

Research Article

## Success of Artificial Insemination in Rural Area Cows

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### Abstract

Biotechnologies of reproduction like artificial insemination were identified as a mean to intensify the local production. Therefore, some factors are still responsible to the failure of their applications in rural area. This survey was conducted from April to June 2023, to evaluate the effects of age, body weight, body condition score (BCS), calving rank, postpartum to artificial insemination interval and feed complementation on the success rate of artificial insemination in females Goudali. Thus, activities took place at two peri-urban livestock farms in the town of Ngaoundéré in the Adamaoua region of Cameroon where 34 cows were concerned. The selected cows have an average of  $8.21 \pm 2.41$  years old, a body weight of  $343 \pm 52.28$  kg, a body condition score of  $3.23 \pm 0.55$ , a calving rank of  $2.21 \pm 1.22$  and a postpartum to insemination interval of  $4.39 \pm 1.40$  months. The induction and synchronization of estrus were carried out based on the PRID-PGF2 $\alpha$ -PMSG protocol which allowed to obtain a synchronization rate of 97.06% and the insemination were done 56-57 hours after the previous operation, with an imported semen of Brahman bull. During rectal palpation and early pregnancy diagnosis based on progesterone level measurement, the result shown that, 20 cows out of 33 inseminated were pregnant. The success rate of artificial insemination recorded was 60.6%. Amongst all the studied factors, analysis shown no significant ( $P > 0.05$ ) effect on the success rate after artificial insemination. Thus, recommendations were made to popularize the use of artificial insemination and early pregnancy diagnosis test as a mean to improve reproductive performances of local farms. However, studies must be done to evaluate the cost of their applications in rural areas.

### Keywords

Artificial Insemination, Biotechnologies, Pregnancy Diagnosis, Success Rate

## 1. Introduction

Livestock breeding constitutes an important activity for populations in rural areas, for whom it plays a socio-cultural, nutritional and economic role in more than 80% of house-

holds [1]. In rural areas, cattle farms consisting mainly of zebu represent one of the most important sources of animal protein and income for local populations. Despite its eco-

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economic importance estimated at 7.2 million head, the Cameroonian cattle herd only contributes around 30% in terms of tonnes of meat and has a deficit of more than 170,000 tonnes of milk per year [2]. These contributions therefore fail to cover the food needs of the local population whose growth rate (2-3% per year) is one of the highest in the world. This paradox is linked to the fact that African local breeds are characterized by low productivity and poor reproductive characteristics [3, 4]. This low productivity of cattle in tropical countries is attributable to numerous factors including food insufficiency, climatic conditions, their low genetic heritage and poor livestock management [5]. In view of this situation, it therefore seems essential to act on each link in the production chain with the aim of intensifying the productivity of local livestock farms. This intensification of cattle production could involve the use of biotechnological tools in a real environment based on methods of controlling reproduction such as artificial insemination. According to Deepak et al [6], artificial insemination is a process in which sperm are collected from the male, processed, stored and artificially introduced into the female reproductive tract at proper time for purpose of conception. It Permits the maximum use of the most valuable breeders at the same time, for significant increase of breeding advance. it's also helps to group pregnancies and promotes the genetic and economic gains through the use of superior genetic bulls. However, the response of local breeds to this act is not really known and may be different from that of the exotic breeds on which the products are tested. In addition, climate seems to have an effect on the physiology of cows, because estrus, which lasts on average 18-24 hours in cattle in temperate climates, is considerably reduced in tropical environments. Indeed, treatments such as PRID® delta vaginal spirals induce fairly high estrus rates and make it possible to remedy numerous cases of anoestrus and subestrus observed in zebu in the tropics [7, 8]. However, numerous studies carried out in Africa have reported low fertility rates in zebus in artificial insemination [5, 9]. Fertilization failures in artificial insemination are therefore associated with several factors (genetic factors and factors linked to the animal's environment). Thus, the present research aims to contribute to a better knowledge of artificial insemination on induced heat, through the analysis of the factors limiting reproduction in Goudali cows.

## 2. Materials and Methods

### 2.1. Study Zone

The present study was carried out from April to June 2023 in two peri-urban livestock farms in the town of Ngaoundéré Vina department, Adamaoua region, Cameroon. The town of Ngaoundéré is located between 7° 0' 19.389" and 7° 0' 21.254" North latitude and 13° 0' 33.51" and 13° 0' 35.51" East longitude and located at approximately 1000-1500 m altitude. Its cli-

mate is humid in the tropical Sudanian climatic domain. The rains are very abundant and fall from mid-March to October with average annual precipitation of 500-1500 mm. The dry season is marked by a wind coming from the North: the harmattan, which transforms into a dry and hot wind.

### 2.2. Animal Material

The selection of cows for artificial insemination was based first on body condition score, then on the duration of the postpartum period. An examination of the genital tract was also carried out on the selected cows. These operations made it possible to isolate 34 Goudali cows, all lactating, which were identified using ear tags attached to their ears. Insemination was carried out with an imported Brahman bulls semen (Mr H Maddox Manso 173) frozen in liquid nitrogen.

### 2.3. Induction and Heat Synchronization

The selection of cows for synchronization consisted of determining: their body condition and their physiological status by examining the genital tract through rectal palpation. At the end of the consultations, cows with a body condition between 2.5-4, with a normal genital tract and not pregnant were retained for synchronization. In addition, the cows had to have a postpartum duration of at least three (03) months. 34 cows were selected for the treatments and divided into three groups (n=12, n=10 and n=12). However, 33 cows completed the treatments and were inseminated.

The heat induction and synchronization protocol consisted of the installation of vaginal spirals (PRID® delta) very early in the morning (6-8 a.m.) to each of the selected cows. The external areas of the genital tract were washed with drinking water and soap then disinfected with Betadine before each operation.

The spirals, once fixed, remained there for eight (08) days. The objective of placing the progesterone coils was to create conditions similar to those induced by the presence of a functional corpus luteum and to block any change in ovarian activity.

An intramuscular injection of 2ml of PGF2 $\alpha$  was given to each cow on the eighth day after the installation of the vaginal coils. The PGF2 $\alpha$  thus injected destroys any possible corpus luteum (in cycled cows) which brings all the cows back to the same ovarian stage.

The day after the PGF2 $\alpha$  injection, the coils were removed in all cows followed by a vaginal wash with Betadine for cows showing signs of vaginal infections (purulent discharge) with the addition of an injection of 'antibiotic. An intramuscular injection of 2 ml of reconstituted solution (3ml of PMSG lyophilisate and 20 ml of solvent) was carried out on the day of removal of the coils in the cows.

### 2.4. Artificial Insemination Methods

All artificial inseminations were carried out 56-57 hours af-

ter the removal of the vaginal coils and the injection of PMSG, following the protocol recommended by the manufacturer (Ceva-animal health). The cows were all inseminated in the afternoon (4-5 p.m.) in order to limit the effect of stress. Insemination was carried out using the recto-vaginal method and the semen deposited at the level of the uterine horns (position 3: intracervical). The operation was carried out in such a way as to avoid any trauma and infection of the cows' genital tract.

## 2.5. Diagnosis of Pregnancy by Progesterone Measurement

Blood samples were taken from the jugular vein on inseminated cows. These samples were taken on the day of insemination, two weeks and three weeks after insemination.

Blood was collected using vacuum tubes (VacUcheck@) which were identified and transported to the laboratory of the Agricultural Research Institute for Development in Wakwa. There they were kept at rest in a refrigerator for 24 hours to promote the formation of serum that was collected into Eppendorf tubes, identified and stored at -20 °C until the progesterone levels were measured.

The quantitative of progesterone in serum was determined by the solid phase enzyme-linked immunosorbent method (ELISA) as described by the commercial Omega Diagnostic kit (panthozyme@progesterone test kit).

## 2.6. Diagnosis of Pregnancy by Rectal Palpation

The assessment of the presence of an embryo by palpation, through rectal excavation was carried out 55 days after artificial insemination. The signs of confirmation of gestation were, an asymmetry of the uterine horns, the consistency of the horns, the presence of a large hardened corpus luteum with a tendency to collapse, the presence of an amniotic membrane (similar to a balloon filled of water) and an increase in the live weight of cows.

## 2.7. Characteristics Studied

The data was collected using cards, on which were recorded: the identification numbers of the cows, the color of the coat of each cow, the dates and times of synchronization and inseminations,

the diagnosis used as well as other observations relating to the activities. The variation factors considered were: the age of the cows, the live weight, the body condition score, the number of farrowings, the calving interval – AI. and the presence of food supplements. The synchronization and pregnancy rates following diagnoses after AI were determined by the following formulas:

$$\text{Synchronization rate} = \frac{\text{Number of cows present at the end of induction/synchronization}}{\text{Number of cows selected} \times 100}$$

$$\text{Pregnancy rate} = \frac{\text{Number of pregnant cows after diagnostics}}{\text{Number of cows inseminated} \times 100}$$

## 2.8. Statistical Analysis

The data collected were analyzed using SPSS version 21 software. Descriptive statistics were used to determine the means and standard deviations. The Chi Square test was used to test the effects of the different variation factors for the pregnancy rate after artificial insemination at the 5% significance level.

# 3. Results and Discussion

## 3.1. Results

### 3.1.1. Physiological Status of Cows After Progesterone Dosage

The progesterone level was used to determine two physiological statuses in inseminated cows: pregnancy and non-pregnancy. Table 1 illustrates the ranges of progesterone levels.

Gestation was characterized by low progesteronemia on the day of artificial insemination ( $\leq 1$  ng/ml), high from two ( $\geq 5$  ng/ml) to three weeks ( $\geq 10$  ng/ml).

Non-pregnancy showed low concentrations of progesterone on the day of artificial insemination ( $\leq 1$  ng/ml), high at two weeks after insemination ( $\geq 5$  ng/ml) which decreased at the third week ( $\leq 2$  ng/ml).

**Table 1.** Physiological status of cows according to progesterone levels.

Physiological status of cows	Progesterone levels (ng/ml) at three intervals after AI		
	Artificial insemination day	At two weeks	At three weeks
Gestation	$\leq 1$	$\geq 5$	$\geq 10$
Non-Gestation	$\leq 1$	$\geq 5$	$\leq 2$

AI: artificial insemination

### 3.1.2. Physiological Status of Palpated Cows

Palpation carried out 55 days after artificial insemination also revealed two physiological statuses. Gestation was characterized by the presence of a large and hard gestational corpus luteum, asymmetry of the uterine horns and the presence of an amniotic sac which bounced when touched. Non-pregnancy was determined by the absence of characteristic signs of gestation and the presence of large follicles.

### 3.1.3. Synchronization Rate and Pregnancy Rate After Artificial Insemination

The total number of cows selected and treated with PRID delta (synchronization) was 34, but 33 cows completed the treatments, representing a synchronization rate of 97.06%.

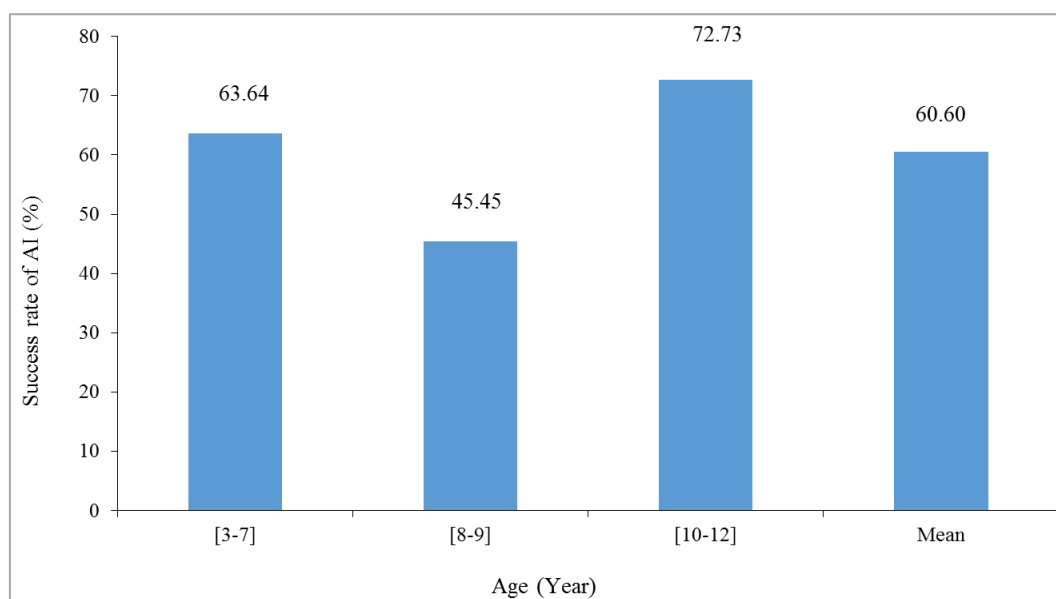
The pregnancy diagnostics used made it possible to identify 20 pregnant cows out of the 33 cows inseminated, i.e. an overall pregnancy rate after insemination of 60.60% (Table 2).

**Table 2.** Status of inseminated cows.

Physiological status of cows	Numbers of cows	Percentage
Pregnant	20	60.60
No-pregnant	13	33.40
Total	33	100.00

### 3.1.4. Effect of Cow Age on Pregnancy Rate After Artificial Insemination

Data relating to the age of the cows made it possible to divide them into three groups. Cows aged 3-7 years and those aged 10-12 years responded better to the act of artificial insemination with pregnancy rates of 63.64% and 72.73% respectively (Figure 1). These rates were high compared to the rate of 45.45% recorded in 8-9-year-old cows. However, no significant difference ( $P>0.05$ ) was observed between these different age groups for the pregnancy rate after artificial insemination.

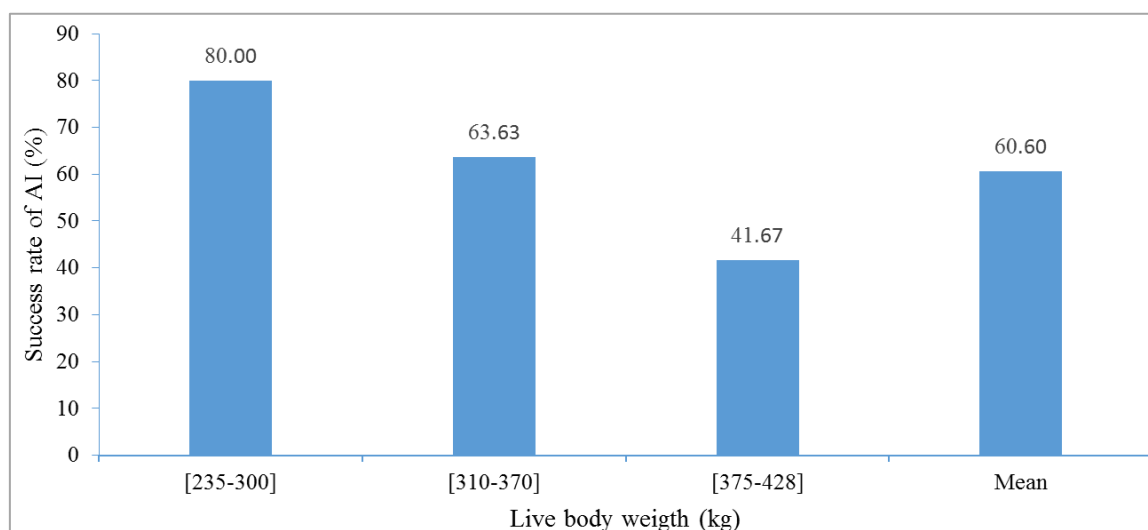


**Figure 1.** Pregnancy rate after artificial insemination depending on the age of the cows.

### 3.1.5. Effect of Live Weight on Pregnancy Rate After AI

Figure 2 shows that the pregnancy rate after AI decreases with increasing live weight in cows. The best pregnancy rate (80%) was recorded in cows weighing between 235-300 kg,

followed by that of cows weighing 310-370 kg which was 63.63%. Heavier cows recorded the lowest pregnancy rate (41.67%). However, the analysis found no significant influence ( $P>0.05$ ) between cow live weight groups for pregnancy rate.

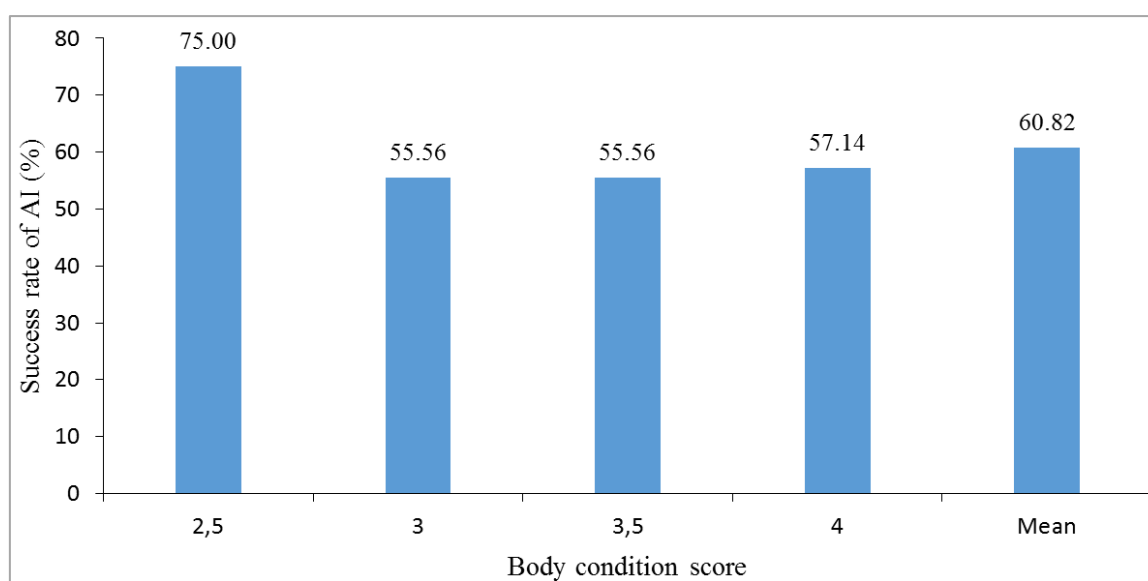


**Figure 2.** Influence of live weight on pregnancy rate after AI.

### 3.1.6. Influence of Body Condition Score (BCS) on Pregnancy Rate

Figure 3 allows us to appreciate the variation in the success rate of artificial insemination depending on the body condition score of the cows. Cows that had a BCS of 2.5 at selection

recorded the highest pregnancy rate (75%) compared to the rates obtained with other BCS values of 3, 3.5 and 4 (55, 56, 55.56 and 57.14% respectively). The analysis showed no significant difference ( $P>0.05$ ) between the different BCS on the pregnancy rate.

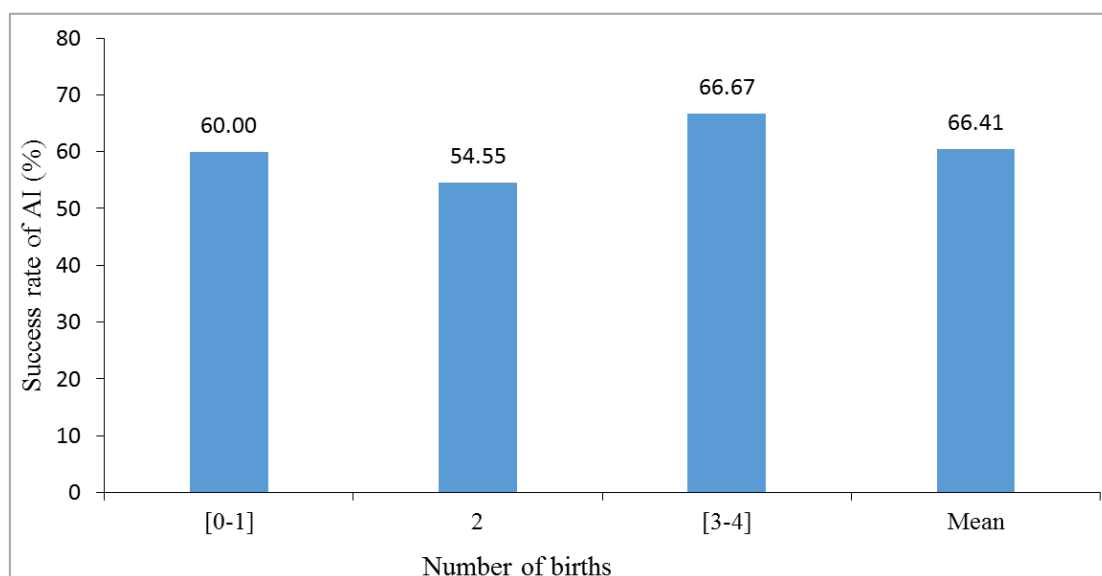


**Figure 3.** Pregnancy rate based on body condition score.

### 3.1.7. Effect of Farrowings Number on Pregnancy Rate After AI

This is the previous calving number of each cow at the time of selection. Cows whose number of births was between 3-4 responded better to artificial insemination with a pregnancy

rate of 66.67%, followed by the nulliparous and primiparous group in which a pregnancy rate was recorded by 60% (Figure 4). Cows having had two births recorded the lowest pregnancy rate (54.55%). However, the number of farrowings had no significant influence ( $P>0.05$ ) on the pregnancy rate.

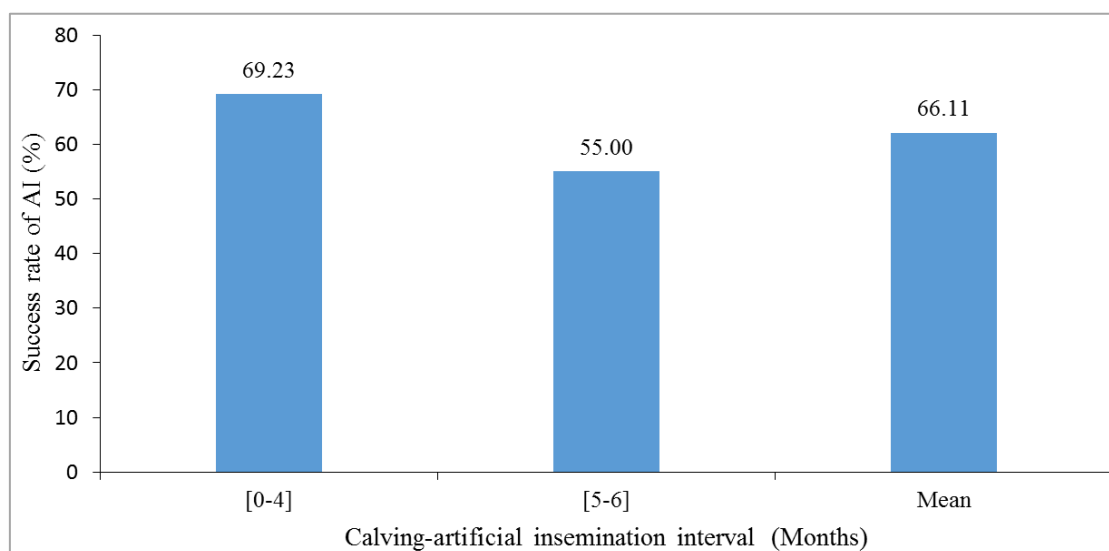


**Figure 4.** Pregnancy rate depending on the number of births.

### 3.1.8. Calving-Artificial Insemination Interval

This interval reflects the number of days elapsed from the last birth until the day of artificial insemination. For this parameter, it was observed that the fertility of cows decreased with the lengthening of this interval (Figure 5). It was better

(69.23%) in cows whose calving-AI interval was less than 5 months (0-4 months) compared to the 55% obtained with the extension of the postpartum period (5-6 months). However, this interval had no significant influence ( $P > 0.05$ ) on the pregnancy rate.



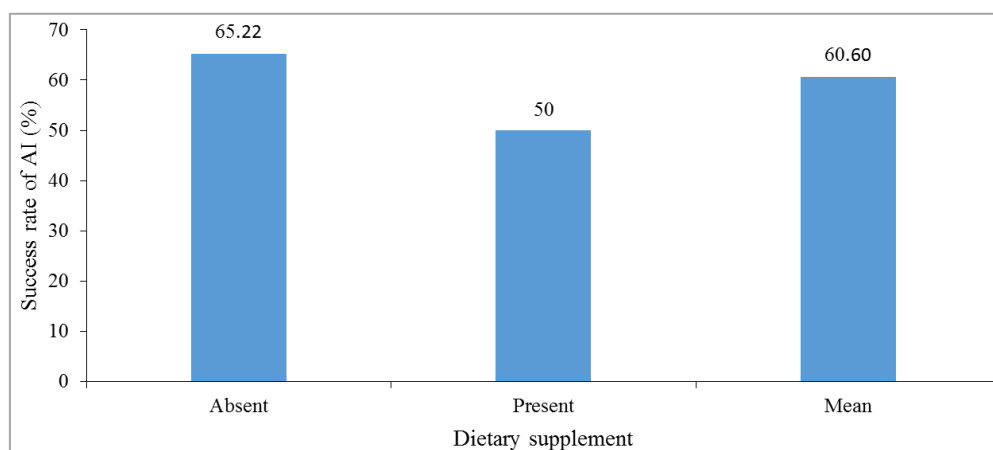
**Figure 5.** Effect of calving-artificial insemination interval on pregnancy rate.

### 3.1.9. Dietary Supplementation

Figure 6 shows that females who had been supplemented recorded a success rate after AI (50%) lower than that of cows

who were on a normal ration during the treatments (65.22%). This dietary supplementation had no significant effect ( $P > 0.05$ ) on the success rate after artificial insemination.





**Figure 6.** Pregnancy rate depending on dietary supplementation.

### 3.2. Discussion

The animal selection involved 34 cows, among which 33 followed the heat induction and synchronization protocol until the end, i.e. a synchronization rate of 97.06%. This rate reflects the effectiveness of the cows' response to hormonal treatments. It shows overall that the females all responded very well to the PRID® delta treatment. This synchronization rate is similar to the 98.04% reported by [10] on Goudali cows with the CRESTAR® method. However, it is higher than the rates of 67% obtained by Tada et al (2010) with PRID® on indigenous smallholder dairy and commercial beef cows and the 66.66% observed by [11] with PRID® on Arab zebus in Chad. The PRID® induction method could therefore solve infertility problems in local cows.

Two physiological statuses were highlighted in cows inseminated after progesterone dosing. Non-pregnant females whose progesteronemia was low on the day of insemination ( $\leq 1$  ng/ml), high two weeks after insemination ( $\geq 5$  ng/ml) and decreasing at the third week after insemination ( $\leq 2$  ng/ml). These results agree with those of [12] who reports that the concentration of progesterone, low on the day of estrus, rises until the 10<sup>th</sup> day post-insemination and then drops suddenly around the 21<sup>st</sup> day after insemination. The presumed pregnant females had low progesteronemia on the day of insemination which increased in the second and third weeks ( $\geq 10$  ng/ml). The application of pregnancy diagnosis by measuring progesterone levels in cows after artificial insemination is an excellent means of monitoring the success of this biotechnology. It is a tool widely used around the world, which allows the optimization of cattle production (helps reduce calving-to-calving intervals). During gestation, the concentration of progesterone remains high. According to [13], circulating progesterone levels is positively correlated to pregnancy rates in animals. These endogenous steroid hormone plays a vital role in the maintenance of the uterus during pregnancy [14].

The diagnosis of gestation by rectal palpation also revealed

two physiological statuses. Its use makes it possible to assess the evolution of the embryo at the start of gestation. This diagnosis also allows an examination of the external and internal genital tracts of cows and to be able to act effectively in the event of anomalies. It limits cases of “false positives” linked to the presence of cystic corpus luteum or anestrus in cows [15].

Pregnancy diagnostics (rectal palpation and progesterone dosage) made it possible to identify 20 pregnant cows out of 33 inseminated cows, i.e. a pregnancy rate of 60.6%. The pregnancy rate also characterizes the success of the PRID® delta treatment and reflects the effectiveness of the act of insemination on the cows. This rate is similar to the 60% rate reported by [16] with the CRESTAR method. However, it is higher than the rates of 45.4% obtained by [12] in the N'Dama breed with the PRID® delta treatment and 59.2% obtained by [17] in the Onkol écow in Rwanda. Opposite the current result, the pregnancy rate of 80% obtained by [18] in Indonesia with the CIDR-PGF2 $\alpha$  method on the Aceh cow is lower. It appears that this pregnancy rate is however consistent with the rates (60 to 70%) recommended in bovine artificial insemination. This result is due to the fact that the inseminations were carried out at the start of the rainy season during which the available fodder does not represent any constraints.

Cow age had no significant influence on pregnancy rate after artificial insemination. This result is similar to that of [19] who found no significant effect of cow age. However, the best results were obtained in cows aged 10-12 years and then in the youngest (3-7 years). This result differs from that obtained by [20] who noted a decrease in the fertility of cows with age. The absence of the influence of age on the pregnancy rate could be justified by the rigor with which the selection of cows was applied (by eliminating very old cows and those which were suspected of being infertile).

The present study showed that the pregnancy rate evolved inversely with the increase in live weight of cows. However, the analysis revealed that the pregnancy rate was not significantly influenced by the live weight of the cows. Cows weighing between 375-428 recorded the lowest pregnancy

rate. This result is similar to that obtained by [10] who noted that cows with a live weight of between 200-350 kg have good fertility in artificial insemination. This could be explained by the fact that reproductive function is hampered in heavier cows due to the accumulation of fat around the ovaries. According to [21] the discharge of LH on which ovulation (and therefore pregnancy) depends is closely linked to the live weight of the animal.

Body condition score (BCS) also had no effect on pregnancy rate after artificial insemination. This would be due to the fact that cows whose BCS is less than 2 and cows that are too fat (BCS greater than 4.5) were eliminated during selection. However, the best pregnancy rate was recorded by cows that had a BCS of 2.5. This result is similar to that of [19] who obtained better pregnancy rates in cows whose BCS was 2.5. That said, this result differs from that reported by [22] according to whom the best rates are obtained in cows with a BCS of 4. [23] noted that the body condition of cows at the time of insemination has a decisive influence on the pregnancy rate and should be monitored at each stage of reproduction.

The pregnancy rate was not influenced by the number of cows giving birth. But heifers and primiparous calves recorded a rate (60%) lower to that recorded in cows which had 3-4 births (66.67%). This observation is in agreement with that of [22] who found no influence of the number of births on the pregnancy rate in Gobra cows. According to the present study, to improve the fertility of a herd, it is better to select animals with lower calving ranks. Nevertheless, these results are in agreement with those of [24] who report a better pregnancy rate with cows having had 3 to 4 births than those having had 2 births.

Pregnancy rate was not significantly influenced by calving-artificial insemination interval. These results are consistent with those obtained by [25] who reported no significant influence of the postpartum artificial insemination interval on the gestation rate. According to [26], after calving, fertility progresses until the 4th month postpartum where it is maintained and then subsequently decreases when the interval is lengthened. This is also explained by the fact that it takes a period of 3 months for uterine involution after calving to be complete. [27] reported that late inseminated cows have poor fertility due to increased risks of embryonic mortality.

Supplementation two weeks before treatments had no significant effect on pregnancy rate. This could be explained by the fact that the selection of cows for inseminations was made at the start of the rainy season during which the growth of the plant offers good fodder resources for the livestock and therefore could be considered as the season favorable for the breeding of cows, the development of the fetus and the preparation for lactation. These results are different from those obtained by [28] in Senegal who noted that the pregnancy rate in cows with high blood sugar is significantly higher as compared to that with low energy source. According to Fortun-Lamothe [29], Energy deficit leads to body mobilization

and reduced reproductive performance. At the period around artificial insemination (one to two weeks after AI), energy deficiency is accompanied by high early embryonic mortality.

## 4. Conclusion and Recommendation

At the end of this study on the effect of age, live weight, body condition score, number of births, calving-artificial insemination interval and dietary supplementation on the pregnancy rate after artificial insemination on heat induced in Goudali cows, the following conclusions were drawn:

The synchronization rate obtained at the end of the induction/heat synchronization treatment was 97.06% and the pregnancy rate recorded after AI was 60.6%. This pregnancy rate was within the range of pregnancy rates recommended in bovine after AI (60-70%). The pregnancy rate after AI was not influenced by any of the factors studied. However, the oldest cows (10-12 years) recorded the best pregnancy rate, this pregnancy rate decreased with the increase in the live weight of the cows. Cows with BCS of 2.5 presented the highest gestation rate. Cows having had less than 3 calvings recorded the lowest pregnancy rates after AI, cows with an AI calving interval of less than 5 months responded better to artificial insemination, finally, dietary supplementation of cows two weeks before synchronization at rainy season did not significantly improve the pregnancy rate. Nevertheless, treatments based on PRID® delta associated with IA can resolve infertility problems in farms in tropical and subtropical areas.

Thus, in-depth studies on methods of heat synchronization and pregnancy control after artificial insemination should be conducted.

## Abbreviations

AI	Artificial Insemination
BCS	Body Condition Score
CIDR	Control Internal Drug Release
PGF2 $\alpha$	Prostaglandin F2-alpha
PRID	Progesterone Release Intravaginal Device
PMSG	Pregnant Mare Serum Gonadotropin

## Acknowledgments

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## Ethical Consideration

This study was carried out in strict accordance with recommendations of institutional guidelines for the care and use of living animals. cows were humanly handled in respect of the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.



## Author Contributions

Herve Tchoffo, Chongsi Margaret Mary Momo, Narcisse Bertin Vemo conceived, designed the research, and reviewed the manuscript.

Nina Fleur Bokop Biamou, Djalil Issa Abdel Ousmane, Mohamadou Adamou, and Ferdinand Ngoula collected the data, carried out data analysis, and wrote the manuscript. All authors read and approved the final manuscript.

## Conflicts of Interest

The authors declare no conflicts of interest.

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