AGRICULTURE & ENVIRONMENT
E-NEWSLETTER
agrinenv.com

Article ID: AEN-2021-02-05-017

Methods of Estrus Detection in Buffaloes

Manisha Sethi[1*], Nadeem Shah[2], Dileep Kumar Yadav[2] and Mohsin Ahmad Parray[1]

[1]MVSc Scholar (Animal Reproduction, Gynaecology & Obstetrics), ICAR-National Dairy Research Institute (NDRI), Karnal, Haryana-132001, India
[2]PhD Scholar (Animal Reproduction, Gynaecology & Obstetrics), ICAR-National Dairy Research Institute (NDRI), Karnal, Haryana-132001, India
[*]*Corresponding author. E-mail: sethimanisha43@gmail.com*

One of the key components for successful Artificial insemination (AI) programs and conception rate improvement in buffalos is the right time detection of estrous. Majorly, estrous is determined in buffalos based on behavioral and physiological signs, such as frequent micturition, bellowing, vulval swelling and cervical mucus discharge. Buffalo being polyestrous are capable of breeding throughout the year. However, in many countries, seasonal variations in breeding activity and consequently calving have been observed. Photoperiod being relatively constant in tropical countries, annual changes in rainfall appear to influence estrous cyclicity, with availability and quality of herbage related to this cyclical reproductive pattern. In dry zones, buffalos kept under free-grazing exhibit ovarian activity some 2–3 months after the onset of monsoonal rains, followed by conceptions that result in 10 months later in the peak calving season. In India, one of the major causes of anoestrus in buffalo is heat stress during the hot summer months which leads to elevated blood prolactin levels which compromise ovarian activity as well as leads to sub-fertility and repeat breeding by decreasing progesterone secretion. Seasonality is influenced by photoperiod and mediated by melatonin secretion.

Behavioural signs of estrus are less overt in buffalo than cattle and homosexual behaviour is also less marked between female buffalos. Main behavioural signs in absence of bull are restlessness, bellowing, and voiding of small quantities of urine, but these are not consistently exhibited by all animals. Bull will show increasing interest when a cow is approaching estrus, and during estrus, the cow will stand to be mounted by the bull. Estrus duration may be shorter and estrous signs may be exhibited only during the night or early morning, during periods of high ambient temperature.

Methods of Estrus Detection:

Estrus detection in the buffalo (*Bubalus bubalis*) can be either conventional or advanced methods. Some validated conventional method includes observation of specific symptoms, use of vasectomized teaser bull in the female herd, use of pedometer, estimation of vaginal electrical resistance, change in female body temperature, and Doka method. Advanced methods include ultrasonography, examining follicular dynamics, estimation of fecal chemical, and estimation of milk constituents with serum oestradiol, cervicovaginal fluid proteomics and detection of Specific saliva ferning pattern.

(I) Conventional methods

1. **Observation of specific symptoms**: Vulval discharge with clear mucus in varying quantities in a recumbent animal is the most reliable single sign of heat in buffalo heifers even in the silent heat. Of the other signs, wall walking (segregation) and bellowing confirm oestrus in more than 84 percent of buffalo with silent estrus. On other hand, the placid response of the animal (to the placing of the palm on the rump and response to light massage of vulval lips) can be observed in more than 86 percent of buffalo with silent heat. Therefore, daily observations of the animal for

these important symptoms of heat a few days before can expect oestrus could make estrous detection a sure success.

2. **Use of vasectomized teaser bull in female heard:** Teaser buffalo bulls which are surgically altered to make them sterile to prevent successful insemination. These bulls can be used to detect silent heat in buffalos. These vasectomized males are fitted with a "chin ball marker" that will mount to females which are in estrus and will leave a mark on the backs. When the animal presses down with its chain on the back or rump region of mounted animals, a spring-loaded valve in the device is opened and fluid is released on the back.
3. **Use of pedometer:** In this method, buffalos are fitted with a pedometer to detect physical activity. In a study, reproductive healthy buffalos were injected with prostaglandin F2α to induce estrus. Further, variants palpations and blood progesterone concentrations were used to monitor corpora lutea response to treatments. At the end of the experiment, only 14% of the animals were detected for oestrus by visual observation but on other hand, 85% of the population was detected for the same by pedometers. This result was also confirmed by palpation which indicated that 70% of the cows were conceived after AI.
4. **Vaginal electrical resistance (VER):** In this method, the changes in baseline VER are used as a tool to detect different stages of the natural estrous cycle in buffalo. The mean VER in buffalo during estrus, metestrus, diestrus, proestrus and anestrus are 32.68 +/- 0.46, 41.26 +/- 1.17, 50.23 +/- 0.55, 43.20 +/- 0.64 and 55.86 +/- 0.57 ohms, respectively. Plasma progesterone level and VER are highly correlated. Fall in plasma progesterone is synchronous to a fall in VER and the correlation (0.65) between them being positive. After ovulation, the VER started rising, which indicates a distinct relationship between VER and ovulation. Thus, VER can be used successfully to predict the stage of the estrous cycle, ovarian status, and ovulation in buffaloes.
5. **Change in body temperature:** High vaginal temperature is correlated with low plasma progesterone (P_4) concentrations (<0.5 ng/mL) during estrus. Whereas, low body temperatures can be observed from a period from PGF2α injection to estrus (38.6 ± 0.3 °C) and around 38.5 ± 0.2 °C at ovulation. Body temperature of animal falls by 0.5^0C on day before estrus. It increases during estrus and falls by 0.4^0C at ovulation.
6. **Doka**: Alveolar compartment of udder stores around 95% of the milk but, due to the small udder cistern in buffalo, for optimal milk ejection, pre-milking stimulation is of utmost importance. As compared to cows, buffaloes have a more prominent cisternal compartment in teats than that in glands. Thus, the suckling of a calf is required for the milk letdown in buffaloes. Buffaloes show a typical symptom of estrus called Doka, where without any external stimuli teat engorgement occurs one to two days before the day of estrus. Doka is ranked as one of the most prominent signs of estrus in buffalo especially in rural India, with an estrous detection efficiency of around 95.1%. Teat diameter is significantly higher at the time of estrus, which indicates its importance as a sign of Doka and incoming estrous. The changes in milk letdown time, milk flow rate, milk fat percentage can be used as signs of Doka for detecting upcoming estrous.

(II) Advanced methods

1. **Ultrasonography:** Ultrasound techniques are important in buffalo reproduction, offering diagnosis tool for the assessment of estrus, pregnancy status, and fetal viability. Determination of ovulation by ultrasound examination can be depicted by the absence of a preovulatory follicle that was present at a previous examination and subsequently confirmed by the development of corpus luteum at the same spot. The ultrasonography is performed at 2-hourly intervals for detection of onset estrus for determination of the exact time of ovulation.
2. **Examination of follicular dynamics:** Follicular activity is associated with estrous behaviour in buffalos. If the ovarian follicular activity is examined by transrectal ultrasonography (TUS), a follicular wave-like pattern can be observed before the ovulation indicates short oestrous cycles. Growth rates and maximum diameters of the ovulatory follicles did not differ between the

ovulations. However, the growth rate for non-ovulatory dominant follicles (DF) can be lower than for the ovulatory follicle. Also, the diameter of all ovulatory follicles can be larger than those of the non-ovulatory DF.

3. **Estimation of fecal chemicals:** Chemical signals are one of the reliable non-invasive methods for estrus detection in mammals. Estrus-specific identification of fecal volatile compounds can give clues about the estrous cycle in buffalo. A volatile compound such as 4-methyl phenol (4mp) and trans-verbenol (tv) was found only in estrus feces. In a study, when these fecal compounds were exposed to the bull and tested for behavioral responses (flehmen and mounting behavior) the bulls exhibit repeated flehmen reaction when exposed to a combination of these two compounds but not in individual exposure. Thus fecal compounds, 4 methyl phenol, and trans-verbenol can be reliable indicators of estrus in buffaloes.
4. **Estimation of milk constituents in relation to serum oestradiol:** Changes in some milk constituents, oestradiol levels and electrical conductivity of vaginal mucus during the peri-oestrous period can be used for the prediction of the timing of estrus in buffaloes. Highly significant acute changes in milk constituents that occur at the time of estrus can be characterized by the peaking of chloride and sodium levels and lowering of potassium and lactose values. The alternation in milk composition, when arranged in decreasing order of magnitude, sodium, is highest, followed by chloride and potassium. Concomitantly, milk lactose can be decreased by 26% compared to baseline levels. Thus, changes in constituents of milk during the peri-oestrous period can be used as a practical non-invasive indicator for estrous detection and prediction of ovulation in buffaloes
5. **Cervico-vaginal fluid proteomics:** Cervicovaginal fluid (CVF) plays a significant role in reproductive performance. It is believed to be a good non-invasive biomarker for various diagnostic purposes. In a study, a comprehensive proteomic analysis of buffalo CVF was performed during the estrous cycle and a total of 416 proteins were identified in the CVF of both estrus and diestrus phases. Out of these proteins, 68 estrus-specific proteins have been found which play a major physiological function of estrus. These appeared proteins belong to stress response, immune response, and the metabolic process eventually, the expression level of heat shock protein-70 remains higher in estrus phase as compared to during diestrus phase. Thus heat shock protein-70 can be used as a biomarker for heat detection in buffalo.
6. **Detection of specific ferning patterns in cervical mucus and saliva:** As an alternative to cervical mucus, saliva fern patterns can be used to confirm the estrus and silent heat in buffaloes. Saliva at estrus shows a typical symmetrical fern-like crystallization pattern with significantly lower fractal dimension values. Salivary estradiol levels and E_2/P_4 ratio are also correlated with estrus and maintain a high value at the estrus stage than those at the diestrus stage.

Conclusion

The biggest bottlenecks in achieving optimum conception rates in dairy animals are estrus detection and proper time of insemination. In the case of buffaloes, the situation is still poor as the animals show silent heat without overt behavioural signs of heat. Various classical and advanced methods of estrous detection can be a suitable tool to achieve a high non-return rate in female buffalos using artificial insemination as a technique of assisted reproductive technologies.