

# Estrus Synchronization and Gestation Rates in Cows under Two Estrus Induction Protocols

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## Abstract

Artificial insemination has been identified as a means to intensify local production and optimize the availability of proteins of animal origin in Africa. Therefore, some factors are seemed responsible to the failure of this application in rural area. This study was conducted from mid-March to June 2018, to evaluate the cow's fertility after artificial insemination, progesterone and oxidative stress profiles according to the estrus synchronization protocols, breed, age and body condition score. 39 cows were concerned and divided into two groups. The first group was made of 9 adult cows (4 - 6 years old) with an average BCS of  $4.44 \pm 0.55$ , a calving rank of  $3 \pm 0.20$  and 10 heifers (2 - 3 years old) with a BCS of  $3.6 \pm 0.52$ . The second group was constituted of 10 adult cows (4 - 6 years old) with an average calving rank of  $2.7 \pm 0.5$  and BCS  $4.2 \pm 0.22$  and 10 heifers (2 - 3 years old) with an average BCS of  $3.8 \pm 0.36$ . The induction of estrus was done using two protocols. The first one with CIDR + Cidirol-CIDR removal + Lutalyse + Cidirol-AI (protocol 1), which allows to obtain an estrus rate induction of 94.47% and a second one with CIDR + Cidirol-CIDR removal + Lutalyse-IA + Cidirol (protocol 2), which gave an estrus rate induction of 70% and was significantly lower from the first one ( $p < 0.05$ ). The cow inseminations were done  $48 \pm 2$  hours after estrus stimulation, with a fresh semen. Analysis have been done for blood progesterone level and oxidative stress indices, within the period of synchronization and after the insemination. The overall success rates of artificial insemination recorded was 69.23 0/0. According to the protocols, the first one gave a significantly higher gestation rate (80%) compared to the second one (50%). No significant effects of oxidative stress parameters on both synchronization and gestation rate were obtained between the protocols. Although, fertility has been affected by the

breed, age and body condition score ( $p < 0.05$ ). Thus, to complete these observations, recommendations were made to improve the knowledge of the impact of total oxidant capacity on the cow's fertility during assisted reproductive treatments.

### Keywords

Artificial Insemination, Gestation, Heat Induction, Oxidative Stress, Synchronization

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## 1. Introduction

In Cameroon, short-cycle animal breeding remains poorly intensified and subject to enormous fluctuations and seasonal variations. It is cattle breeding that contributes the most to the meat supply. This breeding itself faces constraints that are very difficult to overcome. From 7.6 million in 2006, it increased to around 5.8 million in 2013 [1], which is far from increasing the availability of animal proteins. To compensate for this persistent deficit situation, periodic solutions are envisaged such as heavy imports undertaken each year by the State [2]. These imports result in enormous outflows of foreign currency and to this are added health risks linked to the entry into importing countries of products of animal origin fraudulently enriched with dangerous substances [3]. So, it would therefore be essential to act at each link in the production chain with the aim of improving and intensifying the performance of local livestock farms.

Improving the production performance of cattle in Africa could significantly contribute to increasing the availability of proteins of animal origin. It is with this in mind that biotechnological methods of controlling and controlling sexual cycles, such as heat induction and synchronization methods, artificial insemination and embryo transfer have been developed. However, these methods come up against numerous constraints [4] [5], which are of climatic, genetic, dietary, financial and managerial origins.

It is also known that poor breeding conditions and certain heat induction protocols in animals are responsible for female genital tract infections and oxidative stress [6]. In addition, recent data put oxidative stress at the heart of reproductive pathologies [7]. Oxidative stress induces by some animal reproductive biotechnologies could be responsible for low estrus synchronization and gestation rate. Amount methods used in assisted reproduction in cows, Controlled Internal Drug Release (CIDR)-Cidirol provide a consistent progesterone level and maximum number of follicles, ensuring the animal optimal reproductive state at the time of insemination [8].

Oxidative stress negatively impacts the reproductive function of mammals. It affects gametes and their interaction. It affects the differentiation of the zygote, the implantation of the egg in the maternal tract and the viability of the embryos, which impacts on conception rates thus extending calving intervals [9]. It is in-

volved in pathologies such as sepsis, mastitis, respiratory diseases and postpartum disorders such as retained placenta [10]. Oxidative stress also has a negative impact on semen quality in males. The cellular membranes of spermatozoa are very rich in polyunsaturated fatty acids making them very vulnerable to oxidation and peroxidation of lipids, which increases the abnormalities of the intermediate part, with the consequence of a reduction in their mobility. The impact of oxidative stress on spermatozoa could also lead to a reduction in their fertilizing power [7].

Measuring the activity of oxygen radicals makes it possible to characterize oxidative stress. Methods for quantifying oxidative stress involve direct or indirect measurements of oxidants and antioxidants. One of the main indicators of oxidative stress is the level of lipid (fatty acid) peroxidation, characterized by significant amounts of Malondialdehyde (MDA). MDA remains one of the best indexes of cell destruction due to the fact that in adipose cells, it induces mechanisms that tend to peroxidize more quickly than in normal cells [11]. The second indicator of oxidative stress is the activity of antioxidants which act on the redox potential of cells. Only a few studies address the implications of oxidative stress in reproductive pathologies in mammals, mainly in cows in assisted reproduction (artificial insemination and embryo transfer). Thus, this study aims to contribute to better control of the effects of heat induction protocols on fertility after artificial insemination, progesterone profiles and oxidative stress in cows.

## 2. Materials and Methods

### 2.1. Description of the Study Area

The work took place on the farm and in the Physiology laboratory of the Agricultural Research Institute for Development (IRAI) in Bambui (North-West Cameroon), from mid-March to June 2024. IRAD is located in 14 km from the town of Bamenda, in the district of Tubah, department of Mezam North-West Cameroon. This area is located at 05058' North Latitude and 10015' East Longitude and culminates at an average altitude of 1750 m. The vegetation consists of wooded savannah on the heights and gallery forest in the lowlands. In this region, 77% of the population is engaged in agriculture and livestock breeding with a cattle herd of approximately 56,038 heads [1].

### 2.2. Animal Material

The selection of cows was done first on the basis of parity and age which were determined from the farm's breeding records and the body condition score which was obtained by direct observation and interpretation following the grid of Vall *et al.* [12].

An examination by palpation of the genital tract was also carried out on the selected cows, in order to ensure their physiological status (pregnant, empty or cycled). These operations made it possible to isolate 39 empty cows. Which were identified using ear tags. None of these females were lactating at the time of selec-

tion. The cows were fed on cultivated pasture (composed of *Brachiaria ruziziensis* and *Penicetum Purpureum*) and received dietary Supplementation (composed of maize, soya bean meal and CMAV 5%) once a week.

These females were those chosen according to our selection criteria (at least three months after the last birth, body condition score between 3 - 5, parity between 2 - 4 and non-lactating). In terms of health, they were free from tuberculosis and brucellosis, and vaccinated against major epizootic diseases (contagious bovine pleuropneumonia, foot-and-mouth disease).

These cows thus selected were divided into two groups, each subject to a heat induction/synchronization protocol. Each group was subsequently divided into two batches including a batch of adult cows (4 - 6 years old) and another batch of heifers (2 - 3 years old). The inseminations were carried out with fresh Holstein bull semen collected within the Bambui IRAD.

### 2.3. Characteristics of the Selected Cows

The 39 selected cows were divided into two groups:

- The first group consisted of 19 cows. WitMAV 5%\* h a group of 9 adults including 4 of the Borane breed and 5 of the Simmental breed, ages between 4 - 6 years, average body condition score  $4.44 \pm 0.5$  and number of farrowings  $3 \pm 0.22$ . The second batch consisted of 10 heifers including 6 of the Simmental breeds and 4 of the Borane breeds, ages between 2 - 3 years. The average body condition score of heifers at selection was  $3.6 \pm 0.52$ .
- The second group made up of 20 cows. With a batch of 10 cows including 4 crossbreeds (Simmental1  $\times$  Goudali) and 6 Goudali breed cows. The age range was 4 - 6 years, with an average body condition score of NEC =  $4.2 \pm 0.32$  and an average number of farrowings of  $2.7 \pm 0.42$ . And finally a batch of 10 heifers with 5 crosses (Simmental1  $\times$  Goudali) and 5 Borane breed, age between 2 - 3 years. The average body condition score recorded was  $3.8 \pm 0.36$ .

### 2.4. Semen Collection

The semen used for artificial inseminations was collected through a vagina artificial in a Holstein bull at IRAD Bambui. The collected semen was diluted with citrate and egg yolk, packaged in a 0.25 ml dose and refrigerated at 40°C. The characteristics of the sperm were as follows:

- Volume: 9.5 ml.
- Mass motility: 4.
- Individual motility: 80%.
- Sperm concentration:  $1.2 \times 10^9$  sperm/ml.
- Percentage of dead sperm: 15%.
- Percentage of abnormal sperm: 5%.

### 2.5. Induction and Heat Synchronization

Two heat synchronization protocols were each applied to a single group.

- The first group cows ( $n = 19$ ) received on the first day (J 0) CIDR EAZI-BREED vaginal spirals (New Zealand) soaked in 1.55 g of progesterone and an intramuscular injection of 100 mg of Cidirol (Pfizer). On day 7, each of the cows received an injection of 2 ml of Lutalyse (Dinoprost, Pfizer), then all the coils were removed that same day and finally a second injection of Cidirol was given after the coils were removed. The inseminations were carried out  $48 \pm 2$  hours after removal of the spirals.
- The second group ( $n = 20$ ) received on the first day (J0) the installation of the CIDR EAZI-BREED vaginal spirals (New Zealand) soaked in 1.55 g of progesterone and the injection of 100 mg of Cidirol. On day 7, each of the cows received an injection of 2 ml of Lutalyse (Dinoprost, Pfizer), all the spirals were removed that same day. A second injection of Cidirol (Pfizer) was given on the day of insemination. Inseminations were also carried out  $48 \pm 2$  hours after removal of the coils.

## 2.6. Insemination Technique

All artificial inseminations were carried out on average  $48 + 2$  hours after removal of the vaginal coils following the established protocol. Insemination was carried out using the recto-vaginal method and the deposition of the semen was uterine in cows with an open cervix and cervical in those with a closed cervix. The operation was carried out in such a way as to avoid any trauma and infection of the cows' genital tract.

To carry out the actual artificial insemination operation, the cows were led and restricted in a containment corridor. The straws containing the seed were left at room temperature for around ten seconds, protected from the sun. The preparation of the Insemination Gun was done by rubbing on the external body in order to warm it. These straws were introduced into the insemination gun through their end having a double cap. The other heat-sealed end was cut perpendicular to the scissors to ensure maximum sealing with the cap of the insemination sheath. The gun and insemination sheath were covered with a protective plastic sheath that was punctured when the gun was inserted into the cervix.

With a gloved hand, the cervix was grasped through the rectal wall. Crossing the pass was made easier by giving it lateral and vertical movements. Once the pass was crossed, the gun was guided towards one or the other horn. The seed was placed there and the gun slowly removed then disinfected and cleaned for new use. At the end of the insemination each group of cows was separated from the other and concentrate was administered to them.

## 2.7. Progesterone Dosage and Pregnancy Diagnosis

### 2.7.1. Blood Collection and Serum Storage

Blood samples were taken from the tail vein in all cows. These samples were taken in the afternoon and on eight occasions. First throughout the induction/synchronization process and after artificial insemination.

Blood was collected using dry tubes (VacUcheck®) which were identified and transported to the animal physiology laboratory of the Institute of Agricultural Research for Development (IRAD) in Bambui. Blood samples were centrifuged at 3200 rpm for 10 minutes to obtain serum. Blue tips attached to an automatic micropipette (1000 µl) were used to collect the serum which was transferred into Eppendorf tubes, identified and stored at -20°C in the freezer.

### 2.7.2. Progesterone Dosage

Quantitative progesterone assays were carried out by the solid phase immunoenzymatic method (ELISA) as described by the commercial ClinPro progesterone EIA international kit.

To do this, 8 dosages were carried out at different periods of the heat induction/synchronization process and after artificial insemination.

Day 1 which corresponds to the day of installation of the vaginal spirals, Day 3, Day 5, Day 7 which is the day of removal of the vaginal spirals, Day 9 corresponding to the day of insemination, then 15 days after the AI, and finally 21 days after the AI.

According to Bayemi *et al.* [13], the concentrations obtained after dosing with the ClinPro kit International left as follows:

- Progesterone level < 1 ng/ml for non-pregnant cows and may indicate anestrus.
- Progesterone level < 3 ng/ml corresponding to empty cows, indicating the presence of a corpus luteum.
- Progesterone level > 3 ng/ml for cows presumed pregnant.

Non-pregnancy was therefore confirmed for progesterone values lower than 3 ng/ml and the cows were assumed to be pregnant for progesterone values greater than or equal to 3 ng/ml at the end of the dosages, *i.e.* 21 days after the artificial insemination.

### 2.8. Study of Oxidative Stress Parameters

The characteristics of oxidative stress were as follows:

- Serum levels of Malondialdehyde (MDA) and superoxide dismutase (SOD) determined following the methods described by Misra and Fridovich [14] and Nilsson *et al.* [15].

Determination of synchronization and pregnancy rates

Synchronization rate = (Number of cows present at the end of induction/synchronization × 100)/number of cow present

- Pregnancy rates were determined following progesterone measurements.

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

### 2.9. Statistical Analysis

Results were expressed as mean ± standard deviation and comparisons between dependent variables were made by analysis of variance (ANOVA). The Pearson

correlation coefficient made it possible to establish the relationships between the different parameters. SPSS 21.0 software (Statistical Package for Social Sciences) was used for the analyzes and the significance limit set at 5%.

### 3. Results and Discussion

#### 3.1. Results

##### 3.1.1. Evaluation of the Success of Heat Induction/Synchronization Protocols

##### 1) Variation in synchronization rate, serum progesterone concentration and oxidative stress level depending on heat induction/synchronization protocols and age of cows

**Table 1** summarizes the effects of heat induction protocols and cow age on synchronization rate, progesterone concentrations, Malondialdehyde and Superoxide dismutase activity.

**Table 1.** Variation in synchronization rate, serum progesterone concentrations and oxidative stress depending on the protocols and the age of the cows.

Characteristics	Protocol 1		Protocol 2	
	Adults (n = 9)	Heifers (n = 10)	Adults (n = 10)	Heifers (n = 10)
Synchronization rate (%)	100.00	90.00	90.00	50.00
Progesterone (ng/ml)	6.25 ± 4.38 <sup>a</sup>	3.83 ± 2.23 <sup>ab</sup>	1.10 ± 0.53 <sup>b</sup>	7.37 ± 5.82 <sup>a</sup>
		4.75 ± 3.80 <sup>b</sup>	2.75 ± 0.97 <sup>b</sup>	19.50 ± 8.10 <sup>a</sup>
Malondialdehyde (µM/ml)	0.70 ± 0.44	1.91 ± 1.07	2.03 ± 1.32	1.29 ± 0.55
		2.81 ± 2.05	0.86 ± 0.75	1.92 ± 0.73
Superoxide dismutase (µM/ml)	2.06 ± 0.78	5.78 ± 2.97	4.25 ± 2.47	4.89 ± 0.50
		2.68 ± 1.57	3.18 ± 1.16	4.10 ± 1.34
$\rho$	0.134	0.494	-0.027	0.198

(a, b) On the same line, the values assigned the same letter do not differ significantly ( $P > 0.05$ ). The second values correspond to the biochemical parameters of cows which showed no signs of heat. ( $\rho$ ) correlation coefficient between Malondialdehyde level and Superoxide dismutase activity.

At the end of the hormonal treatments of the first synchronization protocol (Cidirol D0 and D7), only one cow out of 19 showed no sign of heat in the hours preceding insemination. The synchronization rate recorded was 94.47%. With percentages of manifestation of heat signs of 100% in adults (n = 9) and 90% in heifers (n = 10) respectively.

Concerning the second protocol (Cidirol D0 and D9), we recorded a synchronization rate of 70% (n = 20), including 90% in adults (n = 10) and only 50% in heifers (n = 10).

Regardless of protocols, the overall synchronization rate was 82.05%. The cows subjected to protocol I reacted better (94.47%) than those of the second (70%). For each Protocol, we also notice that the adults all reacted better than the heifers.

With the heifers of the first protocol in which we recorded a rate of 90% (n = 10) compared to only 50% in the heifers of the second protocol (n = 10).

From the table, it appears that independently of the protocols, the levels of Progesterone, Malondialdehyde and the activity of Superoxide dismutase respectively do not differ significantly between the cows which showed apparent signs of heat and those which did not show any signs of heat.

The percentages of manifestation of heat signs were not significantly influenced by serum progesterone concentrations. However, progesterone concentrations were higher in cows that showed no signs of heat compared to cows in which these signs were observed.

Malondialdehyde concentrations and Superoxide dismutase activity respectively did not differ significantly between cows subjected to the two protocols.

No significant correlation was observed between Malondialdehyde levels and Superoxide dismutase activity. However, negative and non-significant correlations were recorded between progesterone levels and Superoxide dismutase activity respectively in adults of both protocols ( $\rho_1 = -0.178$ ;  $\rho_2 = -0.850$ ).

## 2) Variation in synchronization rate, progesterone profiles and oxidative stress depending on protocol type and breed

**Table 2** presents the pregnancy rates, serum values of progesterone, Malondialdehyde concentration and Superoxide dismutase activity according to the protocols, and the breed of cows which showed signs of heat and those which did not show any signs.

**Table 2.** Variation in synchronization rate, progesterone profiles and oxidative stress depending on protocol type and race.

Characteristics	Protocol 1		Protocol 2		
	Borane	Simmental	Borane	Crossed (S*G)	Goudali
Rate of synchronization (%)	87.25	100.00	40.00	77.7	100.00
Progesterone (ng/ml)	3.96 ± 1.75	4.07 ± 3.07	7.00 ± 5.60	10.41 ± 8.11	
	3.83 ± 2.23		10.66 ± 8.48	11.85 ± 7.45	
Malondialdehyde (µM/ml)	0.70 ± 0.44	1.80 ± 0.88	0.90 ± 0.79	1.15 ± 0.54	
	2.81 ± 2.05		0.84 ± 0.63	1.10 ± 0.82	
Superoxide dismutase (µM/ml)	3.23 ± 2.43	4.38 ± 3.28	2.36 ± 1.25	3.70 ± 1.38	
	3.51 ± 0.92		4.50 ± 1.78	3.75 ± 1.23	
$\rho$	0.448	0.494	0.457	-0.306	0.389

(a, b) On the same line, the values assigned the same letter do not differ significantly ( $P > 0.05$ ). The second values correspond to the biochemical parameters of cows which showed no signs of heat. ( $\rho$ ) correlation coefficient between Malondialdehyde level and Superoxide dismutase activity).

For the first protocol (CidiroI D0 and D7), it appears that the Simmental breed cows all (100%) presented apparent signs of heat compared to the Borane breed cows (87.25) (**Table 2**).

Concerning the second protocol (Cidriol D0 and D9), Goudali breed cows reacted better to treatments (100%) than Borane breed cows (40%).

The Borane and Simmental breed cows subjected to the first protocol reacted better than the Borane cows and the crossbreds of the second protocol.

Malondialdehyde level and Superoxide dismutase activity, respectively, did not differ significantly between cows of different breeds on the one hand between cows which showed signs of heat and those which did not react on the other hand.

No significant correlation was observed between Malondialdehyde levels and Superoxide dismutase activity.

### 3) Variation in synchronization rate, progesterone profiles and oxidative stress depending on protocol type and body condition score

The values of synchronization rates, serum progesterone concentration and oxidative stress according to the protocol type and body condition score of cows are reported in **Table 3**.

**Table 3.** Variation in synchronization rate, progesterone profiles and oxidative stress according to protocol type and body condition score.

Characteristics	Protocol 1		Protocol 2	
	3.5	4 - 5	3.5	4 - 5
Effective	7	12	7	13
Rate synchronization (%)	85.72	100.00	43.00	85.00
Progesterone (ng/ml)	3.80 ± 1.20 <sup>b</sup>	4.38 ± 3.91 <sup>b</sup>	12.67 ± 6.14 <sup>a</sup>	2.68 ± 1.25 <sup>b</sup>
	12.10 ± 10.35		7.50 ± 2.75	17.65 ± 10.27
Malondialdehyde (µM/ml)	1.36 ± 0.97	0.83 ± 0.32	0.98 ± 0.61	1.26 ± 0.75
	2.72 ± 0.89		0.85 ± 0.56	1.17 ± 0.43
Superoxide dismutase (µM/ml)	3.78 ± 2.60	3.90 ± 2.25	2.88 ± 1.15	4.74 ± 3.61
	3.51 ± 1.52		3.68 ± 2.15	3.81 ± 2.30
$\rho$	-0.812 <sup>*</sup>	-0.218	0.435	0.580 <sup>*</sup>

(a, b) On the same line, the values assigned the same letter do not differ significantly ( $P > 0.05$ ). The second values correspond to the biochemical parameters of the cows which showed no sign of heat. ( $\rho$ ) Correlation coefficient between Malondialdehyde level and Superoxide dismutase activity.

**Table 3** shows that the best synchronization rates were obtained in cows with a body condition score between 4 and 5: including 100% for the first protocol and 85% for the second.

As for the second protocol, cows with a body condition score (BCS) equal to 3.5 recorded a synchronization rate lower than that obtained in cows with an BCS between 4 - 5.

According to **Table 3**, low concentration of progesterone was obtained in cows that showed signs of heat compared to cows that did not show. However, the significantly highest progesterone concentrations were observed in cows from the

second protocol (Cidirol D0 and D9) of BCS 3.5 which showed heat.

Malondialdehyde concentration and Superoxide dismutase activity did not differ significantly between cows that showed signs of heat and between cows in which no signs of heat were observed before Artificial insemination.

Negative and significant correlation was recorded between the levels of Progesterone and Malondialdehyde concentrations in cows of protocol I (Cidirol D0 and D7) of BCS = 3.5 ( $\rho = -0.812^*$ ) and positive and significant correlation was recorded between Malondialdehyde level and Superoxide dismutase activity in that of protocol 2 ( $\rho = 0.580^*$ ).

#### 4) Variation in pregnancy rate after artificial insemination

Eight assays were carried out to establish the progesterone profiles of the cows during the synchronization protocols and after artificial inseminations. Confirmation of non-pregnancy was effective at the last dosage, 21 days after artificial insemination.

### 3.1.2. Effect of Induction/Synchronization Protocols and Cow Age on Pregnancy Rates after Artificial Insemination

**Table 4** represents the pregnancy rates and the average progesterone values according to the heat induction protocols and to the age of the cow present.

**Table 4.** Pregnancy rate and progesterone level depending on heat induction/synchronization protocols and cow age.

Characteristics	Protocol 1			Protocol 2		
	Adult (4 - 6 years)	Heifer (2 - 3 years)	Total	Adult (4 - 6 years)	Heifer (2 - 3 years)	Total
Effective	9	10	19	7	13	20
Gestation Rate (%)	100.00	80.00	89.47	60.00	40.00	50.00
Progesterone (ng/ml)	17.73 ± 12.31 <sup>a</sup>	21.80 ± 10.07 <sup>a</sup>	19.77 ± 11.19 <sup>a</sup>	11.25 ± 7.75 <sup>ab</sup>	4.54 ± 3.25 <sup>b</sup>	7.90 ± 5.5 <sup>b</sup>
		1.67 ± 0.90	1.78 ± 0.98	1.21 ± 0.75	1.17 ± 0.88	1.19 ± 0.81

(a, b) On the same line, the values of progesterone assigned the same letter do not differ significantly ( $P > 0.05$ ). The second values of progesterone correspond to those of cows which showed no sign of heat.

Overall, there were 27 pregnant cows out of 39 inseminated, representing a pregnancy rate of 69.23%. According to **Table 4**, cows whose heat was induced from the first Protocol recorded pregnancy rates higher than the rates of those subjected to the Second protocol (Cidirol D9). Regardless of age, the progesterone concentrations of cows in protocol I were significant ( $p > 0.05$ ) higher than those in the second protocol.

### 3.1.3. Effects of Induction/Synchronization Protocols, Breed and Body Condition Score of Cows on Pregnancy Rates After Artificial Insemination

Pregnancy rates after insemination and progesterone values according to heat induction protocols and breed are reported in **Table 5**.

**Table 5.** Effect of induction/synchronization protocols and cow breed on progesterone amounts and pregnancy rates after artificial insemination.

Characteristics	Protocol 1		Protocol 2		
	Borane	Simmental	Borane	Crossed (S*G)	Goudali
Effective	8	11	5	9	6
Progesterone (ng/ml)	19.91 ± 10.98 <sup>a</sup>	19.73 ± 10.30 <sup>a</sup>	5.25 ± 2.50 <sup>b</sup>	6.67 ± 2.70 <sup>b</sup>	15.00 ± 10.67 <sup>a</sup>
	1.35 ± 0.25	1.75 ± 1.15	1.75 ± 1.15	1.25 ± 1.00	1.82 ± 0.79
Pregnancy rate (%)	91.66	87.50	20.00	77.70	33.30

(a, b) On the same line, the values assigned the same letter do not differ significantly ( $P > 0.05$ ). The second progesterone values correspond to empty cows.

Pregnant Borane cows and crossbreeds (protocol 2) recorded significantly lower levels of Progesterone than Goudali cows and those in the first protocol.

The pregnancy rate recorded in Borane cows subjected to the first Synchronization protocol was higher than the pregnancy rate of Borane cows subjected to the second protocol.

### 3.1.4. Effect of Heat Induction Protocols and Cow Body Condition Score on Progesterone Levels and Pregnancy Rates After Artificial Insemination

Post-insemination pregnancy rates and progesterone values based on heat induction protocols and body condition score are shown in **Table 6**.

**Table 6.** Effect of heat induction protocols and cow body condition score on progesterone levels and pregnancy rates after artificial insemination.

Characteristics	Protocol 1		Protocol 2	
	3.5	4 - 5	3.5	4 - 5
Effective	7	12	7	13
Progesterone (ng/ml)	19.77 ± 15.21 <sup>a</sup>	18.80 ± 13.20 <sup>a</sup>	4.48 ± 3.30 <sup>b</sup>	9.15 ± 7.58 <sup>ab</sup>
	2.05 ± 1.58	1.25 ± 0.26	0.45 ± 0.21	1.76 ± 0.74
Pregnancy rate (%)	85.72	92.30	28.75	77.53

(a, b) On the same line, the values assigned the same letter do not differ significantly ( $P > 0.05$ ). The second progesterone values correspond to empty cows.

Regardless of the protocol, the best pregnancy rates were recorded in cows with a body condition score of between 4 and 5. The pregnancy rate of cows with a body condition score of 3.5 in the second protocol was lower than that of those of the first. In these cows with a body condition score equal to 3.5 from protocol 2, the progesterone level was significantly ( $P < 0.05$ ) the lowest compared to those noted in the other cows. The progesterone levels of pregnant cows subjected to the first protocol differed significantly from those of cows in the second protocol.

### 3.1.5. Effect of Heat Induction Protocols, Breed, Body Condition Score and Physiological Status on Variation in Malondialdehyde Levels and Superoxide Dismutase Activity

According to **Table 7**, Malondialdehyde levels and Superoxide dismutase activity did not differ significantly between pregnant and empty breeds regardless of heat induction protocol. However, in empty cows, Superoxide dismutase activity is higher in empty cows compared to pregnant cows.

**Table 7.** Malondialdehyde values and Superoxide dismutase activity according to protocols, cow breed and physiological status after AI.

Characteristics	Protocol			Protocol	
	Borane	Simmental	Borane	Crossed (S*G)	Goudali
Malondialdehyde ( $\mu\text{M}/\text{ml}$ )	$4.19 \pm 1.41$	$1.92 \pm 0.82$	$0.78 \pm 0.12$	$1.08 \pm 0.71$	$1.27 \pm 0.61$
	$0.86 \pm 0.26$	$0.82 \pm 0.31$	$0.66 \pm 0.18$	$1.04 \pm 0.77$	$1.30 \pm 0.97$
Superoxide dismutase ( $\mu\text{M}/\text{ml}$ )	$3.80 \pm 3.23$	$4.32 \pm 2.22$	$2.96 \pm 1.15$	$3.94 \pm 3.25$	$5.18 \pm 2.55$
	$4.25 \pm 2.45$	$7.66 \pm 4.95$	$3.68 \pm 1.38$	$3.68 \pm 2.68$	$7.12 \pm 3.5$
$\rho$	0.484	0.664*	-0.494	0.538	0.582

( $\rho$ ) correlation coefficient between Malondialdehyde levels and Superoxide dismutase activity. (\*) Significant at 0.05.

It appears from **Table 7** that there is a positive and significant correlation between Malondialdehyde concentrations and Superoxide dismutase activity in Simmental cows ( $\rho = 664$ ).

**Table 8** also presents Malondialdehyde values and Superoxide dismutase activity according to protocols, cow body condition score and physiological status after AI.

**Table 8.** Malondialdehyde values and Superoxide dismutase activity according to protocols, cow body condition score and physiological status after AI.

Characteristics	Protocol 1		Protocol 2	
	3.5	4 - 5	3.5	4 - 5
Malondialdehyde ( $\mu\text{M}/\text{ml}$ )	$1.27 \pm 0.76$	$0.67 \pm 0.37$	$0.90 \pm 0.62$	$1.22 \pm 0.99$
	$0.85 \pm 0.24$	$1.80 \pm 0.88$	$1.73 \pm 0.67$	$1.61 \pm 0.72$
Superoxide dismutase ( $\mu\text{M}/\text{ml}$ )	$3.64 \pm 2.45$	$9.50 \pm 6.13^a$	$2.88 \pm 1.15$	$4.91 \pm 3.65$
	$4.56 \pm 3.93$	$7.66 \pm 3.60$	$3.68 \pm 2.15$	$5.58 \pm 2.25$
$\rho$	0.556	0.938**	-0.374	0.530*

(\*) Significant at 0.05; (\*\*) significant at 0.01.

Malondialdehyde levels and Superoxide Dismutase activity respectively did not differ significantly between pregnant and empty cows.

This table also reveals positive and significant correlations between Malondialdehyde concentrations and Superoxide dismutase activity in cows with body condition scores between 4 - 5 from both protocols ( $\rho_1 = 0.938$ ;  $P < 0.01$ ,  $\rho_2 = 0.530$ ;  $P$

< 0.05).

### 3.2. Discussion

Heat induction/synchronization protocols based on the use of progesterone are increasingly being developed in cattle. Some studies [16]-[18]; report that the application of these intravaginal progesterone-releasing devices frequently causes oxidative stress which influences the fertility of animals undergoing artificial insemination. The present study evaluates the implication of certain factors linked to the animal in the oxidative stress induces by Heat induction/synchronization protocols.

After using two CIDR-based protocols, the synchronization rate obtained from the first synchronization protocol (94.47%) was higher than that obtained with the second (70%). This could be justified by the fact that the injection of Cidriol on the day of insemination in the cows of the second group did not have the expected effect (promoting the onset of heat). Especially since Cidriol is a product widely used in the heat synchronization protocol in cattle and containing a synthetic estradiol which promotes the onset of heat and ovulation. This late administration of Cidriol in the cows of the second Protocol would have contributed to its inferiority which would result in a weak manifestation of signs of heat in the cows of this protocol. The synchronization rate of cows in protocol 1 is quite similar to the rate of 98.04% reported by Seydou *et al.* [19] on Goudali cows with the same method, but lower than the 100% rate obtained by Abonou [16] in with the CIDR method on crosses. The synchronization rate obtained is also quite close to the rate of 66.66% observed by Vounparet *et al.* [17] on Arabian zebus with the CRESTAR method which is quite similar to the type of protocol applied to animals in the second group. The results also show that adults in these two protocols (100 and 90%) had better synchronization rates compared to heifers (90 and 50%). This could be explained by the fact that fertility increases in cattle with age and would be quite average in heifers in which the reproductive processors are still established.

It is known that better-fed cows show signs of heat better than cows whose diet is poor. Better synchronization rate was obtained in cows with Body Condition Scores between (BCS) 4 and 5. This could also be explained by the fact that reproductive performance in cattle increases with energy balance and the body condition of females and therefore, cows with better Body Condition Scores would have better fertility. Regardless of the protocols, 90% of the cows of BCS equal 3.5 which did not show signs of heat would be for the majority heifers.

In each group, correlations MDA levels were not significantly elevated. This would have favored the expression of quantities of progesterone coming from the spirals. However, no significant effect was observed on synchronization rate. It is still known that MDA has an effect on the growth process, maturation of follicles but also steroidogenesis and hence the appearance of heat. Also, the activity of MDA is known to increase the production of free radicals. It is also assumed that

it is possible that the increase in MDA levels is local (at the ovarian level) and cannot be significantly detected at the serum level, due to the regulatory activity of SOD.

This observation noted in the present study differs from that of Kuru *et al.* [18] who noted that the quantity of MDA increased significantly after the removal of the spirals until the day of observation of heat, due to significant oxidative activity occurring during the installation and removal of the spirals.

Progesterone profiles were highlighted in cows from both groups. In non-pregnant females, the quantities of progesterone were high on the days following the placement of the coils (5 ng/ml), and reduced the day of estrus for cows in heat (unlike cows which did not show no sign of heat). Despite their decrease, progesterone concentrations remained high two weeks after insemination and reduced at the third week after insemination (<2 ng/ml). These results agree with those of Bayémi *et al.* [13] who revealed that the concentration of progesterone is low on the day of estrus, rises until the tenth day post-insemination and falls around the 21st day after insemination in females diagnosed as non-pregnant. Presumably pregnant females had progesterone concentrations that increased at the second and third week after AI (10 ng/ml). According to Thimonier [20], the concentration of progesterone remains high throughout gestation. 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. This observation is the basis of this pregnancy diagnosis [21].

Regardless of protocols, the overall pregnancy rate was 69.23%. 27 cows out of 39 were diagnosed as pregnant at the end of the Progesterone dosages. The cows subjected to protocol 1 recorded a pregnancy rate of 89.47% unlike the cows subjected to the second in which we noted a pregnancy rate of only 50%. The pregnancy rate of cows from the first protocol was slightly higher than 77.8% reported by Solihati [22] in dairy cattle FH using intravaginal progesterone with estrogen and injection 15 mg PGF2 $\alpha$  intramuscularly and 80% obtained by Siregar *et al.* [23] with the CIDR-PGF2 $\alpha$  method on the Aceh cow.

The pregnancy rate recorded by the cows in the second protocol was below the pregnancy rates recommended in AI (60% - 70%). It is also lower than the rate of 60% reported by Nakrani *et al.* [24] with the CRESTAR method in Holstein cows. Cows in protocol I responded significantly better to AI than those in the second. This is justified by the high rate of heat induction and synchronization caused by the first protocol compared to the second. This would have favored fertility, followed by the better pregnancy rate of group I cows. elsewhere, the low pregnancy rate recorded in group II cows could result from both oxidative stresses induced by insemination and injection of Cidirool at the same day.

Adults in both heat synchronization protocols (100% and 60%) obtained better

pregnancy rates compared to heifers (80% and 40%). With a lower pregnancy rate recorded in heifers in the second group. This is explained firstly by the difference between the induction and heat synchronization protocols followed by the cows in the second group, because a poor luteal phase can influence the fertility of the cows and disadvantage the implantation and development of the conceptus. This observation could also be explained by the fact that fertility and fecundity increase with the increase in the number of births in cows and then reduce when the cows have reached more than 4 births. These results are in agreement with those of Tada *et al.* [25] who report a better pregnancy rate with cows aged between 4 - 6 years.

The lowest pregnancy rate was recorded in Borane and Goudali cows subjected to the second synchronization protocol. This could be justified by the fact that local cows generally have lower fertility than imported breed cows.

The best pregnancy rates were observed in cows which had BCS of 4 and 5. This result is closed to that reported by Dickinson *et al.* [26] according to whom the best rates are obtained in cows with a BCS of 4. Issa *et al.* [27] note 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 best pregnancy rates noted in cows which BCS of 4 and 5 could be justify by appropriate proportion nutrients and good functioning of reproductive organs, necessary for gamete production, fecundation and gestation.

According to Celi *et al.* [28], the oxidative stress attested by the imbalance between MDA level and SOD activity would be the cause of reproductive failures and cases of early embryonic mortality after AI. Serum MDA level and SOD activity were higher in empty Simmental and Goudali cows than in pregnant. This could be justified by the fact that MDA activities were more pronounced during the days preceding estrus in these cows, justifying the high level of oxidative stress responsible for degrading pregnancy. The MDA level and SOD activity were found to be higher in cows with the BCS of 3.5. According to Amel *et al.* [29], oxidative activity would be more pronounced during energy imbalance and notes that oxidative stress would increase with the reduction in body condition score, due to the physiological processes that take place in order to compensate for energy deficits.

#### 4. Conclusion

At the end of this study relating to the evaluation of fertility, progesterone profiles and oxidative stress following heat induction and artificial insemination in cows, the following conclusions were drawn: The synchronization and Pregnancy rates were higher for the first protocol (Cidirol D0 and D7 injections) compared to the second (Cidirol D0 and D9 injections). The best synchronization rates were observed in adults compared to the rates obtained in heifers. Oxidative stress parameters did not significantly affect pregnancy rates. However, we notice a more pronounced imbalance between the concentrations of MDA and SOD in heifers of

group 2 and in cows of Goudali breeds. Thus, future studies should be carried out on strategies to increase the synchronization and Pregnancy rates in cows in case the second protocol (Cidirol D0 and D9 injections) is used.

### Ethical Approval and Consent to Participate

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.

### Conflicts of Interest

The authors declare no conflict of interest.

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