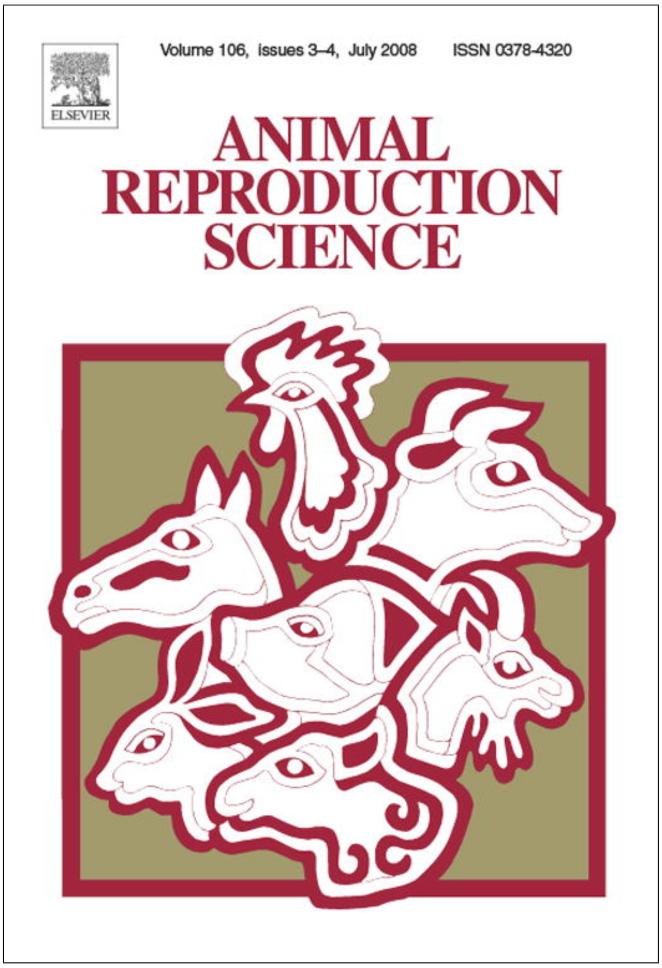
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# Interaction of calf suckling, use of oxytocin and milk yield with reproductive performance of dairy buffaloes

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**Abstract**

Calf suckling and oxytocin injections are commonly used for pre-milking stimulus in dairy buffaloes under field conditions. A study was conducted to investigate effect of these treatments on reproductive performance. Fifty one Nili-Ravi buffaloes were monitored from parturition up to 150 days postpartum through rectal examination. Data on milk yield, body condition score (BCS) and reproductive parameters were recorded weekly. Postpartum ovulation interval (POI) was determined by presence of an ovulation depression or a very soft corpus luteum haemorrhagicum and was confirmed through milk progesterone levels (MPL). Suckling was used to stimulate milk let down, and where the calf had died, injection of oxytocin was resorted to. Milk samples were analyzed for MPL using radioimmunoassay (RIA) and fat; and milk yield was converted to 4% fat corrected milk (FCM). The mean postpartum uterine involution length (PUI) was 34.30 ± 1.33 days. Mean POI was 59.37 ± 4.76 days and mean postpartum estrus interval (PEI) was 69.03 ± 6.03 days. Suckling period averaged 26.40 ± 5.57 days and correlated with POI ($r = 0.19$, $P < 0.01$) and PEI ($r = 0.23$, $P < 0.01$). POI was shortest in buffaloes suckled for one month ($P < 0.05$). Oxytocin was used with a mean dosage of 7.50 IU, delaying placental expulsion time (PET) and POI but shortening PEI. BCS shortened PET, POI and PEI ($P < 0.01$). Mean FCM was 14.50 ± 0.20, ranging from 2 to 35 kg/d; and was higher in estrus group; correlating positively with POI ($r = 0.31$, $P < 0.01$). MPL were 1.37 ± 0.17 ng/ml and increased after ovulation, remaining greater than 1.5 ng/ml from Day 4 to 14 of the estrus cycle, followed by a rapid decline up to next estrus. BCS in buffaloes resuming oestrus was constantly higher than those failing to resume ovarian cyclicity. Live weight, prepartum was 510.0 ± 5.9 kg with a loss of 3.7 ± 2.12 kg, 30 days postpartum. The present study suggests a lower reproductive efficiency of dairy buffaloes under the peri-urban farming system reflected by ovarian cyclicity in 68.63% buffaloes within 150

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days postpartum and silent estrus in 51.5% of the cases. Increasing suckling duration and use of oxytocin delayed POI, however, POI was shortest in buffaloes suckled for one month. The high yielding buffaloes also manifested better reproductive cyclicity; while moderate yielder showed shorter ovulation intervals and higher conception rate.


*Keywords:* Dairy buffalo; Milk production; Reproductive performance; Oxytocin; Suckling; Progesterone

## 1. Introduction

Suckling is commonly used as a stimulus for milk let down under conventional farming in the northwest frontier province (NWFP) of Pakistan, while oxytocin is used for milk let down when a calf dies at a young age (Qureshi, 1995). On poor quality forage, buffaloes can hold their body condition better than cattle. During the period of frequent nursing, serum concentration of prolactin are elevated and are inversely related to the concentration of circulating FSH and LH (Moss et al., 1981). Conflicting results have been reported regarding the effects of oxytocin injections on milk yield in dairy animals (Sagi et al., 1980; Nostrand et al., 1991; Ballou et al., 1993; Knight, 1994). Variations in methodology and experimental design (length of experimentation, dose of oxytocin, whether followed by milk removal or not, etc.) have been blamed for these variable results. For most authors, the increase in milk production associated with the injection of oxytocin, can only be explained by the stimulation of myoepithelial cell contraction to obtain residual milk, thereby limiting the negative effects of feedback inhibitor of lactation (FIL) or increased intra-alveolar pressure on milk secretion (Knight, 1994).

According to Nordin and Jainudeen (1991), the interval from calving to first ovulation averaged 82, 69 and 50 days in Swamp buffaloes experiencing unrestricted suckling, once a day suckling or no suckling, respectively. Abeygunawardena et al. (1995) reported that suckling *ad libitum* in buffaloes resulted in lower conception rate and weaning by Day 60 or 90 did not improve postpartum fertility. Ovarian inactivity has been shown to be the major problem in animals of poor fertility associated with suckling (Perera et al., 1992). Plasma LH levels in lactating Murrah buffaloes which eventually experienced anestrus beyond 90 days postpartum were significantly lower during the second and third weak postpartum than in those cycling within 50 days postpartum (Batra and Pandey, 1983).

The highest and lowest yielding cows had a lower conception hazard than did average yielding cows (Harman et al., 1996). However, there was no effect of milk yield on conception rates after 120 days of lactation. Analysis of data on 389,063 German Simmental cows (Daubinger, 1994) showed that high milk and milk fat yields and a low milk protein yield had an adverse effect on subsequent fertility. The objective of the present study was to determine the interaction among calf suckling, use of oxytocin, milk production and reproductive performance in dairy buffaloes kept under field management.

## 2. Materials and methods

### 2.1. Experimental animals

Fifty one dairy buffaloes in their last two months of gestation were selected at seven private farms located within a radius of 70 kilometers from Peshawar, Pakistan. Past reproductive history

Table 1
Nutrient composition[a] of feeds used for experimental buffaloes

| Feed | Months | Dry matter | Minerals | Crude protein | ME[b] |
|---|---|---|---|---|---|
| Green fodders | | | | | |
| Berseem | Nov–May | 14.43 | 12.94 | 21.03 | 2.67 |
| Sorghum | Jul–Nov | 34.35 | 8.82 | 6.09 | 3.06 |
| Maize | Jun–Nov | 30.00 | 8.91 | 7.51 | 2.95 |
| Wheat | Jan–May | 28.05 | 8.01 | 8.41 | 2.65 |
| Dry roughage | | | | | |
| Maize stovers | Jan–Dec | 95.09 | 6.04 | 3.72 | 1.73 |
| Wheat straw | Jan–Dec | 93.74 | 9.93 | 4.21 | 1.58 |
| Concentrate supplements | | | | | |
| Wheat bran | Jan–Dec | 90.44 | 3.87 | 17.13 | 3.68 |
| Cotton seed cake | Jan–Dec | 91.88 | 5.46 | 24.97 | 5.44 |
| Mustard seed cake | Jan–Dec | 92.01 | 6.79 | 31.61 | 2.93 |
| Maize oil cake | Jan–Dec | 95.38 | 1.94 | 20.50 | 2.72 |
| Commercial conc. | Jan–Dec | 92.60 | 6.68 | 15.66 | 2.98 |
| Dried bread | Jan–Dec | 82.66 | 2.49 | 20.39 | 4.57 |
| Wheat grain | Jan–Dec | 93.00 | 1.32 | 13.00 | 4.78 |
| Beet pulp dried | Jan–Dec | 95.06 | 4.49 | 11.96 | 2.31 |
| Molasses | Jan–Dec | 71.92 | 13.6 | 8.99 | 1.81 |

[a] As % in dry matter.
[b] Metabolizable Energy (MCal/kg DM).

of the selected animals was recorded. The animals were kept under naturally prevailing climatic conditions in sheds attached with open areas. They were stall-fed with green fodder and concentrate supplements, mainly wheat bran and cotton-seed cake. Water was offered two to three times a day. These animals were monitored from parturition 150 days postpartum.

### *2.2. Feeding of animals*

Feed components of the experimental animals comprised green fodders, concentrates and wheat straw with the composition reflected in Table 1. Green fodder during the winter and spring comprised Egyptian clover with higher crude protein and moisture. During autumn and early winter, sorghum and maize were available, having higher levels of dry matter and relatively low crude protein. During late winter and spring, wheat crop was fed. Wheat straw or dried maize stovers were fed throughout the year with an increasing level during winter and early summer. Concentrate feeds comprised wheat bran, cotton seed cake (undecorticated), mustard seed cake, maize oil cake, commercial concentrates, dried bread, wheat grain, beet pulp and molasses and were fed to lactating buffaloes at the same scale, irrespective of milk production, as per conventional practice.

### *2.3. Milk yield and let down practices*

Milking was done twice daily, generally at 0100 and 1300 h through hand milking. Concentrate mixture was offered just before milking and the calf was allowed to stimulate milk let down through suckling. In case of non-availability of calf, a calf from another dam was used for this purpose. If the dam was reluctant to accept suckling by a calf from another dam, oxytocin injection (usually 5 IU, I/M) was used for milk let down. In rare cases (11.8%), a buffalo was milked without the aid of suckling or oxytocin. So, with the advancement of study, the number of dying calves increased; the buffaloes were shifted to oxytocin injections for the purpose of milk let down enhancing the number of such buffaloes from 8 to 33. These observations were used for determination of correlation of use of oxytocin with the PET (placenta expulsion time), lochia discharge, POI (postpartum ovulation interval), PEI (postpartum estrus interval) and PUI (postpartum uterine involution). Sufficient observations are available for both the suckled and oxytocin groups containing 43 and 33 animals, respectively.

Milk yield was recorded (kg/d) once a week until 150th day postpartum. Fresh samples collected once a month and used for milk fat determination using Babcock method (AOAC, 1980). The fat content of buffalo milk is greater than the cow milk (8 versus about 3.5%). Fat contents showed a gradual change with the advancement of lactation and feed changes for each animal and these changes were reflected in their analytical results fairly well. Conversion of the yield to fat corrected milk was a good exercise for expressing the adjusted milk yield of each animal at various intervals. The last milk was collected for the purpose of fat analysis. The yield was converted to 4% fat corrected milk (FCM), as suggested by Morrison (1949).

### 2.4. *Clinical monitoring*

After parturition, rectal examination of reproductive organs was carried out on Days 14 and 21, and then fortnightly, as described by Usmani et al. (1985). Position of the reproductive organs was recorded and the size of Graafian follicle(s), corpus luteum or corpora albicancia, if any was estimated. Estrus detection was made twice daily from 15 days postpartum. In addition to visual signs of vulvular mucous discharge, frequent micturition and bellowing, an intact bull was used for detection of estrus at each farm. This practice was exercised on daily basis and the events were recorded. Standing heat was used as a criterion for estrus confirmation and breeding bulls of proven fertility were used for service. POI was detected by the presence of an ovulation depression or a very soft corpus luteum haemorrhagicum. The POI was confirmed through MPL when the results were made available. MPL remained at the lowest levels on the day of ovulation (<0.10 ng/ml) and this level was used for confirmation of the day of ovulation. Accuracy of rectal diagnosis of corpus luteum was 83.61%; out of which 9.50% observation were false positive and 8.11% were false negative.

### 2.5. *Milk progesterone assay and rectal palpation*

Milk samples were collected every week. Fat layer was removed and 100 ml of 0.1% sodium azide was added to 5 ml of milk sample as a preservative. Samples were stored at −200C until analysis for progesterone assay. RIA was used for determination of MPL, following the procedure of FAO/IAEA (1993). The intra and inter-coefficient of variation were 3.54 and 9.21%, respectively. Evening milk samples were collected once a week and brought to the laboratory in icebox. After removing the fat layer by means of centrifugation (3000 rpm), 100 μl of 0.1% sodium azide was added to 5 ml of milk sample as a preservative. Samples were stored at −20C, until they were analyzed for milk progesterone levels. Milk samples and other assay components were brought to room temperature before the assay was started. Antibody coated polypropylene tubes were labeled for standard, quality control and samples, according to the protocol. Non-coated normal tubes were used for total count. 100 μl of standard, quality control or sample were pipetted into the bottom of the corresponding tube. Then, 1 ml of 125 I-progesterone was pipetted into each tube. The tubes were covered with parafilm and incubated at 4 °C overnight. The next morning, the tubes were decanted vigorously, except for those used for total count. The radioactivity was counted using a gamma counter (Vega-calc (c) NE Technology Limited). The data were analyzed using the Vegacalc software program. The sensitivity (detection limit) of the assay was 0.09 ng/ml.

### 2.6. Body weight and condition score estimation

Body measurement, including the height at withers ($X_1$), girth ($X_2$) and length from pin bone to scapular spine ($X_3$, in inches) were recorded fortnightly. Body weight was calculated using the following formula recommended by Azhar (1976):

$$Y = -1604.790 + (1.268)X_1 + (30.902)X_2 + (3.960)X_3 \quad (\text{for } 6-8 \text{ years old buffaloes}) \tag{1}$$

$$Y = -1263.663 + (8.060)X_1 + (18.924)X_2 + (8.565)X_3 \quad (\text{for } > 8 \text{ years old buffaloes}) \tag{2}$$

where $Y$ is the weight of buffaloes in pounds. Finally the body weight was converted into kg. Body condition score (BCS) was recorded at weekly intervals, using the method described for dairy and beef cows (Peters and Ball, 1987). In this method, the thickness of fat over the lumber and tail area was estimated and assigned a score from 0 (emaciated) to 4 (very fat).

### 2.7. Statistical analysis

The data obtained, were statistically analyzed using general linear model procedures and correlation analysis (Steel and Torrie, 1980). The following model was used for comparing the effects of calf suckling period and fat-corrected milk production levels on various reproductive traits:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + e_{ijk} \tag{3}$$

where $Y_{\text{ijk}}$ is the $k$th observation of the reproductive trait (dependant variable) within the $i$th suckling interval and $j$th production level. $\mu$ is the population constant common to all records; $\alpha_i$ is the effect of $i$th group of suckling interval (0, 1–30 and 31–150 days) on reproductive traits; and $\beta_j$, the effect of $j$th production level of fat-corrected milk (0–10, 11–20 and 20–35 kg/day); $e_{ijk}$ is the random residual term associated with each $Y_{ijk}$.

## 3. Results

### 3.1. Reproductive performance

The mean values (±S.E.) of various parameters of reproductive performance in experimental buffaloes are given in Table 2. The mean PUI was 34.30 ± 1.33 days, ranging from 21 to 74 days. Uterine involution was completed within 35 days in 55.0% buffaloes, 50 days in 85.0%, while within 74 days in all buffaloes. As very few animals (15%) completed uterine involution beyond 50 days postpartum, the mean fell at 34.30 days postpartum. Uterine involution was completed within two months after calving in all animals except two animals; one had dystocia and the other suffered from retained placenta with subsequent metritis.

Table 2
Number of observations (*n*), mean values and standard error (S.E.) of various parameters in buffaloes

| Parameter | *n* | Mean ± S.E. | Range |
|---|---|---|---|
| Reproductive parameters | | | |
| Placenta expulsion time (hours) | 38 | 5.46 ± 0.92 | 0.5–24 |
| Locia discharge (days) | 50 | 6.02 ± 1.24 | 0–34 |
| Postpartum uterine involution (days) | 40 | 34.30 ± 1.33 | 21–74 |
| Postpartum estrus interval (days) | 35 | 69.03 ± 6.03 | 21–147 |
| Postpartum ovulation interval (days) | 43 | 59.37 ± 4.76 | 24–150 |
| Conception rate to first service (%) | 51 | 45.10 ± 6.97 | 0–100 |
| Milk progesterone levels (ng/ml) | 384 | 1.37 ± 0.17 | 0.10–30.69 |
| Calf suckling and milk production | | | |
| Calf suckling period (days) | 43 | 26.40 ± 5.57 | 0–150 |
| 4% Fat-corrected milk (kg/day) | 898 | 14.50 ± 0.20 | 2–35 |
| 4% Fat-corrected-milk (kg/150 days) | 50 | 1989.00 ± 118.37 | 652.43–4797.00 |
| Use of oxytocin (IU) | 33 | 7.50 | 5–25 |
| Body weight and condition score | | | |
| Live weight, prepartum (kg) | 51 | 510.0 ± 5.9 | 416–599 |
| Live weight loss, 30 days postpartum (kg) | 51 | 3.7 ± 2.12 | 1.0–6.0 |
| Body condition score, prepartum | 51 | 2.77 ± 0.10 | 0.5–5.0 |
| Body condition score loss, 30 days postpartum 51 | 0.25 ± 0.08 | 0–5.0 | – |

During 150 days after calving, 68.63% buffaloes were found in estrus, while the remaining 31.37% animals remained anestrous. Mean POI, based was 59.37 ± 4.76 days, ranging from 24 to 150 days. The overall mean PEI was 69.03 ± 6.03 days, ranging from 21 to 147 (Table 3). Based on the observable estrus signs and milk progesterone levels, three types of estrus events were observed, that is, ovulatory estrus (43.9%), anovulatory estrus (4.6%) and silent estrus (51.5%). Fig. 2 shows that BCS in buffaloes resuming oestrus was constantly higher during pre and postpartum periods than those failing to resume oestrous activity. Table 4 shows that BCS correlates negatively with PET and PEI.

The present study suggests a lower reproductive efficiency of dairy buffaloes under the peri-urban farming system reflected by ovarian cyclicity in 68.63% buffaloes within 150 days postpartum and silent estrus in 51.5% of the cases.

Table 3
Correlation of various parameters with reproductive performance in buffaloes (Pearson's correlation coefficients)

| Parameter | Placenta expulsion time | Lochia discharge duration | Postpartum ovulation interval | Postpartum estrus interval | Uterine involution |
|---|---|---|---|---|---|
| Suckling duration | −0.19 | −0.26* | 0.19** | 0.23** | 0.05 |
| Use of oxytocin | 0.40** | 0.02 | 0.17** | −0.18** | 0.02 |
| Fat corrected milk | −0.09 | 0.07 | 0.31** | −0.02 | 0.00 |
| Body condition score | −0.17* | 0.01 | −0.06 | −0.20** | 0.05 |
| Number of observations | 38 | 50 | 43 | 35 | 40 |

* Significant at $P < 0.05$.
** Significant at $P < 0.01$.

Table 4
Effect of calf suckling and production of fat corrected milk on reproductive performance in dairy buffaloes (least square means ± SE)*

| Group | Placenta expulsion time (hours) | Postpartum uterine involution (days) | Postpartum ovulation interval (days) | Postpartum estrus interval (days) | Conception rate (%) |
|---|---|---|---|---|---|
| Calf suckling (days) | | | | | |
| 0 (12**) | 31.9 ± 0.92 a | 32.5 ± 1.33 | 87.8 ± 4.76 a | 97.6 ± 6.03 b | 50.1 ± 6.97 |
| 1–30 (23) | 10.9 ± 0.68 b | 38.8 ± 1.05 | 69.4 ± 5.12 b | 103.2 ± 4.28 b | 47.7 ± 4.56 |
| 31–150 (16) | 8.8 ± 0.74 b | 34.9 ± 0.89 | 93.7 ± 4.51 a | 129.6 ± 5.16 a | 37.1 ± 5.19 |
| Fat-corrected milk production (kg/day) | | | | | |
| 0–10 (17***) | 19.4 ± 0.57 | 37.5 ± 1.14 a | 82.9 ± 3.81 b | 113.1 ± 6.05 a | 25.0 ± 5.78 b |
| 11–20 (28) | 11.7 ± 0.78 | 30.2 ± 0.85 b | 84.0 ± 4.55 b | 101.9 ± 4.22 b | 56.6 ± 6.27 a |
| 20–35 (6) | 5.9 ± 0.46 | 38.9 ± 0.96 a | 104.2 ± 4.64 a | 103.4 ± 5.24 b | 53.7 ± 4.53 a |

* Means within a group in the same column with different letters, differ significantly (P < 0.05).
** Number of calves in each category.
*** Number of buffaloes in each production category.

### 3.2. Effect of suckling and oxytocin on reproduction

Calf suckling period averaged 26.40 ± 5.57 days, ranging from 0 to 150 (Table 2). Increasing suckling duration delayed postpartum ovulation ($r = 0.19$, $P < 0.01$) and estrus ($r = 0.23$, $P < 0.01$) and shortened duration of discharge of lochia ($r = -0.26$, Table 2), but had no effect on placental expulsion time ($r = -0.19$, $P > 0.05$) or uterine involution ($r = 0.05$, $P > 0.05$). Table 4 shows that non-suckled buffaloes had the longest placenta expulsion time ($P < 0.01$) and shortest PEI ($P < 0.01$) as compared to those suckled for one or more than one months. However, POI was shortest in buffaloes suckled for one month ($P < 0.05$). Length of suckling had no effect on uterine involution or conception rates.

Out of the 51 calves born in the experimental herd, only 8 (15.69%) could survive beyond 150 days, while the rest died or were slaughtered, although some of them were born of excellent milk producing dams. In case of death of the calves, the dams were given oxytocin injections for milk let down at a dose rate of 5 IU intra-muscularly, sometimes as high as 25 IU.

Mean value for use of oxytocin for milk let down was 7.50 IU, ranging from 5 to 25 IU (Table 2). The use of oxytocin correlated positively with PET and POI, but negatively with PEI (Table 3). It was also observed that one injection per day shortened PEI, while no injection or two injections per day increased PEI.

The present study suggests that increasing suckling duration and use of oxytocin delays postpartum ovulation, however, POI is shortest in buffaloes suckled for one month.

### 3.3. Effect of milk yield on reproduction

Average daily yield of 4% FCM was 14.50 ± 0.20 kg per animal, while the total milk yield for 150 days postpartum was 1989.00 ± 118.37 kg (Table 2). FCM correlated positively with use of oxytocin ($r = 0.15$, $P < 0.01$) which suggests that milk production is increased by use of exogenous oxytocin, probably by complete evacuation of the udder. FCM was significantly higher in estrus group as compared to anestrus one, during the first two months postpartum (15.09 versus 13.56 kg/day, $P < 0.01$). FCM correlated positively with POI ($r = 0.31$, $P < 0.01$, Table 3). Table 4 shows that moderate yielders had shortest PUI ($P < 0.01$) and PEI ($P < 0.05$) and highest conception rate ($P < 0.01$).

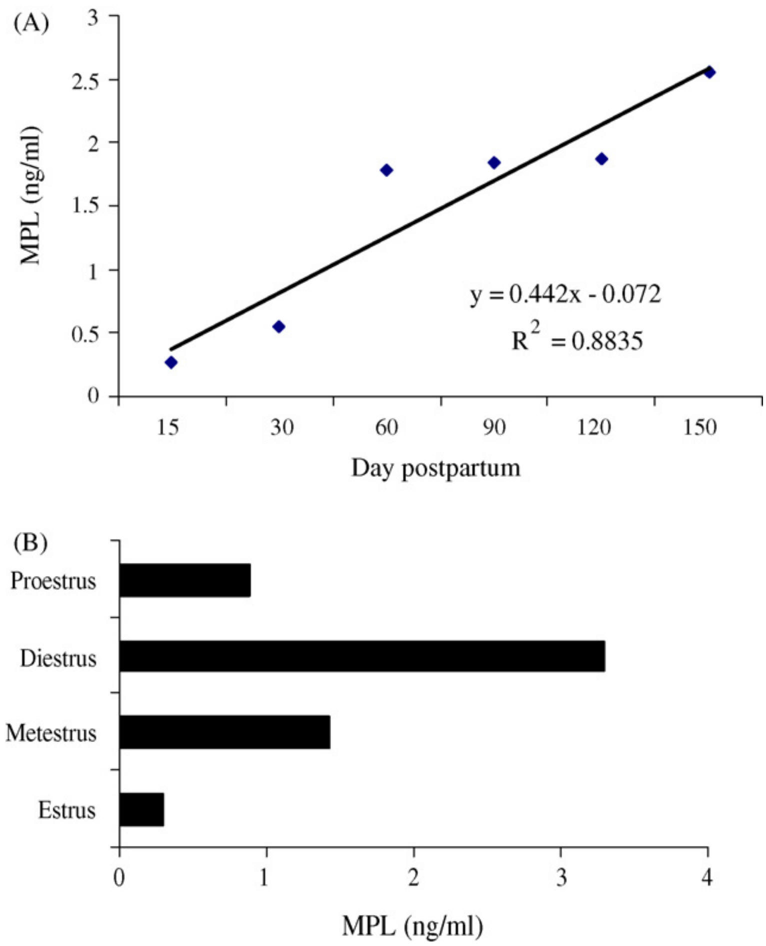


Fig. 1. Milk progesterone levels (MPL) during various reproductive states; (A) postpartum intervals ($n = 384$) and (B) various phases of the estrous cycle ($n = 61$).

These results suggest that the high yielding buffaloes also manifested better reproductive cyclicity, while moderate yielder showed shorter ovulation intervals and higher conception rate.

### 3.4. Milk progesterone levels

MPL showed a mean value of 1.37 ± 0.17, ranging from 0.10 to 30.69 ng/ml. After ovulation the milk progesterone levels increased, remaining greater than 1.5 ng/ml from Day 4 to 14 of the estrus cycle, followed by a rapid decline up to the day of next estrus. During metestrus and diestrus, the values were higher than the proestrus and estrus (Fig. 1-B). Average MPL increased from 15 day postpartum (Fig. 1-A) and remained higher constantly up to 150 days postpartum. There was a slight increase in MPL during the first month postpartum followed by a rapid increase during the second month; a plateau up to fourth month and than again a rapid increase onwards (Fig. 2).

## 4. Discussion

### 4.1. Postpartum uterine involution

The PUI in this study (34.30 ± 1.33 days) was longer than 27.5 ± 8.3 days, reported by Chaudhry et al. (1987), 28.37 ± 1.36 days (Chaudhry et al., 1990) and 26.0 days (Usmani et

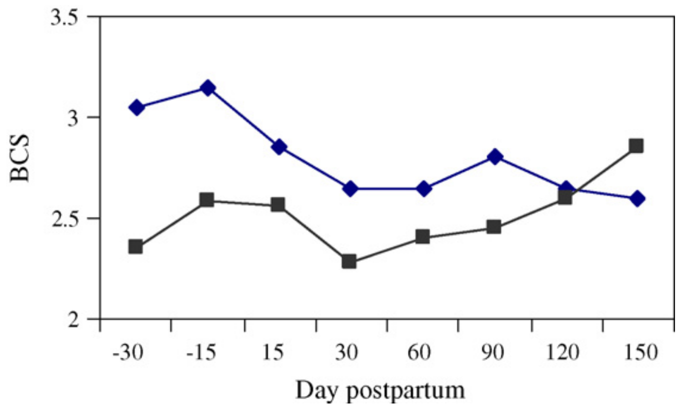


Fig. 2. Postpartum body condition score (BCS) of estrous (♦) and anestrous (■) buffaloes (anestrous buffaloes did not exhibit estrus up to 150 days postpartum while estrous buffaloes exhibited estrus at various postpartum intervals during the same period).

al., 1985) in Nili-Ravi buffaloes at state farms. The accelerated uterine involution at state farms may be due to better housing and feeding conditions and less nutritional stress than the resource-constrained private farmers in the present study. At private farm, the only target is to get maximum milk with minimum investment with little space for housing and poor management. Limited space for free animals' movements may have added to the delayed uterine involution.

Longer time required for completion of uterine involution in buffaloes was reported for Indian (45 days, Roy and Luktuke, 1962; 39 days, Chauhan et al., 1977) and Egyptian buffaloes (40.3 ± 1.2 days, Aboul-Ela et al., 1988). Difference in results among various studies might be due to differences in criteria of recording or methods of examination, seasons of study, breed or managemental practices. El-Wishy (2006) reviewed uterine involution in dairy buffaloes. The distribution of 42 mean values recorded in 32 studies included in the review revealed that 66% were between the 5th and 6th week. Factors such as criteria used for assessing the involution end point, methods of measuring size of the uterus, interval between examinations, climatic and managemental factors contributed to the described spread of values. *Haemolitic streptococci*, *Staphylococci epidermidis*, *E. coli*, *Bacillus* spp. and very infrequently *Actinomyces pyogenes* were isolated during the first 2–3 weeks after normal calving, but all were completely eliminated by Day 28 post-calving (Singh et al., 1997).

These findings suggest that uterine involution completes at an appropriate interval, providing an enabling environment for resumption of ovarian cyclicity and breeding; however, breeding is delayed intentionally due to fear of a drastic decline in milk yield with the onset of pregnancy in dairy buffaloes under field conditions.

### 4.2. *Postpartum estrus interval*

In buffaloes included in the present study, a shorter PEI (69.03 ± 6.03 days) was recorded than that reported by Wahid (1975), who observed that 55% animal showed estrus within 200 days after parturition. In a previous study, 27.7% of 47 Egyptian buffaloes showed normal sequences of estrus and ovulation, 10.6% of which had atretic follicles. Out of 50 ovulations detected, 64.0% were not accompanied by observable signs of estrus (Aboul-Ela et al., 1988).

The shorter PEI recorded in this study might be due to better estrus detection regimes, as the animals were mostly kept on commercial basis for milk production, with a better body condition score. These findings suggests that buffalo resumes cyclicity fairly earlier but due to lack of estrus detection regimes (especially the intact bull), most of the estrus events are falsely declared as silent ovulations.

### 4.3. Effect of suckling and oxytocin on reproduction

As the calves were allowed to suckle two times daily, it probably resulted in adverse effect on resumption of postpartum ovarian activity and increased PEI and POI. Decrease in the duration of lochia discharge might be due to sustained uterine contractions caused by oxytocin released in response to suckling. In agreement with this study, ovarian cyclicity was re-established earlier in non-suckled than suckled river, as well as swamp, buffaloes (El-Fouly et al., 1976; El-Fadaly, 1980; Jainudeen et al., 1984). In Nili-Ravi buffaloes, Usmani et al. (1990) reported that postpartum intervals to uterine involution, resumption of follicular development, first rise in milk progesterone, first palpable corpus luteum formation and first oestrus were longer for limited-suckled buffaloes than for non- suckled buffaloes.

For stimulation of milk letdown, oxytocin is released in response to tactile teat stimulation. The application of a fixed prestimulation of 30 to 60 s before milking has been recommended to ensure immediate and continuous milk flow after the start of milking (Rasmussen et al., 1992). However, recent investigations demonstrated the importance of the udder fill on the course of milk ejection (Dzidic et al., 2004). Therefore, prestimulation time according to the degree of udder fill in individual cows may improve the milking performance. According to previous results, no cisternal milk was available when milking started without prestimulation at a low udder fill (Bruckmaier and Hilger, 2001). At moderate udder fill, cisternal milk was immediately available for milking (Bruckmaier and Blum, 1996) and the alveolar milk ejection started about 70 s after the start of prestimulation, as indicated by the second rise of milk flow (Bruckmaier and Hilger, 2001). In full udders, the amount of cisternal milk was further enhanced (Pfeilsticker et al., 1996) and the lag time until the start of the alveolar milk ejection was further reduced (Bruckmaier and Hilger, 2001). Immediate and continuous milk flow after the start of milking results in a shorter duration of milking (Weiss and Bruckmaier, 2005). A short prestimulation enhances milking stall capacity when milking full udders. If only a small amount of milk is present in the udder, prolonged prestimulation reduces the vacuum load on the teat. In dairy practice, it should be considered that any mechanical impulses to teats before milking, for example, teat cleaning or fore-stripping, cause a prestimulation and hence oxytocin release.

Milk yield in buffalo is lower than cattle because of the little progress made in its conversion to a specialized dairy animal. So the udder is not full and a stronger premilking stimulus is required for milk let down. In the present study, representative of the conventional buffalo farming, the suckling was used by the farmers for milk let down, twice a day for approximately five minutes each time, or it was replaced oxytocin in case of death of the calf.

### 4.4. Effect of milk yield on reproduction

The high yielding buffaloes also manifested better reproductive cyclicity in the present study which may be due to association of the two economic traits. The moderate yielders showed shorter ovulation intervals and higher conception rate, which may be due to the fact that these animals are neither exposed to lactation stress nor to the detrimental effects of excess intake of degradable protein.

Under field conditions in dairy buffaloes used in this study, lactating animals are fed at same scale irrespective of their milk yield and feed requirements. In the present study, Lower milk yield has been shown to be associated with detrimental effects of degradable protein on reproduction in our previous study (Qureshi et al., 2002). In that study we found that crude protein (CP) intake was positively correlated with serum urea levels ($r = 0.22$, $P < 0.01$) and the animals consuming more excess of protein showed anestrus. An almost constant ratio of CP with metabolizable energy of about 12 MJ/g was observed in the animals coming into estrus. Buffalo farmers in the South Asia belong to low socio-economic status and there is no system of ration formulation in relation to the animal's requirements for maintenance and milk production. The farmers try to promote higher milk production by feeding more concentrates. Under such feeding systems, the low milk producers get more protein relative to their requirements, compared to the high producers. The higher intake of protein, mostly degradable, leads to formation of ammonia which is converted into urea in the liver which is an energy consuming process and exerts toxic effects at local and systemic levels. As the milk production increased, more degradable protein was utilized reducing its detrimental effects and resulting in enhanced reproductive performance. So the moderate yielders utilized the protein efficiently and showed better milk yield in addition to better reproductive performance.

Protein is extensively degraded in the rumen or used as an energy source, leaving metabolic residues like ammonia and urea will result (Tamminga, 2006). Such residues may exert metabolic effects that are often detrimental to reproduction and fertility. Ammonia is believed to play a role starting before ovulation, whereas urea mainly interferes negatively after fertilisation. But, urea is also believed to aggravate the severity of NEB and its effect on fertility by preventing or delaying the start of cyclicity. Besides, urea has been shown to lower the pH in the uterine fluid, giving rise to disturbances in follicular development and embryonic growth.

Our results partially support the findings of Harman et al. (1996), who reported that the highest and lowest yielding cows had a lower conception hazard. Slanina and Hlinka (1991) reported that reproductive performance of dairy cows was adversely affected when annual yields were more than $4000 \pm 400$ kg. Reduced fertility in the higher yielding dairy cows is not directly related to increased milk yield but rather to loss of body weight and body condition (McClary, 1991). Investigations in buffaloes had indicated that lactation number and milk yield might have an adverse effects on its fertility (El-Belely et al., 1988). However, Zia-ul-Hasnain (1981) reported that milk yield in the cyclic buffaloes was not different from non-cyclic Nili-Ravi buffaloes. It appears that suckling for one month postpartum was favorable for reproductive performance of dairy buffaloes under field conditions in NWFP, Pakistan, but not beyond that. Moderate milk yield was favorable for reproductive performance than lower or higher yield.

In our previous study, (Qureshi et al., 2002) the prepartum excess ME intake in the oestrus group probably resulted in the accumulation of more body reserves, which supported high postpartum milk production and early resumption of oestrus activity. In the last month prepartum, the ME intake was sufficient to support fetal growth (tissues and fluids).

## 5. Conclusion

The present study suggests a lower reproductive efficiency of dairy buffaloes under the peri-urban farming system, reflected by ovarian cyclicity in 68.63% buffaloes within 150 days postpartum and silent estrus in 51.5% of the cases. Buffaloes needed a stronger premilking stimulation through suckling and use of oxytocin injections. Increasing suckling duration and use of oxytocin delays postpartum ovulation, however, POI is shortest in buffaloes suckled for one month. The high yielding buffaloes also manifested better reproductive cyclicity; while moderate yielder showed shorter ovulation intervals and higher conception rate.

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