



**UNIVERSITI PUTRA MALAYSIA**

**A COMPARISON ON THE SEMEN CHARACTERISTICS AND  
EXTENDERS FOR FREEZING SEMEN OF THE SWAMP,  
RIVER AND CROSSBRED BUFFALO  
(BUBALUS BUBALIS)**

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FOR FREEZING SEMEN OF THE SWAMP, RIVER AND CROSSBRED  
BUFFALO (BUBALUS BUBALIS)**

by

**P. RAMAKRISHNAN**

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By

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January 1990

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A study was undertaken (i) to investigate and compare the effect of chromosome make up on semen characteristics of three buffalo genotypes, viz swamp, 2n=48; river 2n=50; and crossbred, 2n=49; (ii) to investigate the influence of climate on the semen characteristics of the three genotypes; (iii) to study the freezability of the semen of the three buffalo genotypes using four different extenders.

The results showed that in general, the semen quality was better in the swamp buffalo as compared to the river type.



Except for the semen colour, density, volume and spermatozoa concentration, all the other semen characteristics of the crossbred buffalo were inferior to that of the other two genotypes. The lower mean values for mass activity, progressive motility percentage, percent intact acrosome and the higher incidence of total spermatozoa abnormalities in the crossbred buffalo ( $2n=49$ ) semen indicate that the odd number chromosome complement of this genotype has an adverse effect on its semen characteristics.

The reaction time and the ejaculation time were significantly higher in swamp buffalo as compared to the other two genotypes. There was no significant difference in the reaction time between the river and crossbred buffaloes. However, the ejaculation time of the crossbred buffalo was significantly higher than that of river buffalo. The reaction time and ejaculation time were significantly higher in the swamp buffalo during the 'Dry' season. However, there was no seasonal variation in the reaction time and ejaculation time in the river and crossbred buffaloes.

Although an effect of season on semen characteristics of the swamp and the river buffaloes was observed, the quality of semen produced by these two genotypes during the three seasons were within the limit of good semen quality. In the crossbred buffaloes, a significantly higher spermatozoa concentration





and lower percent intact acrosome were noted during the 'Rainy' season.

With regard to the influence of extenders on the post-thaw quality of semen, the overall mean (mean of swamp, river and crossbred) motility at 0 hour post-thaw was significantly higher for semen extended with Tris as compared to those extended with Lactose yolk, Lactose fructose yolk and Triladyl extenders. There was no significant difference in the post-thaw percent intact acrosome among the semen samples frozen in the four types of extenders. Although the overall mean 0 hour post-thaw filter percentage was highest in samples using Triladyl, it did not vary significantly from that using Tris.

With regard to the influence of breed on semen freezing, the overall mean (mean of the four extenders) motility and filter percentage at 0 hour post-thaw were significantly higher in the swamp buffalo as compared to the other two genotypes. The crossbred buffalo recorded a significantly lower overall mean post-thaw motility, percent intact acrosome and filter percentage as compared to the swamp and river buffaloes.

In conclusion, it can be stated that the crossbred buffaloes with odd number chromosome complement are genetically inferior in regard to semen quality, as compared to the pure bred parental types.



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**PERBANDINGAN CIRI-CIRI SEMEN DAN JENIS EKSTENDER UNTUK PEMBEKUAN SEMEN DI ANTARA KERBAU KERBAU SAWAH, SAPI DAN KACUKAN**

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Kajian-kajian telah dijalankan untuk (i) menilai dan membandingkan kesan bilangan kromosoma ke atas ciri-ciri semen tiga jenis genotaip kerbau (sawah,  $2n=48$ ; sapi,  $2n=50$ ; dan kacukan,  $2n=49$ ); (ii) mengesan pengaruh iklim ke atas ciri-ciri semen bagi tiga jenis genotaip ini bagi menentukan kualiti semen pada musim berlainan; dan (iii) menilai kesan pembekuan ke atas ciri-ciri semen kerbau-kerbau ini menggunakan empat jenis pengekal semen (exstender).

Pada keseluruhannya, kualiti semen kerbau sawah didapati lebih baik daripada yang didapati pada kerbau sapi. Kesemua ciri-ciri semen kerbau kacukan kecuali warna, densiti, isipadu



dan kosentrasi spermatozoa didapati kurang bermutu (inferior) dibandingkan dengan genotaip-genotaip lain. Nilai purata terendah bagi ciri-ciri aktiviti mass, motiliti progresif, peratus motiliti, peratus akrosoma kekal dan nilai tertinggi bagi bilangan spermatozoa yang abnormal bagi semen kerbau kacukan menunjukkan bahawa kerbau yang mempunyai bilangan kromosoma ganjil adalah kurang subur dalam pembiakan.

Masa reaksi dan masa ejakulasi kerbau sawah didapati lebih panjang daripada yang didapati pada kerbau sapi atau kerbau kacukan. Tiada perbezaan bermakna didapati bagi masa reaksi di antara kerbau sapi dan kacukan. Bagi masa ejakulasi didapati kerbau kacukan menunjukkan jangkamasa yang panjang daripada kerbau sapi. Pengaruh musim dapat dikesan pada ciri-ciri semen kerbau sawah di mana masa reaksi dan masa ejakulasi adalah lebih panjang pada musim kering. Dibalikannya, musim tidak mempengaruhi parameter-parameter ini bagi kerbau sapi dan kacukan.

Walaupun musim didapati juga mempengaruhi ciri-ciri semen kerbau sawah dan sapi, tetapi didapati kualiti semen yang dihasilkan oleh kedua-dua jenis genotaip ini adalah di dalam nilai kualiti semen yang baik. Kerbau kacukan menunjukkan konsentrasi spermatozoa yang lebih tinggi dan peratus akrosoma yang kekal yang lebih rendah pada musim hujan.



Berhubung dengan kesan ekstender ke atas kualiti semen beku selepas dicairkan (post-thaw), purata keseluruhan (purata bagi kerbau sawah, sapi dan kacukan) motiliti pada jam 0 selepas dicairkan menunjukkan nilai tertinggi bagi semen dikekalkan dengan 'Tris' dibandingkan dengan sampel-sampel dikekalkan dengan 'Lactose Yolk', 'Lactose fructose yolk' dan 'Triladyl'. Tiada perbezaan signifikan pada sampel-sampel semen yang dibekukan menggunakan kesemua jenis exstender. Walaupun peratus turas didapati tertinggi pada jam 0 selepas dicair bagi sampel menggunakan 'Triladyl', ia tidak menunjukkan perbezaan bermakna dari sampel-sampel menggunakan 'Tris'.

Berhubung dengan kesan baka ke atas ciri-ciri semen yang dibeku, purata keseluruhan (purata keempat-empat ekstender) motiliti dan peratus turas pada jam 0 selepas dicair adalah tertinggi sekali pada kerbau sapi. Kerbau kacukan menunjukkan nilai-nilai terendah bagi motiliti, peratus akrosoma kekal dan peratus turas selepas dicairkan.

Kajian ini memberi kesimpulan bahawa dari segi genetik kerbau kacukan dengan bilangan 49 kromosoma mempunyai ciri-ciri semen yang inferior dibandingkan dengan jenis baka tulen.



## CHAPTER I

### INTRODUCTION

The buffalo (Bubalus bubalis) population of the world has been estimated to be between 130 and 150 million. About 94 per cent of the world buffalo population is found in Asia and the Pacific Region (Soni, 1986). It plays a multipurpose role in the agricultural economy of most South East Asian countries.

The buffalo (Bubalus bubalis) is classified by MacGregor (1941) into river and swamp types based on their habitat and use under domestication. The large river type prefers fresh water for wallowing and is used mainly for milk, while the swamp type wallows in muddy water and is used for draught and meat. Recently, the two types were further classified based on their cytogenetic make-up. The river or milk buffalo found mainly in India and Pakistan, has  $2n=50$  chromosomes whereas the swamp buffalo, the common draught animal found in east and south of Burma including Malaysia has  $2n=48$  chromosomes (Rommelt-Vasters et al., 1978). The chromosome complement of the  $F_1$  hybrid (swamp x river) is intermediate ( $2n=49$  chromosomes) to the two parental types (Bongso and Jainudeen, 1979). Further segregation results in two populations ( $2n=49$



and  $2n=50$ ) when backcrossed to river type and two populations ( $2n=48$  and  $2n=49$ ) when backcrossed to the swamp type (Bongso et al., 1984; Harisah, 1988). The inter se mating of the  $F_1$  ( $F_1 \times F_1$ ) produces three populations ( $2n=48$ ,  $2n=49$  and  $2n=50$ ) of  $F_2$  animals (Harisah, 1988; Bongso and Hilmi, unpublished data).

Crossbreeding the swamp buffalo with the larger river types has been practised in Malaysia, China, Thailand and Philippines to exploit the benefit of heterosis and obtain the maximum genetic merit for production characteristics. However, testicular biopsies of the  $F_1$  hybrid obtained via crossbreeding were found to have a large proportion of degenerating spermatocytes and abnormal spermatids. It was suggested that the various synaptic associations resulting in unbalanced gametes during meiosis may be responsible for the degenerating germinal cells, in this genotypes which possesses unbalanced chromosome complement (Bongso and Hilmi, 1983). Fertility has also been shown to be affected in other species such as cattle possessing odd number chromosome ( $2n=59$ ) due to chromosomal translocation (Gustavsson, 1969; Dyrendahl and Gustavsson, 1979). With the present breeding programme and eventual production of  $F_1$ ,  $F_2$  and backcross generations, it becomes important to investigate the effect of unbalanced chromosome make up on fertility in buffalo crossbreds with  $2n=49$  chromosomes. The outcome of this investigation would provide a viable basis for future genetic improvement of the buffalo.

The female buffalo is generally considered a seasonal breeder (Yadava and Kushwaha, 1965; Roy et al., 1972; Hossain and Ahmad, 1973; Harjit and Arora, 1982; Liang et al., 1982). However, there is little evidence to indicate whether the seasonal breeding behaviour is a species characteristic or the result of climate and/or nutritional stress. This behaviour could be related to the fact that in most studies on seasonal effect on the buffalo ejaculates, the semen collected is generally reported to be of lower quality during the summer months (Pandey and Razada, 1978; Gupta et al., 1978; Bajwa et al., 1982; Rajamadedran and Sultanbawa, 1982). In Malaysia, there is scanty information on the semen characteristics of the three local buffalo genotypes (river, swamp and crossbred). Furthermore, there is also a lack of information on the seasonal effect on the buffalo ejaculates. This basic information is useful in formulating a buffalo breeding programme in this country.

The problem of seasonal breeding and the lack of mature and proven buffalo males of the three genotypes in several areas may be overcome by artificial insemination using frozen semen. Artificial insemination is a major tool which can be utilized to enhance buffalo production by exploiting the superior genetic material.

Therefore, the objectives of this study are:-

- (1) To study and compare the raw semen characteristics of the three genotypes of buffaloes as a measure of fertility.
- (2) To study the relationship between climate and semen characteristics of the three buffalo genotypes.
- (3) To evaluate the effect of deep freezing and different extenders on the post-thaw semen characteristics in order to identify the best suitable extender for semen preservation for the three genotypes.



## CHAPTER II

### REVIEW OF LITERATURE

#### Classification of Water Buffaloes

The buffalo (Bubalus bubalis) was classified by MacGregor (1941) into river and swamp types based on their habitat. The river type which is mainly used for milk, prefers fresh water for wallowing, while the swamp type which is used for draft and meat purpose, wallows in muddy water. They are also phenotypically distinct with the river types found mainly in India and Pakistan and introduced into the Near East, Europe, Latin America and Africa. The swamp type indigenous to Southeast Asia extends northward as far as Assam (Mason, 1974). Swamp buffaloes are found in large numbers in Burma, China, Indonesia, Kampuchea, Laos, Malaysia, Philippines, Sri Lanka, Thailand and Vietnam. Swamp buffaloes have also been introduced effectively into Australia, Brazil, Trinidad and several areas of Oceania (Mason, 1977).

Recently the water buffaloes of Asia and Australia have been classified as river and swamp types according to their cytogenetic status. The river type has a chromosome number of 50, whereas the swamp type of most Asian countries possess 48 chromosomes. The Australian swamp buffalo which i

