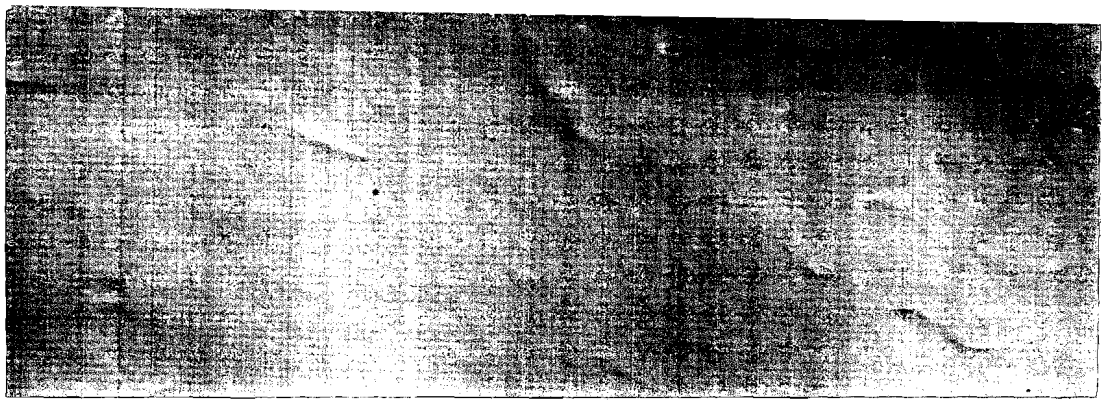

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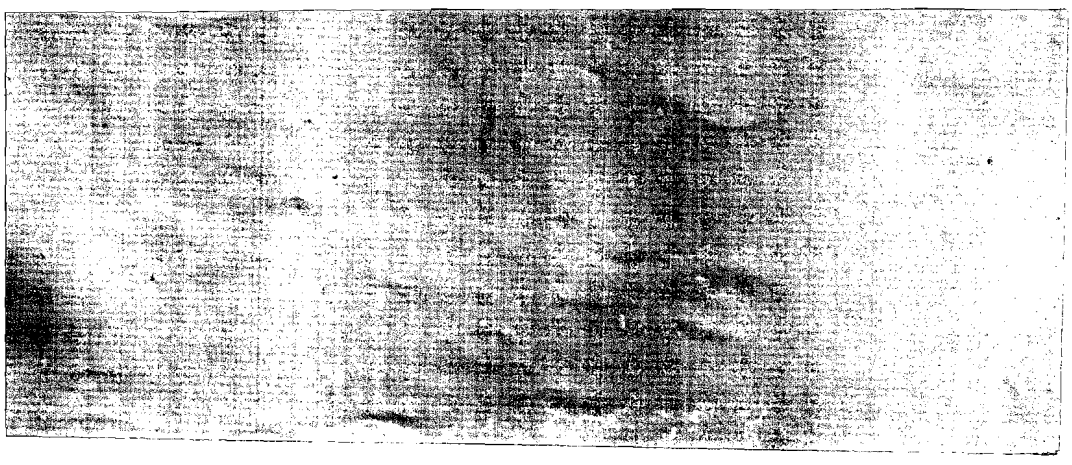

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SYNCHRONIZATION OF OVULATION AS A FIELD TRIAL FOR IMPROVEMENT OF FERTILITY IN BUFFALOES

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ABSTRACT □ A total of 82 postpartum buffalo cows were used in the present work. These animals were assigned into 5 random groups. The 1st one (n=15) was inseminated with frozen buffalo semen during their natural heat and used as a control group. Ovulation in the females of the 2nd group (n=16); 3rd group (n=17); 4th group (n=19) and 5th group (n=16) were synchronized by i.m injections of GnRH and $PGF_2\alpha$. They inseminated at 0 - 8, 9 - 16, 17 - 24 and 25 - 32 hours respectively.Milk progesterone porfile was determined in all groups studied, starting from 0-time of insemination up to 28th days post insemination. The highest pregnancy rates of 66.67 and 55.56% were recorded for the 1st and 4th groups respectively. Meanwhile, lower rates were observed for animal in the 2nd, 3rd and 5th groups respectively. The rest of buffalo-cows did not observed in heat for 3 months post-partum and were considered suboestrous as indicated by rectal palpation of ovarian follicle.

Moreover, irrespective of animals groups and time of insemination the widening of the cervix at the time of insemination might affect the pregnancy rate. It was 56.52%, 42.85% and 6.45% in buffaloes with medium, low and high difficulty to pass the inseminating gun through cervix respectively.

Regarding the level of progesterone hormone in milk, it was very low during estrous phase and very high on 21st to 28th days post insemination in non-returned animals.

INTRODUCTION

Fertility in farm animals is a multifactorial phenomenon being influenced directly or indirectly by management and genetic disposition *(Sirvastava et al., 1998)*. Estrous detection and proper time of insemination consider the best striking single managemental factor for success of artificial insemination program *(Rounasville et al., 1979)*. In buffaloes, the use of artificial insemination is limited due to difficulty in determination of proper time of artificial insemination *(Borghese et al., 1996)*. Difficulties in practicing an artificial insemination in buffaloes are caused mainly by the fact that the buffalo cows have undetected signs of estrus without the presence of teaser bull *(Moiou et al., 1998)*. However, inseminating buffalo cows during

earlier or later times of estrus may result in poor conception ***(Sirvastava et al., 1998).*** Recently, the implementation of timed insemination program eliminate the need for estrus detection ***(Burke et al., 1996; Schmitt et al., 1996; Stevenson et al., 1996 and Parsley et al.,1998).*** The present investigation was designed to determine the optimum time of artificial insemination following the synchrony of ovulation in buffaloes.

MATERIAL AND METHODS

This trial was conducted on 82 postpartum buffalo cows (4-8 years old), belonged to 2 large dairy herds at Berket El-Saba, Menoufia Governorate. These animals were milked twice daily, housed in open yards, maintained under nearly similar feeding and management regimes. The females were thoroughly examined and proved to be free from any detectable genital tract abnormalities. The animals were assigned into one of the following groups: Females in the first group (n=15) used as a control and inseminated with frozen semen during the 2^{nd} half of their natural heat. Buffalo cows in 2^{nd} (n=l6) 3^{rd} (n= 17); 4^{th} (n=18) and 5^{th} (n= l6) groups did not observed in heat for 3 months post-partum and were considered suboestrous buffaloes proved by presence of ovarian structures on rectal palpation. A suggested scheme for synchroniztion of ovulation based on intramuscular injection of 20 mg buserlin (Gn-RH analogue, Receptal, Hochest laboratories) followed 7 days later by 25 mg PGF_2 α I.m (lutalyse, upjohn, Kalamazo, MI, USA), followed 2 days later with a second dose of Gn-RH. (20 mg of buserlin) and inseminated at 0-8; 9-16; 17-24 and 25-32 hours after the second injection of Gn-RH in animals of 2^{nd} , 3^{rd} , 4^{th} and 5^{th} groups respectively. Heat was detected twice daily for females in the first group (natural heat) using tail pulse, vulvar edema and mucous discharge and confirmed by ovarian palpation. The degree of difficulty to pass the inseminating gun through the cervix of buffalo cows in all groups was recorded at time of insemination. Pregnancy was diagnosed rectally 45-60 days after insemination.

Milk sampling and progesterone assay:

Milk samples (20 ml) were collected from all buffalo cows at the time of insemination, then collected once weekly during the 1^{st} month after insemination. The progesterone hormone was extracted from the whole milk by diethyl ether ***(Lamming and Bulman., 1976 and Shawki, 1989).*** The extracted samples were stored at —20C. The progesterone was radio immuno-assayed using 1^{125} ***(Laitinen et al., 1985).***

Data were statistically analyzed using statistical analysis system (**SAS**) (1987).

RESULTS

Results are obtained in Table 1, 2 and 3.

Table (1): Time of artificial insemination and Pregnancy rate in buffalo cows.

Animals group	Time interval* (hours)	Total animal inseminated	Pregnancy rate
First	11-20	15	10 (66.675)[a]
Second	0-8	16	0(0.00%)[b]
Third	9-16	17	6 (35-29%)[a]
Fourth	17-24	18	10 (55.56%)[a]
Fifth	25-32	16	4 (25.00%)[a]

Means with different superscripts are significantly different at level (P<0.05)
*Time interval after onset of estrus in the 1st group and the interval after the second injection of Gn-RH in 2nd 3rd 4th and 5th groups.

Table (2): Time of insemination, degree of difficulty to pass the insemination gun through the cervix and pregnancy rate of buffalo cows.

	High-difficulty		Medium-difficulty		Low-difficulty	
	Total female inseminated	Pregnant females	Total female insemi-nated	Pregnant females	Total female insemi-nated	Pregnant females
First group	3	0(0.0%)[c]	7	4(57.14%)[b]	5	3(40.0%)[bc]
Second group	8	0(0.0%)[c]	4	0(0.0%)[c]	4	0.(0.0%)[c]
Third group	6	0(0.0%)[c]	4	3(75.0%)[a]	7	3(42.86%)[b]
Fourth group	4	1(25.0%)[bc]	6	5(83.33%)[ab]	8	4(50.0%)[b]
Fifth group	10	1(10.0%)[bc]	2	1(50.0%)[b]	4	2(50.0%)[b]
Overall	31	2(6.45%)[B]	23	13(56.52%)[A]	28	12(42.85%)[A]

Means with different small or capital alphabetical superscripts in each category are signifi different at level (P< 0.05).

Table (3): Milk progesterone profile during the first month after insemination of buffalo cows (M ± SE).

	n	At insemination	7th day post insemination	14th day post insemination	21st day post insemination	28th day post insemination
First group	15					
Non return	10	1.88±0.59^{f}	8.86±2.58^{cd}	12.68±3.12^{abc}	15.48±4.46^{abc}	18.66±4.18^{ab}
Return	5	2.44+0.78^{ef}	6.92±2.11^{de}	9.86±2.98^{cd}	2.54±0.81^{ef}	7.48±1.88^{d}
Second group	16					
Non return	0	--	--	--	--	--
Return	16	2.68±0.86^{ef}	7.68±2.92^{de}	9.82±3.24^{cd}	2.84±0.78^{ef}	8.16±2.18^{cd}
Third group	17					
Non return	6	1.92±0.78^{f}	10.14±3.68^{bc}	14.26±3.78^{abc}	16.84±4.12^{abc}	19.46±3.48^{a}
Return	11	2.72±0.82^{ef}	8.54±2.76^{bc}	10.92±2.96^{ac}	2.62±0.78^{ef}	7.24±1.86^{bc}
Fourth group	18					
Non return	10	1.84±0.72^{f}	9.46±3.26^{cd}	13.68±3.18^{abc}	17.18±3.46^{abc}	20.92±3.68^{a}
Return	8	2.56±0.94^{ef}	8.22±2.62^{d}	11.4±82.68^{bc}	2.78±0.88^{ef}	7.84±2.18^{bc}
Fifth group	16					
Non return	4	2.19±0.86^{ef}	9.22±2.86^{cd}	12.82±3.38^{abc}	16.36±2.98^{abc}	18.64±3.22^{a}
Return	12	2.92±0.94^{ef}	7.12±2.38^{de}	9.76±2.84bc	3.16±0.92ef	6.48±1.76^{a}

Means with different superscripts are significantly different at level (P<0.05)

DISCUSSION

Ovulation in buffaloes has been reported to occur 11-18 hours after the end of estrus ***(Kanai and Shimiza, 1986 and Raut and Kadu, 1990).*** Accordingly, the present work indicated that the maximum (66.67%) pregnancy rate was found in females inseminated during the 2nd half of estrus and those inseminated 17-24 hours after the 2nd injection of Gn-RH (55.56%). A much lower values (35.29% and 25.00%) were recorded for the pregnancy rate in females artificially. inseminated 9-16 and 25-32 hours after the 2nd injection of Gn-RH respectively. Buffalo cows inseminated at 0-8 hours after injection of Gn-RH had no pregnancy. These findings are in consistent with a high level of milk progesterone at this time as presented herein and this might be due to the early insemination (i.e long time before ovulation) which occurred immediately after the 2nd Gn-RH injection of the 2nd group of animals. This data supported by the findings of ***Sirvastava et al. (1998).*** Moreover,

the ovulation time in cattle was reported to occur 24-32 hours after the 2nd injection of Gn-RH (***Pursley et al. 1995***), as well as post initiation of estrous phase (***Walker et al. 1996***). The insufficient LH peak and a delayed luteinization are contributing causes of unsuccessful insemination (***Moili et al. 1998***).

Concomitantly with our findings, the maximum conception rate was observed in buffalo cows inseminated 18-24 hours after the onset of heat symptoms (***Singh and Singh, 1988; Vale, 1988; Barile et al. 1996; Grudeli et al. 1996 and Sirvastava et al. 1998***). On the contrary, ***Jacomini (1989)*** and ***Baruseli (1992)*** recording high conception rate in buffalo cows inseminated shortly after the onset of heat. On the other hand ***Pursely et al (1989)*** did not find any significant differences between pregnancy rates of four groups of lactating cows inseminated at 0, 8, 16 and 24 hours after the 2nd injection of Gn-RH, but a low conception rate was observed in caws inseminated 32 hours after the 2nd Gn-RH injection.

In complete agreement with our results, ***Pandit and Chauhan (1998)*** pointed that, milk progesterone assessment was used with great success in detecting the estrus and pregnancy in buffalo cows. The minimum levels of progesterone hormone reported herein were estimated at the time of insemination in all animal's groups particularly those animals inseminated 17-24 hours after the 2nd Gn-RH injection. Moreover, the maximum milk progesterone content was determined on 21st and 28th days post insemination in non returned buffalo cows. Similar results were recorded by ***Shawki et al. (1994)*** in buffalo cows.

In general, the pregnancy rate was affected by the degree of difficulty to pass the inseminating gun through the cervix ***(Grudeli et al., 1998).***

Consequently, our findings revealed that the maximum pregnancy rate (56.52% and 42.85%) were obtained in all groups of buffalo cows with medium and low difficulty to pass the inseminating gun respectively. While the minimum pregnancy rate (6.45%) was found in females with high difficulty. However, the highest pregnancy rates (83.33 and 75.00%) were recorded in buffalo cows with medium difficulty to pass the inseminating gun through the cervix in those animals which inseminated 9-16 and 17-24 hours after the 2nd injection of Gn-RH. This may be returned to the lower values of progesterone content in the milk in these groups of animals at time of insemination with corresponding high levels of estrogen which might result in wide opening of the cervix.

In conclusion, the injection of Gn-RH and $PGF_2 \alpha$ as used in the current study might be agood helpful for treatment of subestrous buffaloes especially when inseminated at 17-24 hours after the 2nd injection of Gn-RH.

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