Short Communication

Development of per vaginal route of serial observations of ovarian activities in buffalo using a simple fiberscope

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Abstract

Six normally cycling Philippine carabaos were selected from a herd after showing normal estrous cycle for two consecutive times. Observations of the ovarian structures were performed with the use of a simple fiberscope (model FS 100). The fiberscope was guided to the pelvic cavity close to the position of the ovary by inserting it through a cannula. To facilitate observation of ovarian structures another individual palpated the ovary gently as it was brought close to the fiberscope tip. The use of a simple fiberscope showed that it was feasible and practical to trace the development and regression of individual follicles with this device. Follicular fluid was transparent and clear in appearance. Corpora lutea on the ovary was less spherical in appearance and was surrounded by a border of luteinized tissue. Only well defined forms with a relatively smooth outline were considered follicles. Follicles as small as 2 to 3mm were clearly viewed. The use of anesthesia to temporarily paralyze the sacro-coccygeal area did not change the estrous cycle length of the experimental animals. The animals still maintained their normal estrous cycle length (averaging 21.5 ± 1.5 days and showed signs of estrus, which included mucus discharge, mounting other animals and being mounted and bellowing).

Keywords: Per vaginal, ovarian activities, buffalo and fiberscope

Introduction

Several methods had been used previously to monitor the ovarian activities especially in cattle. These included marking with India ink (Natton *et al.*, 1981; Dufour *et al.*, 1972), slaughtering on different days of the cycle (Rajakoski, 1960) and the recent is the use of ultrasonography (Ginther *et al.*, 1989a,b; Sirois and Fortune, 1988; Pierson and Ginter, 1984, 1987 a, b, c and d and 1988). While ultrasonography has provided accurate accounts of the growth and regression of ovarian follicles, least

expensive and practical technique has to be developed in places where resources are limiting. In many tropical countries, it would be quite impractical to invest on ultrasound equipment to follow up the dynamics of ovarian follicles in large animals. In the Philippines for example, the desire to study the dynamics of the ovaries of the buffalo has led to a search for a less expensive techniques of accessing their ovaries. This has resulted in the development of techniques of utilizing the flexible fiberscope and also to know the effect

of serial infusion of anaesthetic preparations on the estrous cycle and estrus.

Materials and methods

Experimental animals

Six mature and health Phillippine water buffaloes were selected after having shown two consecutive normal cycles. The animals wee allowed to graze during the day time and corralled at night.

Description of fiberscope

The fiberscope was FS-100 model purchased from Scott Fiber optics, Inc., 122 Charlton Street, Southbridge, M. A. 01550 USA (See Fig. 1). It is 60.96cm in length, 0.800cm in diameter, flexible and has a field view of 600 fixed focus and is accompanied with a right angle viewing attachment. There are two separate fiber optical bundle inside the scope. The 'image' forming bundle contain thousands of fibers precisely arranged at each end so that each fiber is in the same relative position at both ends of the bundle. The 'illumination' bundle of the fibers surrounds the image bundle and carries light from the lamp located in the bundle to the viewing area.

Management/handling of animals

Animals were not fed six hours prior to the observation time. This is important to minimize possible problems of palpating and viewing of the ovaries. Animals were restrained in a chute with fore legs lower than the hind legs to allow most of the digestive organ to move forward leaving the ovaries easy to monitor. Anaesthetic was injected at the epidural space of the last sacral and first coccygral vertebrae. The quantity of aneathetic varied depending on the requirement to paralyze the sacro-coccygral region. The animals are said to be anesthetized if there are no movements when the site of injection is gently touched and if the caudal area is totally relaxed and not stiffened when pulled. Two kinds of anesthetic preparations were used namely: (a) Xylocaine (Astra pharmaceuticals, contains 20mg lignocaine Hcl/ml) and (b) Lidocaine (Elin

pharmaceuticals Inc., contains 20mg lignocaine Hcl/ml).

Insertion of the fiberscope for viewing the ovary

Once the sacro-lumber region was paralyzed, the spreader was inserted into the vagina to allow easy identification for the point of puncture at the floor of the vagina. Stainless steel trocar and cannula of 46cm long and inner cannula diameter of 9.0mm were inserted and the floor of the vagina was punctured with the trocar. Flashlight was used as source of light to guide the point of the trocar for correct positioning and puncture. Both the trocar and spreader were then withdrawn leaving the steel cannula with its inner end lying in the pelvic cavity. The rectum was then cleared of faeces and an operator (palpator) inserted one hand in it and gently lifted the ovaries close to the tip of the cannula. The fiberscope was then inserted into the cannula by another operator (the observer). The distance between the ovary and the tip of the fiberscope should be at least 2cm. This was to allow the observer to clearly see the ovary properly and be able to count and record changes on the ovary. Throughout the study, the same palpator and observer were used to lessen error in measurements during manipulations. It took at most 20 minutes on the average to thoroughly observe one animal. The same procedure was repeated every 3 days beginning on day 3 of the cycle until day 21 or a total of seven observations per animal. In all the subsequent observations, the same vaginal floor puncture was used to bring the cannula and the fiberscope to the correct positioning in order to observe the ovaries (Fig. 2). All animals were monitored for onset of estrus during and after fiberscope. It should be noted that prior to the -in-vivo observation, several practices in the use of the fiberscope were done in-vitro with ovaries collected from the slaughter house.

Results and discussion

The study indicated that it is feasible and practical to monitor the development and

Observation of ovarian activities in buffalo using there scope

Figure 1 *FS100 fiberscope designed for visual inspection of remote or inaccessible location*
Source: scodt fiber optics, INC, USA.

Figure 2 *Follicular observations using a simple fiberscope*

Table 1 *Effect of serial treatments of lignocaine on the amount (mg/kg BW) required to paralyze the sacrolumbar region of buffaloes*

Animal No.	Body wt. (kg)	Days of observation						
		3	6	9	12	15	18	21
16	426	0.47	0.70	0.90	0.84	1.17	1.17	1.51
5	315	0.51	1.27	1.59	1.90	2.22	2.22	2.50
98	280	0.57	0.57	1.79	2.86	3.21	3.43	3.57
122	345	1.16	2.38	3.19	3.48	3.48	2.65	3.77
120	463	0.43	1.08	1.99	2.16	2.25	2.16	2.38
21	365	0.82	1.92	2.47	2.74	3.96	3.18	3.18
Mean $\pm$ SD	0.66 $\pm$.26	1.32 $\pm$.64	1.95 $\pm$.77	2.33 $\pm$.84	2.55 $\pm$.77	2.63 $\pm$.77	2.63 $\pm$.87	2.82 $\pm$.77
% Increment			125.30	31.34	27.90	2.00	3.41	7.13

regression of individual follicles using fiberscope in the water buffaloes. Repeated examinations proved to be a safe procedure since minor rectal injury was induced on the floor of the vagina in the animals used. Also, there was no evidence that repeated examinations and the continuous use of anaesthetic preparations modified the course of a normal estrous cycle.

Locating an ovary with the fiberscope was not difficult after several practice sessions. However, a clear image was not always obtained. Complete evacuation of the rectum was found to be important in the manipulations of the ovaries. Causes of poor image quality were mostly due to the presence of tissues which resulted in a reddish to purplish colour on the viewing end and the movement of the animal as the anesthetic wears out during the course of an examination. Furthermore, the fiberscope showed that the images of the ovary were primarily composed of follicles and corpora lutea. Follicular fluid was transparent and clear in appearance. Corpora lutea on the ovary was less spherical in appearance and surrounded by a border of luteal tissue. Only well defined forms with a relative smooth outlines were considered follicles. Follicles as small as 2 to 3mm were clearly viewed.

The use of fiberscope was consistent with reports of follicular dynamics in heifers (Rajakoski, 1960; Dufour *et al.*, 1972; Matton *et al.*, 1981). These reports were based on marking structures with India ink and

examining slaughter house material and also in cattle by Piersona and Ginther (1985 and 1986) using ultrasonography. Principle point of agreement were (1) growth of a large follicle to an ostensibly ovulatory size followed by regression at approximately mid-cycle, (2) selective accelerated growth of the follicle destined to ovulate of the larger follicles that were not destined to ovulate. It appeared that fiberscopy would permit the sequential monitoring of ovaries from large numbers of buffalo and may help clarify and characterize many aspects of follicular dynamics. Ovulation was detected by the acute disappearance of the larger follicles (>8mm) in an ovary. The corpus luteum or ovulation site was not visible until three days after ovulation. The corpus luteum gave a different pattern than that of surrounding tissues and had a defined border.

The one concern was the amount of anesthetic used in bringing the sacro-coccygeal part of the animal to inactivity temporarily. In this study, the requirements of each animal increased as the days of observation progressed. Table 1 showed that the animals needed 0.66 + 0.26mg lignocaine per kg. body weight on the first day (day 3) of observation and increased to 2.82 $\pm$ 0.77 mg on day 21 or the 7th observation. In some pharmacological test results, approximately 100 ml of a 2 percent solution of lignocaine has been used in cattle without signs of toxicity. Even higher doses up to 400 ml had been tested in horse and cows without any adverse effect (Brader and Pugh, 1977).

Observation of ovarian activities in buffalo using there scope

Table 2 *Estrous cycle length (days) of experimental animals subjected to serial anesthesia treatments and physical manipulation of the ovaries*

Animal No.	Estrous cycle length (days)
16	23
5	20
98	20
122	23
120	20
21	23
Mean $\pm$ SD	21.5 $\pm$ 1.5

Table 2 presented the effect of the serial anesthetic treatments with the effects of the physical manipulations of the ovary in relation to the estrous cycle length of the animals. In this study, it showed that the estrous cycle length of the six waster buffaloes were still within normal. The estrous cycle length ranged from 20 days to 23 days with a mean of 21.5 ± 1.5 days. This may safely be interpreted that the increased amount of anesthetic treatments and tremendous ovarian manipulation did not lengthen or shorten the estrous cycle length.

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