EFFECT OF BULL ON SPONTANEOUS AND INDUCED HEATS IN MEDITERRANEAN BUFFALOES BRED IN ITALY

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This study is the result of an observation effected on 70 buffaloes in the year 1986-1987.

After sexual promiscuity was interrupted from November to April so as to obtain spring calvings, heats not followed by an adequate luteal phase were present in about 33 % of buffaloes investigated (1).

Following a few heats, anoestrus occured and intensified at the approach of spring months.

The acyclic phase resolved when sexual promiscuity was resumed: 55 % and 77 % of buffaloes were found pregnant after 1 and 3 months, respectively.

These findings, confirmed by other 3 trials, allowed us to consider the possible effect of bull on female buffaloes. In a previous paper (2), we pointed out that the reproductive performance of buffaloes may be improved by the presence of a vasectomized bull. In fact, a higher incidence of heats, followed by an adequate luteral phase (68.7 % vs 55.3 %; P ≤ 0.01) as well as a higher fertility (46.9 % vs 27.4 %; P ≤ 0.01) are assessed in comparison with values in control females that were kept far from the male buffalo.

These results have prompted us to carry out further research to verify whether theabove-mentioned positive effects were to be attributed to a lower error incidence as to the choice of the time of insemination.

Therefore, we found it interesting to investigate the intensity of heats and the fertility of buffaloes seen to be buffalo, as compared to those kept under sexual promiscuity conditions and sniffed but not served by the buffalo bull, as well as those seen to be in heat, but bred in paddocs where the bull was not present.

Moreover, in relation to either the presense or the absense of the buffalo bull, we compared the fertility and the incidence of cyclic heats in both spontaneous and indused oestrus as well as oestrus percentages with single and double ovulation, respectively.

Oestrus cases with double ovulation are more difficult, as regards the assessment of the optimum time for insemination.

MATERIALS AND METHODS

The trial started on 1st December 1989 and ended on 15th March of the following year; it concerned 396 female buffaloes bred in 6 farms near the mouth of the Sele river (province of Salerno).

Sexual promiscuity was interrupted on 15th October and buffaloes were divided in two groups.

Each of these groups (B group = presence of the vasectomized bull; W group = absence of the bull) included non-pregnant buffaloes, pregnant lactating buffaloes and buffaloes that calved after 15th October. Groups were arranged by taking into account the farm requirements. In consequense a larger number of animals were present in groups including vasectomized bulls.

Heat was inducedwith alfaprostol (in dose of 8 mg per head) in buffaloes that did not show oestrus signs.

In each farm, observations were made from 8 to 12 a.m. and from 2 to 5 p.m. Buffaloes seen to be in heat in the morning, underwent rectal examination in the afternoon and viceversa.

Once heat was detected, animals were inseminated and explored every 12 hours until ovulation was assessed.

Moreover, 6 or 3 hours were deducted from the time when the fovea was observed, depending on whether a follice with high or low intracavitary pressure had been palpated during the previous exploration.

Buffaloes kept with the vasectomized bull, underwent per rectum examination even when service was not ascertained but heat signs were present or when buffaloes were sniffed by the bull. In these cases it was assumed that service might have occured overnight. Our study was thus based on this criterion, since it is difficult to verify that mating has really occured overnight, due to the present economic conditions of buffalo breeding in Italy.

In each case oestrus signs were recorded on special cards. behavioural changeswere classified according to the following 6 groups: type 1 (numerous feeble signs: isolation, mucous discharge, stare, Flheeman sign towards or from other female buffaloes); type 2 (intense signs: bellowing, homosexual mounting behaviour); type 3 (few feeble signs); type 4 ("silent" heat diagnosed in anoestrous buffaloespresentingboth follicle and uterus tonicity at per-rectum examination).

These first four types of heat (type A) were detected in all buffaloes, kept either in absence or in presence of a vasetomized bull.

With regard to buffaloes of B group, other two types of intense heat were considered and defined as type B: 1) sniffing with Flheeman sign by the male buffalo; 2) service.

This distinction has been made to compare fertility in buffaloes kept under sexual promiscuity conditions, but not detected by the bull, vs those included in the W group.

On the day of heat onset and after 8-10 days from ovulation either a milk or a blood sample was taken to verify whether progesterone values confirmed the state of oestrous and an adequte luteal phase, respectively.

These determinations (ELISA: Kit Immunosystem-SIBAR) were aimed to detect true and "cyclic" heats, "acyclic" heats (i.e. not followed by an adequate luteal phase: 4 ng/ml in milk; 1.5 ng/ml in blood plasma), as well as heats with onset in presence of progesterone and mid-cycle heats.

Oestrus was induced after corpus luteum examination in animals from wich a blood sample was also taken.

Date have been worked out by means of the chi-square test and ANOVA.

RESULTS

Table 1 shows the incidence of different spontaneous heat types in B group (buffaloes kept under sexual promiscuity conditions) and in W group (i.e. buffaloes kept in paddocks without a bull).

A compariison was made exclusively between B group buffaloes that were not detected to be in heat by the bull, and those included in the W group.

In table 1 (second part) B group females with type B heats (i.e. buffaloes sniffed or served by the vasectomized bull) areplaced together with buffaloes wirh type 2 intense heat signs (i.e. bellowing and homosexualmounting behaviour).

Then these animals are compared with those belonging to the W group.

Within type A signs the incidence of behavioural phenomena in B group buffaloes does not differ from that found in the W group animals, although the latter show a higher percentage (23.8 % vs 16.5 %) of silent heats.

However a statistical significance of this difference in values does not result from the chi-square test.

If buffaloes that were sniffed or served by the bull are included in type 2 behavioural changes (intense heat signs), an enhanced liveliness is found in the

B group as compared to the W group: a lower incidence of silent heats (7.4 % vs 23.1 %; P ≤ 0.01) and feeble heats (5.8 % vs 17.6 %; P ≤ 0.01) as well as a higher incidence of intense behavioural changes (78.6 % vs 45.0 %; P ≤ 0.01).

Table 1 also shows the incidence values of heats exhibited in the presence of progesterone (P4); the incidence of these heats averages 11 %.

However, a 3.4 % incidence is observed in buffaloes that are seen to be served by the bull.

The percentage of cyclic females does not differ among groups.

Howevwr, the B group buffaloes that showed intense signs of heat and were sniffed or served by the bull (type 2+B) havebeen found more cyclic than those belonging to the W group, although the latter had the same signs as the B group (66.5 % vs46.3 %; P ≤ 0.05).

Table 2 shows fertility values calculated on the number of A.I. as well as A.I. calculated on cyclic animals.

Buffaloes kept under sexual promiscuity conditions but undetected by the bull while being in heat (type A heat signs) reveal a higher fertility (46.3 % vs 18.9 %; P ≤ 0.01) particularly when intense heat signs occur (78.3 % vs 30.0 %; P ≤ 0.05) and when the latter is calculated on cyclic heats (pregnancies/AIC : 68.9 % vs 32.3 %; P ≤ 0.01).These values suggest that the most positive results obtained do not depend on a better choice of the insemination time but on other factors.

More over, buffaloes with type B heat signs (i.e. sniffing and service) showed a fertility rate which is lower (39.2 %) than that (46.3 %) of buffaloes belonging to the same group but exhibiting type A behavioural changes (i.e. heat signs not followed by sniffing or service by the bull).

Out of 243 heats in buffaloes, 55.1 % of oestrous females were detected by the male buffalo whereas sniffing or service occurred in 23.9 % and in 31.3 % of observations, respectively.

Exclusively in oestrous cases with single ovulation, where dehiscence of the folliclewas assessed it was possible to verify (table 3) that sniffing (Flheeman sign) occurs on average 23 hours before ovulation. A second Flheeman sign was observed in 8.5 % heats (5/59), usually 2.6 hours after ovulation.

Service occurred 17.68 hours before ovulation and was repeated (5/56 = 8.9 % of oestrous cases) at an interval 7.8 hours (before ovulation).

In double ovulations the first Flheeman sign (14 cases) was observed 7.64 h and 36 h defore dehiscence of the first and of the second follicle,respectively. The second Flheeman sign was detected (1 case) 8 h after and 24 h defore rupturing of the first and of second follicle,respectively.

First service occurred (9 cases) 5.89 h and 37.34 h before dehiscence of the first and second follicle,respectively.

Second service (3 cases) came about 45.67 h after and 20 h before repturing

of the first and of the second follicle, respectively.

Results reported above (Flheeman and service time related to ovulation) have to be considered as aproximate values, since standart deviation values are very high.

With regard to the ovulation time in induced heats, exclusively in case of single ovulation, an early dehiscence of the follicle was observed in fuffaloes kept under sexual promiscuity conditions: within 107 h of treatment, dehiscence of the follicle occured on average 87.9 h and 98.8 h in animals belonging to B group and W group, respectively (P ≤ 0.01). In these groups, 86.3 % and 70.3 % of ovulations were recorded within 107 h of treatment (P ≤ 0.01).

Out of 30 pregnancies which occured in induced heat cases with double ovulation, 12 (38.7 %) and 11 (35.5 %) were diagnosed, respectively, in the uterine horn ipsilateral to the ovary where dehiscence of the 1st and 2nd follicle occured; in other 7 pregnancies, the two follicles ruptered at a short interval. In spontaneous heat cases, 85.71 % of pregnancies (12/14) were presented in the uterine horn corresponding to the ovary where dehiscence of the second follice was observed.

Table 4 shows the number of the heats (N), insemination (AI), and inseminations effected on buffaloes subsequentlyfound to be cyclic (AIC); number ofpregnancies (P) in buffaloes of both the B group and the W group, are also reported in same table. Values are recorded with reference to heats characterized by single and double ovulation, respectively; separate values are also reported with regard to spontaneous and alfaprostol-induced oestrous.

In our study, a higher incidence of heats with double ovulation, as described in previous paper (3) was observed in induced heats (24.6 % vs 15.0 %; P ≤ 0.01) and was not affected by the bull's presence.

Fertility rates, calculated on all inseminations as well as on those conserning buffaloes subsequently found to be cyclic, were higher in heats with double ovulation (P ≤ 0.05). This increase in fertility was enhansed in W group buffaloes (22.5 % vs 47.8 %; P ≤ 0.05) as well as in induced heats (40.1 % vs 60.0 %; P ≤ 0.05).

Higher fertility rates (46.6 % vs 27.2 %; P ≤ 0.01) in buffaloes kept in the presence of a vasectomized bull, are generaly recorded in cases of heat with single ovulation (44.5 % vs 22.5 %; P ≤ 0.01).

The effect of bull was less evident in double ovulation. Although this phenomenon seems to occur in spontaneous heats, the chi-square test does not reveal differences due to the limited number of observations (48.0 % vs 22.2 %; N.S.).

DISCUSSION

The presence of the vasectomized bull enhanced the excitement of the group animals, thus facilitating heat detection.

Detection of buffaloes in heat by the bull occurs in 55 % of cases but cannot be used as a reference datum for A.i., when observations are made exclusively in day-time.In fact, buffaloes kept in sexual promiscuity conditions and seen to be in heat (by the researchers) but undetected by the bull show fertility rates which are sightly higher than those observed in females that are detected in heat by the bull.

These observations suggest that the more favourable results obtained in buffaloes bred together with the vasectomized bull, as compared to those kept far from the bull,have to be atributed to the male effect rather than to abetter choise of the A.I. time, at least in this series of trials. Moreover, in some cases, buffaloes had already ovulated when they were sniffed by the bull.

A further evidence of improved fertility is given by our trials on buffaloes kept in sexual promiscuity conditions and inseminated at scheduled intervals (every 24 h until ovulation), after heat was induced with alfaprostol.

The highest incidence of cyclic heats (AIC), in both spontaneous and induced oestrus, is one of the factors which contribute to the increase in fertility, observed in buffaloes kept with the vasectomized bull.

Finally, an interesting result is the improved fertility observed in double ovulations, particulary in induced heats.However, the higher incidence of double ovulations might lead to an unfavourable outcome, unless attention is paid to the fact that, in spontaneous heats, the "most fertile" follicle is the second one. We evidenced this finding in a previous study concerning a lower number of case reports (3).

This rule cannot be applied to induced heats; in fact, the dehiscence of two follicles generally occurs at a 29-hours interval, i.e., a period of time which exceeds the mean life of the spermatozoon in the female's genital system.

Therefore, a careful per-rectum examination is required even in induced heats with regard to both maturation of the follicle and insemination; the latter has to be carried out until ovulation occurs.

CONCLUSIONS

The male presence was an essential factor in this research. We belive that selective breeding has concerned buffaloes more recently than cows.

Moreover, the new breeding methods which do not take into account the physiological requirements of the buffalo species, cannot be easily applied to the

Table 1 – *Numerousness and incidence of different types of oestrous signs, oestrus with onset in the presence of Progesterone (P4) and cyclic oestrus in groups of buffaloes kept with (B) or without (W) vasectomized bulls*

GROUP	HEATS				HEATS WITH P4				CYCLIC HEATS		CYCLIC HEATS/HEATS	
	B		W		B		W		B	W	B	W
TYPE OF SIGNS:	N	%	N	%	N	%	N	%	N	N	%	%
Numerous (Type 1)	20	18.3	13	14.3	1	5.0	1	7.7	13	8	65.0	61.5
Intense (Type 2)	57	52.3	41	45.0	5	8.8	3	7.3	37	19	64.9	46.3
Feeble (Type 3)	14	12.8	16	17.6	3	21.4	6	37.5	10	12	71.4	75.0
Silent heats (Type 4)	18	16.5	21	23.1	3	16.7	1	4.8	10	13	55.6	61.9
TOTAL A	109	100.0	91	100.0	12	11.0	11	12.1	70	52	64.2	57.1
Bull Sniffs	76	31.3			10	13.2			48		63.2	
Bull Serves	58	23.9			2	3.4			42		72.4	
TOTAL B	134	55.1			12	9.0			90		67.2	
Type 1	20	8.2	13	14.3	1	5.0	1	7.7	13	8	65.0	61.5
Type 2+B	191	78.6A	41	45.0B	17	8.9	3	7.3	127	19	66.5a	46.3b
Type 3	14	5.8A	16	17.6B	3	21.4	6	37.5	10	12	71.4	75.0
Type 4	18	7.4A	21	23.1B	3	16.7	1	4.8	10	13	55.6	61.9
TOTAL	243	100.0	91	100.0	24	9.9	11	12.1	160	52	65.8	57.1

Different letters on the some line show significant differences for $P < 0.05$ (small letters) and for $P < 0.01$ (capital letters).

Table 2 Spontaneous heats: pregnancy (P) percentage calculated on the number of insemination (P/A.I.) and insemination on cyclic animals (P/A.I.C.) in 2 groups of female buffaloes kept with (B) of without (W) vasectomized bulls, in relation to the different types of manifestation

GROUP	B AI	B AIC	B P	W AI	W AIC	W P	B P/AI %	W P/AI %	B P/AIC %	W P/AIC %
Type of signs:										
Numerous (Type 1)	13	8	5	9	6	2	38.5	22.2	62.5	33.3
Intense (Type 2)	35	23	18	20	10	3	51.4a	15b	78.3a	30b
Feeble (Type 3)	8	6	3	11	8	4	37.5	36.4	50	50
Silent heats (Type 4)	11	7	5	13	7	1	45.4	7.7	71.4	14.3
TOTAL A	67	45	31	53	31	10	46.3A	18.9B	68.9A	32.3B
Bull sniffs	39	30	15				38.5		50	
Bull serves	40	31	16				40		51.6	
TOTAL B	79	61	31				39.2		50.8	
Type 2 + B	114	85	49	20	10	3	43a	15b	57.6	30
Type 1 + 3 + 4 of A	32	21	13	33	21	7	40.6	21.2	61.9	33.3
TOTAL A + B	146	105	62	53	31	10	42.5A	18.9B	59A	32.3B

Different letters on the same line show significant differences for $P < 0.05$ (small letters) and for $P < 0.01$ (capital letters)

Table 3 Hour-interval between sniffing (Flheeman sign) and service with ovulation as well as between ovulation and treatment with alfaprostol (Y-O) in heats with single and double ovulation

	SINGLE OVULATION			DOUBLE OVULATION					
				1st double ovulation			2nd double ovulation		
	N	x	ds	N	x	ds	N	x	ds
1st Flheeman	59	23.2	21.7	14	7.6	24.1	14	36.0	45.3
2nd Flheeman	5	-2.6	26.4	1	-8		1	28.0	
1st service	56	17.7	28	9	5.9	14.9	9	37.3	47.4
2nd service	5	17.8	15.7	3	-45.7	46.6	3	20.0	28.8
T – O:									
Bull (Group B)	117	87.9	23.2A	44	80.1	20.5	44	106.2	27.9
Without Bull (Group W)	6	98.8	31.1B	15	74.4	20.5	15	111.9	27.8

Different letters on the same column show significant difference for $P < 0.01$

Table 4 Number of heats (N), inseminations (AI), pregnancies (P) and fertility rates (P/AI) subdivided in relation to single and double ovulations in alfaprostol-induced and spontaneous heats in groups of buffaloes kept with (B) or without (W) vasectomized bulls

GROUP	% DOUBLE OVULATIONS: INDUCED HEATS N	INDUCED HEATS %	SPONTANEOUS HEATS N	SPONTANEOUS HEATS %	INDUCED HEATS: SINGLE OVULATION AI	SINGLE OVULATION P/AI %	DOUBLE OVULATION AI	DOUBLE OVULATION P/AI %
B	161	27.3A	243	15.2B	99	48.5A*	36	58.3
W	79	19.0	91	14.3	58	25.9B* A	14	64.3B
	240	24.6A	334	15.0B	157	40.1 a	50	60.0b

GROUP	SPONTANEOUS HEATS: SINGLE OVULATIONS AI	SINGLE OVULATIONS P/AI %	DOUBLE OVULATIONS AI	DOUBLE OVULATIONS P/AI %	SINGLE OVULATIONS AI	SINGLE OVULATIONS P/AI %	DOUBLE OVULATIONS AI	DOUBLE OVULATIONS P/AI %	TOTAL AI	TOTAL P/AI %
B	121	41.3A*	25	48.0	220	44.5B*	61	54.1	281	46.6A*
W	44	18.2B*	9	22.2	102	22.5A* a	23	47.8b	125	27.2B*
	165	35.1	34	41.2	322	37.6a	84	52.4b	406	40.6

Different letters on the same line show significant difference for $P < 0.05$ (small letters) and for $P < 0.01$ (capital letters). Different asterisk letters on the same column show significant differences for $P < 0.01$.

latter, particulary to buffaloes that have always reproduced naturally.

It is likely that both selection and isolation of female buffaloes from the male since puberty will contribute to change behavioral attitudes which, in our study, have affected fertility.

ABSTRACT

The aim of this study was to investigate fertility of buffaloes artificialy inseminated and kept in presence of vasectomized bull (B group) in comparison with that of buffaloes bred in paddocs without vasectomized bulls (W group). Buffaloes belonging to the B group showed a higher incidence of both cyclic heats (68.2 % vs 55.3 %; $P \leq 0.01$) and pregnancies (46.9 % vs 27.4 %). These better results have not to be attributed to a more timely insemination as related to ovulation. In fact, high fertility values were also found in cases of induced heat as well as in buffaloes kept under sexual promiscuity conditions and seen to be in heat (by the researcher) but undetected by the bull. During our trial, a higher incidence of oestrous with double ovulation was found in induced heats, as compared to spontaneous ones (25.5 % vs 14.9 %; $P \leq 0.01$). Fertility rates in oestrous withdouble ovulation were higher (52.4 % vs 37.6 %; $P \leq 0.05$) in comparison with cases of oestrous with single ovulation.

However, this outcome cannot be considered as positive, since a 29-hour interval is generally observed, in induced heats, between dehiscence of the first and of the second follicle, respectively. In this case, repeated observations are required to detect the optimum time of insemination.

BIBLIOGRAPHY

1) Zicarelli L., Campanile G., Infascelli F., Esposito L. (1988)-Riv.Zoot.Vet. 16.1.21-31.
2) Esposito L., Nizza A., Galasso D., Lavorgna L., Zicarelli L. (1990) - Atti XLIV Conv.Naz.SISVet Stresa 27-29 settember (to be printed).
3) Zicarelli L., Campanile G., Infascenelli F., Esposito L., Ferrari G. (1988) - Proceeding of II world buffalo congress (held in India. 12-16 december) Vol. III, 57-62