

BIBLIOGRAPHY

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Adviser: Marlene B. Atinyao, Ph.D.

ABSTRACT

The study was conducted to determine the conception rate of artificially inseminated cows and caracows with or without estrus synchronization in Lagawe, Ifugao from October 2011 to January 2012.

A total number of twenty nine (29) animals composed of 12 cows, 17 caracows were used in the study. The treatments were caracows/cows without estrus synchronization, caracows/cows with estrus synchronization, primiparous caracows/cows with or without estrus synchronization, multiparous cows/caracows with or without estrus synchronization.

Conception rate was highest at 55% in natural multiparous caracows/cows inseminated during natural heat, followed by 50% conception rate in primiparous cows/caracows inseminated during natural heat. Conception rate of cows/caracows inseminated after estrus synchronization were 27.28% for multiparous animals and 0% for primiparous animals.

The main concern in artificial insemination is identification of cows /caracows and the receptivity of farmers in the cordillera to the technology. Estrus syncohonization is a technology yet to be perfected as a tool in artificial insemination in the cordillera.



INTRODUCTION

Artificial insemination is a scientific fertility treatment in which male sperm is collected and artificially implanted in the female reproductive system as a way to aid conception. On farms, this process is used to control breeding among different farm animals. Artificial insemination in cattle and water buffalo has been used to produce genetically superior dairy cows and animals for meat production.

As we understand more about the physiological processes in the food producing animals, we become capable of manipulating these animals to increase their productivity. As new technology develops in agriculture, new industries also develop. The artificial insemination (AI) industry is a classic example. This industry is the result of a quick application of science to agriculture. The primary reason for artificial insemination, and hence this industry, is to speed up the rate of genetic improvement. This is accomplished by greatly increasing the selection differential, wherein one highly selected sire is mated with thousands of females.

Artificial Insemination (AI) in mammals was tried for the first time by Lazzaro Spallanzani, an Italian physiologist in 1780. He inseminated a bitch, which gave birth to three pups. Ivanoff of Russia used AI in horse and sheep in the beginning of the 20th century. Thousands of sheep and cows were inseminated in Russia in 1930. In 1939, Kumasen of India claimed to have started using AI at Palace Dairy Farm, Mysore, India. It was then followed by IVRI in 1942 thru the guidance of P. Bhattacharya and was generally used as regular practice among cattle and buffaloes (Spallanzani,1803)

The Philippine Carabao Center (PCC) makes use of the technique as a major tool in the massive upgrading program of the female carabaos, which are in the hands of the



farmers. Because of this, AI has been use. This has been the first choice because of its practicality. Later, the Center has done a lot of coordinative efforts to invite LGU technicians and others to undergo Artificial Insemination and Pregnancy Diagnosis training. Initial equipment like liquid nitrogen tank, AI gun and other supplies were put in place. The Water Buffaloes and Beef Cattle Improvement project (WBBCIP) has supported the AI activities in the country. Some important equipment for the semen processing laboratory was provided through the WBBCIP. JICA provided equipment and experts as support for better AI training the use of ultrasound machines for the monitoring of ovary and logistic support for the implementation of AI.

Department of agriculture in the local government unit are very supportive on the program given by the Bureau of animal industry and Philippine Carabao Center (PCC-CLSU). In the cordillera artificial insemination experience was lack of information dissemination because some farmers don't know the said program.

The information gathered may serve as basis to future AI progress. It also an evaluation of the AI efforts in the Cordillera. The result of the study is useful for students in showing the success rate of AI in the Cordillera.

The study aimed to determine the conception rate of cows and caracows with and without estrus synchronization in artificial insemination and to determine problem encountered in artificial insemination.

This study was conducted on October 2011 to January 2012 at different barangays of Lagawe, Ifugao.



REVIEW OF LITERATURE

The major site of sperm storage is the caudal portion or tail of the epididymis. This part of the tract has a relatively wide lumen in which high concentration of spermatozoa are stored. The tail of the epididymis contains 70% of the total number of spermatozoa in the excurrent duct, whereas the vas deferens contains only 2% (Amann, 1981).

Prostate gland is source of sperm cell antiagglutinin; seminal vesicles are the primary source of fructose, citric acid and ascorbic acid. Mann (1964) found that the level of fructose in seminal vesicle secretion was 0.84 to 1 g/100 ml. Citric acid was 1.0 g/100ml and ascorbic acid was 2-14 mg/100ml.

There are different protocols like Ovsynch in which GnRH is injected 7 days prior to PGF2 α treatment, causes development of follicles or ovulation of the dominant follicle depending on the stage of the estrous cycle. The second GnRH is administered 48h post PGF2 α injection (Bodsteiner *et. al.* 1996)

It has been suggested that AI and other forms of ART could be useful for genetic conservation and preservation of rare breeds. Many of these technologies have been successful to some degree in research setting but none have produced result sufficient to effect population wide improvements in genetic management (Morrow *et al.*, 2009).

As previously mentioned, the ability of cryopreserved spermatozoa retain their fertilizing ability varies widely between the species. One recent advance has been the introduction of dimethylsulphoxide and the amides formamide and dimethylformamide as cryoprotectants in place of glycerol. These molecules seem to function, better than glycerol for some individuals whose spermatozoa do not freeze well. One explanation for this



observation is that these molecules are smaller than glycerol and the refuse may cause less damage when they penetrate sperm membrane (Holt, 2000).

The gonadotropin releasing hormone (GnRH) and prostaglandin (PGF2 α .) method of estrous synchronization has proven to be very successful in synchronizing estrus in cattle and buffaloes (Amaya-Montoya *et. al*, 2007).

Studies carried out by Dhaliwal *et. al*. (1988) revealed that PGF2 α is effective in inducing estrus. In some of the synchronization protocols GnRH has been included along with PGF2 α , either single GnRH injection prior to PGF2 α (seven days earlier to PGF2 α injection) and for the other one double GnRH injections seven days prior to PGF2 α and second injection , 48h post PGF2 α . administration.

Estrus synchronization programs improve reproduction efficiency by reducing the length of breeding and calving seasons and increasing calf weaning weights. Artificial insemination technique can also be used more efficiently. Prostaglandin (PGF2 α) causes CL to regress during the responsive phase and a consequent decrease in the levels of progesterone leading to the development of follicles of the next wave (Galina and Orihuela, 2007).

Although many studies have been carried out with PGF2 α alone (Brito *et. al*.2002; Battista *et. al*. 1984) or in combination with GnRH, the use of artificial insemination technique has been successfully used in breeding farms for genetic improvement of animals. In buffalo there is difficulty in identification of estrus manifestations and for application of A.I. at the accurate time



METHODOLOGY

Materials

The materials that were used in conducting artificial insemination are: AI Gun, disposable plastic AI sheaths, disposable plastic shoulder length gloves, surgical gloves, paper towels, semen straw, scissors/cutters, and semen straw forceps, thermometer, thawing jar, lubricant (mild soap), disinfectant like isopropyl alcohol, AI record forms are the important contents of AI kit (Figure 1). Other AI paraphernalia that was bring in conducting the study are brush, apron/cover-all, pail, syringes and emergency medicines, disposable plastic waste bags. The most important equipment that was needed to conduct AI is the liquid nitrogen tank containing the (LN₂) and frozen semen. The lutalyse hormone was used in synchronization of the animal.

Preparation before Conducting Artificial Insemination

Before conducting artificial insemination there was been a communication with Provincial Veterinary Office together with the Local Government Unit to inform local barangay officials to disseminate the information about artificial insemination. The farmers should be instructed to make a chute to be used for restraining the animal.

The treatments were as follows:

T₀ – caracows/cows without estrus synchronization

T₁ – caracows/cows with estrus synchronization

T₃ – primiparous caracows/cows with or without estrus synchronization

T₄ – multiparous cows/caracows with or without estrus synchronization





Figure 1. Mother tank and contents of AI kit

Steps Prior to Artificial Insemination

The following steps were followed before artificial insemination:

1. Identifying the animal. The breeding information should be checked before conducting artificial insemination.

2. Determining body condition. The body condition of the animal is one of the most important factors in choosing animals for insemination. Animals with body score of 3 to 4 were inseminated.

The body score of animal was determined using the following:

- Body Score 5- Animal in over condition with fat.
- Body Score 4- Animal in excellent condition with well- covered hip and pin

bones and clean cut barrel indicating well fleshed ribs.

- Body Score 3- Animal in moderate condition with hip and bones, and ribs discernible but not prominent
- Body Score 2- Animal in poor condition and relatively better than the animal with body score 1
- Body Score 1- Animal in very poor condition, very thin with prominent scapula, hip and pin bones and ribs.



Figure 2. Determining the body score of the animal

3. Restraining of animals. The animal should be restrained in a chute preferably under shed, to protect the semen from heat of the sun, and to protect both technician and the animal from being harmed (Figures 3 and 4). The animal should be handled gently before, during and after insemination as rough treatment may reduce the chances of conception.



Figure 3. Restraining the caracow in the chute Figure 4. Restraining the cow in the chute

4. Preparation of technicians. Before inseminating the cow, following steps was followed:

- Wear appropriate AI attire like cover all apron and boots
- Trim long finger nails
- Wear shoulder length gloves before wearing surgical gloves to keep fingers fitted
- Lubricate gloved hand before inserting into the rectum

5. Examination of animal through rectal palpation. The following procedure was followed:

- a) Lift the animal's tail to free the gloved arm of any disturbance in entering the rectum.
- b) Examine the animals through recto-vaginal method.

c) Remove feces as much as necessary to perform the required manipulations but avoid excessive in and out motion because this allows air to rush into the rectum that can cause ballooning. It is difficult to work through the rectal wall if this happens.

c) When ballooning occurs, gently slap or pat the rectal wall to stimulate the caracow to strain, expelling the air.

d) Locate the cervix and the uterus. Familiarize with the structure of the cervix and distinguish it from the other parts of the reproductive tract. Pick the cervix up and hold hollow of the hand with middle finger extended.

e) Allow fingers to lie where the horns of the uterus come together. The two horns of the uterus are joined together by the intercornual ligament. The point at which the horns separate is called bifurcation.

f) Push the finger down between the horns and carefully examine each horn to determined indications of pregnancy.



Figure 5. Rectal palpation through recto vaginal method together with the farmer

Steps in Artificial Insemination

The following procedure was followed during insemination:

1. Preparation of Semen for Insemination

- a) The semen straw was removed from LN₂ tank (Figure 6)
- b) The stopper of the LN₂ tank was uncapped and removed then the canister holding the straw to be used was quickly located.
- c) The canister was lifted only as high as necessary (at least 1 ¼ inches from the top) to safely pick the desired straw goblet up. Place the handle between the first and second fingers with palm toward the LN₂ tank.



Figure 6. Removing of semen straw in the LN₂ tank

2. Thawing the Straw. The following was observed:

- a) To obtain high fertility, the frozen semen straw was thawed in warm water (38 to 42 C) for 15-20 seconds.
- b) Straw thermos or thawing jars was used (Figure 7).

- c) The thawed semen straw was dried with clean paper towel.
- d) The end that has straw powder seal should be cut off using semen straw scissor or cutter.



Figure 7. Thawing the semen straw in the thawing jar

3. Preparation of the AI gun and sheath. When working with the AI gun (syringe with plunger) and sheaths, the following should be observed:

a) AI Gun

- The gun should be disinfected with isopropyl alcohol.
- It will be wiped dry.

b) AI sheaths

- The sheath should be properly stored to avoid contamination.
- The sheath should be made sure is not deformed or warped.

4. Preparation of semen straw and AI gun. After cutting the powdered end of the semen straw, the cut end was inserted into the green adapter. Always handle the sheath

near the open end and never handle the area that enters the animals reproductive tract pull back the plunger about 6 inches(13 cm). The sheath containing the straw was placed over the end of the AI gun and slowly pushed over the barrel. The straw goes into the barrel of the syringe (plug end toward the plunger) while the sheath goes over it.

5. Inseminating the cow through recto-vaginal method.

a) Locating the cervix. The animal was now ready for insemination, hold the cervix in your hand.

b) Inserting the AI gun. The area around the vulva and inside the lips was wiped as clean as possible with a new, dry paper towel. This helps keep inside organs free from feces discharge the other external contaminants. With the gloved hand inside the rectum, slight backward and downward pressure was exerted with the wrist so that the vulvar lips will open (Figure 8). This method makes AI gun insertion easier.



Figure 8. Inserting AI gun through the vulva at a 35⁰ to 45⁰ angle

6. Cleaning up after AI. The sheath containing the semen straw should be released from the AI gun holding with the gloved hand. The gloves were removed and then the sheath was broken into smaller pieces. This way, all used disposable materials was

inside the glove allowing easy clean up. Used materials were disposed immediately and properly. If AI is done in the villages, plastic waste bags was brought for storage of wastes accumulated during AI and disposed properly before leaving the area. The breeding record was completed immediately after insemination. The reusable instrument such as AI gun, semen straw scissors, forceps, was cleaned, rinsed with warm water then dried.

The AI gun was wiped with isopropyl alcohol to sanitize. Special attention was be given in cleaning the “O” ring and upper parts of AI gun since the come into contact with the vulva during insemination. Before reuse, the instrument was wiped dry with paper towel. Hands were also being rinsed and dried after insemination. This should protect animals as well as oneself from transferring any disease.

Before leaving the insemination area, trash was discarded properly and footwear was disinfected as well as bottom of inseminating kit and other AI paraphernalia with disinfectant.



Data Gathered

1. Age of the animal. Period in which the animal is mature for copulation.
2. Type of heat (Estrus synchronization or Natural heat). Distinguish if an animal is injected with Lutalyse hormone or Natural heat.
3. Breed of the animal. A variety of domesticated animal within the species (Figure 9)
4. Body score of animal. Distinguish the body of animal from limb to head if she had the capacity to implant a young in the womb.
5. Number of calving. Number of young calves that had been delivered.
6. Date of last estrus. Period when an animal give sign for the acceptance of a male animal.
7. Problems encounter. To find out the unknown problem in the experiment.
8. Other observation. Recognizing other problem encountered in the experiment.



Figure 9. Crossbred car

Data Computed

1. Conception rate. This was computed by the following formula

$$\text{Conception Rate} = \frac{\text{Number of Cows/Caracows Pregnant}}{\text{Number of Cows/Caracows Served}} \times 100$$



RESULTS AND DISCUSSION

Conception Rate

Conception rate of artificially inseminated cows and caracows with or without estrus synchronization and parity are presented in Table 1.

Results showed that the conception rate of multiparous cows/caracows inseminated during natural heat was 55.5%, 50% for primiparous cows/caracows inseminated during natural heat. Estrus synchronized primiparous cows/caracows had 0% conception rate, while estrus synchronized multiparous cows/caracows had a conception rate of 27.28%. From these observations, there, is a higher chance of an artificially inseminated cow/caracow to become pregnant if insemination is done during natural heat rather than after estrus synchronization.

Table 1. Conception rate (%) of artificially inseminated cows and caracows with or without estrus synchronization.

TREATMENT	NO. BRED	NO. PREGNANT	CONCEPTION RATE
Estrus Synchronization			
Primiparous	3.0	0.0	0.00 %
Multiparous	11.0	4.0	27.28 %
Natural Heat			
Primiparous	6.0	3.0	50.00 %
Multiparous	9.0	5.0	55.50 %
Total	29.0	12.0	



Previous local data on estrus synchronization and artificial insemination in large ruminants, including dairy cattle and carabao, indicated 10% to 12% average conception rate with 2.5 inseminations needed impregnate the animals. A higher conception rate of 27.28% was obtained in multiparous cow/caracows with single insemination after estrus synchronization in this study.

Application of artificial insemination however resulted in a 33% conception rate on the first service alone. At 33% conception rate per service, repeat breeders for the second and third time, also those pregnant animals from the first service achieved the aggregate 70% calf drop.

Problems Encountered

The first problem encountered in the conduct of the study was in identifying experimental units. Some of the available cows and caracows were observed to have reproductive abnormalities that included injured cervix, endometritis, pyometra, infection in the vulva and vagina, cystic ovaries, hence were not used in the study.

Another problem was the difficulty in getting the approval and cooperation of the owner of cows and caracows for their animal to be used as experimental units. While artificial insemination is an old technology, farmers in the Cordillera still opt for natural mating. There is even greater resistance in introducing estrus synchronization. One of the reasons for this is lack of understanding and appreciation on the procedure being introduced. It is important that in order to attain high success rate, in this case high conception rate from artificial insemination and estrus synchronization, farmers must fulfill some responsibilities. Some of these responsibilities include proper care and management of the animal especially when it is pregnant and bringing the animal to a pooling place for



the different procedure that include estrus synchronization, artificial insemination and pregnancy diagnosis through rectal palpation.

The 0% conception rate in primiparous cows/caracows with estrus synchronization may be attributed to many factors, One of which is the size of the cervix of primiparous cow, The cervix of primiparous cows is small and difficult to find, hence it is difficult to properly guide the AI gun with the cervical opening. This often resulted to depositing the semen in the vagina. Unlike with the multiparous cows/caracow have bigger cervix that can easily found and held for manipulation making the insertion of the AI gun easier thereby depositing the semen in the cervix or uterus. This ensures a better success rate in AI. Method in the use of prostaglandin to estrus synchronization. Prostaglandin is expensive and the supply is limited in the Philippines. PCC-CLSU opted for the single dose method, where prostaglandin is introduced to an animal and then mated days after injection.

This practice may contribute to the low conception rate with estrus synchronization. According to Mitchell *et al* (2004), a prostaglandin is effective to inducing estrus in mid cycle cows (day 6 to 17 of the estrus cycle). Cows on day 1 to 5 and on 18-21 are affected, that is where two injection system is recommend.

It could be that in the single system, the cows/caracows were injected with prostaglandin during the days when the Corpus Luteum is not responsive to the action of prostaglandin (days 1 to 5 and days 18 to 21). In such case inseminating the cows after 3 days will not result in fertilization.

Conception rate of female animal may then be increase if we suggest introducing double dose in synchronizing female animal for a greater chance of animal to become in heat or giving signs of estrus.



SUMMARY, CONCLUSION AND RECOMMENDATION

Summary

The study was conducted to determine the conception rate of cows and caracows with and without estrus synchronization in artificial insemination, determined problem encountered in artificial insemination. The study was conducted last October 2011 to January 2012 at different barangays of Lagawe Ifugao.

A total number of twenty nine (29) heads of animals 12 heads of cow, 17 heads of caracow was used in the experiment. There were 4 treatments used caracows/cows without estrus synchronization ,cows/cows with estrus synchronization, primiparous caracows/cows with or without estrus synchronization, multiparous cows/caracows with or without estrus synchronization. Result showed that multiparous cows/caracows inseminated during natural heat had the highest conception rate of 55.5% , followed by primiparous cows inseminated during natural heat with a conception rate of 50%. Estrus synchronized multiparous cows/caracows had a lower conception rate of 27.28% while estrus synchronized cows had a conception rate of 0%.

Some of the challenges of doing artificial insemination in the Cordillera include identification of cows and caracows for artificial insemination, the cooperation of farmers, and information dissemination on estrus synchronization and artificial insemination.



Conclusion

Based on the study in conducting artificial insemination, I therefore conclude that higher conception rate can be achieved if AI is done during natural heat than using estrus synchronization. Another is lualyse hormone is very expensive and not locally available to be used in conducting artificial insemination.

Recommendation

I recommend that in achieving high conception rate, it is better and more accurate if the female animal is on natural heat during the artificial insemination than using the estrus synchronization.



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