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2. To provide literature search and photocopy services
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BUFFALO BULLETIN
ISSN : 0125-6726

Buffalo Bulletin is published quarterly in March, June, September and December. Contributions on any aspect of research or development, progress reports of projects and news on buffalo will be considered for publication in the bulletin. Manuscripts must be written in English and should not exceed 2,000 words or the equivalent inclusive of tables and illustrations. Short communications should not exceed the equivalent of 1,000 words. Every article should have a short abstract (not more than 250 words) complete in itself and understandable without reference to the paper and its author should be identified by name, title research organization. Two copies, one of which must be the original of the typescript and illustrations.

Editor

S. Sophon

Subscription price (out of Thailand) :

$ 35 per year

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USE OF BUFFALO CALF SERUM (BCS) AS A PROTEIN SUBSTITUTE FOR IN VITRO MATURATION OF BUBALINE OOCYTES

Suresh Kumar, Shiv Prasad and S.N. Maurya

ABSTRACT

The effect of buffalo calf serum was studied on oocyte maturation in vitro. The investigation suggested the beneficial effect of cheaply available buffalo calf serum as a protein substitute for in vitro maturation of bubaline oocytes. A maturation rate of 46.3% could be obtained with BCS in presence of hormones (FSH + LH + E2) in Ham’s F-10 while the similar value for TCM-199 was 48.25%.

INTRODUCTION

In almost all studies of mammalian in vitro maturation (IVM), the basic medium is supplemented with serum and hormones. The maturation medium and the selection of protein supplements and hormones for IVM play an important role in subsequent in vitro fertilization (Totey, 1992). The commonly used commercially available serum is highly costly and contributes heavily to the cost of in vitro produced embryos (Singh and Dhanda, 1999). The serum from buffalo calf is cheap and easily available; hence, the present investigation was aimed to study the in vitro maturation of bubaline oocytes with buffalo calf serum as a protein substitute in the maturation medium.

MATERIALS AND METHODS

The ovaries of slaughtered buffaloes were collected in 0.9% normal saline supplemented with antibiotics (Streptomycin 6,000 μg/100 ml, and penicillin G 10,000 IU/100 ml). The ovaries were transported at 25-30 °C to the laboratory within 1 h of slaughter. The ovaries were washed three times in Dulbecco’s Phosphate Buffer Saline (DPBS) and then twice with 60% alcohol and then stored in warm DPBS till processing for collection of oocytes. The oocytes were collected in TLI-Hepes by aspiration method with the help of an 18 gauge needle fitted to a glass syringe.

The oocytes were matured in TCM-199 or Ham’s F-10 medium supplemented with buffalo calf serum (10%) and hormones (FSH, 1 μg/ml, LH10 IU/ml and E2 1 μg/ml) singly or in combination. The cumulus oocyte complexes (COCs) were cultured for 26 h at 39 °C under 5% CO2 in air and 95% humidity.

The maturation of oocytes was evaluated by cumulus mass expansion, enlargement of perivitelline space and extrusion of the 1st polar body. Some of oocytes were fixed overnight with acetic acid and ethanol (1:3) and stained with aceto-orcein stain and assessed for metaphase-II (M-II) phase formation.

The statistical analysis was done using the ‘Z’ test as per Snedecor and Cochran (1967).
RESULTS AND DISCUSSION

The maturation rates obtained by using buffalo calf serum (BCS) in the presence or absence of hormones in TCM-199 and Ham's F-10 medium are presented in Table 1 and Table 2, respectively. The addition of BCS in TCM-199 showed significant improvement (P<0.05). The addition of FSH or E2 alone improved the maturation rates markedly in TCM-199 but the differences were non-significant (P>0.05). However, LH or LH + E2 or LH + FSH or FSH + E2 showed significant increase in TCM-199. When LH + FSH + E2 was used in the presence of BSC, a significantly higher rate of maturation (48.25%) was observed. When BCS alone was used in Ham's F-10 medium or BCS in the presence of either LH, or FSH or E2, the improvement was non-significant, but addition of any two hormones showed higher rates of maturation with a maximum of 46.25% maturation when LH + FSH + E2 was used in the presence of BCS.

In almost all studies of mammalian in vitro maturation, the basic medium was supplemented with serum, crystallised albumin or estrus serum. Fetal calf serum has been used efficiently for oocyte maturation. Buffalo estrus serum has also been used for in vitro maturation of buffalo oocytes (Totey et al., 1993; Bhatt, 1995; Singh and Dhanda, 1999) but the use of buffalo calf serum is not reported in literature. Addition of serum during maturation of oocytes is reported to prevent "zona hardening" and enhance the maturation and fertilization in addition to providing a rich source of supplemental protein (Eppig and Schroeder, 1986). The rate of oocyte maturation in the presence of BCS was lower than in the previous reports of Totey et al. (1993) and Bhatt (1995) obtained with BES in buffaloes and Younis et al. (1989) in bovines. The variation in maturation rates may be due to the source of the sera or the quality of oocytes used for maturation. These findings suggest that sera may contain factors that promote the acquisition of developmental competence of oocytes during in vitro maturation. Beneficial role of the serum source in the culture medium has been also reported by Sanbuissso and Threlfall (1985), Down et al. (1986), Totey et al. (1993) and Bhatt (1995). The addition of hormones singly or in combination has a marked beneficial effect on oocyte maturation but addition of LH + FSH + E2 improved the maturation rate significantly (P<0.05). Similar findings have been observed by Totey et al. (1993) and Bhatt (1995) in buffaloes.

It can be concluded from the present investigation that buffalo calf serum can be used as a protein substitute for in vitro maturation of buffalo oocytes and addition of hormones significantly improved the maturation rates in the presence of Buffalo Calf Serum (BCS).

ACKNOWLEDGEMENT

The authors are thankful to the Dean of the College of Veterinary Sciences for providing necessary facilities and also to the CSIR for providing financial assistance during the course of this investigation.
Table 1. *In vitro* maturation of bubaline oocytes: effect of buffalo calf serum (BCS) and hormones in TCM-199.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Medium</th>
<th>Serum</th>
<th>Hormone</th>
<th>Cultured (No.)</th>
<th>Matured (No.)</th>
<th>Maturation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>TCM-199</td>
<td>-</td>
<td>-</td>
<td>362</td>
<td>92</td>
<td>25.40^a</td>
</tr>
<tr>
<td>2.</td>
<td>TCM-199</td>
<td>BSC</td>
<td>-</td>
<td>602</td>
<td>189</td>
<td>31.39^b</td>
</tr>
<tr>
<td>3.</td>
<td>TCM-199</td>
<td>BCS</td>
<td>LH</td>
<td>298</td>
<td>118</td>
<td>39.59^c</td>
</tr>
<tr>
<td>4.</td>
<td>TCM-199</td>
<td>BCS</td>
<td>FSH</td>
<td>240</td>
<td>88</td>
<td>36.66^bc</td>
</tr>
<tr>
<td>5.</td>
<td>TCM-199</td>
<td>BCS</td>
<td>E₂</td>
<td>250</td>
<td>94</td>
<td>37.60^bc</td>
</tr>
<tr>
<td>6.</td>
<td>TCM-199</td>
<td>BCS</td>
<td>LH + E₂</td>
<td>295</td>
<td>128</td>
<td>43.38^cd</td>
</tr>
<tr>
<td>7.</td>
<td>TCM-199</td>
<td>BCS</td>
<td>LH + FSH</td>
<td>290</td>
<td>117</td>
<td>40.34^c</td>
</tr>
<tr>
<td>8.</td>
<td>TCM-199</td>
<td>BCS</td>
<td>LH + FSH + E₂</td>
<td>800</td>
<td>386</td>
<td>48.25^d</td>
</tr>
<tr>
<td>9.</td>
<td>TCM-199</td>
<td>BCS</td>
<td>FSH + E₂</td>
<td>264</td>
<td>105</td>
<td>39.77^c</td>
</tr>
</tbody>
</table>

Values with non-identical superscripts differ significantly (P<0.05)
Table 2. *In vitro* maturation of bubaline oocytes: effect of buffalo calf serum (BCS) and hormones in Ham's F-10 medium.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Medium</th>
<th>Serum</th>
<th>Hormone</th>
<th>Cultured (No.)</th>
<th>Matured (No.)</th>
<th>Maturation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ham's F-10</td>
<td>-</td>
<td>-</td>
<td>156</td>
<td>40</td>
<td>25.64^a</td>
</tr>
<tr>
<td>2.</td>
<td>Ham's F-10</td>
<td>BCS</td>
<td>-</td>
<td>120</td>
<td>381</td>
<td>31.66^ab</td>
</tr>
<tr>
<td>3.</td>
<td>Ham's F-10</td>
<td>BCS</td>
<td>LH</td>
<td>150</td>
<td>53</td>
<td>35.33^b</td>
</tr>
<tr>
<td>4.</td>
<td>Ham's F-10</td>
<td>BCS</td>
<td>FSH</td>
<td>150</td>
<td>48</td>
<td>32.00^ab</td>
</tr>
<tr>
<td>5.</td>
<td>Ham’s F-10</td>
<td>BCS</td>
<td>E₂</td>
<td>140</td>
<td>45</td>
<td>32.14^ab</td>
</tr>
<tr>
<td>6.</td>
<td>Ham’s F-10</td>
<td>BCS</td>
<td>LH + FSH</td>
<td>148</td>
<td>62</td>
<td>41.89^bc</td>
</tr>
<tr>
<td>7.</td>
<td>Ham’s F-10</td>
<td>BCS</td>
<td>LH + FSH +E₂</td>
<td>800</td>
<td>370</td>
<td>46.25^c</td>
</tr>
<tr>
<td>8.</td>
<td>Ham’s F-10</td>
<td>BCS</td>
<td>FSH + E₂</td>
<td>150</td>
<td>60</td>
<td>40.00^bc</td>
</tr>
<tr>
<td>9.</td>
<td>Ham’s F-10</td>
<td>BCS</td>
<td>LH + E₂</td>
<td>145</td>
<td>60</td>
<td>41.37^bc</td>
</tr>
</tbody>
</table>

Values with non-identical superscripts differ significantly (P<0.05)
REFERENCE


**Continue from 61**


Economic losses of dairy products due to spoilage by yeasts have been increasing in European companies because of the reduced use of preservatives, packaging in modified atmospheres, or new formulations that do not strictly control the growth of these organisms. This study reports the results of a survey of yeast species and populations in 145 samples of cow and buffalo dairy products collected in some regions of Southern Italy. Yeasts were isolated from 74% and 57% of cow and buffalo products, respectively. *Candida inconspicua* was the predominant species in unripened products from cow's milk, while *C. famata* was detected in medium and long-term ripened dairy products, mostly in association with other yeasts and with moulds belonging to the genus *Penicillium*. For dairy products produced from buffalo milk, *C. inconspicua* was the most important yeast frequently isolated from dairy products. Total yeast populations ranged from $5 \times 10^2$ to $5 \times 10^4$ cfu/g, indicating a good hygienic quality of the products. The isolation of *C. albicans* from one stracciatella sample is noteworthy, as this yeast represents a potential contamination by human. Even though yeasts are considered as environmental contaminants, the occurrence of some of them in dairy products at high levels could represent a risk for human health, in particular for immunocompromised patients.

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MULTIPLE BIRTHS IN BUFFALO (*BUBALUS BUBALIS*)

M. Mazhar Ayaz¹, Muhammad Dilpazir Khan² and S.U. Rahman³

INTRODUCTION

Multiple births like twins in female cattle are not very commonly reported and its incidence is very low (Morrow, 1986) but the phenomenon is even rares in buffalo. This clinical article reports the case of one female buffalo that parturated triplets on her first delivery after being provided some help during her natural parturition process.

CASE REPORT AND HISTORY

The owner of a female buffalo accessed the clinic for assistance in relieving the dystocia of his animal. While giving the case history, he added that his female buffalo had been having difficulty in delivering her calf for three hours.

The animal was approached, and restrained. After being properly disinfected, and sufficiently lubricated with simple oil, the hands were put into the birth canal of the animal. Upon palpation, it was revealed that animal had triplets in her womb. The cervix and vagina of the animal was sufficiently oiled with simple oil and delivery was made easy by giving some help with the hand and correcting the position of the head of the foetus. After half and hour of effort, the animal gave birth to the triples, one female buffalo calf and two male buffalo calves one after and other. They were healthy and physically normal behavior.

DISCUSSION

Triplets are very uncommon in female buffaloes and the phenomenon become even more rare when the animal is primiparous. The reason for triplets might be the shedding of more than one ova or the accidental division or cleaving of the fertilized ova. Dystocia or difficult parturition due to multiple births is common in cattle (Noakes, 1997, Srivastava, 1997 and Boden, 1999) but no cases of triplet birth have been reported in buffalo so far.

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1. Department of Veterinary Parasitology
2. Department of Animal Reproduction
3. Department of Veterinary Microbiology
   University of Agriculture Faisalabad, 380340, Pakistan.
INBREEDING IN A NILI-RAVI BUFFALO HERD

K. Thevamanoharan¹, W. Vandepitte¹ and K. Javed²

INTRODUCTION

Pure breeding and selection practiced for the improvement of performance traits of buffaloes sometimes may result in increased levels of inbreeding in the herds. Inbreeding above certain level is generally considered to cause deterioration in health, vigour and growth rate of the animals, consequently lowering their productive and reproductive performance. Thus the study of the level of inbreeding and the evaluation of the extent of inbreeding depression (if any) would be useful in the formulation of future breeding strategies. As the herd of Nili-Ravi buffaloes maintained at the Livestock Experiment Station, Bahadarnagar, Okara, Pakistan, was known to be closed to outside breeding, the present project was planned to study the level of inbreeding in this herd.

MATERIALS AND METHODS

Pedigree records of 1,810 animals of Nili-Ravi breed of buffaloes collected at the Livestock Experiment Station, Bahadarnagar, Okara, Pakistan, during 1950 and 1998 were used in the present study. The identification numbers of buffaloes were used to trace their pedigree backward up to the base population. The resulting pedigree data consisted of both male and female sides of pedigree and date of birth of each animal. The birth dates were used to sort data by age, the oldest animal coming first. Animals were then numbered consecutively from 1 to N, unknown parents being identified as zero. The coefficient of inbreeding of each animal was calculated using DFREML set of computer programmes (Meyer, 1991).

RESULTS AND DISCUSSION

Pedigrees of the animals were traced back to the base population of buffaloes to calculate the level of inbreeding. Analysis of the pedigree records of 1,810 animals having identification for the extent of inbreeding revealed that only 14 animals (0.78%) were inbred with an average inbreeding of 25%. Out of the total 92 sires used, no one was found inbred. Most frequent value for this category of animals was 0. About 38% of the animals (702) did not have sire identification, while the number of animals for which dam identification was missing was 902. One of the main reasons for the low level of inbreeding in the present herd was incompleteness of pedigrees especially for animals born in earlier years. This contention is substantiated by the findings of Khan (1986), who

1. Center for Animal Genetics/Selection, Minderbroederstraat 8, University of Leuven, Belgium.
2. Livestock Production Research Institute, Bahadarnagar, Okara, Pakistan.
studied the effect of inbreeding in a closed herd of Nili-Ravi buffaloes in Pakistan which was kept during 1940-84. It was reported that the pure breeding program resulted in mild inbreeding, and only 17 buffaloes had an inbreeding coefficient of 25% or above. It was further reported that although milk yield in the first lactation and milk yield expressed as most probable producing ability (MPPA) was decreased due to inbreeding, the regression analysis did not reveal any significant effect.

As there were only 14 animals inbred, the effect of inbreeding on various productive and reproductive traits in the present herd could not be evaluated. Although the level of inbreeding in this herd of Nili-Ravi buffaloes is not alarming, mating of closely related animals should be avoided in future.

ACKNOWLEDGEMENTS

We would like to thank the Director, Livestock Production Research Institute, Bahadarnagar, Okara, Pakistan, for granting permission to use this data and Dr. K. Meyer for the use of her DFREML computer program.

REFERENCES


ANTIBIOTIC FEED SUPPLEMENT IN BUFFALO CALF RATIONS
ENHANCES GROWTH, SANS AFFLICTIONS

Y. Annaji Rao and K.C. Lalitha

To raise healthy buffalo calves, we have to surmount many a hurdle in their management. The calf is more prone to environmental upheaval and susceptible to variations in stress and strain imposed on the calf by either man or nature. The factors that mostly account for its debility and degradation include low planes of nutrition, poor managerial practices, hazards due to infectious and contagious diseases, and much less to the effect of ecto and endoparasites.

It has been established through various researchers that calf mortality rate in buffalo calves has been alarmingly high; 60 to 70 % of calves die during the first two months of life, followed by 1.6 to 8.5 % deaths reported between 2 1/2 to 5 1/2 years, and 6.5 to 8.0% between 5 1/2 years to 14 1/2 years age group. This unqualified observation indeed affirms the importance of calf management, at birth in particular.

Antibiotic feed supplementation is a means to annihilate disease-causing bacteria in the intestinal lumen of the growing calf. It has been established that small quantities of antibiotics will arrest the growth of pathogens in the intestinal tract. Generally known antibiotics that are normally used in the calf feeds include:

1. Aureomycin (Chlore-tetracycline)
2. Terramycin (Oxytetracycline)
3. Pencillin
4. Bacitracin
5. Chloromycetin (Chloramphenicol) and
6. Streptomycin

The calf gains a higher rate of growth with the elimination of the infectious bacteria. The following are the possible beneficial effects that the calf will derive by feed supplement treatment with antibiotics.

1. In addition to destroying infectious bacteria, the antibiotics will increase the activity of beneficial bacteria to synthesize B_{12} vitamin, which stimulates calf appetite, better feed utilisation, and thereby promotes calf growth.

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2. They effectively compete with other organisms and make the nutrients available to the calf.

3. They make the intestinal walls thinner and thus the food nutrients in the gut are better absorbed.

In particular, aureomycin supplementation in calf rations is significant when there had been much problem of scours noticed among classes of calves. It has drastically reduced the incidence of diarrhoea in calf pens. The beneficial effects of oral administration of the antibiotics will be visible from birth to three months age only, during which period the calves have only one stomach functional (Monogastric). Beyond the third month, it is unadvisable to continue feeding the antibiotics because all the stomachs become working thereafter. The calves at the third month onwards should be provided with ad libitum access to succulent, nutritious and luscious fresh green fodders.

However, in adult lactating buffaloes there has not been observed marked difference in increased milk production or higher fat percentage by this treatment. On the contrary, antibiotic feeding to high-yielding dairy buffaloes is complicated by possible adverse effects on beneficial rumen bacteria and even larger residues of the antibiotics are also being excreted through milk.

By and large, the antibiotics keep all digestive upsets at bay and thus such calves gain good growth and continue to grow actively even up to the age of six months. Antibiotic-fed calves generally grow faster than non-antibiotic ones. Antibiotics help the calf to overcome certain stress conditions and improve its general growth performance. They also stimulate the pituitary gland to produce more growth promoting hormones. However, it should be borne in mind that the positive effects of feeding antibiotics are reduced if the degree of sanitation in the calf’s surroundings is poor. These antibiotics will supplement the action of digestive juices in the intestine of calf which are capable of killing or at least preventing the rapid growth of harmful organisms. Antibiotics should not be used indiscriminately.

They must not be regarded as a panacea, nor given in too low doses. It must be remembered that aureomycin-resistant strains of B-coli have been recovered in the faeces of calf scours.

The usual rate of antibiotic feeding is in the range of 15-20 mg for around 45 kg live body weight of calf per day. The antibiotic may be added to the daily milk feeds of the calf. Milk replacers or calf starters could also be used as vehicles for feed supplementation. Ten grams per ton is the normal recommended dose for machine processed feeds. The class and age of livestock and conditions under which the calf was born will determine the relative efficacy of the antibiotic feed that will produce good results.

As stated above, the feed additives are functionally effective to check internal parasites and disease-causing organisms. As a result, since these are only feed additives, it is imperative that they are to be supplied together with adequate nutritious feeds containing minerals and vitamins to produce faster growth of the calf. The entire program goes away if the administration of the treatment is not undertaken with meticulous care, because the just-born calf is here the subject.

There is sample experimental evidence to show that feeding a mixture of two or more antibiotics may be better than any single antibiotic alone. Further, experiments indicate that aureomycin and terramycin are by far superior to other antibiotics to produce higher growth rates in dairy heifer calves.
In the case of aureomycin, there are instances in which when fed to healthy calves (like those of the Mediterranean type) in dosages as high as 80 milligrams per calf per day from the 4th to 116th day of age aureomycin hydrochloride produced the following results.

1. A gain of 10 to 30% in calf weight.

2. Markedly improved the appetite.

3. Significant decreases in calf scours and

4. Smoother hair coat.

In this practice, it is important to remember that the weight, size and condition of the heifer at maturity is more important for breeding than the age of heifer alone.

Buffalo calves up to the age of one month, and especially during the first week of its life, requires greater care and attention. Once they pass this critical period, they compare favourably with their adult counterparts in verve, vigour and vitality.

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**RESEARCH ABSTRACTS**

**MANAGEMENT AND PRODUCTION**


The effect of vacuum packaging on the physical qualities namely, pH, water holding capacity (WHC), colour and moisture percent of buffalo beef (*Bubalus bubalis*) packed in four different packaging material and stored at different periods under chiller storage (5 ±2°C) and freezer storage (-10°C) was studied. Buffalo beef packed under vacuum, in multilayer (ML) material and stored up to 120 h at 5±2°C recorded the lowest pH. Buffalo beef packed under vacuum, in multilayer material and stored up to 72 h at 5±2°C had the highest WHC and moisture percent. Highest Munsell colour-Hue and Value were noticed in buffalo beef packed in ordinary method, in low density polyethylene (LDPE) monolayer and stored up to 72 h and highest Munsell colour-Chroma was noticed in buffalo beef packed under vacuum, in LDPE monolayer and stored up to 120 h at 5 ±2°C. Buffalo beef packed under vacuum in multilayer material and stored up to 60 days at -10°C recorded lower pH, higher WHC and moisture percent. Highest Munsell colour-Hue, Value and Chroma were noticed in buffalo beef packed under ordinary method, in LDPE monolayer and stored up to 30 days at -10°C. Beneficial effects by maintaining lower pH, higher WHC and moisture percent were reported in buffalo beef packed under vacuum and in multilayer material. But the muscle colour improved when packed under ordinary method and in LDPE material. pH, WHC muscle colour and moisture percent decreased on increase in duration at chiller and freezer storage but only Munsell colour-Chroma increased on increase in duration at chiller storage.

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**FEEDING AND NUTRITION**

J.L. Chaudhary, L.R. Gupta* and A.B. Mandal*.  
*Department of Animal Production, Rajasthan Agricultural University, Bikaner,  

Fifteen lactating Murrah buffaloes in early stage of lactation were divided into three groups of five animals each on the basis of milk yield (8.9 kg) and body weight (472.66 kg), and were allotted to three dietary treatments viz. T1-40 kg berseem (*Trifolium alexandrinum*) + ad libitum wheat straw (*Triticum aestivum*) + concentrate, T2-20 kg berseem + ad libitum wheat straw + concentrate and T3-5 kg berseem + ad libitum wheat straw + concentrate as per requirement. The milk yield and fat content in milk were affected (P<0.05) by levels of green fodder given to buffaloes. The average 6% FCM yield was 11.73, 10.18 and 9.96 kg, in T1, T2 and T3 groups, respectively. Higher (P<0.05) milk yield was recorded in T1 than T2 and T3 groups, however, there was no difference in milk yield of T2 and T3 groups. The same trend was observed in the case of fat content of milk. DCP and TDN consumed per kg milk produced was significantly (P<0.05) less in group T3. Similarly net protein and net energetic efficiency was significantly (P<0.05) higher in T3 group followed by T2 and T1 group. The voluntary water intake per kg dry matter intake and per kg milk yield lowest (P<0.05) in T1 followed by T2 and T3 group.


Osmotic active agents like sodium bicarbonate and sodium chloride increase the dilution rate/ouflaw rate, which improves animal performance. Keeping this in view, the present studies were conducted on four rumen-fistulated buffaloes (2 to 2.5 years). They were fed wheat straw + concentrate mixture as per requirements, group A. Sodium chloride was added daily for one month at a rate of 50 and 100 g/head for groups B and C, respectively. Feed intake was not affected in group B but reduced significantly in group C. Animals consumed more water in both treatment groups, possibly to maintain the water electrolyte balance. Rumen water kinetics studies also showed an increase in the outflow rate and rumen volume in group C only, where sodium chloride was supplemented at 100 g/head daily. Total population of bacteria and protozoa were not affected in group B, but their number reduced significantly in group C, which might be due to an increase in flow rate in this group. Holotrichs protozoa were affected more than the entodinomorphs. These results indicate that 50 g sodium chloride head daily is an optimum level to promote better feed utilization in buffaloes.


Three healthy fistulated male buffalo calves of 1 1/2 to 2 years of age were fed ration comprising of concentrate and wheat straw in 35:65 ratio, once daily for a period of three weeks. After the adaptation period, rumen liquor samples were collected from each individual animal before feeding (0 h) and 2, 4 and 6 h post feeding for two consecutive days. The same animals were then maintained on the following experimental diet under 3 separate trials; Basal diet + 300 g arrowroot starch (Treatment I); Basal diet + 300 g gur (Treatment II); Basal diet + 300 g sucrose (Treatment III). The data showed an increase in total volatile fatty acid concentration in all animals fed supplemented diet as compared to those maintained on basal diet alone. Total protozoa
numbers increased with starch supplemented diet and decreased with gur and sucrose supplementation. Microbial protein synthesis in rumen decreased in animals fed with carbohydrate supplements.


Forty five male buffalo calves of about 6-7 months of age were selected and allotted to 3 groups of 15 each. The animals of group I were fed individually as per ICAR standard. Group II and III were fed on 25% higher and 25% less CP. There was significant (P < 0.01) effect of protein level on nitrogen balance. Endogenous nitrogen losses were 0.32 g/100g DMI in faeces and 0.11 g/kg W^{0.75}/day in urine. The CP and DCP requirement for buffalo calves at 100-150 kg, 151-200 kg and 201-250 kg body weight were estimated as 3.37 and 1.12, 2.69 and 0.03 and 3.91 and 1.03 g/kg W^{0.75}/day, respectively. The DCP requirements for maintenance and growth observed at 75, 100, 125, 150, 175, 200, 225, 250, 275 and 300 kg body weights by digestion trial and factorial methods were 225, 240, 250, 270, 280, 320, 335, 360, 380 and 395 g/day and 182, 200, 200, 208, 220, 258, 268, 286, 305 and 315 g/day, respectively. For practical feeding purposes it is more appropriate to use the DCP requirement determined by the feeding trial method. Various feeding standards have been developed for feeding of cattle in the world (NRC, 1978; ARC, 1980; I.C.A.R. 1985), but no systematic feeding standards have been developed for feeding buffaloes. Recently it has been tried to established the feeding standards for feeding country buffalo which now gaining popularity in India (I.C.A.R., 1985; Pathak and Verma, 1993). Hence an attempt to determine requirement suitable for economically feeding of male Murrah buffalo calves under eastern agroclimatic conditions of Uttar Pradesh has been made in this study.

**HEALTH AND DISEASES**


Antibodies to *Neospora caninum* were assayed in sera of 222 female water buffaloes from Ribeira Valley of Sao Paulo Stage, Brazil, using an indirect fluorescent antibody test (IFAT) and *Neospora agglutination test* (NAT). IFAT antibodies were found in 64% of buffaloes with titers of 1: 25 (42 buffaloes), 1: 50 (53 buffaloes), 1: 100 (31 buffaloes), 1: 200 (10 buffaloes), 1: 400 (3 buffaloes), or ≥ 1: 800 (3 buffaloes). NAT antibodies were found in 53% of buffaloes; in titers of 1: 40 in 52 buffaloes, 1: 80 in 27 buffaloes, 1: 160 in 21 buffaloes, and ≥ 1: 320 in 17 buffaloes. Results indicate a high prevalence of *N. caninum* exposure in water buffaloes in Brazil and warrant an investigation of the role of *N. caninum* as an abortifacient in water buffaloes.

Fifteen clinically healthy Murrah male buffalo calves (70-120 kg in weight, and 6 months to one-year-old) were randomly divided into three groups of five animals each. Group I served as the control. Calves in groups II and III were given cypermethrin and deltamethrin in repeated subacute oral doses of 0.25 and 0.2 mg/kg daily in 50 ml of water, respectively, for 21 consecutive days. Cypermethrin toxicity was mild to moderate, with animals displaying thick eye discharge, sunken eyes, muscle twitching, staggering gait, salivation, diarrhoea and loss of body condition from day 14-17 onwards. In comparison, deltamethrin resulted in mild toxicity, producing only eye discharge and salivation. Repeated oral administration of cypermethrin and deltamethrin significantly inhibited plasma cholinesterase activity up to 44.1 and 30.7%, and increased plasma levels of aspartate aminotransferase to 24.9 and 25.5% alanine aminotransferase to 19.8 and 28.7%, alkaline phosphatase to 39.0 and 28.2%, and acid phosphatase to 48.5 and 31.2%, respectively. The relatively greater effect of cypermethrin and deltamethrin on acid phosphatase than alkaline phosphatase indicates that these synthetic pyrethroids have more marked effects on lysosomes in the animals. The findings demonstrate the relatively stronger toxicity of cypermethrin compared with deltamethrin after repeated exposure in buffalo calves.


Genetic characterisation of two pathogens, namely foot and mouth disease (FMD) virus and Mycobacterium bovis, isolated from African buffalo (Syncerus caffer) in Southern Africa was used to determine the origin of buffalo in situations where the source of infection was obscure. By determining the phylogenetic relatedness of various FMD virus isolates using partial sequencing of the main antigenic determinant, VP1, the origin of buffalo moved illegally to the non-endemic region of South Africa was traced to the Kruger National Park (KNP) where FMD is endemic in the buffalo population. Comparative analysis of the 'genetic fingerprints' of bovine tuberculosis isolates from buffalo and cattle has aided in tracing the original source of infection of buffalo populations in the KNP. Furthermore, these analyses have assisted in tracing the origin of infected animals that have been moved to other parts of South Africa.


Arthritis was induced by intra-articular injection of 1.0 ml turpentine oil into the left radiocarpal joint in 20 clinically healthy male buffalo calves. The animals were randomly divided into four groups of five animals each. Untreated animals in Group A served as control. Fresh autogenous synovial fluid, fresh autogenous synovial fluid in combination with 20 mg prednisolone-acetate and 40 mg gentamicin sulfate, and fresh homogenous synovial fluid were intra-articularly administered to treat buffalo calves in Groups B, C and D, respectively, on the 7th, 15th and 21st day after arthritis was induced. As a result of treatment, there was a general increase in synovial fluid, a decrease in total leukocyte count and synovial lymphocytes, and an increase in neutrophils. Viscosity and mucin precipitate quality score remained significantly lowered at different intervals. The results demonstrate the role of corticosteroids in the treatment of the induced arthritis, however, it must be considered that treatment using fresh synovial fluid transfusions alone was also satisfactory compared with the control group.
REPRODUCTION


This study was done to determine if superovulation and embryo collection could have an effect on the milk production in the buffalo. Parous cyclic, Murrah-type buffaloes between 70 to 175 day postpartum were superovulated between days 9 and 11 of the synchronized oestrous cycle with a total of 600 mg NIH-FSH-P1 in a twice daily descending dose schedule for 5 days. Luteolysis was induced by two injections of luprostrol (PGF) at seventh and eighth injection of FSH and animals were inseminated 4 times at 12 h intervals starting 36 h after PGF administration. Non-surgical embryo collection was performed on day 5.5 to 6 after the onset of superovulatory oestrus. Daily milk production was recorded during 15 days before initiating superovulatory treatment (BT), during treatment upto embryo collection (12 days, DT) and up to 15 days after embryo collection (AT). Mean daily milk production was 7.56±0.21, 6.65±0.23 and 6.25±0.18 kg during these periods. Regression coefficients of milk yield on days in milk, estimated separately for the three periods, revealed that the significant fall in daily milk yield before treatment period was arrested during treatment and after treatment periods. Comparison of regression slopes between three periods revealed that superovulation and embryo collection had no effect on milk production in the buffalo.


The principal objective of this study was to derive an improved procedure for cryopreservation of swamp buffalo (Bubalus bubalis) spermatozoa. Experiments were conducted to determine effects of cooling rate, intermediate plunge temperature and warming rate on motility and acrosome integrity of spermatozoa. Spermatozoa were obtained from three bulls (three ejaculates/bull) and were subjected to nine cooling conditions before being frozen in liquid nitrogen: cooling at 10, 20, or 30°C/min each to -40, -80, or -120 °C before being plunged into liquid nitrogen. The spermatozoa frozen under a given condition were then thawed either at 1,000 or 200°C/ min. Cooling rate, intermediate temperature and warming rate significantly affected survival of spermatozoa obtained from the three bulls. Cooling spermatozoa from 4 to -120°C either at 20 or 30°C/ min yielded better progressive motility compared to other cooling conditions (50 versus 30%). Rapid warming was superior to slow warming. In an additional study, motility and fertility of spermatozoa frozen after being cooled to -120 °C at 20 °C and 30 °C/min and those frozen by a standard protocol used routinely for semen processing were assessed. Progressive motility of cryopreserved spermatozoa cooled at 20°C and 30°C/min was 40 %, while that of spermatozoa cryopreserved using a standard protocol was 25%. A total of 178 buffalo cows were inseminated with cryopreserved spermatozoa obtained from one bull, and their pregnancy status was assessed 60 days later by rectal palpation. Out of the 60, 26 (43%) and 23 of 58 (40%) cows inseminated with sperm cooled at 20 and 30°C/min, respectively, became pregnant, whereas 17 of 60 (28%) cows inseminated with sperm frozen by a standard protocol became pregnant. This study demonstrates that an effective cryopreservation procedure for buffalo spermatozoa can be derived by systematic examination of various cryobiological factors.

The time during which the oocytes are left at postmortem condition significantly affect the developmental competence of oocytes. In this study, the timing of sequential changes during 1st meiotic division and the subsequent fertilization, MPN formation and cleavage capability of swamp buffalo oocytes aspirated at 2 h and 6 h post slaughter were evaluated. In Expt. 1, oocytes recovered 2 h and 6 h after slaughter respectively were cultured and fixed at 0, 6, 9, 12, 15, 18, 21 and 24 h to evaluate meiotic progression. Initial observation on the occurrence of the different meiotic stages at each culture time point for oocytes recovered 6 h after slaughter indicate quite an advanced stage of nuclear progression compared to oocytes recovered 2 h after slaughter. In Expt. 2, oocytes were cultured for 24 h before in vitro fertilization. The resulting sperm penetration, MPN formation and cleavage rate were higher even if not significantly different when oocytes were aspirated 6 h vs 2 h after slaughter. This indicates that the time difference utilized has not caused enough follicular changes to affect the developmental competence of the oocytes. Nevertheless, the results of the study show that swamp buffalo oocytes acquire meiotic competence before in vitro maturation. Therefore oocytes aspirated from ovaries at 2 h to 6 h postmortem condition can be used for the production of early stage swamp buffalo embryos in vitro.


In three experiments we studied the baseline and changes in VER during different natural estrous cycle stages (n=146) in ovarian structures and in plasma progesterone during estrus induced by prostaglandin injection (n=16) and the VER at insemination (n=90) in an attempt to predict estrus, ovulation and the best VER range for inseminating buffaloes for optimum conception. The baseline VER was classified on the basis of ovarian findings and estrous cycle stages. The mean VER during estrus, metestrus, diestrus, proestrus and anestrus was 32.68±0.46, 41.26±1.17, 50.23±0.55, 43.20±0.64 and 55.86±0.57 ohms, respectively. There was a significant difference (P<0.01) between the VER except those between metestrus and proestrus. The ANOVA for VER over estrous cycle stages showed a highly significant (P<0.01) effect of stage of estrous cycle on VER in buffaloes. The percent decrease in VER was more pronounced from diestrus to estrus. In the second part of the study plasma progesterone profiles and the appearance of estrus in buffaloes induced to estrus using two dose schedules and routes of PGF2α administration showed that luteolysis and estrus induction was slower in the 10 mg IVSM route (Intra Vulvo Submucosal) (only 60 % animals evinced estrus in 48 to 72 h) as compared to the 25 mg IM route (83.33% evidenced estrus in 48 to 72 h). Fall in plasma progesterone was synchronous to a fall in VER, the correlation (0.65) between them being positive and significant (P<0.01). After ovulation the VER started rising, showing a distinct relationship between VER and ovulation. By using VER, an additional 36.6% of the buffaloes could be detected in estrus. In the third part of the study, insemination of buffaloes induced to estrus (n=11) and normal-
estrus buffaloes (n=79) showed that the overall conception rates to single insemination when the buffaloes were inseminated at the VER range of 26 to 30, 31 to 35 and 36 to 40 ohms were 81.48, 58.97 and 16.66%, respectively. Buffaloes showing VER from 31 to 35 ohms and 36 to 40 ohms also evidenced atypical and Null fern pattern in the cervicovaginal mucus. The study proved that VER can be used successfully to predict the stage of estrous cycle, ovarian status and ovulation; and insemination at a low VER distinctly improves the conception rates in buffaloes.


The morphological development of the buffalo placenta along the pregnancy, particularly on months 4-5, 7-8 and 9-10 was studied. Concerning the number of the placentons, we found an increase during early and mid-pregnancy and a decrease during late pregnancy, although their size progressively increased from early to late stages. In all pregnant periods we found placentons of different shapes and sizes, with a gross structure similar to the bovine placenta, but more flattened. The materno-fetal interface was closely appositioned and extremely irregular formed by fetal villous tree that indented the maternal epithelium. The trophoblast consisted of a simple cellular layer containing individual and binucleated cells. The axis of the villous tree and maternal folds were both fundamentally formed by loose connective tissue, however, the endometrial stroma was richer in collagen fibers. In comparison to early phases of the pregnancy, the villus trees on months 9-10 were much more branched and the feto-maternal junction contained regions of hematomes and crythrophagocytosis. Ultrastructurally, an intense vascularization of the fetal villous could be seen as well as binucleated cells presenting a well developed granular endoplasmic reticulum and Golgi complex associated to abundant vesicles, indicating intense activity on protein synthesis.


To evaluate the proper time for artificial insemination (AI) using fixed time schedule, 56 Mediterranean lactating buffaloes were used. Starting from February until the end of April the animals received a PRID (Progestrone Releasing Intravaginal Device) for 10 days plus PMSG and PGF_2 \alpha (analogue) on 7th day in order to control oestrus. Buffaloes were inseminated using two different schedules: 3 AI at 48, 72 and 96 h (26 animals) versus 2 AI at 72 and 96 h (30 animals after PRID removal. Starting from 15 days after the insemination the buffaloes were kept together with a breeding bull and then naturally mated at the following oestrus. The conception rate (CR) after PRID treatment was 48.32 % to AI and 69.6 % at the following oestrus (AI + natural mating), confirming that the use of PRID improves pregnancy rate in the low breeding season. The two different AI schedules utilised showed a better result in the CR after AI with 2 AI schedule than with 3 AI one (56.7 % vs. 38.5 %). In both AI schedule groups PRID was able to induce a good synchronisation as inferred from the status of uterus and ovaries registered at the insemination times: the maximum uterine tone was found 72 h after PRID removal and the presence of a soft and fluctuating follicle was recorded in the 89.3 % of buffaloes within 96 h after PRID removal. We can conclude that 72 and 96 h from PRID removal could be proper time for AI in synchronised buffalo cows.
To evaluate the possibility to induce fertile oestrus in prepuberal heifers using a PRID (Progesterone Releasing Intravaginal Device) in order to breed them during the low breeding season (March-September period), 45 buffalo heifers aged 19.8±1.3 months were used. The animals were assigned (n=15/group) to three groups: A, B (treated groups) and C (control group). At the start of the trial (March), all heifers were non cycling. The animal of groups A and B received a PRID for 10 days plus PMSG (group A: 1000 IU; group B: 750 IU) on the 7th day, were inseminated (Al) using frozen-thawed semen at 48 and 72 h after PRID removal and then naturally mated at the oestrus following AI. The other animals (group C), kept with a bull and naturally mated, were used as controls. PRID was useful in inducing oestrus (66.6% in group A and 73.3% in group B). The conception rate (CR) to AI was similar in the two treated groups: 40% in group A and 33.3% in group B; when including the remaining trial period (AI + natural mating in treated; natural mating in controls), the CR of treated animals was 66.6% (both in groups A and B), while it was 33.3% in controls. PRID plus PMSG treatment of heifers, regardless of PMSG utilised dose, is able to induce fertile oestrus in non-cycling heifers. This could have an economic impact on buffalo production because a greater proportion of heifers could be bred early. Moreover, with this treatment, it is possible to overcome the problem of oestrous detection and increase the effectiveness of AI programmes in buffalo heifers.


The arrangement and ramification of the arteries and veins of the umbilical cord and the placentas of 34 buffaloes, with fetal ages of between 5 to 10 months, were studied. Placentas originated from adults, of non-defined breed, from the states of Para and Maranhao, Brazil. The umbilical cord, had a length of 26 cm and contained the urachus, two umbilical arteries and two umbilical veins with inter-arterial and intervenous anastomoses, funicular vessels and vasa vasorum. In the umbilical funiculus, 82.35% of anastomoses were inter-arterial (single or double) and 17.34% are intervenous, both being transversal and convergent.
Funicular branches were also noted, particularly arising from the funicular anastomoses in 42.85% of observations. The cotyledonary placenta of the buffalo possessed cotyledons, the majority of which were oval, and more numerous in the pregnant uterine horn (where large cotyledons predominated) than in the non-pregnant uterine horn (where small cotyledons predominated). The umbilical arteries of the pregnant and non-pregnant uterine horns divided into collateral branches and terminal cotyledonary, intercotyledonary and mixed distals. The umbilical veins were formed from cotyledonary, intercotyledonary and mixed tributaries and roots. The types of arteriocotyledonary and venocotyledonary arrangements were also observed and the number of cotyledons and their sizes were noted, in addition to the arterial and venous vessels that connected to them.

**PHYSIOLOGY**


Eighteen pregnant buffaloes in the third and fourth parities were used in this study. The animals were divided into three equal groups, according to their body weight. Animals of the first (G1) and second (G2) groups were given daily 6 and 12 g of niacin/head, respectively. Supplementation was given 4 weeks antipartum and continued until week 12 postpartum. Animals of the third group (G3) served as controls. The results showed that buffaloes which were given niacin had an increase (P<0.05) in plasma glucose, hemoglobin, and total protein concentrations. Plasma sodium and cholesterol concentrations decreased as a result of niacin supplementation as compared with the control group, with no difference due to the level of niacin. The results indicate that animals in G1 and G2 groups consumed more (P<0.01) food and more (P<0.05) metabolizable energy. They produced more (P<0.05) fat corrected milk and tended to have a shorter (P<0.01) interval from calving to detected estrous. They also required less (P<0.01) services per conception and had shorter (P<0.01) days open as compared with the control. It was concluded that supplementation of niacin at 6 g/h/day led to improvement of the productive and reproductive performance of pregnant buffaloes.


The relative association of light-dark cycles with circulatory prolactin in buffaloes was studied in controlled light chamber conditions which consisted of three different phases of (i) Natural day and night photoperiods (11L:13D; L-light hours, D-dark hours), (ii) Induced 16 h darkness and 8 h light (8L:16D) and (iii) Induced 8 h dark and 16 h light (16L:8D). The temperature during various light-dark cycles of all the 3 phases was almost constant, and sufficient illumination was provided in the chamber to make the simulated light intensity similar to daylight. Mean blood plasma prolactin levels in six non-lactating Murrah buffaloes measured in the samples taken twice a day at the end of dark and light cycles on alternate days was significantly higher in the samples taken after dark than after light cycles in both 8L:16D and 16L:8D phases. The values in these two phases were 89.31±11.02 and 39.80±4.97 ng/ml after dark cycle and 50.86±8.21 and 25.91±2.27 ng/ml in the sample taken after light cycle, respectively. In natural day-night photoperiod phases also the levels were higher in the samples taken after dark (57.55±6.83 ng/ml) than in light hour cycle (46.08±10.89 ng/ml).
We studied the infiltration of different subsets of immune system cells in the ovarian parenchyma of Egyptian buffaloes during follicular and luteal phases of the estrous cycle. All subsets of leukocytes infiltrated significantly more into corpora lutea (CL) than into Graafian follicles (GF) (P<0.01) except for plasma cells that were abundant in the GF but not observed in the CL. The number of macrophages, lymphocytes, neutrophils and eosinophils were significantly greater in mature CL than in corpora hemorrhagica (CH) or regressing CL. Moreover, the regressing CL showed significantly more macrophages, lymphocytes and neutrophils than the CH. Large antral follicles were infiltrated with larger number of leukocytes than growing preantral atretic follicles. Macrophages and neutrophils observed in large antral follicles were significantly more abundant in the theca externa than the theca interna (P<0.01). Only plasma cells were significantly greater in number in the theca interna (P<0.01). Leukocytes infiltrated significantly more into large mature follicles than large, growing, preantral atretic follicles (P<0.01). Results of this study reveal the calling of leukocytes in a significant numbers inside the ovarian tissue of buffaloes around the time of ovulation and at luteolysis. It is possible that leukocytes with their powerful bioactive cytokines (IL-1, TNF-α, GM-CSF, and INF-γ) may assist in ovarian functions such as ovulation and luteolysis.

The effect of different concentrations of three antioxidants on phagocytic and kill activities of blood polymorphonuclear leukocytes (PMN) isolated from buffaloes during the peripartum period (4 weeks before to 7 weeks after parturition) was investigated in this study. Two concentrations of β-carotene and vitamin A (10⁻⁴ and 10⁻⁵ M) and one concentration of Se (10⁻⁵ M) were used. Phagocytic activity of PMN treated with β-carotene (10⁻⁵ M) significantly enhanced (P<0.05) after parturition (Week 0 until Week 3), whereas the kill activity of the same cells significantly (P<0.05) increased before and after parturition (at Weeks -4, -3, -2, 0, 1, 2 and 3). The concentration of β-carotene (10⁻⁵ M) enhanced phagocytosis of PMN only at Weeks 0 and 1 and kill activity at Weeks -4, -3, -2, 0, and 1. Selenium (10⁻⁵ M) significantly (P<0.05) enhanced phagocytic activity of PMN starting from parturition (Week 0) until Week 3 postpartum. Kill activity increased significantly both before (Weeks -4, -3 and -2) and after (Weeks 0, 1, 2, 3 and 4) parturition. Vitamin A (10⁻⁴ M) significantly enhanced phagocytic activity of PMN at Weeks 0, 1, and 2, whereas, the concentration of β-carotene (10⁻⁵ M) increased phagocytic activity only at Week 0. Kill activity of PMN increased significantly (P<0.05) at Weeks 1 and 0 (10⁻⁵ M). These results demonstrate that β-carotene and selenium significantly enhanced phagocytic and kill activities of PMN isolated from buffaloes around parturition in vitro. Vitamin A enhanced phagocytosis and kill activities but not to the same extent as β-carotene and selenium. Apparently, the in vitro killing activity of PMN is a distinctive function from phagocytosis and both activities may be enhanced by the use of essential nutrients, especially during the peripartum period. Moreover, β-carotene is more effective as an antioxidant than vitamin A in enhancing the activities of phagocytic cells.

**BREEDING AND GENETICS**

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**Genetic diversity between Italian, Greek and Egyptian buffalo populations.** *Livestock Production Science* (2001) 70: 203-211.

Genetic diversity of three buffalo populations of the Mediterranean area: Italian, Greek and Egyptian, was estimated on the basis of allele frequencies at 13 polymorphic microsatellite loci: CSSM33, CSSM36, CSSM38, CSSM43, CSSM47, CSSM60, CSSM70, BMC1013, DRB3, ETH03, ETH121, ILST005 and RM099. The number of detected alleles per locus varied from two (ILST005) to 19 (ETH03). Major differences in the three populations were found at loci BMC1013, DRB3, CSSM47, CSSM60 and CSSM70, while, at the other loci, allele frequency distribution was similar in the three national samples, which have the same alleles at the highest frequency. Average gene diversity over all loci was 0.624. Across-loci average gene diversity increased with the number of alleles. Observed average heterozygosity was 0.135, 0.151 and 0.158 in the Italian, Greek and Egyptian populations, respectively. The degree of differentiation between the Italian and Greek buffaloes was 0.031±0.015; differentiation between the Egyptian and each of the other two populations was 0.070±0.020. Estimation of distance by isolation among geographical areas, within population, gave indication that inbreeding rates calculated on the basis of 13 microsatellites describe well the geographical distribution of the three populations.


Caseins are milk proteins existing in several molecular forms (alphaS1, alphaS2, beta and kappa) with variant alleles of each. Kappa-casein variant B is reported to be favorable for milk quality and considered to be included in breeding strategies of dairy animals. PCR-RFLP is a fast and efficient method for discrimination between different K-casein variants and is also useful for genotyping bulls. DNA samples from four different buffalo breeds were subjected to PCR amplification using bovine K-casein primers (K1: 5'-CAC GTC ACC CAC ACCCACATT C - 3' & K2: 5'- TAA TTA GCC CAT TTC GCC TCT GT - 3') and the PCR product of 379-bp was digested with Hind III and Hinf I. The PCR products from all the DNA samples showed only two bands 225- & 154-bp on Hind III digestion and only 288- & 91-.

**IMMUNOLOGY**

A.A. Ramadan, S.A. Selim, H.M. Hassan, M.A. Wahba. *Immunobiology and Immunopharmacology Unit, Pathology Department Animal Reproduction Research Institute, 5 Hadayk El-Aharm St., P.O. Box 12556, Giza, Egypt.* **Immune regulation of ovarian function in buffaloes (Bubalus bubalus).** *Theriogenology* (2001). 55: 661-669.
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BUFFALO BULLETIN
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