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Response of cattle to different dosage of follicle stimulating hormone with Pgf2 alpha followed by Artificial insemination (AI)

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Abstract

This study assessed the effectiveness of Follicular Stimulating Hormone (FSH.) to compare the different dosage of FSH specifically to determine which of the different dosage of FSH could trigger more ovarian response and in the subsequent estrous cycle in terms of Manifestation of estrus; Multiple ovulation of follicles; Number of corpora lutea; Multiple pregnancies; Multiple birth; Number of animal return to estrus and Multiple abortion. Results show that animals treated with 5ml of FSH plus PGF2 alpha had the highest number of ovulated follicles, corpora lutea developed and pregnancies from the first treatment of FSH and in the subsequent cycle with an average mean of 2.58 ± 0.144, 1.58 ± 0.14 and percentage mean of 50.0 ± 25.0 respectively followed by Treatment 1 had a mean of 2.25 ± 0.43, 1.5 ± 0.25, 41.67 ± 14.43, and the least was observed in Treatment III (4ml physiological solution plus PGF2 alpha) with a mean of 1.0 ± 0.0, 41.67 ± 14.43 respectively. On the other hand, Treatment I had one (1) twin birth delivered equivalent to 20 percent of the pregnant animals.

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Introduction

Induction of follicular development and multiple ovulations in bovine animals and other mammalian prior to fertilization and embryo transfer has been widely practice in the developed countries using exogenous hormones like FSH (Eldens, 1978). This hormone is very effective in terms of superovulatory response to recipients, the repeatability of use and antibodies formations are not a problem (Becker *et al.*, 1986, Guilbault, and Brassard (1985).

One of the potential applications of this technique is the induction of multiple fetuses or twinning in cattle after natural or artificial service. This method compliments the embryo transfer technology in increasing calf crop without enormous capital investment (Sreenan and other 1975). The use of FSH and other superovulatory hormones at standard dose has been proven to induce follicular growth and ovulation in various species of animals especially in cattle (Becker *et al.*, 1986).

One of the objectives of this technology is to produce genetically potential in terms of meat, milk production and other genetic merits. It is on this context that this study was conducted with intentions to know the effect of the different dosage of FSH on multiple fetuses after artificial insemination that could trigger more ovarian response and in the subsequent estrous cycle in terms of Manifestation of estrus; Multiple ovulation of follicles Number of corpora lutea; Multiple pregnancies; Multiple birth; Number of animal return to estrus and Multiple abortion.

Materials and methods

The experiment was conducted at Cagayan State University Biotech Cattle Herd Piat campus and in the different barangays of Piat and Tuao. The materials used in this study: experimental animals, FSH, PGF2 alpha, mother tank, field tanks, liquid nitrogen (Ln2), frozen semen, AI gun and sheaths, syringe, disinfectant and gloves.

Experimental Design and Treatments

The Randomized Complete Block Design (RCBD) with three (3) replication per treatment were used to analyze and evaluate the influence of using different levels of FSH for the induction of multiple births in cattle followed by artificial insemination. The age of the experimental animals was considered as basis for blocking.

The experimental treatments of the study were the following:

Treatment I – 2ml of FSH plus PGF2 alpha

Treatment II – 5ml of FSH plus PGF2 alpha

Treatment III – 4ml physiological solution plus PGF2 alpha (control)

Experimental Procedures

a. Experimental Animals

a1. Identification and selection of the experimental animals

Breedable animals of the CSU Biotech cattle herd and selected farmers animal in the different barangays of Piat and Tuao were used in this study. Animals found with active corpus luteum (CL) in their ovaries (through rectal palpation) were considered. Likewise, Age was one of the important factors considered, since young cows have more active ovaries than the old ones. Age of the experimental animals ranged from 4 to 9 years with 1 to 5 calving record. Moreover, body condition and other reproductive defects of the animals were also noted during the selection.

a2. Source and description of frozen semen used

Frozen semen of Brahman bulls used in this study were processed and produced at the National Artificial Breeding Center-BAI, Manila. Semen was processed last April 1999 with the corresponding number of bulls (454, 474, 458, 510 and 323) as the source of semen. The standard number of sperm count per unit volume of semen in determining the optimum fertility (the sperm density perml of fresh bull semen) ranged from about a hundred thousand to as many as three (3) billion with an average of one million, with a post thaw motility of 30 to 40 percent to insure 25 to 30 percent conception rate.

a.3 Care and management of experimental animals (pre-treatment of FSH & Post AI)

Experimental animals (cattle) from the Biotech herd for FSH and PG treatment were allowed to graze daily in a wide pasture area of CSU Biotech extension ranch, Piat, Cagayan. After a whole day grazing, the animals were herded in the night corral every afternoon and they were given mixed feed supplements e. g. rice bran, corn bran, vitamins and molasses. Salt was also supplied alternately. Likewise, water was provided to the animals the whole year round. In the farmers level, tethering of animals were the common practices of farmers in the area. Gathering of highly nutritious grasses, giving feed supplements and salt for their animals are rarely done by farmers.

b. FSH administration

Selected experimental animals were randomly distributed in the different treatments. Twelve (12) animals were assigned in each treatment with four (4) animals per replicate. In Treatment I, animals found with cycling corpus luteum (CL) through rectal palpation in their ovaries from day10 post estrus were injected with 2ml of FSH administered at constant dosage of .5ml for four (4) consecutive days plus 2ml of PGF2 alpha given on the third day along FSH injection. While in Treatment II, similar procedures were followed using 5ml of FSH and this was injected at 1.25ml for four days. In Treatment III, (control) physiological solution was used at a dose of 1ml for four consecutive days and 2ml of PGF2 alpha was also injected on the third day. Observation of estrus and breeding was done simultaneously (Fig. I).

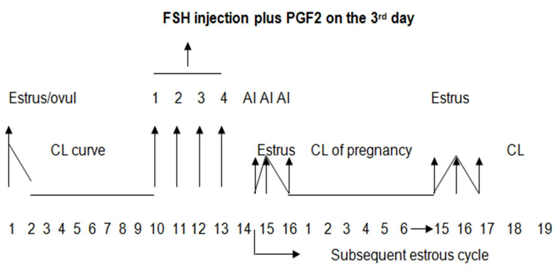

Fig. 1. Graghical mode of FSH/PGF2 alpha administration and AI.

c. Estrus monitoring and breeding (AI)

After the injection of FSH and PGF2 alpha to the experimental animals for four consecutive days, they were closely monitored for their estrus occurrence. Animals that exhibited heat were bred three times. The first breeding (AI) was done as heat observed and follow-up AI (second breeding) was also done after 12 and 24 hours from the first breeding service to insure higher pregnancy.

Table 1. Summary of event in FSH administration and breeding in cattle.

Day of Cycle	Treatments		
	I (FSH)	II (FSH)	III (Physiological solution)
10	0.5ml	1.25ml	1.0ml
11	0.5ml	1.25ml	1.0ml
12	0.5ml AM	1.25ml AM	1.0ml AM
	plus 2ml	plus 2ml	plus
13	PGF2 alpha	PGF2 alpha	2ml PGF2
14 – 16	PM	PM	alpha PM
Onset of estrus	0.5ml	1.25ml	1ml
Onset of estrus	Estrus	Estrus	Estrus
+ 12h	Inseminate	Inseminate	Inseminate
Onset of estrus	Inseminate	Inseminate	Inseminate
+ 24 h	Inseminate	Inseminate	Inseminate

- Monitor any possible occurrence of estrus in the subsequent estrous cycle
- Pregnancy diagnosis was done 3 months post breeding

e. Monitoring of ovulated follicles after FSH and PGF2 alpha injection

Ovarian palpation was done to all animals given with FSH and PGF2 alpha. Their ovaries were examined for ovulation through rectal palpation (J. F. Hasler, A. D. McCauley *et al.* 1985) after the last AI service or end of estrus. This was done to determine the number of follicles that ovulated.

f. Monitoring of corpora lutea post breeding

Animals injected with FSH and PGF2 alpha in the different treatment were monitored. Their ovaries were examined (through rectal palpation) to determine the number of corpora lutea developed. This was done from 8 – 15 days post AI.

g. Pregnancy determination

All experimental animals in the different treatments were bred through artificial insemination. Pregnancy diagnosis through rectal palpation was done 3 to 4 months post breeding. This was done to determine the number of pregnant animals, non-pregnant and presence of fetuses.

Results and discussion

A. Number of animals that exhibited estrus after FSH and PGF2 alpha treatment

Table I. Mean percent of animals that exhibited mucous discharges after FSH and PGF2 alpha treatment.

Treatment	N	B1	B2	B3	Total	Mean
I – 2ml FSH plus PGF2 alpha	12	100	100	100	275	91.67
II - 5ml FSH plus PGF2 alpha	12	100	100	100	300	100
III – PGF2 alpha	12	75	75	50	200	66.7
G. TOTAL					775	
G. MEAN						86.11
CV = 4.15%						

Presented in Table I, Animals in Treatment II (5ml of FSH plus PGF2 alpha) attained the highest number that showed copious mucus discharges with a mean of 100 percent followed by treatment I (2ml of FSH plus PGF2 alpha) with a mean of 91.67 and the least was Treatment III (4ml physiological solution plus PGF2 alpha) with a mean of 66.67 percent.

Analysis of variance shows no significant difference among treatment means. However, it could be inferred that injection of gonadotropins (FSH) 10 days post estrus triggered follicular development (Hasler, Mc Cauley *et al.* 1985) associated with the surge of gonadotropins (FSH & LH) and estrogen production during this period. The high percentage response of estrus observed from the animals injected with FSH was due to its direct stimulatory effect in the developing follicle. Animals treated with PGF2 alpha alone resulted in a lower number of animals that manifested estrus. This was due to the poor stimulatory effect of the compound (PGF2 alpha) in inducing follicular development (Rahil *et al*,1988)

B. Number of follicles ovulated after FSH and PGF2 alpha Injection

Table 2. Average mean ± SE of follicles ovulated post injection of FSH and PGF2 alpha.

Treatments	N	R1	R2	R3	Total	Mean ± S.E.
T1-2ml FSH	12	2.75	2.0	2.0	6.75	2.25 ± 0.43 [a]
T2-5ml FSH	12	2.5	2.75	2.5	7.75	2.58 ± 0.44 [a]
T3-PGF2 alpha	12	1.0	1.0	1.0	3.0	1.0 ± .0 b
G. TOTAL					17.5	
G.MEAN						1.944
CV = 14.54%						

Value (mean ± S.E) with different superscript differ significantly (P<0.01)

Table 2, Animals in Treatment II injected with 5ml of FSH attained the highest number of follicles ovulated with an average mean of 2.58 ± 0.43 followed by Treatment I injected with 2ml of FSH with a mean of 2.25 ± 0.144, while animals injected with prostaglandin (Treatment II) obtained the least number of follicles ovulated with an average mean of 1.0 ± .0.

Analysis of variance shows significant difference (*P<.01)* among treatment means. Comparison among treatments indicates that T2 differed significantly with T3 and significant difference was also observed between T1 and T3. This fig. illustrates that given higher dosage of FSH to the animals resulted to more follicles ovulated as shown in Treatments I and II. This is similar to the findings of Becker *et al.* (1986), using higher level of FSH administered in decreasing dose over a period of four days plus PGF2 alpha injected on the third day followed by insemination that resulted to a higher ovulation and more fertilized eggs The animals injected with prostaglandin (Treatment III), had the lowest number of follicles ovulated due to its indirect stimulatory effect on the growth and development of follicles (Leon and Chupin 1984).

C. Number of Animals Pregnant after Artificial Insemination

Table 3. Mean percent of animals pregnant post injection of FSH and PGF2 alpha followed by artificial breeding.

Treatments	N	R1	R2	R3	Total	Mean ± S.E.
T1-2ml FSH	12	50	25	50	125	41.67 ± 14.43
T2-5ml FSH	12	75	50	25	150	50.0 ± 25.00
T3-PGF2 alpha	12	25	50	50	125	41.67 ± 14.43
G. TOTAL					400	
G. MEAN						44.44
CV = 14.8%						

As shown in Table 3, the animals in Treatment II (injected with 5ml FSH plus PGF2 alpha) obtained the highest number of pregnancies with a percentage mean of 50.0 ± 25.0 compared to treatment I and II with an equal percentage means of 41.67 ± 14.43.

Analysis of variance shows no significant differences among treatment means. It was observed that despite the less effect of prostaglandin (Treatment III) to stimulate follicular development in the ovary and ovulations, the pregnancy rate did not differ much with those injected with FSH (Treatment 1I and I). The low average conception rate obtained in all the treatments were associated with the compounded factors, mainly the fertility of females served, semen quality, nutrition and season effects (Nebheerong 1991). According to Diop, and Bousquet *et al.* (1985), delayed insemination was thought contributory to the lack of fertilization. He also mentioned that the presence of abnormal ova and embryos in animals inseminated after ovulation suggest that the genital tract environment are not suitable for optimal production of normal fetuses

At present, evidence gathered that part of the problem of low pregnancy rate was related to the incidence of short luteal phase, a case where the function of corpus luteum is terminated as early as day 6. This would mean that the developing follicle destined to ovulate and subsequently become CL has not been exposed sometime to progesterone environment before ovulation. Thus the CL that develops would regress early. Based on data from few animals, the incidence of short luteal phase can be as high as 52.9 percent (Duran *et al.,* 1990) in animals induced with PGF2 alpha with body condition score of medium to poor.

D. Number of corpora lutea developed post injection of FSH and PGF2 alpha in the subsequent estrus

Table 4. Average mean ± SE of corpora lutea developed post injection of FSH and PGF2 alpha followed by AI in the subsequent estrus.

Treatments	N	R1	R2	R3	Total	Mean ± S.E.
T1-2ml FSH	12	1.75	1.25	1.5	4.5	1.5 ± 0.25 [a]
T2-5ml FSH	12	1.5	1.5	1.75	4.75	1.58 ± 0.14 [a]
T3-PGF2 alpha	12	1.0	1.0	1.0	3.0	1.0 ± .0 [b]
G. TOTAL					12.25	
G. MEAN						1.361
CV = 12.2%						

Value (mean ± S.E.) with different superscript differ significantly (P < 0.05)

As presented in Table 4, the animals injected with 5ml of FSH plus PGF2 alpha (Treatment II) attained the highest number of corpora lutea developed with a mean of 1.583 ± 0.44 followed by Treatment I injected with 2ml of FSH with mean of 1.5 ± 0.25 while Treatment III given 2ml of prostaglandin obtained the lowest number of corpora lutea developed with a mean of 1.0 ± 0.

Analysis of variance shows significant differences (P<.05) among treatment means. Treatment II differed significantly with Treatment III and significant difference was also observed between T1 and T3. It could be inferred that the development of corpora lutea in the subsequent estrous cycle could be attributed to progesterone priming made possible by the first injection of FSH. The latter stimulate the growth of more follicles destined to ovulate in the succeeding cycle, followed by more corpus luteum formation (Duran 1990). In this event, it is interesting to note that chances of multiple ovulation and more fertilized ova could be expected in the subsequent estrous cycle (Treatment II and I), and likewise, the possibility of multiple birth.

E.Number of animals returned to estrus after artificial breeding

Table 5. Mean percent of animals returned to estrus after artificial breeding.

Treatments	N	B1	B2	B3	Total	Mean ± S.E.
T1-2ml FSH	12	50	75	50	175	58.33 ± 14.43
T2-5ml FSH	12	25	50	75	150	50.0 ± 25.0
T3-PGF2 alpha	12	75	50	50	175	58.33 ± 14.43
G. TOTAL					500	
G. MEAN						55.55
CV = 9.69%						

As reflected in Table 5, Animals in Treatment II and Treatment III, obtained the highest number of animals returned to estrus with an equal mean of 58.33 ± 14.43 followed by Treatment II with a of mean of 50.0 ± 25.0, this would imply that T2 was the most effective in terms of pregnancy rate among the treatment observed.

Analysis of variance shows no significant difference among treatment means in all the treatments tested. It was observed that despite the less effect of prostaglandin (T3) to stimulate follicular development in the ovary and ovulations, the number of animals returned to estrus after the first AI service did not differ much with those injected with FSH (Treatment II and I).

One major factor that caused the high return of estrus in all the animals artificially inseminated was associated with the fertility of the female served, quality of semen used and environmental factors. However, there are other inherent factors that may affect the fertility of the animal, the body condition as influenced by poor management, nutrition and season effect thus, in most cases there will be failure of fertilization (Nebheerong 1991).

Number of animals pregnant aborted

Table 6. Percentage number of pregnant animals aborted.

Treatments	N	R1	R2	R3	Total	% aborted
T1-2ml FSH	12	1	0	0	0	20.0
T2-5ml FSH	12	0	0	0	0	0
T3-PGF2 alpha	12	0	0	0	0	0
G. TOTAL	36	1	0	0	0	0
G. MEAN						20.0

6.25% aborted from the total population of pregnant animals

Table 6, of the sixteen (16) animals pregnant, one (1) in Treatment I aborted with an equivalent value of 20 percent, or with an overall average value of 6.25 percent aborted based from all animals pregnant. Nevertheless, no animals were monitored aborted in Treatment 2 and 3.

No attempt to analyze statistically the difference between treatments, however, reports showed that incidence of abortion in pregnant animals in all type of herd production was relatively high due to several factors that affect the developing fetus. As cited by Bearden and Fuguay (1992), when several ova were produced followed by fertilization, space limitations in the uterus usually result in embryonic mortality, but this is not always the case, instead too many implantation occurred and later resulted in abortion. Moreover, death of one bovine fetus in a multiple fetation pregnancy results in death and expulsion of all fetuses contained within the same anastomosed placenta, a unique characteristics of bovine pregnancy (Echternkamp 1981). As reported by Van Rensburge (1991), the greater concentration of progesterone was associated with the number of CL. He also mentioned that abortion occurs if CL produced inadequate progesterone level during gestation, thus survival of the fetus depends on CL to produce more progesterone.

Number of animals with multiple calves

Table 7. Percentage number of animals with multiple fetuses.

Treatments	N	R1	R2	R3	Total	% Multiple birth
T1-2ml FSH	12	1	0	0	1	20,0
T2-5ml FSH	12	0	0	0	0	0
T3-PGF2 alpha	12	0	0	0	0	0
G. TOTAL		1	0	0	1	0
G. MEAN						20.0

As reflected in Table 7, of the five (5) animals conceived in Treatment I, one (1) animal delivered twins which is equivalent to 20 percent of the animals pregnant. Treatment II and III show no multiple birth monitored from the pregnant animals. It could be inferred that giving 2ml of FSH will elicit twinning in cattle. This was associated with the stimulation of FSH to induce more follicles to grow followed by multiple ovulation that have resulted to more fetuses developed and production of twin calves. On the other hand, it could be deduced that T2 have ovulated several eggs (Table II) due to the action of FSH but only one oocyte was fertilized which resulted to single calf born. The release of viable number of oocytes should correspond on the ovulated number of follicles which is an important consideration in a successful fertilization (Cruz *et al.,* 1988). Several hypotheses have been advanced to explain that incidence of such ovarian abnormality which is relatively high in induced animals (Cruz *et al.,* 1988). The data in this case would support the hypotheses that premature induction results in abnormal luteal function, since most of the animals treated with FSH had delivered a single offspring. In this results, a possibility also exist that some of the follicles that responded to the FSH and the LH surge were not fully mature (Cruz *et al.,* 1988). On the other hand, cows that delivered twin calves have shorter gestation period of 260 days compared to the 286 days for cows with single calves, the latter was within the normal gestation length established in cattle.

Conclusion

Results of the study showed that Treatment II attained the highest number of ovulations, corpora lutea developed and pregnancies with a mean of 2.58 ± 0.144, 1.58 ± 0.14 and 50.0 ± 25 respectively; followed by Treatment I and III with a corresponding mean of 2.25 ± 0.43, 1.5 ± 0.25, 58.33 ± 14.43, 1.0 ± .0, 1.0 ± .0 and 41.67 ± 14.43 respectively. On the other hand, Treatment I had one (1) twin birth delivered equivalent to 20 percent of the pregnant animals. Based from the finding of this study, using lower dose of exogenous hormones (FSH) in Treatment I was found very effective in the induction of multiple fetuses. This is due to its high stimulatory effect in inducing more follicular development that resulted to multiple ovulations from the first cycle and in the subsequent estrous cycle. This would mean that multiple fetuses or birth is now possible using FSH administered in four (4) days after the onset of estrus particularly day 10 to 14 of the luteal phase. This was attributed largely by the action of exogenous hormones that stimulated more follicles to grow and ovulate and followed by the corpora lutea formation that secretes progesterone essential in maintaining pregnancy. Moreover, considering the remarkable effect of the gonadotropin to induce multiple births, a follow up study is recommended using more number of animals given the same level of FSH to increase more twinning rate, including the use of fresh semen in lieu of frozen semen. On the induced scheme to produce more calves followed by AI, Treatment I, is likewise recommended, considering the high net income generated and more offspring the dam could produce in a life time.

References

Becker JF. 1986. Isolation and use of a porcine FSH to improve the quality of superovulation in cattle – Dept. of animal reproduction, Brussels, Belguim, page 13.

Castillo C. 2000. Synchronization of ovulation in crossbred dairy heifers using gonadotrophin-releasing hormone agonist, prostaglandin F2alpha and human chorionic gonadotrophin or estradiol benzoate 2000 Jan **33(1),** 91-101.

Cruz LC, Duran PG. 1988. Ovarian response and pregnancy rate of carabaos treated with PGF2 alpha followed by GNRH and AI, Paper presented at Phil. J. Vet and animal science.

Diop PEH, Bousquet D, King WA. 1985. Artificial insemination and fertilization in superovulated heifers, Universite de Montreal – Canada, page 18.

Diraneh E. 2009. GnRH injection before artificial insemination (AI) alters follicle dynamics in Iranian Holstein cows African Journal of Biotechnology **8(15),** 3672-3676.

Diskin MG, Donagh TMC, Sreenan JM. 1984. The experimental induction of twin calving in beef cows by embryo transfer. Agricultural Institute, Galway, Ireland, page 19.

Duran PG, Cruz LL. 1990. Occurrence of ovulation and estrous length of cyclic buffaloes after PGF2 alpha. Paper presented at Phil. J. Vet. And Animal Science **16,** 72.

Echternkamp SE. 1981. Fetal development in cows with multiple fetuses. U.S. Department of Agriculture, Agricultural Research Services Roman L. Hruska. U.S. Meat Research center, Clay center, Nebraska 68933 USA, page 20.

Eldens. 1978. Superovulation and estrus synchronization in cattle. Embryo Transfer Technology and its Application in Developing Countries, page 10.

Guilbault IA, Brassard P. 1985. Superovulation by continuous infusion of follicle stimulating hormone (FSH) in cattle. Clinique Veterinaire de Coaticook Inc., Canada, page 23.

Hasler JI, Cauley ADMc. 1985. Effect of donor-embryo-recipient interaction on pregnancy rate in large scale bovine embryo transfer program. Department of Animal Science Cornell University, Ithaca, USA, page 265.

Leon D, Chupin D. 1984. Comparison of different methods to induce luteal regression during superovulation in cows. Reproductive physiology, Monnale France, page 31.

Nebheerong P. 1991. Effects of storage on the quality of frozen semen. Carabao reproduction in the Philippines, page 82.

Ocilon G. 2011. Treatments to Optimize the Use of Artificial Insemination and Reproductive Efficiency in Beef Cattle under Tropical Environments Veterinary Medicine International, Volume 2011, Article ID 923053, pp.10

Rahil Tayyab, Rashid Ahmad Chaurhry, Khan LH, Ahmed W, ARSLAN M. 1988. Superovulation response and progesterone levels in Nilli-Ravi buffaloes. Dept. of Animal Reproduction, College of Veterinary Science, Labhore, Pakistan, page 76.