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Controlled Breeding in the Asiatic Buffalo (*Bubalus bubalis*)

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Abstract

Recent developments in exogenous hormone therapy to control and facilitate breeding in domestic buffalo cows (*B. bubalis*) are reviewed. Oestrus in domestic animals was synchronized satisfactorily during the normal breeding season by both of the standard treatments with prostaglandins or progestagens initially developed for use in *Bos taurus* cattle. Anoestrous cyclic cows treated with prostaglandin showed synchronized oestrus and conception rates similar to those recorded in normal cyclic animals, though the responses may have depended to some extent on increased intensity of observation of oestrus. Seasonally acyclic animals treated with progestagens and pregnant mare serum gonadotrophin also showed synchronized oestrus and conception rates equivalent to those recorded in cyclic animals, but these observations did not include prepubertal heifers or cows in the early stages of post-partum anoestrus. Controlled breeding did not overcome a general problem of low conception rates at spontaneous oestrus. Further investigations of controlled breeding should incorporate other management strategies, such as better feeding and reduced heat stress, which are known also to improve buffalo reproduction.

Introduction

There are in excess of 130 million domestic water buffaloes (*Bubalus bubalis*) used variously for draught power, milk production and meat production in some 25 countries ranging from south-east Asia and China through the Indian subcontinent and the Near East to southern Europe. Small populations are also found in northern Australia, the Caribbean, and South America. A full description of the distribution and productivity of various breeds of water buffalo has been presented by Cockrill (1974). A rapid expansion of knowledge of buffalo production since that time has not been gathered together in a single source, and the background information on buffalo cow reproduction given below has been taken from Cockrill (1974) unless specified otherwise. The purpose of this paper is to summarize recent developments in exogenous hormone therapy to control breeding in buffalo heifers and cows.

Normal Reproduction in *B. bubalis*

Hormonal regulation of the oestrous cycle in buffalo cows is similar to that in cattle (Dobson and Kamonpatana 1986). A rapid decline in circulating progesterone concentration associated with luteolysis of the corpus luteum is accompanied by an increase in plasma oestradiol-17β concentration and followed by a surge of luteinizing hormone secretion over a short period around the time of onset of oestrus (Kanai and Shimizu 1984; Avenell *et al.* 1985). Oestrous cycles range in length from 17 to 24 days with an average of 22 days, the duration of oestrus is mostly in the range 12–24 h, and ovulation occurs some 12–18 h after the end of oestrus (Jainudeen 1983). External signs of oestrus are not as obvious in the buffalo as in the bovine, however, and difficulty in detecting oestrus is widely recognized as a major breeding problem.

Buffalo cows are capable of breeding at any time of the year, but seasonal variation in breeding activity is commonly found in many regions due to periods of suboestrus (ovulation

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Table 1. Oestrus and conception in buffalo heifers and cows treated with prostaglandin $F_{2\alpha}$ or progesterone, or their synthetic analogues

Author	Treatment[A]	No. of animals[B]	No. of treated animals detected in oestrus (%)[C]	No. of oestrous animals that conceived (%)[C]
	(a) Cyclic animals			
Jainudeen (1976*a*)	A:X	23c	91	48
Jainudeen (1976*b*)	A:X	16c	88	36
Perera *et al.* (1977)	B:Z	12h	92	30 (33)
Rao and Rao (1977)	D:Y	20h	80 (25)	31 (40)
Prasad *et al.* (1978)	A:Y	18h	83	47 (44)
Rao and Rao (1978)	B:Z	40c	100 (20)	52 (50)
Pathiraja *et al.* (1979)	B:Z	57c	96	39
Prasad *et al.* (1979*a*)	A:Y	18h	72	54
Prasad *et al.* (1979*b*)	B:Y	17h	88	53
Rao and Rao (1979*a*)	D:Z	50c	96 (60)	44 (47)
Singh *et al.* (1979)	A:Y	12c	92	45
Rajamahendran *et al.* (1980)	A:X	18c	17	67
	C:X	16c	77	80
Rao (1981)	C:Z	50c	96	46
Avenell (1982)	B:Y	16c	81	69
Khurana and Gupta (1982)	A:Y	22h	64	21 (17)
	B:Y	7h	86	17
Rao and Rao (1983)	C:Z	150c	93	41
	C:Z[D]	200c	75	25
El-Menoufy *et al.* (1986)	B:X	25c	92	52 (48)
	(b) Suboestrous animals			
Rao and Rao (1979*b*)	A:Z	36c	94	44
Singh *et al.* (1979)	A:Y	16c	75 (92)	25 (45)
Khurana *et al.* (1981)	A:Y	66c	77	29 (30)
Chauhan *et al.* (1982)	A:Y	8c	88	28
	A:X	17c	76	54
	B:Y	6c	83	20
	B:X	11c	54	67
	(c) Anoestrous animals			
Rao and Sreemannarayana (1983)	D:Y	50h	98	15
	D:Y	25c	100	36
Singh *et al.* (1983)	C:Z	30c	38 (0)	55
	D:Z	30c	70	71
Henniawati *et al.* (1985)	B:X	9h	22	50
	C:X	9h	67	17
Rao *et al.* (1985)	C:Y	15c	20 (15)	33 (33)
	D:Y	34c	62	52

[A]Hormone treatments: A = single injection prostaglandin; B = two spaced injections of prostaglandin; C = progestagen alone, D = progestagen with PMSG. Insemination methods: X = natural service, Y = artificial insemination relative to onset of oestrus, Z = artificial insemination at fixed times after treatment.
[B]h = heifer, c = cow.
[C]Parentheses show results from untreated animals.
[D]Treated during a season of normally low reproductive activity.

without oestrus) or anoestrus (ovarian inactivity) associated with high ambient temperatures and/or poor nutritive conditions. These factors, together with a long gestation period of 320–340 days (Jainudeen 1983), contribute to the problems of late sexual maturity (first calving at 3·5–4·5 years of age) and long calving intervals (1·5–2 years) which are considered to be the main causes of low reproductive rates.

Reasons for Controlled Breeding

The main stimulus for the development of controlled breeding in buffalo cows has come from the introduction of artificial insemination programs. These have been developed primarily for breed improvement, but have a secondary role in some countries such as Indonesia and Thailand in counteracting reproductive problems associated with insufficient numbers of breeding bulls among widely dispersed cow populations. As well as the convenience of inseminating at synchronized oestrus, controlled breeding has the potential advantage of reducing problems of oestrous detection by allowing fixed-time insemination or reducing the period of intensive observation needed to detect oestrus. The application of controlled-breeding techniques to suboestrous and anoestrous animals, irrespective of artificial insemination programs, has the potential to reduce age at first calving in heifers and calving intervals in mature cows.

Results of Controlled-breeding Investigations

Because considerations of controlled breeding have come later to buffaloes than to most other domestic ungulates, emphasis has been not on the development of new technology but on the application of known techniques of synchronizing ovulation and oestrus through the use of prostaglandin $F_{2\alpha}$ or progesterone, or their synthetic analogues. Various reports of treatments with exogenous hormones to control breeding in buffalo heifers and cows are presented in Table 1. Results from small numbers of animals given uncommon treatments such as oestradiol after prostaglandin injection or intra-uterine administration of prostaglandin (Chauhan *et al.* 1982) have not been included. Treatments which did not significantly affect the results such as comparisons between prostaglandin injection at different luteal stages of the oestrous cycle (Prasad *et al.* 1979*a*), between prostaglandin $F_{2\alpha}$ and synthetic analogue (Prasad *et al.* 1979*b*), and between different doses of prostaglandin (Jainudeen 1976*a*; Khurana *et al.* 1981; Khurana and Gupta 1982) have been pooled for simplicity of presentation. There were no clear differences in the degree of synchrony of oestrus between the various observations. Results refer only to synchronized oestrus recorded over periods of up to 10 days (and most commonly between 2 and 4 days) after prostaglandin injection or progestagen withdrawal, and do not include any subsequent returns to service.

With few exceptions, the levels of oestrus recorded throughout Table 1 can be considered satisfactory for animals in which detection of oestrus is generally considered to be a problem. It is uncertain whether or not hormone treatments enhanced the expression of oestrus in cyclic animals since there were few direct comparisons with untreated controls. More comparisons between treated and control animals were available for conception than for oestrus because several reports of conception rates did not include the relevant oestrous data. Although the number of comparisons is still relatively small, the absence in all cases of significant differences between treated and control animals indicates that the relatively low conception rates evident throughout Table 1 represent a general characteristic of buffalo production and are not a particular consequence of controlled breeding.

Some of the possible sources of variation between results in Table 1 could not be assessed because they were not specified in all observations. Variations in a range of management practices known to influence breeding efficiency in untreated cows (Harinadharao *et al.* 1979) would also undoubtedly affect responses to hormone treatments. Breed of cows was specified in most though not in all cases, but there is no evidence from these data, or from other sources, of fundamental differences in reproductive physiology between the different breeds. The season at which cyclic cows were treated was rarely specified, yet the differences recorded by

Rao and Rao (1983) between mature cyclic cows treated during the season of generally low reproductive activity and those treated during the normal breeding season indicate that this could have a marked effect on the outcome. Lactation *per se* may not affect reproduction (Ford 1982), but the observation by Pathiraja *et al.* (1979) that conception rates in cows which had calved 60–90, 90–120 and 120–150 days before prostaglandin treatment were 17, 36 and 56% respectively indicates that unspecified post-partum interval could contribute to variation in results.

Methods used to detect oestrus either were not specified or included varying combinations of behavioural observation (standing for mounting by bulls, increased restlessness, vocalization, or increased micturition) and clinical examination (changes in uterine tone, relaxation of the cervix, cervical mucus, or condition of the vaginal mucosa). This undoubtedly accounted for some of the variation between different observations. The 17% incidence of oestrus recorded from cow-standing behaviour by Rajamahendran *et al.* (1980) stands out as an exceptionally poor result for cyclic cows, yet the 83% incidence of oestrus recorded from both behavioural and clinical observations by Prasad *et al.* (1978) would have been only 11% if they had relied on cow standing behaviour alone. At the other extreme, nearly one-third of the 96% of cows detected in oestrus solely from clinical examination by Pathiraja *et al.* (1979) showed insufficient dilation of the cervix to allow passage of the inseminating pipette, and such cows would not necessarily be included within the definition of oestrus used by other authors.

Four possible sources of variation other than those discussed above can be identified from the data in Table 1 : (i) hormone treatment — single or double prostaglandin injection, or progestagen with or without pregnant mare serum gonadotrophin (PMSG); (ii) reproductive state (cyclic, suboestrous or anoestrous); (iii) maturity (heifer or cow); and (iv) method of insemination (natural service, artificial insemination relative to onset of oestrus, or artificial insemination at fixed times after treatment). It is difficult to assess the possible effects of these variables without some form of summary, but insufficient data are available to consider them all in a single interaction matrix. Instead, weighted means of pooled observations have been used first to assess the different hormone treatments in cyclic, suboestrous and anoestrous animals, and then to assess effects of maturity and method of insemination independently. Apparent differences between pooled means which are not necessarily balanced for other variables have been interpreted conservatively, with a possible consequence that some small but real differences have been disregarded.

Responses in Cyclic Animals

Weighted means for incidence of oestrus and conception rate respectively were 72 and 41% after a single injection of prostaglandin (seven observations with 127 animals), 94 and 41% after two injections of prostaglandin spaced 11 or 12 days apart (seven observations with 174 animals), 88 and 36% after treatment with progestagen alone (four observations with 416 animals), and 91 and 41% after treatment with progestagen and PMSG (two observations with 70 animals). These means show no clear differences between prostaglandins and progestagens as basic methods of synchronizing oestrus in cyclic buffaloes. The tendency towards a lower mean incidence of oestrus after a single injection of prostaglandin is not readily explicable because all of these animals had a palpable corpus luteum at the time of treatment. Also there was no evidence in suboestrous cows, discussed below, of any difference in incidence of oestrus after one or two prostaglandin injections. In any case, two spaced injections would normally be the preferred treatment because it caters for animals which are not in the luteal phase of the oestrous cycle at the first injection. If cost-saving became more important than the problem of detecting oestrus, however, a satisfactory outcome could probably be achieved by inseminating all cows detected in oestrus after the first injection, and giving a second injection only to those that did not respond to the first.

There was no indication from the limited data available that the effectiveness of progestagen treatment was influenced by type of progestagen or mode of administration. Nearly half of the animals contributing to the apparently lower mean conception rate in cows treated with progestagen without PMSG were from a single group treated during a season of normally low ovarian activity (Rao and Rao 1983), and there was no indication that progestagen treatment for cyclic cows in other circumstances would be enhanced by PMSG injection at progestagen withdrawal.

Responses in Suboestrous Animals

Pooled data for suboestrous cows treated with a single injection of prostaglandin (five observations with 143 cows) and with two spaced injections of prostaglandin (two observations with 17 cows) showed weighted mean incidences of oestrus of 82 and 64% respectively, and conception rates of 35 and 46% respectively. No examples of progestagen treatments for suboestrous cows were noted. Comparisons between single and double prostaglandin injections were of little relevance because of the small numbers of animals given the latter treatment. The responses to both generally showed little difference from those already described for cyclic cows.

There is no obvious reason why suboestrous cows showing regular cycles of ovulation without oestrus, and therefore presumably subject to normal endogenous prostaglandin secretion, should suddenly show oestrus after exogenous prostaglandin treatment. Jellinek and Avenell (1982) found no difference in the duration of prostaglandin-synchronized and spontaneous oestrus, though there was a non-significant tendency for cows to be mounted by vasectomized bulls more frequently during the former. Kamonpatana *et al.* (1979), on the other hand, reported that oestrous behaviour was less pronounced after prostaglandin treatment than one cycle later. No reports of prostaglandin treatment in suboestrous cows included control groups of untreated animals, and other factors such as increased intensity of observation could have contributed to the improved oestrous response. Harinadharao *et al.* (1979) recorded oestrous in 75% of cows in an improved management situation which included intensified observations for signs of oestrus, but no hormone treatments, during a period when no cows in a traditional management system were detected in oestrus.

Responses in Anoestrous Animals

Investigations of controlled breeding in anoestrous animals have concentrated on the use of progestagens, since there is no expectation of responses to prostaglandins in animals that do not have an active corpus luteum. The single report of oestrus and conception after prostaglandin treatment in anoestrous heifers (Henniawati *et al.* 1985) was considered to represent spontaneous activity rather than a response to prostaglandin. Weighted mean results from anoestrous animals treated with progestagens alone (three observations with 54 animals) and with progestagens followed by PMSG (four observations with 139 animals) showed incidences of oestrus of 38 and 84% respectively, and conception rates of 40 and 36% respectively. In contrast to the situation in cyclic animals, oestrus was induced more reliably in anoestrous animals by treatment with progestagens together with PMSG than by treatment with progestagens alone. Although weighted means from all observations showed no substantial increase in conception rate after PMSG, the two direct comparisons (Singh *et al.* 1983; Rao *et al.* 1985) both indicated that PMSG after progestagen treatment in anoestrous cows increased conception rate as well as incidence of oestrus.

These results refer mostly to seasonal anoestrus. The heifers studied by Rao and Sreemannarayana (1983) were all more than 4 years old, and their response to progestagen treatment at this age is unlikely to represent advanced onset of puberty. The cows studied by Rao and Sreemannarayana (1983) and Rao *et al.* (1985) were all more than 6 months post partum at the time of treatment. Other results not included in Table 1 indicate that progestagen treatments are not particularly effective in the earlier stages of post-partum anoestrus (Jainudeen *et al.* 1983), nor when anoestrus is a consequence of inadequate nutrition (I Gede Putu *et al.* 1986).

Effects of Maturity

Evidence from Rao and Sreemannarayana (1983) that anoestrous heifers treated with progestagen and PMSG showed a similar incidence of oestrus but lower conception rate than anoestrous cows given the same treatment appears to have general application. Pooled results from all data in Table 1 (weighted means from 10 observations with 182 heifers and from 25 observations with 967 cows) show a similar average incidence of oestrus of 81 and 83% for heifers and cows respectively, but a lower average conception rate in heifers (30%) than in cows (41%). This probably reflects a general tendency for lower fertility in young animals rather than a specific consequence of controlled breeding.

Effects of Insemination Method

Chauhan *et al.* (1982) found a lower incidence of oestrus but a higher conception rate from natural service than from artificial insemination in suboestrous cows treated with prostaglandin. This too appears to apply more generally. Pooled results from all data in Table 1 (weighted means from nine observations with 144 animals for natural service, and from 26 observations with 1005 animals for artificial insemination) show average incidences of oestrus of 70 and 84% and average conception rates of 51 and 38% from natural service and artificial insemination, respectively. A lower incidence of oestrus might be recorded for natural service than for artificial insemination because the former is absolutely dependent on the sometimes weak expression of behavioural oestrus, whereas the latter can use clinical as well as (or instead of) behavioural signs of oestrus to select animals for insemination. Lower conception rates from artificial insemination than from natural service could simply be a reflection of the relatively recent development of artificial insemination for buffalo cows, though in some cases it might also be related to reliance on clinical examination for oestrous detection. Thus Pathiraja *et al.* (1979) reported conception rates of 17 and 49% in oestrous cows which had closed and open cervices respectively at the time of insemination, and Rao and Sreemannarayana (1983) reported conception rates to artificial insemination of 11, 30 and 38% in cows classified as showing weak, normal or pronounced signs of oestrus respectively.

Artificial insemination at fixed times after hormone treatment appeared to be as effective as artificial insemination relative to onset of oestrus. The two methods of insemination have not been compared within a single observation, but pooled data from Table 1 show an average incidence of oestrus and conception rate of 87 and 39% respectively from fixed-time insemination (weighted means from 10 observations with 655 animals) and 79 and 35% from insemination relative to onset of oestrus (weighted means from 16 observations with 350 animals). All reports of fixed-time insemination used two inseminations, 72 and 96 h after prostaglandin injection or 48 and 72 h after progestagen withdrawal. Insemination practices related to onset of oestrus, where described, included both a single insemination and two spaced inseminations. Insufficient data were available to show any clear differences in conception rates from single or double inseminations, or from insemination with fresh, chilled or frozen semen.

Conclusions

There is ample evidence that oestrus in cyclic buffalo heifers and cows can be synchronized satisfactorily during the normal breeding season by treatments with either prostaglandins or progestagens. Oestrus and conception rates from the two types of treatment were similar, and any choice between them would be largely a matter of convenience and/or cost. There is no firm evidence that synchronizing treatments enhance the expression of oestrus, but detection of oestrus nevertheless may be facilitated by the reduced time period over which observations of oestrus need to be continued. Alternatively, they allow artificial insemination at fixed times after treatment without reference to oestrus. Conception rates from artificial insemination, based on numbers of animals detected in oestrus, were similar from fixed-time and oestrus-related inseminations, though both tended to be lower than conception rates from natural service.

The problem of suboestrus (ovulation without oestrus) can be overcome by treatment with prostaglandin. Whether or not progestagens would also be effective has not been investigated. There is no clear line of distinction between the specific condition of suboestrus and the more general problem of weak expression of oestrus in buffaloes, and intensified observations of oestrus as well as prostaglandin injection may be necessary for the successful treatment of suboestrous cows.

No investigations of hormone treatments to advance the onset of puberty in young heifers have bene noted. Limited investigations of hormone treatments in cows during the early stages of post-partum anoestrus have not been successful. It is clear, however, that progestagen treatments combined with PMSG injection at progestagen withdrawal can successfully induce oestrus and conception in seasonally anoestrous heifers and cows. This represents a possible means of reducing age at first calving in heifers and calving intervals in mature cows.

There remains a problem in both hormone-treated and untreated control animals of low conception rates, which rarely exceeded 60% and were most commonly less than 50% of animals detected in oestrus. This apparent barrier to further improvement in controlled-breeding techniques might best be investigated by combining future studies of hormone treatments with other known methods of improving reproduction in buffalo cows such as better feeding, reduction of heat stress, and greater intensity of oestrous dectection.

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