

## FOLLICULAR GROWTH PATTERN IN BUFFALOES SYNCHRONIZED TO ESTRUS WITH PROGESTERONE IMPREGNATED INTRAVAGINAL SPONGES

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**Abstract:** A study was conducted in regular cyclic Murrah buffaloes which were free from any anatomical and reproductive disorders. Progesterone impregnated polyurethane intravaginal sponges containing 1.5g of natural micronised progesterone were aseptically inserted into vagina. These sponges were placed *in situ* for 9 days and removed manually on the 10<sup>th</sup> day. Ultrasonography of the ovaries was done in all the animals on Day 0 (day of insertion of the sponge), Day 3, 10 (day of removal) and at induced estrus transrectally using a real time ultrasound scanner equipped with a linear array 5- 7.5 MHz frequency transrectal transducer. Blood samples collected during these days were analyzed for progesterone levels by radioimmunoassay method. All the animals retained vaginal sponges up to 10 days. There was no abnormal discharge noted upon removal of the sponge and all the animals were induced to estrus within 48 to 72 hours after sponge removal. Ultrasonographic study revealed that on day of insertion of sponge, all the animals had corpus luteum. No dominant follicles were noticed in these animals. On day-3, corpus luteum was present with no appreciable further growth from day - 0. No dominant follicles were noticed in these animals. On day of sponge removal, all the animals had a developing dominant follicle. On the day of estrus, all the animals showed the presence of a well developed mature graffian follicle of size ranging from 10 x 7 mm to 12x 11 mm. The progesterone hormone assay revealed that the mean plasma progesterone level on day-0 was 3.7±0.70 ng/ml, which increased to 10.4±1.29 ng/ml on day-3. On the day of sponge removal, the progesterone level was 2.62±0.28 ng/ml which further decreased to 0.47±0.08 ng/ml at estrus. The results indicate that the progesterone sponges helped in maintaining the progesterone levels with peak values reaching on day 3 of insertion of sponge. During the period of intra vaginal sponge the

follicular waves were inhibited and corpus luteum was maintained. Nearing day 10, the progesterone levels showed a decreasing trend which led to the follicular growth stimulation and so a dominant follicle could be observed on day 10 in all the animals.

The present study shows that progesterone impregnated intravaginal sponges could be used to regulate the duration of the corpus luteum and follicular turnover thereby this device can be used to synchronize estrus in buffaloes.

**Keywords:** Buffaloes, oestrous synchronization, progesterone sponges, follicular waves.

## Introduction

Buffaloes contribute more than one third of the total milk production in Asia and the Asian countries produce over 96% of the world's total buffalo milk output. In India, buffaloes contribute to food security through 60 million tones of milk and more than 1 million tones of meat, besides work energy for agricultural purposes. They are found in widely differing geographical conditions, which suggests that this species is adaptable to wide range of environmental conditions. The productivity of buffalo is considerably affected by the inherent problems such as poor reproductive efficiency which is mainly due to late maturity, poor expression of estrus (particularly in summer, irregular estrus cycle length, anestrus, inactive ovaries and long postpartum intervals. Besides genetic makeup, several factors such as nutrition, management, environment, physiology, pathology and psychology affects the conception rate in buffaloes both in farm and field conditions and pose serious threat to profitable dairy farming (Hiremath, 2013). Various estrus synchronization techniques have been tried for improving the fertility of buffaloes.

In the present study, progesterone impregnated intravaginal sponges were used to synchronize estrus in buffaloes. Since limited information is available on the changes in the ovarian structures and progesterone levels during the treatment period, the present study was undertaken to evaluate the efficiency of intravaginal sponges in eliciting changes in the follicular growth pattern and progesterone levels.

## Materials and Methods

The study was conducted in six graded Murrah buffaloes (1<sup>st</sup> and 2<sup>nd</sup> parity non lactating and regular cycling) maintained at the Centralized Embryo Biotechnology Unit of Department of Animal Biotechnology, Madhavaram, Chennai. All the selected animals were free from any anatomical and reproductive disorders.

Ultrasonographic study of the ovarian structures was done transrectally using a real time ultrasound scanner equipped with a linear array 5-7.5 MHz frequency transrectal transducer, in all the animals prior to the insertion of the vaginal sponges. Progesterone

impregnated polyurethane intravaginal sponges, 8 X 5 cm (prepared in the Department of Veterinary Physiology, VC&RI, Namakkal) containing 1.5g of natural micronised progesterone were aseptically inserted intravaginally using speculum. The vaginal sponges were placed in situ for 9 days and removed on the 10th day.

Blood samples were collected and ultrasonography of the ovaries were done on Day 0 (day of insertion of the sponge), Day 3, 10 (day of removal) and Day 12 (post removal period). Plasma was separated and stored at -21 °C until further analysis of progesterone. Plasma progesterone levels were analyzed by radioimmunoassay using <sup>125</sup>I labeled antigen antibody coated tubes and standards procured from Immunotech, France. The radioactivity was measured in the Stratec gamma counter (Germany) available in the Department of Veterinary Physiology, VC& RI, Namakkal.

### **Results and Discussion**

All the animals retained vaginal sponges up to 10 days. There was no abnormal discharge noted on removal of the sponge and all the animals were induced to estrus within 48 to 72 hours ( $56 \pm 3.82$  hours) after the removal of the sponges. These observations are in agreement with those of Hiremath, 2013 who reported that the estrus signs were noticed within 48h in true anestrous buffaloes after the removal intravaginal progesterone device (EAZI Breed CIDR) which was placed for 10 days.

### **Ultrasonographic studies on ovarian status**

On the day of insertion of the sponges (Day 0) all the animals had corpus luteum with size ranging from 10x7 mm to 18x13 mm. No dominant follicles were noticed in the animals. On Day 3, corpus luteum was present in the animals with no appreciable further growth from Day 0. No dominant follicles were noticed in these animals. On the day of removal of the sponges, all the animals had a developing dominant follicle with size ranging from 7x4 mm to 11x 9 mm. Later on the day of induced estrus all the animals showed the presence of a well developed mature graffian follicle of size ranging from 10 x 7 mm to 12x 11 mm.

### **Plasma progesterone levels**

On the day of insertion of sponges, the mean plasma progesterone value was  $3.7 \pm 0.70$  ng/ml which showed increasing trend on the day 3 having a mean of  $10.4 \pm 1.29$  ng/ml. On the day of removal of the sponges the plasma progesterone values decreased to  $2.62 \pm 0.28$  ng/ml. Two days after the removal of the sponges, the progesterone values further decreased to

0.47±0.08 ng/ml. The results agree with the findings of Singh *et al* 2006 who reported that at 24h post CIDR insertion mean progesterone registered a significant increase in cows (13.94±1.41 ng/ml) and there after a progressive decline reaching 3.35±0.92 ng/ml and 0.24±0.68 ng/ml on day 7 and 48h post CIDR withdrawal respectively. These values are also in agreement with those reported by Sarvaiya, *et al* (1993)

The results indicate that the progesterone sponges helped in maintaining the progesterone levels with peak values reaching on day 3 of insertion of sponge. During the period of intra vaginal sponge the follicular waves were inhibited and corpus luteum was maintained. Nearing day 10, the progesterone levels showed a decreasing trend which led to the follicular growth stimulation and so a dominant follicle could be observed on day 10 in all the animals. In agreement with previous studies (Savio *et al*, 1988, 1990; Sirois and Fortune, 1988; Knopf *et al*, 1989), normal plasma concentrations of progesterone from the corpus luteum plus additional progesterone from the sponge resulted in consistent follicular turnover at midcycle.

Finally after the removal of the sponge, the progesterone values decreased as the presence of a mature graffian follicle was present in all the animals. All the animals exhibited estrus signs between 48 to 72 hours with a mean value of 56±3.82 hours after the removal of the progesterone source. The follicular development and exhibition of estrous in buffaloes after sponge removal suggests that the increasing level of progesterone released from the intravaginal sponges was absorbed through the vaginal mucus membrane. This increased concentration of progesterone sensitizes hypothalamic-pituitary system. Elevation of progesterone before the onset of ovarian cyclicity works a primer sensitizer for initiation at the level of hypothalamus and pituitary. (Singh, 2003)

## **Conclusion**

Dominant follicle development, preovulatory follicular growth and oestrous synchronization in cattle result from the interplay between concentration of plasma progesterone, stage of the follicular wave and time of luteolysis. More precise timing of onset of oestrus can be expected when progesterone withdrawal is coincident with the presence of a fully mature dominant follicle. The present study shows that progesterone impregnated intravaginal sponges could be used to regulate the duration of the corpus luteum and follicular turnover thereby this device can be effectively used to induce and synchronize estrus in cattle and buffaloes.

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**Table 1.** The mean plasma Progesterone levels and the mean size of the follicles during the period of the insertion of progesterone impregnated intravaginal sponges

<b>Day</b>	<b>Mean plasma progesterone levels (ng/ml)</b>	<b>Mean size of the corpus luteum</b>	<b>Mean size of the follicles</b>
Day 0 On the day of insertion of sponge	3.7±0.70	14.5x10.2 mm ± 1.89	3.5 x 2.0 mm ± 1.2
Day 3 On the day 3 of insertion after of sponge	10.4±1.29	12.0 x 9.5 mm ± 1.14	3.8 x 2.5 mm ± 0.98
Day 10 On the day of removal of sponge	2.62±0.28	5.5 x 4.0 mm ± 0.73	9 .5 x 7.0 mm ±1.36
Two days after the removal of sponge removal	0.47±0.08	3.4 x 3.2 mm ±1.21	11 x 9 mm ± 0.86