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Mammary cisternal size, cisternal milk and milk ejection in Murrah buffaloes

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The internal arrangement of the mammary gland cavity system, cisternal and alveolar milk fractions and the characteristics of milk ejection were investigated in buffaloes. Twenty-four Murrah buffaloes in three different stages of lactation and of two age groups were used. Continuous ultrasound cross-sections during milk ejection induced by exogenous oxytocin were performed to record the latency period of milk ejection. Buffaloes had small cisterns and the cavity area in the teat and gland regions were not significantly different ($P>0{\cdot}05$). The animals had long teat canals ($3{\cdot}1\pm0{\cdot}1$ cm), longer in the hind than fore quarters. Cisternal milk yield was low ($0{\cdot}17\pm0{\cdot}01$ kg) and cisternal fraction was only $4{\cdot}9\pm0{\cdot}1$% of the total milk. The cisternal area (cm^2) was $69{\cdot}6\pm4{\cdot}6$, $51{\cdot}61\pm4{\cdot}8$ and $26{\cdot}01\pm4{\cdot}8$ while the cisternal yield (kg) was $0{\cdot}32\pm0{\cdot}05$, $0{\cdot}18\pm0{\cdot}05$ and $0{\cdot}05\pm0{\cdot}05$ in early, mid and late lactation, respectively. A close correlation ($r=0{\cdot}87$, $P<0{\cdot}05$) existed between the ultrasound cisternal area and cisternal milk yield. The latency period of induced milk ejection was similar to that reported for cows (25 ± 1 s) and was negatively correlated with milk yield ($r=-0{\cdot}75$, $P<0{\cdot}05$). Milk ejection occurred shortly after elevated oxytocin concentrations were present. Delayed milk ejection reported earlier in this species must therefore be due to the absence of cisternal milk and delayed oxytocin release. An increase in teat length and circumference at milk ejection was also evident in the ultrasound cross sections.

Keywords: Buffalo, cisternal milk, milk ejection.

The proportion of milk stored in the udder cistern before milking has substantial consequences for optimal machine milking in a given species. It is generally accepted that milk is continuously shifted into the cisternal cavities during the intervals between milkings in cows and sheep (Jatsch & Sagi, 1978; Knight & Dewhurst, 1994a; Pfeilsticker et al. 1996). Before milking, the amount of milk in the cisternal cavities is 20–40% of the total milk stored in the udder, and is usually smaller in cows than in goats and sheep (Bruckmaier et al. 1994b; Knight & Dewhurst, 1994a; Bruckmaier & Blum, 1998). To extract this milk fraction, it is only necessary to overcome the barrier of the teat sphincter. In contrast, the alveolar milk fraction must be actively shifted into the cisternal cavities via the milk ejection reflex to be available for milking. In buffaloes, milking is assumed to be possible only after milk ejection. No cisternal milk is available after cannulation of teats (Alieve, 1970). Palpation of the udder indicates the absence of a mammary cistern (Lind et al. 1997).

Presence of cisternal milk in cows and goats prior to milk ejection makes the teats firm and stiff for application of the teat cup liners. If it is lacking during cluster attachment, the applied vacuum may cause collapse of milk ducts thus inhibiting milk let-down during further milking and complete emptying of the udder in cows (Bruckmaier & Hilger, 2001). The buffalo has become a major milk-producing animal in many developing countries. The study of the anatomical structure of the mammary gland and the pattern of milk accumulation and storage can help to improve techniques and routines for machine milking in this species.

This investigation addressed the anatomical arrangement of the mammary tissue and the distribution of milk fractions in the udder, which is a prerequisite for machine

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milking. In addition, the latency period of milk ejection after oxytocin injection was investigated, as well as the teat dimensions before and after milk ejection in buffaloes of the Murrah breed.

Materials and Methods

Animals

The twenty-four clinically healthy buffaloes (*Bubalus bubalis*) of the Murrah breed used in the experiment were from the herd of the B.G. Chitale Dairy Pvt Ltd (Research and Development Wing, Sangli, India). The buffaloes were in their second to sixth lactation, and their body weights ranged from 500 to 700 kg. Three groups of eight animals in early (weeks 3–14), mid (weeks 16–30) and late (weeks 32–45) lactation each were selected. The selected animals were additionally classified into younger (2–3 lactations) and older (4–6 lactations). All buffaloes were loose-housed in a byre on a daily ration of fresh roughage including sugar cane, maize and alfalfa. Concentrates were given via automatic computerized feeding stations, according to individual production levels. Animals were offered concentrate during machine milking in a 2×5 tandem parlour (DeLaval, Tumba, Sweden).

Ultrasound measurements

B-mode ultrasonography was used to determine the area of the cisternal cavities (teat and gland) and the length of the teat canal. The udder was immersed in a water bucket as described by Bruckmaier & Blum (1992). A 6-MHz linear array rectal probe (Philips Medical Systems India Ltd) was used. This probe created a cross-sectional image 9 cm wide and up to 9 cm deep into the mammary tissue. Two of these sections were placed side by side on the screen to give a composite image. The probe was applied laterally to each quarter, with the teat cistern in longitudinal direction as the scan axis. The probe was moved along this axis until the cross-section with the largest cavity area was found.

In each animal the right fore and hind quarters were recorded. Teat and gland cistern size (area) and teat canal length were measured from the ultrasound pictures, by means of a digitizing tablet in combination with a personal computer program (Sigma-Scan^R, 1988). At the end of ultrasound measurements 2 i.u. of oxytocin (Paratoxin^R vet 10 i.u./ml; Pharmacia & Upjohn Animal Health AB, Helsingborg, Sweden) was administered intravenously while ultrasound imaging continued. The start of milk ejection was recognized by the typical whirling effects of milk constituents and the concomitant enlargement of cavity area in the gland cistern (Bruckmaier & Blum, 1992). Time from oxytocin injection until commencement of milk ejection (latency period of milk ejection) was recorded. To study the changes of teat dimensions during milk ejection, ultrasound measurements of the right fore and hind quarters were repeated after milk ejection.

Determination of cisternal milk fraction

The cisternal milk fraction available to the milking machine was determined while milk ejection due to the release of endogenous oxytocin was prevented with an intravenous injection of oxytocin receptor blocking agent (Atosiban; 10 mg/animal; Ferring Research Institute AB, S-200 61 Malmö, Sweden). Atosiban was administered 2 min before the start of machine milking, without any pre-milking udder preparation (Wellnitz et al. 1999). To abolish the oxytocin receptor blockade and to obtain all the alveolar milk, a supraphysiological intravenous injection of 10 i.u. oxytocin was administered. Cisternal and alveolar portions were recorded using a mobile milk flow recorder (Lactocorder, WMB, Balgach, Switzerland) which was especially adapted to low milk-flow rates ($<0{\cdot}05$ kg/min). The accuracy of the lactocorder was tested by comparing the milk yields with those weighed on electronic scales. The difference between the systems was <5 % in all milkings (data not shown). Cisternal milk fraction (%) was defined as (cisternal milk fraction $\times$ 100)/total milk yield.

Teat dimensions before and after milk ejection

Teat length and the teat girth of the right fore and hind quarters were measured by means of a cotton thread and metal rule. Measurements were taken before and after milk ejection. Teat length was measured by stretching a cotton thread (2–3 mm diameter) from the teat base to the teat tip and the length was then marked and read immediately on the metal rule. Similarly teat girth was measured at approximately the middle of each teat with the help of the thread and was then marked on the rule and read. A vernier calliper and direct measurement with a metal rule was avoided because the touch of the metal irritated the animals and this could cause teat injuries (Saxena, 1973; Akhtar et al. 1999). The purpose of these measurements was to measure the teat dimensions and the increase in teat length and teat girth upon milk ejection.

Experimental design

All measurements of cisternal areas, cisternal volumes, latency period of milk ejection in response to exogenous oxytocin and teat dimensions were performed once in the afternoon at 16.00–17.30 (11–12 h after the preceding milking). To avoid carry-over effects from the previous treatment the animals were studied in experimental groups of six each in random sequence. Two from each stage of lactation underwent ultrasound measurements while, in another two, cisternal volumes were measured; the remaining two had no treatment. Each treatment day was followed by a rest period of three milkings after which the next treatment was performed in random sequence. In 6 d all animals received all treatments. All ultrasound and cisternal fraction measurements were performed in a special treatment area using a bucket milking machine

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(DeLaval, Sweden; operational vacuum 55 kPa, pulsation rate and ratio 70 cycles and 65 : 35 respectively). Teat dimensions before and after milk ejection were measured on a separate day during routine milking in the parlour.

Statistical analysis

Unless stated otherwise, results are presented as least squares means ± SE. The general linear model (Minitab™ Statistical Software, Release 13 for Windows® 95/98 and Windows NT®, 2000) was used for analysis of variance based on least squares means. Effects of stage of lactation, number of lactations and lactational production, udder quarter (fore or hind quarter) and organ (teat or gland cistern) were tested. Tukey's test was used to test treatment differences for significance ($P<0{\cdot}05$). Model 1 was used for the analysis of variance in the calculation of cisternal area.

$$\text{Model 1:}\quad Y_{ijklm}=\mu+m_i+t_j+q_k+p_l+(m\times t)_{ij}+(m\times t\times q)_{ijk}+(m\times t\times q\times p)_{ijkl}+e_{ijklm}$$

where m_i is the fixed effect for the age i (i=1,2), t_j is the fixed effect for lactation stages j (j=1,2,3), q_k is the fixed effect for the udder quarter k (k=1,2), p_l is the fixed effect for the organ l (l=1,2), $(m\times t)_{ij}$ is the fixed effect of age lactation stage interaction, $(m\times t\times q)_{ijk}$ is the fixed effect for the age × lactation stage × udder quarter interaction and $(m\times t\times q\times p)_{ijkl}$ is the fixed effect for the age × lactation stage × udder quarter × organ interaction.

Model 2 was used for the analysis of variance in the calculation of cisternal, yield, cisternal fraction, total yield and latency period of milk ejection.

$$\text{Model 2:}\quad Y_{ij}=\mu+m_i+t_j+(m\times t)_{ij}+e_{ijk}$$

where m_i is the fixed effect form age i (i=1,2), t_j is the fixed effect for lactation stages j (j=1,2,3) and $(m\times t)_{ij}$ is the fixed effect of age × lactation stage interaction.

The terms e_{ijkm} and e_{ijk} represent the normally distributed error. Interactions are symbolized with an (×). Interactions of the fixed effects were excluded from the model when they were not significant.

Changes of teat length and girth as well as ultrasound measurements before and after milk ejection were tested for significance ($P<0{\cdot}05$) by using a two-sample t test. Pearson's coefficients of correlation and linear regression between the traits were also calculated.

Results

Cisternal area

Teat and gland cisternal areas of the right udder half were not significantly different from each other in front and rear quarters and, similarly, the total cisternal areas of the fore and hind quarters were not significantly different (Fig. 1, Table 1). On the ultrasound cross-section several pockets of numerous ducts emptying into the teat instead of a single cisternal cavity were observed (Fig. 2). Mean teat and gland cisternal areas irrespective of quarters were 10·7 ± 1·9 and 13·1 ± 1·1 cm^2 respectively. Total cisternal areas in early, mid and late stages of lactation differed significantly from each other ($P<0{\cdot}05$; Table 1). Early lactation animals had the largest cisterns, followed by animals in mid and late lactation. Older buffaloes had larger total cisternal areas ($P<0{\cdot}05$) than young ones. The mean length of the teat canal was significantly greater ($P<0{\cdot}05$) in the

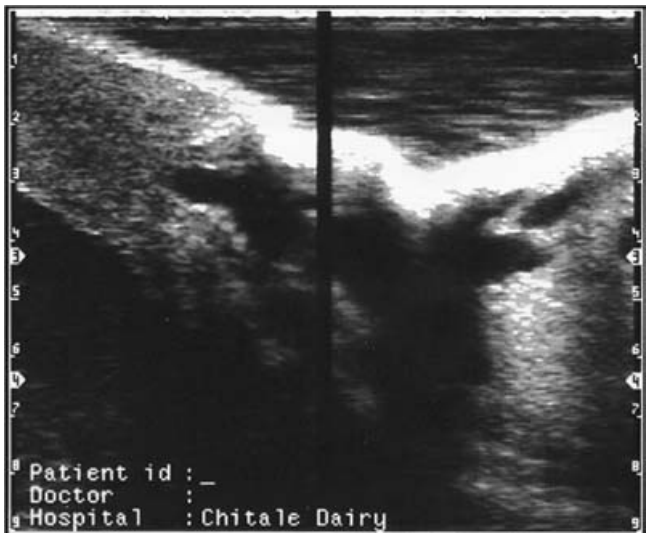


Fig. 1. Ultrasound cross-section of the right hind quarter of a Murrah buffalo where the cisternal cavity area is more prominent in the teat region.



Fig. 2. Ultrasound cross-section of the right fore quarters of the udder of a Murrah buffalo showing several pockets of numerous ducts emptying directly into the teat instead of a single cisternal cavity.

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Table 1. Mammary gland cisternal area in Murrah buffaloes (n=24) in three stages of lactation and two age groups

Values are least square means ± SE

Animals	Fore teat cistern (cm^2)	Fore gland cistern (cm^2)	Hind teat cistern (cm^2)	Hind gland cistern (cm^2)	SEM	Total cisternal size (cm^2)
Early lactation (8)†	$15{\cdot}22^{A}$	$15{\cdot}43^{A}$	$17{\cdot}73^{A}$	$21{\cdot}16^{A}$	1·62	$69{\cdot}67 \pm 4{\cdot}64^{A}$
Mid lactation (8)	$9{\cdot}84^{AB}$	$14{\cdot}05^{AB}$	$13{\cdot}69^{AB}$	$14{\cdot}14^{AB}$	1·81	$51{\cdot}61 \pm 4{\cdot}81^{B}$
Late lactation stage (8)	$5{\cdot}46^{B}$	$7{\cdot}11^{B}$	$5{\cdot}84^{B}$	$7{\cdot}17^{B}$	1·81	$26{\cdot}01 \pm 4{\cdot}81^{C}$
Younger animals (15)	8·73	11·14	10·67	12·29	1·21	$42{\cdot}94 \pm 3{\cdot}44^{A}$
Older animals (9)	11·62	13·26	14·16	16·05	1·62	$55{\cdot}25 \pm 4{\cdot}52^{B}$
All animals (24)	9·57 ± 2·10	11·85 ± 1·40	11·84 ± 3·09	14·29 ± 2·20	—	47·56 ± 11·10

† Figures in parentheses are numbers of animals
ABC: means without common superscript within columns and within fixed effects (lactation stage and age) are significantly different (P<0·05)

hind teats than the fore teats (3·7 ± 0·2 and 3·0 ± 0·1 cm), and ranged from 1·9 to 4·3 cm. Overall mean teat canal length was 3·1 ± 0·1 cm. Teat canal length did not differ between stages of lactation or between old and young animals (data not shown).

Cisternal milk

Cisternal milk yield (kg) was highest in early lactation and decreased as lactation advanced and was significantly higher in early than in late lactation (P<0·05; Table 2). Older animals had a higher cisternal fraction (%) than younger ones. Cisternal fraction (%) did not differ significantly between the stages of lactation and was lowest in animals in late lactation. There was a close correlation between the total cisternal area derived from the ultrasound and cisternal milk yield (r=0·87; P<0·05).

Latency period of milk ejection

The latency period of milk ejection was shortest in early lactation and longest in the later stages (Table 3). There was no difference between old and young animals. The latency period of milk ejection was negatively correlated with milk yield on the day of the experiment (r=–0·75; P<0·05), whereas the latency period of milk ejection and the stage of lactation were positively correlated (r=0·93; P<0·05).

Teat and cistern dimensions before and after milk ejection

Teat length and teat circumference showed large variations among individual animals. Prior to milk ejection the hind teats were significantly longer (P<0·05) than the fore teats. Teat length and teat girth in the fore teats were significantly higher (P<0·05) in old than in young animals (Table 4), whereas these traits did not differ significantly between stages of lactation. There was a significant increase (P< 0·05) in teat length and teat girth after milk ejection. When gland cistern areas were observed in the ultrasound image (Fig. 3, Table 4), they were shown to increase significantly (P<0·05) during milk ejection.

Table 2. Cisternal fraction of milk in Murrah buffaloes (n=24) in three stages of lactation and two age groups

Values are least square means ± SE

Animals	Total milk yield (kg)	Cisternal fraction (kg)	Cisternal fraction (%)
Early lactation (8)†	$5{\cdot}60 \pm 0{\cdot}35^{A}$	$0{\cdot}32 \pm 0{\cdot}05^{A}$	5·87 ± 1·31
Mid lactation (8)	$3{\cdot}07 \pm 0{\cdot}36^{B}$	$0{\cdot}18 \pm 0{\cdot}05^{AB}$	7·11 ± 1·36
Late lactation (8)	$1{\cdot}56 \pm 0{\cdot}36^{C}$	$0{\cdot}05 \pm 0{\cdot}05^{B}$	3·11 ± 1·36
Younger animals (15)	3·63 ± 0·35	0·15 ± 0·03	3·80 ± 1·06
Older animals (9)	3·20 ± 0·34	0·22 ± 0·04	6·93 ± 1·28
All animals (24)	3·40 ± 0·88	0·17 ± 0·01	4·9 ± 0·80

† Figures in parentheses are numbers of animals
ABC: means within a column and without a common superscript are significantly different (P<0·05)

Table 3. Latency period of milk ejection in Murrah buffaloes (n=24) in three stages of lactation and two age groups

Values are least square means ± SE

Parameter	Latency period of milk ejection (s)
Early lactation (8)†	$21 \pm 0{\cdot}54^{A}$
Mid lactation (8)	$25 \pm 0{\cdot}61^{B}$
Late lactation (8)	$29 \pm 0{\cdot}61^{C}$
Younger animals (15)	25 ± 0·40
Older animals (9)	25 ± 0·54
All animals (24)	25 ± 0·73

† Figures in parentheses are numbers of animals
ABC: means without a common superscript within a column are significantly different (P<0·05)

Discussion

Cisternal area

Cisternal areas measured in single quarters were only half the values in cows (around 40–45 cm^2) (Bruckmaier & Blum, 1992). Both teat and gland cistern areas were smaller in buffaloes than reported for cows (Bruckmaier

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Table 4. Influence of milk ejection on teat dimensions in Murrah buffaloes (n=24) in younger and older animals

		Mean ± SE			
Trait	Time	Younger animals (15)†	Older animals (9)	All lactations (24)	% increase after milk ejection (all lactations)
Fore teat length (cm)	Before milk ejection	$8{\cdot}25 \pm 0{\cdot}43^{aA}$	$10{\cdot}30 \pm 0{\cdot}86^{b}$	$9{\cdot}02 \pm 0{\cdot}45^{C}$	+13·4
	After milk ejection	$9{\cdot}75 \pm 0{\cdot}50^{bB}$	$11{\cdot}56 \pm 0{\cdot}89^{b}$	$10{\cdot}42 \pm 0{\cdot}47^{D}$	
Hind teat length (cm)	Before milk ejection	$10{\cdot}71 \pm 0{\cdot}37^{aC}$	$13{\cdot}07 \pm 0{\cdot}61^{b}$	$11{\cdot}80 \pm 0{\cdot}39^{C}$	+10·2
	After milk ejection	$12{\cdot}45 \pm 0{\cdot}47^{aB}$	$14{\cdot}31 \pm 0{\cdot}65^{b}$	$13{\cdot}15 \pm 0{\cdot}41^{D}$	
Fore teat circumference (cm)	Before milk ejection	$9{\cdot}74 \pm 0{\cdot}48^{a}$	$11{\cdot}27 \pm 0{\cdot}50^{bA}$	$10{\cdot}31 \pm 0{\cdot}38^{C}$	+13·3
	After milk ejection	$10{\cdot}97 \pm 0{\cdot}51^{a}$	$13{\cdot}47 \pm 0{\cdot}57^{bB}$	$11{\cdot}90 \pm 0{\cdot}45^{D}$	
Hind teat circumference (cm)	Before milk ejection	$11{\cdot}23 \pm 0{\cdot}29^{A}$	$12{\cdot}44 \pm 0{\cdot}80$	$11{\cdot}68 \pm 0{\cdot}361^{C}$	+12·1
	After milk ejection	$12{\cdot}71 \pm 0{\cdot}45^{B}$	$14{\cdot}26 \pm 0{\cdot}77$	$13{\cdot}29 \pm 0{\cdot}42^{D}$	
Fore gland cisternal area (cm^2) as seen in the ultra sound image	Before milk ejection	$8{\cdot}73 \pm 1{\cdot}10^{aA}$	$9{\cdot}11 \pm 1{\cdot}80^{aA}$	$8{\cdot}87 \pm 0{\cdot}95^{C}$	+65·9
	After milk ejection	$24{\cdot}82 \pm 2{\cdot}00^{bB}$	$28{\cdot}17 \pm 2{\cdot}60^{bB}$	$26{\cdot}07 \pm 1{\cdot}60^{D}$	

† Figures in parentheses are numbers of animals
ab: means without a common superscript within a line and between animal age are significantly different (P<0·05)
ABCD: means without a common superscript within column and between before and after milk ejection are significantly different (P<0·05)



Fig. 3. Ultrasound cross-section of the right fore cisternal area before and after milk ejection of a Murrah buffalo.

& Blum, 1992). In buffaloes the cisternal area is comparatively more conspicuous in the teat region, in contrast to what has been reported for cows. The gland cistern area showed several pocket-like areas, which are only partly seen in cows (Bruckmaier & Blum, 1992). This is in line with the findings of a recent study in Egyptian (riverine type) buffaloes (El-Ghousien et al. 2002). As reported for cows, cisterns were larger in early stages of lactation than in mid and late stages, owing to the intramammary pressure and udder fill. Again as reported for cows, cisternal areas were larger in older buffaloes because of the stretching effect in older animals (Bruckmaier & Blum, 1992; Bruckmaier et al. 1994b; Bruckmaier & Hilger, 2001).

Teat canals were longer in this study than reported for cows (0·5–1·3 cm; Geishauser & Querengässer, 2000; Neijenhuis et al. 2001). This pronounced difference partly explains why buffaloes are reported to be hard to milk (Bhagat et al. 1992), and also explains why higher vacuum levels have been used in machine milking of buffaloes (Marathe & Whittlestone, 1957; Alieve, 1969; Badran, 1992). Therefore it is also important to perform stripping towards the end of machine milking of buffaloes (Pazzona, 1989). Longer teat canals and a stronger teat sphincter (Uppal et al. 1994) are also reasons for a lower incidence of mastitis in buffaloes than in cows (Sastry et al. 1988; Singh & Singh, 1994).

Cisternal milk

Mean cisternal yield and fraction in buffaloes was less than 25% of that reported for cows (Bruckmaier et al. 1994b). The cisternal milk fraction in buffaloes decreased as lactation proceeded while older animals had larger cisternal milk fractions; this has also been reported for cows (Mielke, 1969; Knight & Dewhurst, 1994b; Pfeilsticker et al. 1996). In buffaloes, as in cows, the cisternal fraction of milk depends principally on inherent anatomical features, such as cisternal cavity area (teat and gland) and alveolar tissue size. The close correlation between the cisternal area obtained from ultrasound cross-sections and cisternal milk fractions further confirms that most of the cisternal milk is stored in the teat cistern area, which is the major part of the total cisternal cavity. In buffaloes, since cisternal sizes are small and almost 95% of the milk is predominantly available as the main milk yield (alveolar milk), it is imperative that this milk is removed as completely as possible by complete milk ejection and an efficient milking technique. Incomplete milk removal causes immediate production losses and apoptosis in the mammary epithelium (Stefanon et al. 2002). Therefore, milk yield drops considerably when calves are separated from the dam owing to weaning or calf death and in some buffaloes milk

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production ceases completely (Russev et al. 1961; Alieve, 1969; Gangwar, 1976; Alim, 1982; Pathak, 1992). On the other hand, increasing the frequency of milking, from two to three times a day, caused a 9–10% increase in milk yield in buffaloes (Dash et al. 1976; Ludri, 1985). In cows three-times daily milking increased milk yield by about 10–15% (Henderson et al. 1983). Cows with small cisternal portions respond more to thrice-daily milking than cows with larger cisternal reservoirs (Dewhurst & Knight, 1994). Since buffaloes have small cisterns they are likely to be more adapted to frequent milk removal, i.e., suckling, instead of twice-daily milking only. The influence of milking intervals on milk ejection and milking efficiency in buffaloes needs further study.

Latency period of milk ejection

The latency period of milk ejection in buffaloes induced by exogenous oxytocin at a physiological level is similar to that reported in cows and goats commencing at about 0·20–0·25 s (Schams et al. 1984; Gorewit & Gassman, 1985; Bruckmaier & Blum, 1992; Nickerson, 1992). The close correlation between the latency period of milk ejection and the quantity of milk extracted or degree of filling of the mammary gland also accords with reports for cows (Bruckmaier et al. 1994a; Bruckmaier & Hilger, 2001). This is probably why buffaloes in late lactation, have a longer latency period of milk ejection. Hence, the delayed milk ejection in response to teat stimulation reported in buffaloes is obviously not due to a delayed response of the myoepithelial cells to the hormone (Russev et al. 1961; Alieve, 1969; Gangwar, 1976; Alim, 1982; Pazzona, 1989). Thus in buffaloes, the alveolar area is mainly involved in the accumulation of milk. The degree of filling, time until release of oxytocin and sustaining of milk ejection will therefore have a direct impact on the commencement of milk flow and emptying of the udder. In practical terms, stage of lactation, milking frequency, stimulation and milking procedures will directly influence milking efficiency in buffaloes.

Teat and cistern dimensions before and after milk ejection

Teat length and girth increased by 12–13% in the fore teats and 10–12% in the hind ones. Comparatively large volumes of cisternal milk within the udder cistern and teat cisterns in cows and goats prior to milk ejection make the teats firm and stiff for application of the milking device. Even in cows, if the milking machines are attached while the teats are still limp and therefore small, or at the end of milking, the milking vacuum will suck the teats too deep ('crawl') into the teat cups. This causes closure of the udder-teat passage, which can result in incomplete milking (Schams et al. 1984; Nickerson, 1992; Bruckmaier & Hilger, 2001). In buffaloes, owing to the low cisternal milk fraction and the low intrammary pressure prior to milk ejection, the teats are collapsed and flaccid prior to milk ejection. Application of the milking unit at this stage will cause the teats to creep deep into the teat cup liners, pinching off the milk passage at the annular duct. The vacuum in the teat cistern approaches the vacuum in the long milk tube, possibly leading to mechanical irritation of the tissue and consequently to stress, resulting in inhibition of milk ejection as occurs in cows (Bruckmaier & Blum, 1998). With the usual volumes of cisternal milk in the case of goats and cows, the milking machine can be applied with only a rather short stimulation (30 s to 1 min). Further stimulation is provided by the pulsating action of the liners while emptying the cisternal volume. This, along with the flow-sensitive receptors in the teat canal and cisterns, further intensifies stimulation resulting in complete milk ejection (Lefcourt, 1982; Schams et al. 1984). In buffaloes, the small cisternal size and fraction make it crucial to elicit milk ejection before machine milking begins. Equipment manufacturers should bear these aspects in mind when designing a milking unit for buffaloes.

This study showed that udder anatomy and the internal arrangement of the mammary tissue, cisternal fraction of milk and teat canal length are quite different in buffaloes as compared with dairy cattle, and that there is also a marked increase in the teat dimension on milk ejection. These results have implications for the machine milking of buffaloes. Buffaloes have species-specific requirements for milk ejection and complete milking owing to their udder anatomy. Conditioning for machine milking, pre-milking stimulation routines and start of machine milking after milk ejection could help to improve milking procedures in buffaloes.

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