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FACTORS AFFECTING THE EFFICIENCY OF ARTIFICIAL INSEMINATION IN CATTLE AND BUFFALO IN PUNJAB, PAKISTAN

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ABSTRACT

The main objective of this study was to identify the factors that affect the success of artificial insemination (A.I) services under field conditions. The data from a total of 459 inseminations were analyzed. The effects of farm, animal, semen/bull and A.I. technician on conception rate were studied. Milk progesterone concentration was used as an indicator of conception. Milk samples were collected from animals at day 0, 11 and 22 post-insemination and analysed for progesterone (P_4) concentrations using radioimmunoassay (RIA) kits. Results indicated that the overall conception rate through A.I. under field condition was 29%. Among the farm-related factors, only region had a significant effect on conception rate ($P<0.01$). Nutrition had a significant effect on conception ($P<0.01$). Housing system and the time interval from first heat signs to A.I. had no effect on the conception rate ($P>0.05$). Animals inseminated within 24 hours after the onset of estrus had a higher, though statistically insignificant, conception rate than those inseminated after 24 hours. Among the animal-related factors, species, milk production, body condition score (BCS), lactation state, heat signs and uterine tone had a significant effect on conception rate. The conception rate in buffaloes was higher than in cattle ($P<0.05$). Animals with the higher BCS had a better conception rate than those having lower condition. Conception rate in the milking animals was more than the dry ones ($P<0.05$). Animals showing the passage of mucus from external genitalia ($P<0.05$) and marked uterine tone ($P<0.01$) showed better conception rate. Among the semen/bull related factors, bull breed, semen type, quality and source had a marked effect on conception rate. The conception rate was higher with the semen of cross bred and buffalo bulls ($P<0.05$). The conception rate with liquid semen was high and low with frozen semen ($P<0.01$). Good quality semen yielded higher conception rate than poor quality semen ($P<0.01$). The conception rate varied significantly due to A.I. technician ($P<0.01$). In conclusion, there is a tremendous scope to improve the existing A.I. technology in field through improved management of both animal and farm, supply of high quality frozen semen and enhancement in insemination skill of A.I. technicians.

Key Words: Milk progesterone, artificial insemination, cattle, buffalo, conception rate

INTRODUCTION

Artificial Insemination (AI) in cattle and buffaloes has potential benefits in genetic improvement and costs of production. The degree to which these benefits are realized depends upon the efficiency of the AI system. The latter is influenced by many factors including the efficiency of estrus detection (Foote, 1975), nutrition (Ferguson and Chalupa, 1989), environment and stress factors (Braden and Moule, 1964).

In Pakistan, at the moment, about 4% adult buffaloes and about 14% cattle are bred through AI and the rest are served through natural mating or even remain not served due to shortage of bulls (Ahmad and Saji, 1997). The overall conception rate through AI in cattle and buffaloes ranges from 38 - 62% (Saji, 1984). Problems in the field related to AI services include poor awareness of farmers, unskilled technician, irregular supply of liquid nitrogen, long distance, inappropriate detection of heat and insemination at the wrong time. The early diagnosis of cyclicity and pregnancy can also improve the efficiency of AI services. Radioimmunoassay of progesterone in milk as an aid to such diagnosis has been demonstrated (Ball and Pope, 1975) and shown to be a good method of early pregnancy diagnosis (Laing and Heap, 1971). The present study was conducted to investigate the efficacy of AI in Punjab, Pakistan using the radioimmunoassay of progesterone and to identify the factors responsible for poor fertility under field conditions.

MATERIALS AND METHODS

Animals and sites

A total of 459 animals, including 280 cows and 179 buffaloes, brought for AI during 1994 and 1995 were assessed. The farmers were interviewed about their farm management and breeding history of their cows or buffaloes at the time of insemination. The body condition score was recorded at the time of insemination for each animal (1 to 5 scale; 1= thin and 5= obese) (Butler and Smith, 1989). The study was conducted at four different locations, viz., Bahadurnagar, Daultala, Faisalabad and Gujar Khan. Eighteen A I technicians participated in the study.

Bulls and semen

Semen from bulls of different species and breeds (Nili-Ravi buffalo, Sahiwal, Dhanni, Friesian, Jersey and crossbred cows) was used in this study. Frozen semen (extender: Sodium Citrate, freezing method: vapor freezing) produced by the Semen Production Unit (SPU), Government of the Punjab, was used for insemination of buffaloes and cows at Artificial insemination Center (AIC's), Daultala and Gujar Khan. At the University of Agriculture, Faisalabad, fluid semen (extender based on skim milk) was used. Based upon sperm motility and acrosome morphology, semen was categorized either as good (motility: >50%, % intact acrosomes:>70%) or not good (motility: <50%, acrosomes intactness: ,<70%).

Milk sampling and processing

Milk samples (5 ml) were collected at day 0 (day of insemination) and days 11 and 22 post AI from animals in a tube containing sodium azide tablet (0.1 g; Merck, Germany). These samples were brought to the laboratory in an ice cooler (4°C), centrifuged at 3000 r.p.m for about 15 minutes and placed in refrigerator for 15 minutes. Fat was removed and skim milk was collected in storage vials. The samples were stored at -20 °C until used for progesterone assay.

Milk progesterone radioimmunoassay

Concentrations of progesterone (P_4) in milk were assayed using radioimmunoassay (RIA) kits. Standards of P_4 prepared in skim milk ranged from 0-40 nmol/L in concentration. The assay used was a non-extraction solid phase type utilizing antibody coated tubes and I^{125} - labeled P_4. Sensitivity of the assay was 0.125 nmol/L Maximum binding of the assay ranged from 38 to 47%. The inter- and intra-assay coefficients of variation were 15 and 10%, respectively.

Statistical analysis

The proportionate data on effect of farm (region, feeding system, housing system, and timing of insemination), animal (species, milk production, body condition score and lactation state), estrus sign (heat signs, type of mucus, swelling of vulva and uterine tone) species/ breed (bull breed/ species, type of semen, semen quality and source of semen) and technician (level of education, site of deposit, and passage of A I gun) on conception rate was analysed using Chi-square statistics (Steel and Torrie, 1980). The overall data was organized and calculated by using Artificial Insemination Database application (AIDA), software provided by the FAO/IAEA.

RESULTS AND DISCUSSION

Milk progesterone and reproductive characteristics

A total of 181 animals were available in which three milk samples could be collected. This constituted about 40% of the total animals surveyed. Conception rate to a single insemination was 35% in buffaloes and 38% in cattle. Concentration of milk progesterone was low on Day 0, high on Day 10-12 but declined on Day 22-24 in 11% of buffalo and 8% cattle. These animals did not become pregnant. Perhaps in these fertilization failure or early embryonic death occurred. Of 181 animals, 90 had at least one sample that showed intermediate value, therefore, additional clinical data was required to assist in the diagnosis.

The results based on two milk samples revealed that 52 percent of cows and buffaloes were inseminated at an ovulatory cycle, 14% had either anestrous or short luteal phase while 1.5% had luteal cyst. Another, 1.5% animals were inseminated during luteal phase. On the basis of one milk sample it was noted that the percentage of cows and buffaloes that were inseminated at a time other than the luteal phase was 79, while 4% animals were inseminated during luteal phase.

The objective of the present study was to assess the efficiency of milk progesterone assay as a tool for early pregnancy diagnosis and for monitoring reproductive changes in cattle and buffaloes bred through AI. Progesterone concentration in milk has shown to be a good method for early pregnancy diagnosis (Ball and Pope, 1975). This method seems to be much more advantageous under field conditions than ultrasound scanning of uterus (Pierson and Ginther, 1984), or detection of pregnancy related B protein (Kiracofe *et al*., 1993). Comparatively fewer number of animals included in this survey belonged to an organized farm

(n = 89) and larger number were from individual owners (n = 361). Perhaps, this is the main reason for collection of fewer milk samples. Furthermore, non-availability of animals during the day (as they are usually out for grazing) and farmer's lack of awareness for the potential benefits of early pregnancy diagnosis may be the factors responsible.

The difference in the fertility rate between cattle and buffalo could most likely be due to the species differences. In buffaloes, timing of insemination still needs to be defined. Pregnancy rate after first insemination is highly variable in cattle, averaging no more than 45% nationally in USA (Fetrow *et al*., 1988). However, under controlled farm conditions conception rate through A.I. ranged from 40 to 60% in buffalo (Saji, 1984). On the basis of pattern of milk progesterone in animals after breeding, it is believed that the early embryonic death ranges from 8 to 11% in cattle and buffalo which is similar to some of the previous data (Boyd *et al*., 1969, Ayalon, 1978). Factors that can affect embryonic loss include genetics, environment, nutrition, disease, time of insemination and ovarian, oviductal and uterine factors involving synchrony or hormonal imbalance.

In the present study, 52% percent of the animals in which two milk samples were collected showed an ovulatory cycle, 14% of the animals were probably in anestrous when bred, whereas in 31% percent animals nothing could be deduced. This fairly large proportion of the animals is indicative of limitations of the assay system for assessing reproductive functions. Insemination of cows that are not in estrus results from inefficient or inaccurate detection of estrous (Hawk, 1978).

Factors affecting the efficiency of artificial insemination.

In this study, overall conception rate for four locations was 29% (132 conception from 459 inseminations), using the frozen semen at three locations and fluid semen at fourth location. The conception rate observed in cattle and buffaloes following A.I. varied significantly amongst regions ($P<0.01$; Table 1). It was highest in Faisalabad and lowest in Gujjar Khan. Conceptions tended to be higher in animals whose ration was supplemented with concentrates and lower in those fed on roughage and/or grazing (Table 1). The conception rate was not affected by the housing system (Table 1), but there was a higher tendency in tie-stalls than in loose-barn animals. Timing of insemination in relation to onset of heat signs did not influence conception rate significantly, (Table 1), although it tended to be higher in animals that were inseminated at the optimum time (within 12- 24 hours after onset of heat signs). However, number of animals inseminated 24-48 hr after onset of the heat was too small to draw any conclusion.

The variation in the conception rate due to regions in this study can be attributed to differences in quality of frozen semen, thawing procedures and handling of liquid nitrogen containers. It may be noted that at Faisalabad chilled semen was used which usually gives better results (Shabbir et al., 1982). Other contributing factors for this variation might be nutrition and management of the animals and level of interest by the owners. The feeding of concentrate supplement to cattle and buffaloes appeared to improve the conception rate in this study. Poor nutrition is frequently referred as the cause of inadequate fertility (Dominguez, 1995). In Pakistan, under nutrition is the main limiting factor for livestock productivity. Qualitatively, protein supply throughout the year is subject to much wider fluctuations than the energy supply. Furthermore, mineral deficiencies could also be considered as one of the factors as only sodium chloride is being fed commonly as a mineral supplement. Poor fertility in the undernourished animals of this survey might be due to some of these factors.

The trend towards a high conception rate in tie-stalls than in loose-barn animals is in contrast to previous report where freedom of movement in loose-barn system not only increased the intensity of heat signs (Kiddy, 1977) but also stimulated the onset of the cycle after parturition. Differences in the nutrition between the two systems could possibly explain this difference. Alternatively, close and vigilant watch of the heat signs by the owners might have led to the proper timing of insemination in tie-stalled animals. In this study, a higher though statistically non-significant, conception rate was observed when animals were inseminated within the 24 hours after the onset of estrous. Approximately 90% of the animals were inseminated within the 24 hours after the onset of estrous. It suggests that farmers have become conscious of the need to get their animals bred at proper time during the estrus period. It is generally recommended that the animals should be inseminated between the middle and the end of the estrous period. The inseminations carried out after ovulation, result in lower pregnancy rates (Daas, 1970).

Table 1. Effect of farm on conception rate

Parameters	Conception rate (%)	Number pregnant/ Number of services
Regions		
Bahadurnagar	22.5[a]	20/89
Daultala	25.0[a]	27/108
Faisalabad	39.6[b]	84/212
Gujjar Khan	2.0[c]	1/50
Feeding system *		
High	30.7[a]	127/414
Low	10.6[a]	5/45
Housing system		
Loose barn	23.3[a]	24/103
Tie stall	30.3[a]	108/356
Timing of insemination		
Optimal (12-24 hr after onset of heat signs)	29.4[a]	127/432
Late (24-48 hr after onset of heat signs)	18.5[a]	5/27

*High: Roughages + concentrates + grazing Low: Roughages + grazing
Different superscripts within parameters differ significantly (P<0.05)

The conception rate was related to the animal's milk production (P<0.05). It was better in high milk producers than in low milk producers (Table 2). The conception rate was influenced significantly by BCS (P<0.05) (Table 2), being higher in animals with better condition than in animals having poor condition. The conception rate was also affected significantly by the lactation state of animals (P<0.05). It was lowest in dry animals and highest in those which were in milking and were being suckled (Table 2). Decreased conception rate in low milk producers might be due to the fact that such animals are not fed concentrates. The results did show that concentrates also improve conception rate. There are studies showing that conception rate is lower in high milk producer (Nebel and McGilliard 1993) but this is unlikely to be a contributing factor in these Pakistani cows.

Most of the animals of this study were in the middle range of condition score (2-3) and their conception rate was about 30%. This finding is consistent with the trend towards a higher pregnancy rate in animals that were fed a concentrate supplement. In a previous study (Butler and Smith, 1989), cows that lost from one-half to one BCS had a higher (53%) first service conception rate than 17% in cows losing more than one score. Body condition score is clearly an important tool under our conditions for monitoring and improving reproductive efficiency.

Nature of heat signs significantly affected the conception rate (P<0.05; Table 3). Maximum conception rate was observed in animals that passed mucus at the time of insemination. Conception rate did not differ due to type of mucus or swelling of external genitalia noted at the time of insemination. The degree of uterine tone at the time of estrus had a profound effect on conception rate of animals (P<0.01; Table 2). It was high when the tone was marked and low when the tone was slight.

One of the most important elements of herd management is timely detection of estrous. Negligence in detection of estrous may give rise to a wide variety of problems related to the fertility of animals (Foote 1975; Britt, 1977). Cows that have previously been inseminated and failed to conceive will often pass unnoticed due to inadequate estrous detection. This results in an unduly long service period (Esselmont and Ellis, 1974). It is suspected that many estrous cycles passed unnoticed resulting in long service period in our animals. This situation reflects the problem of estrous detection in buffaloes and cows. The intensity of heat signs had a significant effect on conception rate. Behavioral attributes, mucus consistency and uterine tone specifically affected conception rate. Based

Table 2. Effect of animal on conception rate

Parameters	Conception rate (%)	Number pregnant/ Number of services
Species		
Cattle	27.1[a]	76/280
Buffalo	31.3[b]	56/179
Milk production		
Upto 5 lit	21.1[a]	32/152
Over 5 lit	32.5[b]	100/307
BCS *		
1 (thin)	10[a]	3/31
2	29[b]	60/208
3	29.3[b]	58/198
4	47[c]	8/17
5 (Fat)	60[c]	3/5
Lactation state		
Dry	6.5[a]	2/31
Milking and suckling	31.3[b]	77/246
Milking only	29.4[b]	52/177
Suckling only	20[b]	1/5

*Body condition score (1-5) adapted from Butler and Smith (1989)
Different superscripts within parameters differ significantly (P<0.05)

Table 3. Effect of estrus signs on conception rate

Parameters	Conception rate (%)	Number pregnant/ Number of services
Heat signs		
Bellowing	28.7[a]	38/134
Mounting	14.7[b]	11/75
Mucus	37.7[c]	78/207
Restless ness	9.4[b]	3/32
Standing	20[b]	2/10
Type of mucus		
Clear	31[a]	123/397
Purulent	15[a]	3/20
Turbid	28.6[a]	4/14
None	7.1[a]	2/28
Swelling of vulva		
Marked	32.3[a]	20/62
Slight	28[a]	106/378
None	31.6[a]	6/19
Uterine tone		
Marked	35.7[a]	91/255
Slight	19.7[b]	38/193
None	27.3[a]	3/11

Different superscripts within parameters differ significantly (P<0.05)

upon the conception rate, the passage of mucous though external genitalia and high uterine tone were found to be the most reliable heat signs.

Buffaloes showed a higher conception rate than cattle (P<0.05; Table 2). Overall, it was 31% in buffaloes and 27 % in cattle. These results are in accordance with findings of Ali *et al*., (1985). Similarly, in another study (Chohan *et al*., 1992) conception rate was 33% in buffaloes under controlled farm condition. Research is needed on the reproductive physiology of buffaloes to determine factors that enhance fertility level of our cattle under our conditions. Lowered conception rate in this study might be due to poor management, improper quality of semen and its handling. Within species the pregnancy rate was higher in crossbred cows than Sahiwal and others except Jersey which had fewer animals. Type of semen markedly influenced the conception rate (P<0.01; Table 4). The chilled semen yielded better conception rates than frozen semen. The difference in conception rate due to the source of frozen semen was also significant (P<0.01; Table 4). The pregnancy rate obtained from semen produced by the SPU was lower than that from semen produced by University of Agriculture, Faisalabad. These findings are supported well (Shabbir *et al*., 1982; Sharma and Shahni, 1988). Freezing of semen causes damage to the sperm cells (Abhi, 1980). The conception rate was clearly influenced (P<0.05; Table 4) by the quality of semen. As anticipated, it was high with good quality semen and low with poor quality semen. The conception rate varied significantly due to AI technician (P<0.05; Table 5) and ranged from zero to 100%. The conception rates were higher when inseminators were professional degree holders (DVM) and lower when done by the Diploma holders.

The main reason for variation in the pregnancy rate due to source of semen could be that most of the inseminations carried out at the University clinic used the chilled semen. The quality of frozen semen is one of the most influential factors to establish the conception rate in the field (Saacke *et al*., 1980).

The skill of A I technicians is yet another factor that influences the success of AI in the field. In the present study, the conception rate varied significantly due to AI technician as has been found by Singh and Singh (1989). In our conditions, the technician's skill in terms of handling of the liquid nitrogen container, thawed semen and insemination techniques need to be evaluated. Their performance should be monitored on the basis of conception rates rather than number of inseminations performed.

In conclusion, the data indicate that the overall conception rate in the field is low and there is a tremendous scope to improve the existing AI program in the country. This study clearly indicates that factors like region, species, milk production, body condition, lactation state, heat signs, bull breed, semen quality, and

Table 4. Effect of species/breed and semen on conception rate

Parameters	Conception rate (%)	Number pregnant/ Number of services
Bull breed/Species		
Buffalo	31.3 [a]	56/179
Cross bred	42.4 [a]	36/85
Dhanni	12.5 [c]	1/8
Friesian	25 [a]	5/20
Jersey	50 [b]	3/6
Sahiwa	19.3 [b]	31/161
Type of semen		
Chilled	41.5 [a]	78/188
Frozen	19.9 [b]	54/271
Semen quality*		
Good	35.4 [a]	107/302
Not good	15.9 [b]	25/157
Source of semen		
SPU**	19.9 [a]	53/266
Univ. Dept.	40.9 [b]	79/193

* Good: >50% sperm motility, >70% acrosomes intactness
Not Good: < 50% sperm motility, <70% acrosomes intactness
**SPU: semen production unit, Qadirabad, Punjab, Pakistan
Different superscripts within parameters differ significantly (P<0.05)

Table 5. Effect of A.I. technician on conception rate

Parameters	Conception rate (%)	Number pregnant/ Number of services
A.I. Technician		
1	100[a]	3/3
2	26.3[a]	5/19
3	23.1[a]	9/39
4	30[a]	9/30
5	50[a]	4/8
6	66.7[a]	2/3
7	60[a]	6/10
8	20.8[a]	15/72
9	20[a]	1/5
10	29.4[a]	5/17
11	41.7[a]	10/24
12	81.7[a]	17/21
13	26.5[a]	19/68
14	0[a]	0/3
15	25.0[a]	8/32
16	2[a]	1/50
17	30.2[a]	13/43
18	50[a]	6/12
Level of education		
Professional/Degree	34.2[a]	97/284
Technician/Diploma	20.0[a]	35/175
Site of semen deposit		
Cervix	35.7[a]	10/28
Uterus	28.3[b]	121/428
Vagina	33.3[b]	1/3
Passage of pipette		
Difficult	9.1[a]	1/11
Easy	29.3[a]	130/444
Impossible	25.0[a]	1/4

Different superscripts within parameters differ significantly (P<0.05)

skill of AI technician are critical and significantly affect the conception rate obtained in the field through AI.

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