy for ICAR from December 7 to December 21.

January 11, 1989

The time in this consultancy was divided between the planned buffalo embryo transfer experiment, and attending the World Buffalo Conference in New Delhi at the request of Drs. Acharya and Mudgal.

Objectives

1. To investigate 3 different superovulation treatments.
2. To estimate survivability of embryos following freezing and storage at 190°C.
3. To ascertain pregnancy rates following transfer of half embryos produced by micro-manipulation.
4. To investigate the problems or recovery and transfer of buffalo embryos.
5. To observe the stages of development of buffalo embryos.

Justification

Reports to date on buffalo embryo transfer have been few, with small numbers. The experiments have been mostly carried out by relatively inexperienced technicians in the field of embryo transfer. Thus it is difficult to ascertain if problems are due to the unique physiology of buffalo or the inexperience of the operators. There have been no reports on the success of freezing and splitting buffalo embryos.

Materials and Methods

Forty two buffalos were superovulated by protocols described in my previous report. Embryo recovery and transfer were carried out by standard procedures (Elsden et al 1976).

Results and Discussion

There were no differences in the 3 superovulation treatments so the results are combined from the 16 buffalo collected by this consultant (Table 1). Eleven other buffalo were collected prior to the consultants arrival, however problems arose due to inexperience, therefore they were not included. Thirteen other buffalos were not detected in heat after the FSH and PG treatment and were not bred or collected.

TABLE 1

Buffalo Embryo Transfer: Response to FSH and collection results

	Buffaloes collected	Corpora lutea	Follicles	Transferable embryos	Degenerate embryos	Unfertilised ova
Total	16	33	13	12	0	6
Mean		2.0	0.8	0.8	0	0.4

There were insufficient embryos of high quality to permit experimentation with freezing and splitting. But several significant observations were made: buffalo do not respond as well as cattle to FSH, producing fewer corpora lutea and follicles, fewer embryos, but a similar percentile of unfertilised eggs.

The buffalo, being not very responsive to FSH, is similar to the horse in this aspect. Researchers in Bulgaria and the Asian countries are reporting similar results. It would appear changes in protocol make no difference which is a similar experience undergone in cattle during 1970s and early 1980s. Possibly the buffalo is refractory to FSH which could be a genetically inherited predisposition in most of the animals preventing a response which if this is true will never change. As we found in the cow, the addition of GnRH or HCG to the FSH treatment produces no convincing evidence of improved responses. Since there is very little to be achieved without embryos, new approaches to the problem may need to be addressed by Indian scientists if embryo transfer is to be of any use to improve or multiply superior animals.

Collecting Embryos

The techniques used to collect embryos from the donors presented no problems. In fact, catheterising the cervix is easier than in Bos indicus cattle. Catheters used in cattle are quite satisfactory and it is unnecessary to waste time and money on futile attempts to devise new catheters as has been suggested. If a technician has problems then it is inexperience. The uterine horns of the buffalo are usually tightly coiled which has been suggested as causing an impediment when washing embryos out of the uterus. However, if the balloon of the catheter is placed in the uterine horn at the level or anterior to the palpable bifurcation of the horns, medium reaches the uterine tip (where embryos are located at this time) and returns at sufficient speed to wash out

eggs and embryos. Fluid is held under sufficient pressure so as to expand the uterine tip up to the utero-tubal junction, thus this junction area must be able to hold medium under pressure (as in the bovid). So it is unlikely that eggs will be flushed in a retrograde manner up into the oviducts. In order to partly overcome the tight coiling of the buffalo uterine horn, it is possible (after administering an epidural) to hold the horn near the utero-tubal junction and raise it. Held in this position, fluid can be palpated as it enters this section of the uterus and effective drainage is attained. We found when developing non-surgical collection of cow embryos that a minimum of five "flushes" were necesaary to remove all eggs, ensuring the uterus is completely emptied in between each "flush" - so the same technique was applied to the buffalo.

Examination of embryos

Few embryos were collected, therefore the following statements have to be regarded with some caution and more work is necessary. Our observaitons are not in agreement with previous work (Drost and Elsden, 1985; Alaxiev etal 1988).

Embryos collected on Day 5.5. (estrus = Day 0) were loose morulae and one compact morula, on Day 6.0 compact morulae and one loose morula and on Day 6.5 early blastocysts and one poor quality loose morula (Table 2). In 1985, we reported the buffalo embryo appeared to be at least one day ahead of the cow embryo in morphological development. The Bulgarian reported even faster development of the buffalo embryo reporting latched blastocysts on Day 6, and only 17-32 cell embryos on Day 5. If this was the case, then in just 24 hours their embryos matured from loose morulae to compact morulae to early blastocysts and finally blastocysts. This occurence seems most unlikely and has not been reported in other farm animals. Certainly it does not agree with our observations. Since it can be difficult to observe first standing estrus in buffalo, possibly there is a problem in accurate heat detection.

Table 2

Buffalo Embryo Morphology and Age

DAY	5.5	6.0	6.5
	2 x loose morulae	1 x loose morula	1 x loose morula
	1 x compact morula	3 x compact morulae	1 x early blastocyst
			3 x blastocysts

All buffalo embryos were darker in color than normal cow embryos, and appear to have longer blastomeres at all stages of development. They are approximately the same size as cow embryos at equivalent stages of development. The zona appeared about 0.5mm thicker and a few zonae were completely surrounded by a fine granulated mucus which made them difficult to locate. No deterioration in embryo quality was observed for the 2 to 6 hours incubation in modified PBS at room temperature, contrary to our earlier report in 1985. Thus it would appear PBS is a suitable medium for the collection and short term culture of buffalo embryos.

Transfer of buffalo embryos into recipients

Cervices of buffalos were no problem to catheterize with an IMV transfer pipette. Location of corpora lutea in most case was not difficult, although some were very small and rose only 1 or 2mm above the stroma of the ovary, making diagnosis uncertain. Due to the tone of uterine horns, it is difficult and often impossible to place the embryo past the greater curvature of the horn. In most cases embryos were deposited just anterior to the palpable bifurcation of the uterine horns. In cattle this position does not appear to lower pregnancy rates.

Conclusions

1. Some difficulty in accurate heat detection.
2. Poor response to the gonadotropins when superovulating donors.
3. Increased tone of uterus causing some problems in inexperienced hands.
4. New approaches to obtain embryos are necessary. Two suggestions: a) single egg collection with embryo splitting; and b) cloning 5 day embryos (Possibly available in 2 to 3 years).

Future Experiments

I. Single embryo collection of buffaloe coupled with emrbyo splitting since superovulated donor average 1 embryo/treatment.

A) Superovulating every 60 days averaging 1 embryo/collection.

Days	60+60+60+90*	=	270 days
Embryos	1+ 1+ 1+ 1	=	4
Half Embryos	- split 3	=	6
Pregnancy		=	3 or 4
			==========

B) Single embryo collection for 270 days

Every 21 days for 270 days		= 13 chances
At 70% success rate		= 9 embryos
Split	6	= 12 halves +
		= 3 wholes not split
Transfers		= 15
Pregnancies		= 6 or 7, possibly 8

Other advantages of single embryo collection are:

1) No FSH
2) No Prostaglandin
3) No subsequent chances of inferitility
4) If splitting is successful in the buffalo then pregnancies are doubled.
5) Much lower costs.

In February 1989 we hope to start the above experiment and compare this to superovulating with equine FSH (I have already mailed this FSH to Drs. Mudgal and Jain at Hisar).

II. Freeze embryos for storage.

Once again the Indian scientists were concerned because they still have not received their embryo transfer drugs and equipment. They now have been waiting for 2 years.

The Director of CIRB is anxious to keep the momentum going with the buffalo embryo transfer experiments, hence a second experiment was planned in late February.