Research Article

Studies on Factors Affecting Pregnancy Rate after Treatment of Anestrus Buffaloes in Chitwan District

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Abstract

This study was designed to determine the effects of age, parity, feeding practice, Body Condition Score (BCS), blood nutritional parameters, types of anoestrus, and length of anoestrus on pregnancy rate of anoestrus buffaloes after their treatment with ovsynch protocol followed by fixed time artificial insemination. Blood samples were collected on the day ovsynch protocol and analyzed. The mean values of the nutritional parameters between different groups were compared by Students t test and pregnancy rate was compared by Fisher's exact test or Chi-square test. The overall pregnancy rate in buffaloes was 28.6%, when checked on day 41 and 64.3% on day 102 of FTAI. Blood glucose, cholesterol, calcium and phosphorus level was found to be significantly higher (p<0.05) in pregnant than in non-pregnant buffaloes. Pregnancy rate was higher in silent estrus buffaloes. Buffaloes with BCS ≥3 had higher pregnancy rate than those with BCS < 3. Similarly, pregnancy rate was higher in lactating than in non-lactating buffaloes. While young buffaloes of age <10 year responded better than old buffaloes of age ≥10 year. Buffaloes which were free from gastrointestinal parasitic infection had higher pregnancy rate. In conclusion, ovsynch protocol produces a good pregnancy in anoestrus buffaloes; however, state of true anoestrus, older age and poor BCS with poor nutritional management adversely affect the pregnancy outcome. The role of blood nutritional parameters requires further clarification.

Keywords: Pregnancy; Anestrus; BCS; Buffalo; Oysynch; Nutrition

Introduction

Reproduction is an important factor in economics of animal production. The buffalo (*Bubalus bubalis*) is given the name "Black Gold of South Asia" where more than 95% of the buffalo milk is produced (Javaid *et al.,* 2009). Buffaloes play a prominent role in overall social development by maintaining sustainable food producing system and power for agricultural operations in developing countries (Nanda and Nakao, 2003; Barile, 2005).

Absence of estrus/anestrus is the most common single cause of infertility observed in rural buffaloes. It is also a big reproductive problem in modern dairy cow production worldwide. Anestrous and inactive ovaries lead to longer postpartum interval in buffaloes. In Nepal, it has commonly recognized that buffaloes have severe reproductive

problems (Sah and Nakao, 2010). Majority of cases of infertility in buffalo presented in infertility camps were of anestrous condition (Sankhi, 1999). Infertility in buffalo is mostly due to anestrous condition (Devkota, Bohora, and Yamagishi, 2012). True anestrous and silent estrous conditions are prevalent among Nepalese anestrous buffaloes (Sah and Nakao, 2010). Incidence of anestrous is higher in the buffaloes than in cattle. Anestrous accounted 52.7% cases in cattle and 69.4% cases in buffaloes indicating dysfunction of ovaries (Singh and Sahni, 1995).

Ovsynch is a protocol consisting the first injection of GnRH (20mcg) I/M on randomly selected day of oestrus cycle which either causes ovulation of matured follicle and start new follicular wave or there may be follicular wave present spontaneously, followed by $PGF_{2\alpha}$ injection (25mg) I/M on 7th day which induces luteolysis of newly formed CL and the second injection of GnRH given 9th day induces ovulation of mature follicle (Pursley *et al.*, 1995).

Anestrus buffaloes are conventionally treated with vitamin-mineral mixture (Vit-M) supplements with the variable effects. But nowadays, more veterinarians have started to use hormones, such as $PGF_{2\alpha}$ and GnRH, for the treatment of anestrus in buffaloes in Nepal. But treatment of anestrus using hormones only is not enough to get satisfactory level of pregnancy rate. There are many factors which directly affect pregnancy rate in livestock that are herd-level management factors (such as methods of husbandry, feeding, estrus detection, semen handling and transition cow management), environmental factors (such as season, temperature, humidity) and cow-level factors (such as age, BCS, parity, blood nutritional parameters, post parturient problem, disease events, milk yield and genetics) (Lucy, 2001). Tesfaye *et al.,* (2015) stated that BCS and age of the cow, management system and AI service performance affect the reproductive performance of the smallholder dairy farms. Thus, to fulfill the demand of milk and meat production from dairy sector or buffalo, increased reproductive performance/pregnancy is mandatory and to increase the pregnancy rate or fertility in livestock it need to understand the factors affecting it. So, this study reveals about the factors which affect the pregnancy rate in buffalo such as hormonal disorders, nutritional causes, environmental causes, infectious conditions, BCS and parity after the various heat synchronization treatment.

Material and Methods

Study Area

This study was carried out in Chitwan District of Nepal with Average annual maximum temperature of the district is 32.2°C and minimum temperature 18°C with relative humidity 83%. AFU livestock farm of Sharadanagar VDC and private farms of adjoining Shivanagar VDC of Chitwan district as shown in Fig. 2 were selected for research study. This area is part of tropical section of Chitwan district. Number of buffalo (118,955) is higher than that of cattle (74, 609) among which 48,932 are milking in Chitwan district (DLSO, 2014)
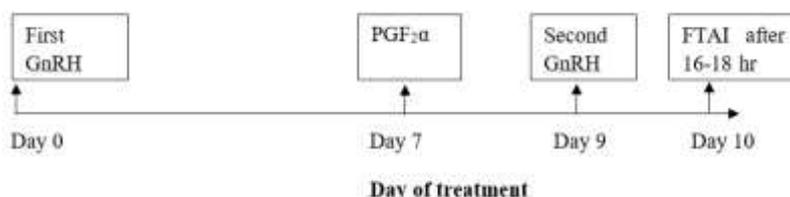

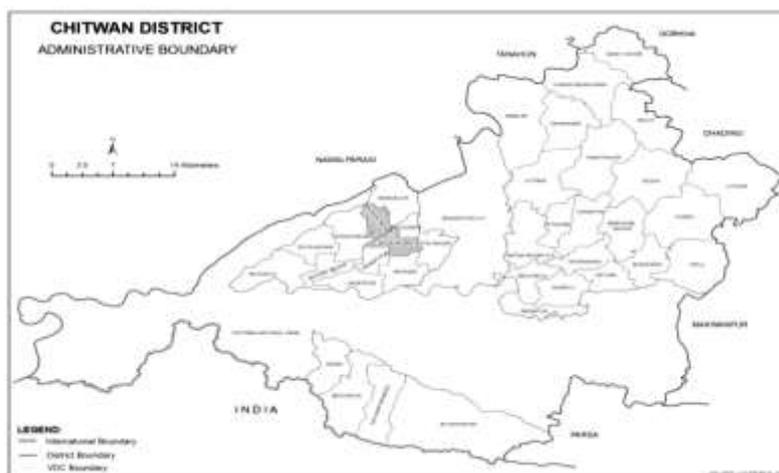**Fig. 1**: Schematic representation of ovsynch protocol

Fig. 2: Study site in map of Chitwan district

Materials and Methods

Hormones

Consultan injection 10 ml (a product of ASKA Japan) as depicted in Plate 1: Each ml of Consultan injection contains 50 microgram fertirelin.

Zenoadin C 20 ml (a product of ZENOAQ, Japan) as depicted in plate 1: Each ml of Zenoadin C contains 0.250 mg cloprostenol. (Figure 3)
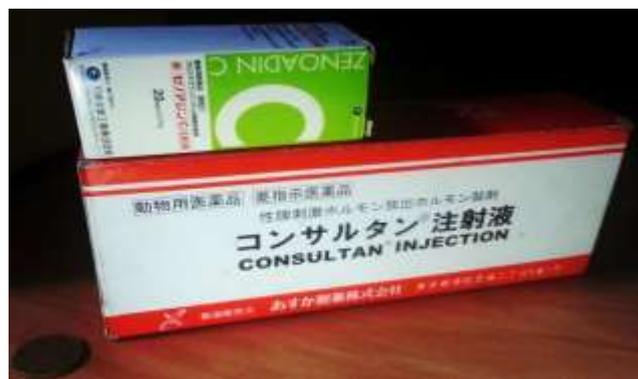

Fig. 3: Hormones used in experiment

Data Collection and Classification of Buffaloes

A data record sheet was developed covering reproductive, managemental and nutritional aspects, as presented in appendix 1 and record sheet was filled, data were entered in Microsoft excel 2007. From history record of anoestrus, 6 buffaloes from IAAS farm and 8 buffaloes from shivanagar VDC were selected randomly and examined by trans-rectal palpation of genital organs. Informations based on rectal palpation were verified with per rectal ultrasonography. On gynaecological examination, colour of vulva, diameter of cervix, its contraction and consistency, diameter of uterine horns, its contraction and consistency and size of both ovaries, its follicular condition, presence or absence of CL etc were examined and cattle were classified on the following basis.

- **True anoestrus**: Those buffaloes which have ovaries without detectable corpus luteum and without follicles larger than 5 mm.
- **Subestrus (silent oestrus):** Those buffaloes which have large follicles and/or detectable CL on the ovaries.
- **Length of anestrous period:** Parous buffaloes were classified into 2 groups

Length of Anoestrus period	<10 months
	≥ 10 months

- **Body condition score (BCS):** Buffaloes were classified into following two groups based on the BCS recorded following the procedure as presented in website of Elanco Animal Health.

BCS(Body condition score)	Buffaloes with BCS < 3.0
	Buffaloes with BCS BCS ≥3.0

- **Milking status:** Buffaloes were categorized into following two groups

Milking Status	Lactating
	Non-lactating

- **Parity:** Buffaloes were classified into two groups:

Parity	Heifers
	Parous

Managemental condition: Buffaloes are classified into 2 groups

Managemental condition	University management (Fed with straw and little green grass)
	Farmer's management (Fed with green grass ,straw and concentrate)

- **Age:** Buffaloes were classified into 2 groups:

Age	✓ Young - <10 years
	✓ Old - ≥10 year

Blood Collection and Biochemical Analysis

- Blood samples were collected in chilled heparinized tubes of buffaloes under the investigation (10 mL, BD Vacutainer; Becton, Dickinson, and Company, Franklin Lakes, NJ, USA) on the first day of ovsynch protocol by vein-Punture through the jugular vein.
- Tubes were centrifuged (3000 rpm for 15 min) and serum were harvested according to the standards methods (coles, 1986) and 12 × 75 mm polypropylene tubes and stored at -20 °C to get clear serum.
- Serum samples were drawn in dry clean capped tubes and sent to medical lab to determine the parameters (i.e. Glucose, Total protein, Cholesterol, Ca and P) using specific kits that was analyzed by automatic biochemical analyser.
- Glucose was measured after oxidizing it to gluconic acid and hydrogen peroxide in the presence of glucose oxidase. Hydrogen peroxide was added that reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a red colored quinoeminine dye complex. Intensity of the colour formed was measured that was directly proportional to the amount of glucose present in the sample.
- Calcium was measured in an alkaline medium that combines with o-cresolphthalein complexone to form purple coloured complex. Intensity of the colour formed is directly proportional to the amount of calcium present in the sample.
- Total proteins were measured in the alkaline medium that binds with cupric ions present in the biuret reagent to form a blue-violet coloured complex. The intensity of the colour formed is

directly proportional to the amount of proteins present in the sample.

- For measuring the cholesterol level, esterase was added that hydrolyes esterified cholesterol to free cholesterol. The free cholesterol is oxidized to form hydrogen peroxide which further reacts with phenol and4-aminoantipyrine by the catalytic action of peroxidase to form a red colored quinoneimine dye complex. Intensity of the colour formed was measured that is directly proportional to the amount of cholesterol presnt in the sample.
- Phosphorus was determined spectophometrically by applying molybden blue using Coral clinical systems (Tulip group) B-2440 Geel, Belgium.

Fecal Examination

Sedimentation procedure is used for fecal examination. It is primarily used to detect eggs or cysts that have too high a specific gravity.

- 2 gram of fecal is mixed with tap water in a cup or beaker. Strain the mixture through a tea strainer into a centrifuge tube.
- Centrifuge tubes and centrifuge is balanced the sample at about 1500 cycle/ minute or (rpm). If a centrifuge is unavailable, allow the mixture to sit without disturbed for 20-30 minutes.
- The liquid is poured off in the top of the tube without disturbed the sediment at the bottom.A small amount of the top layer of sediment is tranferred to a slide. If the drop is too thick, dilute it with a drop of water.
- A coverslip is placed to the drop and examined under the microscope.

Fixed Time Artificial Insemination at Tenth Day of Ovsynch Protocol

Fixed time artificial insemination was done at 7 am of tenth day of Ovsynch protocol 18 hours after second injection of Consultan and 63 hours after Zenoadin C injection. Frozen semen of Murrah breed from regular government supply by NLBC was used through DLSO, Chitwan for AI. AI gun was easily passed in some of the buffaloes due good relaxation of cervix and not in others. Semen was deposited in body of uterus in those animals in which gun was easily passed. In buffaloes in which passing of gun was difficult, semen was deposited in between the cervical rings.

Pregnancy Diagnosis at 41 Days of AI

Pregnancy was diagnosed at forty-one day after AI by ultrasonographic scanning by using 7.5 MHz rectal probe.

Pregnancy Diagnosis at 102 Days of AI Including Breeding of Subsequent Natural Estruses

All buffaloes were scanned for pregnancy with ultrasonograph and rectal palpation for confirmation of maintenance of pregnancy in previously pregnancy positive diagnosed buffaloes and for diagnosis of pregnancy of subsequent breeding in previously pregnancy negative diagnosed buffaloes.

Statistical Analyses

Non-parametric test i.e. χ^2 test and Fisher's exact test in IBM SPSS Statistics version 20 were utilized to know the effect of different factors on pregnancy rate. Students' t-test assuming equal variance, at 5% level of significance was used to compare mean value of different blood nutritional parameters of pregnant and non-pregnant animals using MS-EXCEL-2007. Similarly, MS-EXCEL-2007 was used for data entry and making different diagrams.

Results

Pregnancy Rate

Among total 14 buffaloes, 4 (28.6%) buffaloes were found to be pregnant when checked at day 41 of FTAI i.e. on 1st time AI, while 9 (64.3%) buffaloes were found to be pregnant when checked at day 102 of FTAI i.e. on 2nd time AI, which include both positive at 41 day and second time AI of negative pregnancy at 41 day of FTAI.

Blood Nutritional Parameters in All Buffaloes (N=14) Used in The Experiment

Blood nutritional parameters of all buffaloes used in the experiment was given in the Table 1. The range of different nutritional parameters obtained from this experiment was very similar with range explained by Abd Ellah *et al.,* (2014). Abd Ellah *et al.,* (2014). Serum biochemical and haematological reference intervals for water buffalo (Bubalus bubalis) heifers'.

Table 1: Blood nutritional parameters in all buffaloes used in the experiment

Parameters	Mean ± SD	Range
Glucose (mg/dl)	75.8 ± 19.8	38-95
Total protein (g/dl)	6.6 ± 0.4	6-7.6
Cholesterol (mg/dl)	60.1 ± 9.1	40-70
Calcium (mg/dl)	8.3 ± 2.1	4-11.1
Phosphorus (mg/dl)	6.2 ± 2.3	2-9
Ca:P	1.5 ± 0.5	0.8-2.5

Comparative Study of Blood Nutritional Parameters Between Pregnant and Non Pregnant Buffaloes

The mean (±SD) values of various nutritional parameters of pregnant and non-pregnant buffaloes are shown in Table 2. It was found that blood glucose level, cholesterol level, calcium level and phosphorus level of non-pregnant buffaloes were significantly lower than pregnant buffaloes. Protein level was also lower in non-pregnant buffaloes than pregnant ones but not significantly lower. When these nutritional parameters of pregnant and non-pregnant buffaloes were compared separately with the average value of all experimental buffaloes then no parameters were found to be significantly different.

Effect of Various Factors On Pregnancy Rate After Treatment with Ovsynch Protocol When Pregnancy Checked at 42 Day and 102 Day of FTAI

Among various factors, no factors were seen significantly associated with the pregnancy outcome. It might be due to small sample size. When we see the odd ratios of different factors with pregnancy outcome at 42 days of FTAI, It can be said that milking status and management conditions are more associated with pregnancy outcome than other factors. So it can be said that lactating condition and farmers' management/good feeding management played major role in pregnancy outcome (Table 3).

But the odd ratios of different factors with pregnancy outcome at 102 days of FTAI, then we found age factors was more associated with pregnancy outcome than other factors i.e. young age/<10 year played major role in pregnancy outcome. Then after parity (parous buffaloes) was found to be associated with pregnancy outcome.

Table 2: Comparative study of blood nutritional parameters between pregnant and non-pregnant buffaloes

Parameters (mg/dl)	Mean ± SD			P value a and b	P value a and c	P value b and c
	Mean of total n=14 (a)	Pregnant n=9 (b)	Non-pregnant n=5 (c)			
Glucose	75.8 ± 19.8	83.7 ± 11.8	61.6 ± 24.6	0.3	0.25	0.04**
TP (g/dl)	6.6 ± 0.4	6.3 ± 1.5	5.2 ± 1.6	0.6	0.4	0.25
Cholesterol	60.1 ± 9.1	63.6 ± 6.3	53.8 ± 10.6	0.3	0.2	0.05**
Calcium	8.3 ± 2.1	9.3 ± 1.4	6.5 ± 2.2	0.2	0.1	0.007***
Phosphorus	6.2 ± 2.3	7.1 ± 1.6	4.5 ± 2.2	0.3	0.15	0.02**
Ca:P	1.5 ± 0.5	1.4 ± 0.5	1.6 ± 0.5	0.7	0.6	0.5

Note: ** Significantly different ($P \leq 0.05$)
          *** Highly significantly different ($P \leq 0.01$)

Table 3: Effect of various factors on pregnancy rate when checked at 41 day and at 102 day of FTAI

Basis of categorization	No. of Animal	Pregnancy rate (No.) at 42 day	Odd ratio	P value	Pregnancy rate (No.) at 102 day	Odd ratio	P value
Type of anestrous			0.43			0.31	1.00
True	9	22.2 (2)		0.58	55.6 (5)		
Silent	5	40 (2)			80 (4)		
Length of anestrous			2			0.31	1.00
< 10 months	9	33.3 (3)		1.00	55.6 (5)		
≥ 10 months	5	20 (1)			80 (4)		
BCS			1.5			0.33	0.58
< 3	6	33.3 (2)		1.00	50 (3)		
≥3	8	25 (2)			75 (6)		
Milking status			3			3	0.58
Lactating	8	37.5 (3)		0.58	75 (6)		
Non lactating	6	16.7 (1)			50 (3)		
Parity			1.33			0.19	0.5
Heifer	3	33.3 (1)		1.00	33.3 (1)		
Parous	11	27.3 (3)			72.7 (8)		
Age			2			5.25	0.27
Young	9	33.3 (3)		1.00	77.8 (7)		
Old	5	20 (1)			40 (2)		
Mgmt. condition			0.33			0.33	0.58
University mgmt.	6	16.7 (1)		0.58	50 (3)		
Farmers mgmt.	8	37.5 (3)			75 (6)		
Total	14	28.6 (4)			64.3 (9)		

Effect of Blood Nutritional Parameters On Pregnancy Rate Checked at 42 Days and 102 Days of FTAI

When blood nutritional parameters were analyzed with pregnancy rate, It was found that blood total protein level have greater tendency to be associated with pregnancy outcome than other factors according to, p-value and when pregnancy was checked at 42 days of FTAI, but it was found that blood phosphorus level have greater tendency to be associated with pregnancy outcome when pregnancy was checked at 102 days of FTAI but they were not significantly associated.

When we use the odd ratios to describe the relationship then it was found that blood total phosphorus level ≥ 4.5 (mg/dl) had played major role in pregnancy outcome than all other factors in both of time of pregnancy checked. Then after blood total protein ≥ 7 (g/dl) and blood glucose level ≥ 75 (mg/dl) respectively were found to be responsible for pregnancy outcome when pregnancy was checked at 42 days of FTAI but when pregnancy was checked at 102 days

of FTAI it was found that after blood phosphorus level, blood cholesterol level ≥ 60 (mg/dl), glucose level ≥ 75 (mg/dl) and blood total protein ≥7 (g/dl) and blood calcium level ≥ 8.5 (mg/dl) were found to be responsible for pregnancy outcome respectively. The detail description was given in Table 4.

Influence of Gastrointestinal Parasite On Pregnancy Rate of Anestrus Buffaloes After Treatment

Among all 14 anestrus buffaloes, 8 were found to be negative and 6 were found to be positive on gastrointestinal parasite infection when their fecal examination was done. Among negative, 6 (75%) were found to be pregnant after ovsynch treatment of anestrus while among positive only 2 (33.33%) were found to be pregnant and it was not significantly lower than negative/non-infected buffaloes. This showed that there was not significant relationship between gastrointestinal parasite infection and pregnancy rate (Table 5).

Table 4: Effect of blood nutritional parameters on pregnancy rate checked at 42 and 102 days of FTAI

Basis of categorization	Number of animal	Pregnancy rate (N)at 42 day	Odd ratio	P value	Pregnancy rate (N) at 102 day	Odd ratio	P value
Glucose							
<76 (mg/dl)	5	20 (1)	0.5	1	40 (2)	0.19	0.26
≥ 76	9	33.3 (3)			77.8 (7)		
Total protein							
< 7 (g/dl)	9	11.1 (1)	0.08	0.09	55.5 (5)	0.31	0.58
≥ 7	5	60 (3)			80 (4)		
Cholesterol							
< 60 (mg/dl)	3	33.3 (1)	1.3	1	33.3 (1)	0.18	0.5
≥ 60	11	27.3 (3)			72.7 (8)		
Calcium							
<8.5 (mg/dl)	6	33.3 (2)	1.5	1	50 (3)	0.33	0.58
≥ 8.5	8	25 (2)			75 (6)		
Phosphorus							
< 4.5 (mg/dl)	4	0 (0)	0	0.25	25 (1)	0.08	0.095
≥ 4.5	10	40 (4)			80 (8)		
Ca:P							
< 1.5	7	50 (2)	1	1	57.1 (4)	0.8	1
≥ 1.5	7	50 (2)			71.4 (5)		

Table 5: Influence of gastrointestinal parasite on pregnancy rate of *anestrus* buffaloes within treatment

Parasitic infection	No. of buffalo treated	No. of buffalo pregnant (%)	P-value
Negative	8	6 (75)	
			0.27
Positive	6	2 (33.33)	

Discussion

It was found that there was low pregnancy rate i.e. 28.6% on 1st AI, checked on 41 day of FTAI while pregnancy rate was greater i.e. 64.3% on 2nd AI, checked on 102 day of FTAI. This finding was similar to the finding of Rabidas and Gofur (2017), who found 31.96% (31/97) pregnancy rate in indigenous river type buffaloes of Bangladesh after treatment with ovsynch protocol in 1st AI. Yotov *et al.* (2012) found 37.5% pregnancy rate on anestrus buffaloes of Bulgaria with ovsynch protocol. Low pregnancy rate on 1st AI of ovsynch of this research might be due to true anoestrus buffalo which is not fertile and hormone induces conjunctive fertile oestrus cycle in next cycle.

In this study blood serum glucose level, cholesterol level, Calcium and Phosphorus level of pregnant and non-pregnant buffaloes were found to be significantly different (P<0.05) i.e. higher in pregnant buffaloes than in non-pregnant buffaloes while total protein and Ca:P level were not found to be significantly different. The similar finding was obtained by Yotov *et al.* (2012), who also found significantly higher blood glucose, cholesterol, Calcium and Phosphorus (p<0.05) in pregnant buffaloes than non-pregnant buffaloes in Bulgaria. Present study showed that glucose, calcium, phosphorus and total cholesterol levels in blood could have an impact on the subsequent reproductive performance of buffaloes after the ovsynch treatment

Present study found that silent estrus buffaloes which were cyclic and contain palpable CL in their ovary, showed higher pregnancy rate than true anoestrus which were non cyclic and contain no palpable CL in their ovary. It is generally accepted that malnutrition and an excessive negative energy balance are the main causes of true anoestrus/inactive ovary and delayed ovarian function after calving (Butler, 2000; Lucy, 2008) which can't response properly to the hormones given externally. While silent estrus mostly has good BCS and can response to the hormones given externally and become pregnant. This study showed that pregnancy rate was higher in buffaloes having more than 10 months of anoestrus period than less than 10 months of anoestrus period when pregnancy was checked at 102 days of FTAI. Similar finding was obtained by Devkota *et al.* (2013) who found highest pregnancy rate (37.5%) in buffaloes showing 7-12 months of anoestrus and found lowest pregnancy rate (12.5%) in buffaloes having 2-6 months of anoestrus and >12 months of anoestrus. Similar finding was obtained by Hoque *et al.,* (2014), in cattle in Bangladesh.

Regarding BCS, it was found that pregnancy rate was higher in buffaloes having BCS ≥3 than having BCS <3 but it was not significantly higher when pregnancy was checked at 102 days of FTAI. Similar finding was obtained by Devkota *et al.,* (2013) who found highest pregnancy rate (40%) in buffaloes with BCS=2.5-3.5 and lowest pregnancy rate

(0%) in buffaloes with BCS <2.5 (p<0.05). Similar findings were obtained by Anzar *et al.,* (2003) in Pakistan in cattle and buffaloes who found conception rate was significantly influenced by BCS (P<0.05), being higher in animals with better condition than in animals having poor condition. The pregnancy rate was found to be higher in parous buffaloes than heifers when pregnancy was checked in 102 days of FTAI but no significantly different. Similar finding was obtained by Hoque *et al.*, (2014) in cattle in Bangladesh who found highest pregnancy rate (57.4%) in cows of parity 1-2 and lowest pregnancy (45.0%) in cows of parity 0/heifer. However, the variation in pregnancy rates among different parity of cows was not significant (p>0.05). Similarly, Devkota *et al.,* (2013) found that buffaloes with ≥4 parity have highest pregnancy rate (30%) than 1-3 parity (20%) and lowest in the heifer (10%) after treatment with PGF2α in Nepal. Similarly, Khan et al. (2015) reported higher conception rate in cows at second and third parity than that of cows at zero parity (nulliparous).

In this study, pregnancy rate was found to be higher in lactating/milking buffaloes than non-lactating but it was not significantly higher. Similar finding was obtained by Anzar *et al.,* 2003, in Pakistan in cattle and buffaloes who found that conception rate was affected significantly by the lactation state of animals (P<0.05). It was lowest in dry animals i.e. 6.5% and highest in those which were in milking and were being suckled i.e. 31.3%. But there are other studies showing that conception rate is lower in high milk producer (Nebel and McGilliard, 1993). Hoque *et al.,* (2014) in cattle in Bangladesh found that the pregnancy rate in cows yielding >2-5 L milk was the highest (62.1%) and the pregnancy rate in cows yielding 1-2 L milk was the lowest (49.4%). However, the variation in pregnancy rates among cows of different milk yields was not significant (p>0.05). Similarly, Sarder and Hossain (2001) found no variation in pregnancy rates between high and low yielding indigenous cows. In contrast, conception rates are higher in cattle with higher milk production than that with lower milk production (Shamsuddin *et al.,* 2006). The positive effect of high milk yield on conception rate may be explained by the fact that the high yielding cows received more attention of owners, balanced diet and reared under good management programme (Shamsuddin *et al.,* 2006). In present study, the pregnancy rate was found to be higher in young buffaloes with age <10 year than with age≥ 10 year. Hoque et al. (2015) in cattle in Bangladesh found that cows of 3-5 years of age showed the highest (56.1%) pregnancy rate while the cows of 2-3 years of age showed the lowest (47.1%).

Gastrointestinal parasite infection has been common problem to cause poor production and sub fertility in buffaloes. Nearly a half of buffaloes referred to the infertility camps were positive for helminthes. In present study the infected buffaloes showed a lower pregnancy rate than those without infection. Similar result was obtained by

Devkota *et al.,* (2013) who found significantly higher pregnancy rate in buffaloes with negative gastrointestinal parasitic infection than infected ones (76.9%).

Conclusion

This study showed the pregnancy rate obtained in buffaloes of AFU farm and Shivanagar VDC after their treatments with ovsynch protocol. It has compared the blood nutritional parameters of pregnant and non-pregnant buffaloes to check whether they have any role on pregnancy outcome and it has analyzed various management and individual factors to check whether they effect on pregnancy rate. The current study revealed that ovsynch protocol produces a good pregnancy in anoestrus buffaloes, however state of true anoestrus, older age and poor BCS with poor nutritional management adversely affect the pregnancy outcome. The role of blood nutritional parameters requires further clarification.

Acknowledgement

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