

Efficacy of Hormonal (PGF and eCG) Treatment on the Reproductive Performance of Muturu Cows Crossed with White Fulani Semen under Natural Grazing Condition: A Research Work

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Abstract— An experiment was conducted at Abakaliki in Ebonyi State during the period of dry season (less rainfall) and onset of rainy season between the months of (February-May) at three locations. Two locations were for the control while the other was used for the synchronized. A total of 100 breeding cows of muturu cattle breed were used for the study. A multi-stage selection process was used on the basis of previous calving history. All animals selected were subjected to scanning with drumniski i-scan ultrasound scanner to ascertain pregnant and non-pregnant cows. All non-pregnant cows were further scanned to ascertain their ovaries for growing follicles hence forming our basis for cyclicity. 100 breeding animals selected were randomly allocated to two groups of 50 animals each for the control and the synchronized. All animals in each group were managed on semi-intensive production system where the cows have access to pasture and water.

The Fixed Time Artificial Insemination (FTAI) protocol was initiated on random days of the estrous cycle of the animal designated as day 0, 7 and 10 respectively. The cows each received CIDR (controlled internal drug releasing device). On day 7, the CIDR was removed and 1ml of PGF analogue (cloprostenol) and 2ml of eCG was administered intramuscularly to each animal. The Kmacs and vasectomised bulls introduced into the herd helped in estrous detection. On day 10, second dose of GnRH was administered intramuscularly to each animal in the morning and 12 hours later, all animals were inseminated using semen from NAPRI crossbred white Fulani bull. Results indicated that, 46 animals out of 50 retained their CIDR in the period of 7 days. 92% of the animals showed vaginal discharge at CIDR removal. The on-set of estrous was 31 hrs after eCG and PGF administration and lasted for 54 hrs. It took the animals a shorter period of time (32 hrs) to come on heat after treatment. There was increase

in the follicular size at each treatment (Day 0, Day 7 and Day 10) respectively. The animals had 100% ovulation rate and 46% had multiple ovulation which could be attributed to the effect of eCG on the follicles. The hormonal treatment increased the follicular size and as well induced the growth and development of pre-ovulatory follicles for ovulation unlike in the natural state where we had only 64% ovulation rate.

Keywords—Hormones, Reproductive Performance, PGF, eCG, Muturu cows, Synchronization, White Fulani.

I. INTRODUCTION

Artificial Insemination (AI) is one of the best alternatives to introduce new genes into an indigenous herds; however, the peculiarity of their temperament and the tendency to show short oestrus (many of them during the night) greatly affects the effectiveness of genetic improvement programmes. Therefore, the most useful alternative to increase the number of females that are inseminated is the use of protocols that allow for AI without the need for heat detection, usually called fixed – time Artificial Insemination (FTAI) (BO' et al., 2003).

Besides, the development of protocols to advance the resumption of cyclicity during the early post-partum period has a great impact on beef production and will allow for the inclusion of a significantly larger populations of animals into genetic improvement programmes.

Consistent pregnancy rates in suckled cows, fertility in the successive cycles and the overall pregnancy rates at the end of the breeding season, have been shown to be improved by the use of progestin devices at the beginning of the breeding season. Furthermore, exogenous control of luteal and follicular development has facilitated the application of assisted reproductive technologies, by offering the possibility of planning programmes without the necessity of

oestrus detection and may provide the opportunity to improve the reproductive performance of muturu cattle in Nigeria.

Given the stressful environment such as high, temperatures, and humidity, ectoparasites and predominant low quality forages, artificial insemination is one of the best alternatives to introduce new genetics into the muturu herds, however, only a small percentage (if any) are subjected to AI. Among the main factors that affect the extensive use of AI in the muturu herd are: those related to nutrition, management, labour intensive, time-consuming, and inefficient oestrus detection. Moreover, geography environment economics and social traditions are contributing factors for a lower use of reproductive technologies. The most useful alternative to significantly increase the number of animals involved in AI programmes is the use of protocols that allows for AI without the need for oestrus detection, usually called Fixed-time AI (FTAI) protocols (BO et al., 2003). The combination of low and variable oestrus response and high incidence of anoestrus common in animals grazing tropical grasses (2) explain the wide variability in oestrus response and pregnancy rates after PGF treatments (Galina and Arthur, 1990; Moreno et al., 1986; Kerr et al., 1991; Alonso et al., 1995; and Piriheiro et al., 1998). These studies emphasize the need for treatments that control follicular and luteal development to obtain high pregnancy rates to FTAI without the necessity of oestrus detection.

Furthermore, treatment protocols should be capable of inducing oestrus and ovulation in anoestrus animals.

Under favourable conditions, a cow has the potential to produce one calf per year, with an interval of 12 months between calvings. However, suckled beef cattle under grazing conditions often have a high incidence of postpartum anoestrus, which extends the calving to conception interval and consequently, negatively affects their reproductive performance (Baruselli et al., 2004). eCG treatment increased plasma progesterone concentrations and pregnancy rates in suckled cows treated during postpartum anoestrus. Therefore, eCG treatment may be an important tool for increasing pregnancy rates at FTAI, reduce the postpartum period, and improve reproductive efficiency.

One of the main advantages of implementing FTAI programmes is that, more cows can be impregnated earlier in the breeding season to genetically improved bulls, resulting in heavier weaning weights (Cutaia et al., 2003b). Optimal reproductive efficiency is crucial to increase net returns. However, variability in response to the traditional PGF-based hormonal treatments and the time and effort required to perform oestrus detection, have limited the

widespread application and success of these technologies. The incorporation of techniques designed to control follicular wave dynamics and ovulation have reduced problems associated with oestrus detection.

Hormonal protocols have been developed to resolve some of the reproductive challenges of the *Bos indicus* Cattle and allow artificial insemination, which is the main strategy to hasten genetic improvement in commercial beef ranches. Most of these treatments use exogenous sources of progesterone associated with strategies to improve the final maturation of the dominant follicle, such as temporary weaning and exogenous gonadotropins. These treatments have caused large impacts on reproductive performance of beef cattle reared under tropical areas.

It is against this backdrop that this work is designed to determine the efficacy of Hormonal (PGF and eCG) treatments on the reproductive performance of Muturu cows crossed with while Fulani semen under natural grazing conditions.

II. MATERIALS AND METHODS

This study was conducted at Abakaliki, in Ebonyi State, Nigeria. Abakaliki is situated within the Southeastern part of Nigeria. It exist on latitude 6°40'N, 7°30' and Longitude 5°40' and 6°45'E., and 91.44m above sea level in the derived Savannah of South eastern agro-ecological zone of Nigeria. The minimum and maximum temperatures of the area are 27°C and 31°C respectively (Ofomata, 1995).

At the moment, Ebonyi State houses the majority of the muturu breed of cattle in the whole of Nigeria as a country. The experiment was carried out during the period of dry season (less rainfall) and on-set of rainy season between the months of February – May). The experiment was conducted at three different locations. One location for the control was at the cattle breeding and multiplication centre of Ebonyi State University, Abakaliki; while the other two locations were located in the villages under natural pastures that were used for the synchronized.

A total of 100 cycling and non-lactating breeding cows of muturu cattle were used for the study. A multi-stage selection was conducted in order to select animals to be used for the experiment. The criteria were on the basis of previous calving history of cows which was provided by the farmers throughout the study, all cows were allowed to graze on natural pastures during the day (07.00 – 18.00h) and restricted at night in Paddocks or Tethered at homes in their villages. The predominant forage species on the natural pastures was panicum maximum, sporobolus pyramidalis imperata cylindrical and centrosema among

others. All cows were allowed unrestricted access to mineral salts and water.

All cows selected were subjected to scanning using Drumniski i – Scan Ultrasound scanner to ascertain pregnant and non-pregnant cows. All non-pregnant cows were further scanned to ascertain the status of their ovaries for growing follicles, hence, forming our basis for cyclicity. 100 breeding animals selected were randomly allocated to two groups of 50 cows each for both the control and the synchronized. All cows in each group were managed on semi-intensive production system where the cows have access to pasture and water.

The local prevailing environment in which the animals are reared was not altered in order to investigate if the hormonal treatment can improve their reproductive performance in their natural environment. All the cows were ear tagged for proper identification and record keeping. Acaricides was used on the animals to treat tick infestation. Generally, all practices and procedures adopted, including; injections, blood collections, ultrasonography and inseminations were performed preserving the animals' welfare.

The Fixed Time Artificial Insemination (FTAI) protocol was initiated on random days of the estrous cycle of the animals designated as Day 0. On this Day 0, the ovaries of the animals were scanned with the I-scan ultrasound to record the diameter of the follicle. Follicle diameter (FD) was obtained by averaging perpendicular measurements of the cross sectional diameter for each follicle.

Each of the cows received CIDR (Controlled Internal Drug Releasing device), Pfizer Animal health, impregnated with 1.38 progesterone) insert into their rectum with the aid of CIDR applicator, Bovi gel was applied on the applicator for easy penetration. 2ml of blood was collected from the jugular vein using syringes and put into plain tubes placed in the ice. 1ml of gonadotropin agoist (ovurelin, Gonadotropin acetate injection, Mamac Laboratories LTD NIZ) was administered intramuscularly to each cow.

On day 7, the CIDR was removed. Discharges was observed following the removal of CIDR as it appeared that the CIDR was longer than the length of their vagina, so the discharges could have been as a result of irritation. 1ml of PGF analogue (cloprostenol) and 2ml of eCG (equine chorionic gonadotropin – Norvomom-Syntex Ltd) was administered intramuscularly to each animal.

Each procedure for ultrasonography of the ovaries and blood collection as done previously on Day 0 was repeated and the Kmacs were placed on the (tail end) hips of the animals at the same time.

The Kmacs and Vasectomized bulls introduced into the herds helped in estrus detection. The animals were monitored for estrus twice daily at the hours of 8.00am – 10.00am and 4.00 pm – 6.00pm. A change in the colour of the Kmacs placed on the animals signifies that the animal has been mounted. This action was the basis for estrus detection.

Standing estrus which was defined as the moment of receptivity for mounting as shown by these cows formed the basis for onset of estrus and the moment the animal refuses to be mounted formed the basis for cessation of estrus (B.O et al., 2016). The interval between the onset of estrus and cessation of estrus was recorded as duration of estrus. Kmacs that fell off was always replaced.

On day 10, second dose of GnRH (Gonadotrophin releasing hormone was administered (1ml of ovurelin – Gonadorelin acetate injection) intramuscularly to each animal in the morning and 12 hours later, all animals were inseminated which was 66 – 72 hours after PGF and eCG treatment.

The semen used for the experiment was purchased from National Animal Production Research Institute, Zaria, Kaduna State, Nigeria. The semen was obtained from proven sound breeding bull of white Fulani. The semen was thawed by bringing it out from the ice and exposing to warmth for 5 minutes and then, it was loaded into the semen straws and inseminated into the animals. Similar procedure for scanning and blood collection as done previously in day 0 and 7 was repeated. All the cows were inseminated twice pm/am rule.

Scanning of the ovaries for ovulation was done on days 10 and 12. Ovulation was measured as the rupturing of the largest follicle observed previously. Ovaries showing more than one corpus luteum (CL) indicates that more than one follicle ruptured. All animals observed with multiple corpus luteums were recorded. Pregnancy diagnosis was carried out on day 45 after the insemination using I-scan ultrasound scanner.

Pregnancy per artificial insemination was defined as the number of cows that were pregnant divided by number of animals inseminated multiplied by 100.

$$(\text{Percentage Pregnancy}) = \frac{\text{No of cows pregnant}}{\text{No of cows inseminated}} \times \frac{100}{1}$$

All the blood samples collected during the experiment were refrigerated at 4°degrees during the first 4-5hours and then centrifuged (300 x g x 20 minutes) and stored at minus 20° degrees until time for assay.

III. RESULTS AND DISCUSSION

The results of the study showed that 46 cows (46%) retained their CIDR throughout the 7 days. Owing to the fact that the CIDR was longer than the length of the vagina, irritations and not being well placed may have led to some of the animals not being able to retain their CIDR for the seven days period. 46 cows out of 50 which represents 92% of the animals showed vagina discharge at CIDR removal. There is the need for manufacturers to consider the breed/strain of animals in their production.

The on-set of estrus was observed in 31 hours \pm 3.00 after eCG and PGF administration, lasted for a period of 54 \pm 5.00 hours. Although, there are numerous controlled breeding protocols in beef and dairy herds, a thorough understanding of estrus cycle physiology and follicular wave dynamics in particular, is necessary before one attempts manipulation of the estrous cycle.

The duration of estrus was recorded as interval between on-set and cessation of estrus. Due to the resetting of the estrous cycle with the hormonal treatment, longer duration of estrus was observed. It took the cows a shorter period of time (32 hours) to come on heat after the treatment. Kastelic (1990) had maintained that, duration of the estrous cycle is approximately 20 days in 2-wave cycles and 23 days in 3-wave cycles. The dominant follicle present at the time of

luteolysis will become the ovulatory follicle, and emergence of the next wave is delayed until ensuring ovulation.

Various reports have described the use of intravaginal progestagens, accompanied by the administration of equine chorionic gonadotrophin (eCG), formerly called pregnant mare serum gonadotrophin (PmSG), following progestagen withdrawal to synchronize estrus during the normal breeding season (Menegatos, et al., 1995), induce estrus out of season (Karatzas, et al., 1997) and improve the ovulation rate (Greyling and Van Niekerk, 1990; Pandleten et al., 1992). eCG is a placental glycoprotein hormone prepared from the serum of pregnant mares (Abecia et al., 2012). However, the use of eCG involves more input costs for the producer. Progesterone and its analogues have an inhibitory effect on the release of the luteinizing hormone (LH) from the anterior pituitary so that the endocrine events that influence the withdrawal of progesterone, estrus and ovulation occur at a predictable time (Labouef et al., 1998). Probably the most important effect of eCG is the stimulation of the growth of the dominant follicle that consequently increases ovulation rate (SaFilho et al., 2010). There was increase in follicular size at each treatment (Day 0, Day 7 and Day 10).

Table.1: Classes and Follicular Sizes of Muturu Cows after (eCG and PGF) Treatments

Classes	Sizes	Day 0	Day 7	Day 10
Small	<11mm (8-10)	21	-	13
Medium	11 – 14mm	26	06	24
Large	>14mm	09	44	11

The animals were responding to each treatment from 13mm \pm 5.05 to 18mm \pm 5.46 upto 12mm \pm 6.92. At day 7, the follicular size had increased and by day 10, most of the largest follicles observed previously were ruptured and those large follicles that did not rupture on day 10, ruptured on day 12. It goes without saying that follicle sizes could be categorized into 3 classes as depicted in Table 1.

Several studies have also characterized follicular – wave dynamics in *Bos indicus* cattle and indicated that *Bos indicus* cattle have smaller diameters of the dominant follicles and corpus luteums (B.O et al., 2003) and lower serum progesterone concentrations (Segerson et al., 1984) relative to those of *Bos Taurus* Cattle. Previous studies also showed that the diameter of the dominant follicle at the time of deviation has been reported to be smaller in Nelore (6.0 to 6.3mm) Sartorelli et al., (2005) than in Holstein (8.5mm) cattle (Ginther et al., 1996). Furthermore, the diameter at which the dominant follicle acquired the capacity to ovulate

in response to a treatment with PLH (Lutropin – V, Bioniche Animal Health Canada) in Nelore heifers was found to be between 7 and 8.4mm (Gimenes et al., 2005a); whereas, it was 10mm in Holstein Cows (Satori et al., 2001).

The proportion of animals with follicles in the small class on day 0, must have grown to acquire preovulatory capacity which could be seen in day 7 where the proportion of animals with medium and large classes increased. For the proportion of animals with large follicles on day 0, the follicles may have ruptured and a new follicular wave initiated with new recruitment of follicles which may be the reason(s) why we had small proportions of animals with medium class of follicles.

At day 10, the proportion of animals with small class of follicles increased compared to day 7 with smaller proportion of animals with large class of follicle. The proportions of animals with follicles in the small class on

day 0 must have grown to acquire preovulatory capacity which could be seen in day 7, where the proportion of animals with follicle sizes of medium and large class increased.

For the proportion of animals with large follicles on day 0, the follicles may have ruptured and a new follicular wave initiated with new recruitment of follicles which may be the reason why we had small proportion of animals with medium class of follicles. Similar trends must have orchestrated the various classes of follicular sizes observed in day 7 and 10 respectively. Most of the large follicles must have ruptured and a new follicular wave initiated which gave rise to the small medium and large classes of follicles observed.

Table 2 showed that the muturu cows had 100 percent ovulation rate and 23 (46 percent) had multiple ovulation. Prostaglandin F₂ or (PGF) has been the most commonly, used treatment for synchronization of oestrus in cattle (Odde, 1990). However, the variable interval from PGF treatment to expression of oestrus and ovulation (Kastelic and Ginther, 1991) makes oestrus detection essential to attain high pregnancy rates in Artificial insemination programmes.

The combination of low and variable oestrus response and the high incidence of anoestrus common in animals grazing tropical grasses explain the wide variability in oestrus response and pregnancy rates, after PGF treatments (Galina and Arthur 1990; Alonso et al., 1995; Pinheiro et al., 1998). These studies emphasized the need for treatments that control follicular and luteal developments to obtain high

pregnancy rates to fixed time Artificial Insemination (FTAI) without the necessity of oestrus detection.

The multiple ovulation recorded could be attributed to the effect of eCG treatment on the follicles. The hormonal treatment increased the follicular size and as well induced the growth and development of the preovulatory follicle for ovulation unlike in the natural condition where we recorded only 64 percent ovulation rate. eCG treatment increased plasma progesterone concentrations and pregnancy rates in cows. Therefore, eCG treatment may be an important tool for increasing pregnancy rates at FTAI, to reduce the postpartum period, and to improve reproductive efficiency in muturu cows.

It is important to note that these results have been achieved only when cows were gaining body condition during season. If drought conditions of lack of feed prevent cattle from improving body condition during the breeding season, pregnancy rates will most probably be 35% percent or less, even after the administration of eCG (Mararia et al., 2006).

In this work, the pregnancy rate observed was 84 percent; 42(50) of the cows inseminated were pregnant although, the remaining 8 non-pregnant cows ovulated. Timing of insemination may be a contributing factor, whereas, 36 percent pregnancy rate was recorded for the cows under natural method. BO et al., (2005) had previously observed similar trend in their work in which they reported that eCG treatments increased pregnancy rates (eCG: 49.5% Vs no eCG: 40%; $P < 0.05$) in cows that only had follicles at the time of progestin device insertion.

Table.2: Result of Muturu Cows on Co-synchronization Treatments

Variables	Co-Synch	Treatments
Vaginal discharge	46 (50)	92 percent
On-set of oestrus	31.09 ± 3.00hours	
Duration of oestrus	54.10 ± 5.00 hours	
Cessation of oestrus	86.04 ± 6.56 hours	
Ovulation rate	50 (50)	100 percent
Multiple ovulation	23 (50)	46 percent
Pregnancy rate	42 (50)	84 percent

IV. CONCLUSION

Variability in response to the traditional PGF-based hormonal treatments and the time and effort required to perform oestrus detection, have limited the widespread application and success of these technologies. The incorporation of techniques designed to control follicular wave dynamics and ovulation has reduced problems

associated with oestrus detection. Treatments with progestin – releasing devices like eCG and PGF have provided possibilities for the application of FTAI in muturu cows and to advance the resumption of cyclicity in cows. Fertility and overall pregnancy rates have been shown to improve with the use of progestin – releasing devices in cows on an increasing plane of nutrition.

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