



Endocrine Profiles of Oestrous Cycle in Buffalo: A Meta-analysis

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ABSTRACT : A meta-analysis was conducted to summarize the results of studies which have described the profiles of hormones during the oestrous cycle in buffalo using a fixed effect model and a random effect model. Plasma progesterone concentrations were lowest (0.30 ± 0.06 ng/ml) during the peri-oestrous phase and increased ($p = 0.067$) through the early luteal phase to a maximum concentration (1.94 ± 0.03 ng/ml) during the mid-luteal phase. Circulating plasma inhibin and estradiol concentrations were lowest (0.31 ± 0.01 and 11.04 ± 0.13 ng/ml) during the mid-luteal phase, increased through the late luteal phase to maximum concentrations (0.44 ± 0.02 and 22.48 ± 0.32 ng/ml) during the peri-oestrous phase. Plasma FSH concentrations were lowest during the early luteal phase and increased through the mid-luteal phase to a maximum concentration during the peri-oestrous phase. Peripheral prolactin concentrations were lowest during the late luteal phase and increased to a maximum concentration during the peri-oestrous phase which then declined ($p = 0.716$) during the early luteal phase. Peripheral plasma cortisol concentrations decreased from 2.68 ± 0.14 ng/ml during the early luteal phase to 1.43 ± 0.27 ng/ml during the mid-luteal phase ($p < 0.001$) which then increased to 2.06 ± 0.17 ng/ml during the late luteal phase. Plasma T_3 concentrations decreased from the late luteal phase to the peri-oestrous phase ($p < 0.001$) which then increased during the early luteal phase. T_4 concentrations increased from the late luteal phase to the peri-oestrous phase which then decreased during the early luteal phase. (**Key Words :** Meta-analysis, Buffalo, Fixed Effect Model, Random Effect Model, Oestrous Cycle)

INTRODUCTION

Buffalo is the principal dairy animal in the developing countries of Asia and the mainstay of the Indian dairy industry, contributing over 60% of the total milk production. India produces about two-thirds of the world's buffalo milk and nearly half of the world's buffalo meat (FAOSTAT, 2005). Problem of silent heat coupled with late maturity, poor expression of estrus, irregular oestrous cycle, seasonality in breeding, anestrus, low conception rate, long postpartum interval, repeat breeding are some of the major constraints in buffalo productivity and improvement through artificial breeding (Madan, 1990). Silent estrus is one of the major impediments in understanding reproductive parameters and assisted reproduction in this species (Mondal et al., 2003b; Mondal and Prakash, 2003c; Mondal et al., 2008b). Buffalo tends to exhibit overt signs during night or early morning and most farmers are ignorant of physical signs of oestrous. The inherently suboptimal functioning of hypothalamo-hypophysial-gonadal axis and consequently low circulating hormones might be

responsible for higher incidence of inactive ovaries and true anestrus in buffalo. Oestrus detection is also difficult in buffaloes due to lack of expert personnel, variation in duration of oestrous and reluctance of some teaser bulls to mate. Therefore, an understanding of oestrous expression mechanism in terms of endocrine modulations will form the basis for elucidating the causes of lower reproductive efficiency in buffalo.

The different phases of reproductive cycle are regulated by intricate sequential events and interaction between hypothalamic releasing factors from the pituitary and sex steroids. Lack of integration or synchronization and endocrine imbalances at any phase of the sequence may result in reproductive failure. Progesterone as a marker for monitoring of functional status of corpus luteum and diagnostic tool for identifying ovarian condition such as oestrous confirmation, silent oestrous, lack of cyclicity have been reported (Foote et al., 1979; Claus et al., 1983; Mondal and Prakash, 2002; Mondal et al., 2003b; Mondal and Prakash, 2003c; Mondal et al., 2008b). Estrogen induces behavioural symptoms of oestrous by its action on the central nervous symptom (Hafez, 1974). Pituitary FSH is essential for development and maintenance of ovarian follicles in single and multiple ovulating species (Taya et al.,

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1991). Inhibin, produced by granulosa cells of ovarian follicles, is a glycoprotein hormone which suppresses production and/or secretion of FSH through negative feedback at pituitary level (Burger, 1992). In the last two decades considerable attention has already been focused on reproductive endocrinology as a means to identify specific problems and devise means to augment productive performance. Meta-analysis is a statistical tool that allows the quantitative integration of results from published studies that involve certain amount of differences, such as physiological status of animal, experimental design and measurement methods among other. Results from single classical experiment cannot be the basis for a large inference space because the conditions under which observations are made in a single experiment are generally very narrow. A single experimentation measures the effect of one or a very few factors while maintaining all other factors as constant as possible. Often the experiments are repeated by others to verify the generalization and repeatability properties of the observations that were made and to challenge the range of applicability of observed results and conclusions. There are lots of laboratory-to-laboratory variations and there is a need to summarize the results across all published studies describing the profiles of

various hormones in buffalo. The objective of this study was to integrate and consolidate statistically the endocrine factors regulating the cyclicity in buffalo by conducting a meta-analysis of published results.

MATERIALS AND METHODS

Articles published were sought using the on-line journal databases Web of Science (ISI, UK), CAB (CABI Publishing) and VET CDs. Articles were also found by cross-referencing citations in retrieved articles. No unpublished study was considered. About 45 peer reviewed studies elaborating the hormone profiles were searched and 25 studies which satisfied the following criteria were included in meta-analysis (Table 1). The criteria for selecting the studies were (i) oestrous cycle, (ii) buffalo (iii) steroid and protein hormones and (iv) radioimmunoassay. Given a vast quantity of heterogeneous literature, the type of items that were collected include the characteristics on the report of the study (such as author, year and source), the study itself, research design (experimental or observational, treatment assignment mechanism or sampling mechanism, attrition rate or non-response rate).

After testing for significance of heterogeneity of studies

Table 1. Studies on endocrine profiles during oestrus cycle considered for meta-analysis

| Sl. No. | Author | Year | No of animals | Hormones studied |
|---------|--------------------|---------|---------------|--|
| 1 | Arora and Pandey | 1982 | 5 | Progesterone, Estradiol, LH |
| 2 | Ahmed et al. | 1977 | 14 | Progesterone |
| 3 | Bachalaus et al. | 1979 | 18 | Progesterone, Estradiol |
| 4 | Batra and Pandey | 1982/83 | 17 | Estradiol, LH |
| 5 | Batra et al. | 1979 | 5 | Progesterone |
| 6 | Batra et al. | 1980 | 10 | Estradiol |
| 7 | Kamboj and Prakash | 1993 | 6 | Progesterone |
| 8. | Kanai and Shimizu | 1984 | 5 | LH, Progesterone, Estradiol |
| 9 | Khurana and Madan | 1985 | 6 | T3 and T4 |
| 10 | Kumar et al. | 1981 | 10 | Progesterone, Estradiol, LH, T3, T4 |
| 11 | Madan et al. | 1993 | 6 | Progesterone |
| 12 | Palta et al. | 1997 | 5 | Inhibin |
| 13 | Rao and Pandey | 1982 | 6 | Progesterone |
| 14 | Rao and Pandey | 1983 | 6 | Estradiol, LH |
| 15 | Mondal and Prakash | 2002 | 5 | Progesterone |
| 16 | Mondal et al. | 2003a | 5 | Progesterone, Inhibin |
| | | 2003b | 5 | Inhibin |
| | | 2003c | 5 | Progesterone |
| 17 | Mondal et al. | 2004 | 5 | Progesterone |
| 18 | Mondal et al. | 2008a | 5 | FSH |
| | | 2008b | | |
| 19 | Pahwa and Pandey | 1983 | 12 | Progesterone, Estradiol |
| 20 | Pahwa and Pandey | 1984 | 11 | Prolactin |
| 21 | Palta et al. | 1996 | 6 | Inhibin, Progesterone |
| 22 | Sharma et al. | 1999 | 10 | Progesterone, Estradiol FSH, T3, T4 |

based on chi-square test and τ^2 (heterogeneity co-efficient), fixed effect model or random effect model was selected for integrating the results. When heterogeneity was insignificant, fixed effect model by Inverse variance method was used with zero heterogeneity co-efficient, otherwise a random effect model by DerSimonian and Laird Method. Random effect model allows the study variation by co-efficient of heterogeneity to some extent while integrating results and in the present study, the maximum τ^2 is allowed was 20 so as to avoid the larger heterogeneity among the studies (Glass, 1976; Cooper and Hedges, 1994; Eugene et al., 2004; Sauvart et al., 2008).

Inverse-Variance Method: The Inverse-Variance Method (IV method) was used to pool either binary, continuous or correlation data. This approach has wide applicability since it can be used to combine any estimate that has standard error available. The effect size or mean are combined to give a pooled estimate (denoted by θ) by calculating weighted average of the treatment effects from the individual studies as follows.

$$\theta_{IV} = \frac{\sum w_i \theta_i}{\sum w_i}$$

Where the weights w_i are calculated as,

$$w_i = \frac{1}{SE(\theta_i)^2}$$

That is, the weight for the i^{th} study is equal to its precision of the estimate.

The standard error of θ_{IV} is given by,

$$SE(\theta_{IV}) = \frac{1}{\sqrt{\sum w_i}}$$

The heterogeneity statistic (denoted by Q_w) is given by,

$$Q_w = \sum w_i (\theta_i - \theta_{IV})^2$$

The Q_w follows chi-square distribution with $(k-1)$ degrees of freedom, where k is the number of studies included in the meta-analysis.

DerSimonian and Laird Method: The DerSimonian and Laird method (DL method) of meta-analysis is based on the random effects model. Under the random effects model, the assumption of common effect is relaxed, and the effect size or mean θ_i are assumed to have a normal distribution with mean θ and variance τ^2 . The usual DL estimate for τ^2 is given by,

$$\tau^2 = \frac{Q_w - (k-1)}{\sum w_i - \frac{\sum w_i^2}{\sum w_i}}$$

where, Q_w is the heterogeneity statistic, and the weights w_i are calculated as in the IV Method, and k is the number of studies. The τ^2 is set to zero if $Q_w < (k-1)$. In this approach, the weights for each study effect size w_i are as given below.

$$w_i' = \frac{1}{SE(\theta_i)^2 + \tau^2}$$

The pooled estimate is given by,

$$\theta_{DL} = \frac{\sum w_i' \theta_i}{\sum w_i'}$$

With standard error.

$$SE(\theta_{DL}) = \frac{1}{\sqrt{\sum w_i'}}$$

The heterogeneity statistic and its test of significance is as given in the IV method.

RESULTS

Peripheral plasma concentrations of progesterone ranged between 0.3 ± 0.06 to 1.94 ± 0.03 ng/ml during oestrous cycle in buffalo. Plasma levels which were lowest (0.30 ± 0.06 ng/ml; $\tau^2 = 0.0125$) during perioestrous phase increased to 0.47 ± 0.07 ng/ml ($p = 0.067$) during early luteal phase and then further to 1.94 ± 0.03 ng/ml ($\tau^2 = 0$; $p < 0.001$) during mid luteal phase. The concentration declined thereafter to 1.24 ± 0.02 ng/ml ($\tau^2 = 0$) during late luteal phase. Plasma estradiol concentrations were maximum (22.48 ± 0.32 pg/ml; $\tau^2 = 15.93$) during perioestrous phase and declined ($p < 0.001$) to 11.04 ± 0.13 pg/ml ($\tau^2 = 0$) during mid luteal phase and increased to 12.29 ± 0.21 pg/ml ($\tau^2 = 0$; $p < 0.001$) during late luteal phase.

Plasma concentrations of FSH were found to range between 0.98 ± 0.13 to 2.45 ± 0.33 ng/ml. FSH concentration were lowest (0.98 ± 0.13 ng/ml; $\tau^2 = 0$) during early luteal phase and increased to 0.99 ± 0.22 ng/ml ($\tau^2 = 0$; $p = 0.969$) during mid luteal phase. The concentration further increased to 1.55 ± 0.27 ng/ml ($\tau^2 = 0$; $p = 0.1252$) during late-luteal phase and then to the peak level of 2.45 ± 0.33

ng/ml ($Tau^2 = 0$; $p = 0.0483$) during perioestrous phase. Peripheral plasma inhibin and cortisol concentrations decreased from 0.44 ± 0.02 and 2.68 ± 0.25 ng/ml during perioestrous phase to 0.34 ± 0.01 ($Tau^2 = 0.003$; $p < 0.001$) and 2.68 ± 0.14 ng/ml ($Tau^2 = 1.12$; $p = 0.999$) during early luteal phase which then declined to 0.31 ± 0.01 ($Tau^2 = 0.0009$; $p = 0.061$) and 1.43 ± 0.27 ($Tau^2 = 2.07$; $p < 0.001$) ng/ml during mid luteal phase, respectively. The concentrations then increased to 0.39 ± 0.02 ($Tau^2 = 0.05$; $p = 0.004$) and 2.06 ± 0.17 ($Tau^2 = 0.36$; $p = 0.057$) ng/ml, respectively during late luteal phase. Plasma prolactin concentrations ranged between 219.28 ± 25.4 to 245 ± 23.3 ng/ml throughout the oestrous cycle. Peripheral prolactin concentrations were lowest (219.28 ± 25.4 ng/ml) during late luteal phase and increased to a maximum concentration (245 ± 23.3 ng/ml; $p = 0.552$) during perioestrous phase which then declined to 227.7 ± 30.19 ng/ml ($p = 0.716$) during early luteal phase in buffalo. Fixed effect model was used as the heterogeneity was not statistically significant and Tau^2 was zero.

In the present study, mean plasma T_3 concentrations have been reported to vary between 1.04 ± 0.04 to 1.45 ± 0.09 ng/ml and T_4 concentration between 44.78 ± 0.64 to 48 ± 1.38 ng/ml during the oestrous cycle. Plasma T_3 concentrations were 1.45 ± 0.09 ng/ml during the late luteal phase which then decreased ($p < 0.001$) to 1.04 ± 0.04 ng/ml during perioestrous phase. The concentration then increased to 1.12 ± 0.05 and 1.12 ± 0.04 ng/ml during early luteal and mid luteal phase, respectively. T_4 concentrations were 46.54 ± 1.11 ng/ml during late luteal phase and increased to ($p = 0.414$) to 48 ± 1.38 ng/ml during perioestrous phase. The concentrations then decreased to 44.78 ± 0.64 ng/ml ($p = 0.039$) during early luteal phase followed by an increase to 49.8 ± 0.64 ng/ml ($p < 0.001$) during mid luteal phase of oestrous cycle. Meta-analysis using the fixed effect model was used in both T_3 and T_4 concentrations as the heterogeneity between the studies considered is not statistically significant ($p > 0.05$).

DISCUSSION

To the best of our knowledge and belief, this is the first study to report the meta-analysis of hormonal profiles of oestrous cycle in buffalo. Meta-analysis is used increasingly as a method of summarizing data that tests the same hypothesis in several different published studies. The validity of a choice of meta-analytical methods depends on pattern of variability (heterogeneity) observed in the study results. However, there is no empirical guidance currently available to judge which methods are appropriate in which circumstances so as to yield better results. Therefore, the

present study has made an attempt to provide appropriate meta-analytical methods to deal with the hormone profiles during oestrous cycle in buffalo.

In the present study, plasma progesterone concentrations, which were lowest during the perioestrous phase, increased through early luteal phase to a maximum concentration during mid luteal phase in Murrah buffaloes. These results are in agreement with earlier observations in cattle (Schomberg et al., 1967; Gupta and Pope, 1968) and buffaloes (Bachalaus et al., 1979; Arora and Pandey, 1982; Mondal et al., 2003a; Mondal et al., 2004) in terms of minimum level on the day of oestrous with gradual rise to the higher levels during luteal phase and then declining to basal level at subsequent oestrous. The cyclic pattern of progesterone concentrations in jugular plasma follows the known changes in corpus luteum function in buffalo during oestrous cycle. Like cattle (Mares et al., 1962), the decline in progesterone levels towards the end of the cycle and a sharp rise during luteal development suggest that functioning of corpus luteum can be monitored by progesterone determination. Similarly in cattle and buffalo that exhibited overt oestrous and silent oestrous, progesterone level was lowest during perioestrous phase and increased to maximum concentration during mid luteal phase (Mondal et al., 2003c; Mondal et al., 2003d).

Plasma inhibin, FSH and estradiol concentration were lowest during the mid luteal phase, increased through the late luteal phase to a maximum concentration during the perioestrous phase. The patterns of inhibin concentration agrees with earlier report in cattle (Kaneko et al., 1995) and buffalo (Palta et al., 1997; Mondal et al., 2003a; Mondal et al., 2003b) in terms of an increase in inhibin levels during the follicular phase of oestrous cycle. The follicular growth occurs in a wave-like pattern, with a predominance of two waves in cattle (Pierson and Ginther, 1988; Ginther et al., 1989) and buffalo (Manik et al., 1998). The first and second waves start at Days 1 and 11, respectively (Day 0 = Day of ovulation) in cattle (Pierson and Ginther, 1988; Ginther et al., 1989) and at Days 0.20 and 9.20, respectively, in buffaloes (Manik et al., 1998). Each wave is marked by the emergence of a dominant follicle and a cohort of subordinate follicles. The concentrations of plasma FSH have been reported to be high prior to the emergence of each follicular wave and remain low during the growing phase of a dominant follicle (Kaneko et al., 1995). In the present study also, the highest circulating concentrations of FSH were observed at Day 0 (day of oestrous). Following a decrease thereafter, circulating FSH concentrations rose again and reached high concentrations on Days 7 in buffalo prior to the emergence of the second wave. The concentrations of inhibin have been reported to reach a high level with the emergence of each wave, in the growing

phase of the dominant follicle and decrease when the dominant follicle ceases to grow or ovulates (Kaneko et al., 1995). In the present study also, such a trend was observed with the dominant ovulatory follicle, as evident from the high concentrations of inhibin on Day-2. Such a clear trend could not be observed for the dominant non-ovulatory follicle probably because the data were not clustered around initiation of wave 1, due to non-availability of the follicular data. Our results showed that plasma estradiol concentrations were maximum during oestrous phase and declined during mid luteal phase which agrees with earlier reports of Batra and Pandey (1982/83) and Samad et al. (1988) wherein the concentration of estradiol 17β following luteolysis increased and reached to its peak value either a day before or on the day of oestrus. After attaining the peak concentration, estradiol declined within 2 days following estrus. Thereafter estradiol fluctuates at lower levels throughout the luteal phase of the cycle except on day 4 and 10 where minor peaks have been recorded. These minor peaks might have resulted from waves of follicular growth. Buffaloes have been reported to undergo two or three waves of follicular growth during oestrous cycle with the second wave occurring during days 10-11 of the cycle (Barnselli et al., 1997). The prooestrous rise of estradiol may be associated with triggering of LH release by positive feedback on hypothalamo-hypophyseal axis (Batra and Pandey, 1982;1983). Prooestrous rise in estradiol secretion after progesterone withdrawal is considered to be a prerequisite event for the initiation of both behavioral oestrous and preovulatory LH surge in most livestock.

In the present study, peripheral plasma cortisol levels were high during oestrous phase in buffalo which agrees with earlier report of Kumar et al. (1991) and Madan et al. (1993). The high levels may be attributed to stress caused by increased physical activity and stress of oestrous. Plasma T_3 concentrations was maximum during late luteal phase which subsequently decreased during oestrous phase whereas T_4 concentrations increased during oestrous phase. Vadodoria et al. (1978) reported a lower thyroid activity immediately after ovulation with the peak activity at the beginning of oestrous. High level of T_3 during proestrus may possibly be due to stress caused by hyperactivity. Peak T_4 level was observed at the onset of oestrus. This may be due to high estradiol level which stimulates thyroid gland activity by directly acting on it with the intervention of pituitary gland. Estrogen has been found to stimulate TSH release from pituitary gland (D'Angelo and Fisher, 1969) causing elevation of T_4 during oestrous.

In conclusion, the hormonal profiles during oestrous cycle presented in this paper are more accurate and precise compared to the individual studies as meta-analysis reduces the standard error by increasing the sample size.

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