

Assessment of Fertility, Progesterone Profiles and Oxidative Stress Parameters after Estrus Induction and Artificial Insemination in Cows

Hakoueu Flora¹, Ndzi Elvis Ndukong¹, Nsadzetsen Gilbert Adzemye¹, Mbiba Hassanu Fanadzenyuy¹, Kinso Ambrose Limnyuy¹, Kouamo Justin², Ngoula Ferdinand³, Bayemi Henri¹

¹ Institute of Agricultural Research for Development (IRAD), Bamenda, Cameroon

² School of Veterinary Medicine and Sciences, University of Ngaoundere, Cameroon

³ Department of Animal Production, Faculty of Agronomy and Agricultural Sciences, University of Dschang, Cameroon

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Abstract

Artificial insemination (AI) has been identified as a means to intensify local production and optimize the availability of proteins of animal origin in Africa. Some factors are seemingly responding to the failure of this application in rural areas. This study was conducted to evaluate the fertility, progesterone profiles, and oxidative stress parameters after estrus induction and artificial insemination in cows according to age, breeds, and body condition score after the implementation of two estrus synchronization protocols. The induction of estrus was realized using two protocols with each protocol corresponding to one group of cows. Oxidative stress parameters were studied by measuring the concentrations of malondialdehyde and superoxide dismutase. Protocol 1 was applied with CIDR+Cidirol-CIDR removal+Lutalyse+Cidirol-A, which resulted in a synchronization rate of 94.47%, and the second protocol with CIDR+Cidirol-CIDR removal+Lutalyse-IA+Cidirol (protocol 2), which gave an estrus rate of 70% and was significantly different from the first one ($p < 0.05$). The overall success rate of artificial insemination recorded was 69.23%. With respect to the protocols, protocol 1 gave a significantly higher gestation rate (80%) compared to protocol 2 (50%). Fertility was affected by the breed, age, and body condition score ($p < 0.05$). The later injection of the Cidirol (Day 9) in protocol 2 induced the lowest rate of fertility after artificial insemination. The present study also suggests that estrus synchronization methods and artificial insemination contribute to the variation of oxidative stress parameters such as malondialdehyde and superoxide dismutase which leads to an imbalance resulting in oxidative stress.

Keywords: Artificial insemination, Cidirol, Gestation, Oxidative stress, Synchronization

Introduction

Livestock rearing constitutes one of the most important activities for rural populations. It plays a crucial role in more than 80% of the sociocultural, economic, and nutrition welfare of these populations (1). In these rural areas, cattle husbandry is principally made of zebu, which represents one of the first sources of animal proteins and revenue. Demography evolution and individual consumption growth predictions suggested that by the year 2020, it will be a need to produce more than 220 billion liters of milk and 100 million tons of meat in developing countries to satisfy the demand for animal proteins (2). However, these objectives seem difficult to achieve in Africa. Although Africa possesses 16.7 % of the worldwide bovine herd, her production remains low with a milking potential which hardly reaches 4.6 % of worldwide burdens (3). In addition, even if the Cameroonian bovine herd is estimated at 7.2 million, it contributes just 30 % of the national meat production and still has a deficit of 17000 tons in her milk production per year (4). This contribution does not satisfy the food needs of a nation with an annual growth rate of between 2 to 3%, which is one of the highest in the world.

Importation is used as a means to temporarily resolve this situation despite the sanitary risks that come with it.

The low productivity of bovine in Cameroon is caused by many factors such as insufficient feeding, poor climatic condition, animal genetic, and poor livestock management which could be associated with oxidative stress. View this situation; it is a necessity to improve all the steps involved in the animal production chain. The intensification of local production could pass through the utilization of assisted reproductive techniques (ART) in livestock such as estrus induction and synchronization, artificial insemination (AI), and embryo transfer. According to Robert and Gray, AI is the simplest means of improving the productivity of African bovine breeds. It is the most efficient tool of genetic material diffusion widely used around the world (5). However, the transfer of these technologies in real areas faces numerous difficulties that delay their expansion. Synchronization methods are applied to control the reproduction and facilitate the management of livestock (6). Nevertheless, in Africa, the response of local breeds is not well known and differs from exotic

*Corresponding author: Hakoueu Flora, Institute of Agricultural Research for Development (IRAD), Bamenda, Cameroon. E-mail: hakoueuf@yahoo.fr

breeds in which these techniques are usually tested. Many factors (endogenous and exogenous) also affect the application of these techniques. In addition, because all products used in the synchronization methods are purchased abroad, it makes the application very expensive in Africa. It is therefore important to find the right way to apply these techniques at low cost with high efficacy in local settings.

Oxidative stress could be one of the problems associated with AI because these techniques usually involve introducing exogenous factors into the natural system of animals. Oxidative stress is a situation characterized by the cellular imbalance between the production of oxidants and their neutralizing antioxidants. This stress increases with deficiency of the immune system. Oxidative profiles are exploited to control the sanitary and nutritional status and to monitor the reproductive performances of animals. It is known that oxidative stress has numerous negative impacts on the development and the expression of gametes. It also affects the growth and development of the eggs in mammals (7, 8). Oxidative Stress implies pathologies like preeclampsia, septicemia, mastitis, respiratory diseases, and postpartum disorders in cows. Oxidative stress also affects semen quality in bulls. Indeed, spermatozoa cell membranes are rich in polyunsaturated acids, rendering them vulnerable to lipid peroxidation which increases the abnormality of the intermediary peace, leading to reduction of their mobility and fertility power (9). The measure of the free oxygen radical activities enables the characterization of oxidative stress. The principal indicator used is the level of lipid peroxidation characterized by increasing Malondialdehyde (MDA) blood concentrations as the end product. The second indicator used is the level of blood total antioxidants which act on the cell redox potential. Per our knowledge, data on the effect of oxidative stress in bovine reproductive function after AI is not yet available as is the case in assisted reproductive technologies (ART) in human reproduction.

It is in this context that this study was carried out to compare the efficacy of two protocols using CIDR vaginal spirals as a base and evaluating the variation of progesterone concentration and oxidative stress parameters during the process of estrus induction and AI, considering internal factors such as breed, age, and body condition score of cows.

Materials and methods

Period and Study Area

The present study was carried out during the period from March to June 2021 at Bambui Regional Centre of the Institute of Agricultural Research for Development (IRAD).

Selection and management of experimental cows

A total of 39 non-pregnant and healthy cows were used in this study. These cows were selected according to their health status (only cows with good health) depending on the evaluation of general animal health status and the rectal palpation.

Cows were grazing in rotation on pastures from 9 AM to 4 PM and they were receiving *ad libitum* forage (*Pennisetum clandestinum*, *Stylosanthes*, and *Bracharia sp*) and hay (*Bracharia sp*) when they were in the pens. Water supply was *ad libitum* in concrete water troughs placed in pasture or in pens. Each cow received 4 kg (2kg morning at 7 AM and 2kg evening at 5 PM) of concentrate made of 38% of corn, 30% rice or wheat bran, 30% cottonseed cake, 1% bone meal, and 1% common salt. The calculated chemical composition of the

concentrate showed a rate of 74.6% for digestible total nutrients and 16 % for crude protein. They were all subjected to the semi-intensive system and were divided into two groups and each group was further divided into two lots.

Group 1 was constituted of 19 cows with the first lot of 9 adults (4 Borane and 5 Simmental). The age interval was 4-6 years old, mean body condition score (BCS) = 4.44 ± 0.50 and mean number of calving (NC) = 2.70 ± 0.42 . The second lot was made of 10 heifers (4 Borane and 6 Simmental), with an age interval between 2-4 years and mean BCS = 3.60 ± 0.52 .

Group 2 was constituted of 20 cows with the first lot of 10 adults, made of 4 crossbreeds (Simmental-Gudali) and 6 Gudali. The age interval was 4-6 years, mean BSC of 4.20 ± 0.32 and a mean NC of 2.70 ± 0.42 . The second lot of 10 heifers was made up of 5 crossbreeds and 5 Gudali, with an age interval of 2-4 years and a mean BSC of 3.80 ± 0.36 .

Estrus induction and synchronization protocols

Two protocols were applied for the estrus induction and synchronization (one protocol per group of cows) using CIDR (Controlled Internal Drug Release), Cidriol (GnRH), and lutalyse (PGF2 α), at different times for each protocol. Figures 1 and 2 show the application scheme of the two protocols. It consisted of each protocol of the intra-vaginal application of CIRD for 8 days and intramuscular injection of 1ml of Cidriol (GnRH) at the time of application of CIRD. An injection of 5ml of lutalyse (PGF2 α) and 1 ml of Cidriol was given intramuscularly at the time of removal of CIRD in protocol 1 whereas in protocol 2 only 5ml of lutalyse (PGF2 α) was administered when CIRD implant was removed. Cows were inseminated using the recto-vaginal method 24 hours after withdrawal of CIRD in protocol 1 and 48 hours in protocol 2 using a sample of semen collected from Holstein bull. After insemination, each cow received 1 ml of Cidriol. Semen samples having 85%, of individual motility, of spermatozoa concentration of 1.2×10^9 sp/ml, 15% of dead spermatozoa, and 5% of abnormal spermatozoa were diluted into citrate and egg yolk, packed in doses of 0.25 ml, and cooled at 4°C.

Blood sample collection and serum conservation

Blood samples of cows were collected from caudal veins with dry tubes (vacUcheck®) in the morning to avoid stress. These collections were realized along with the estrus synchronization protocols, on the day of artificial insemination and after artificial insemination. The blood tubes were labeled and carried to the animal physiology laboratory of the Institute of Agricultural Research for Development of Bambui. Blood samples were centrifuged at 3200 rounds/minute to obtain serum. This serum was put into labeled Eppendorf tubes and conserved at -20°C.

Assay of progesterone concentration and pregnancy testing

The variations of progesterone concentration induced by the application of the two protocols and by artificial insemination were compared within and between the two groups of animals. A quantitative assay of progesterone was done using ELISA which permitted us to obtain the absorbance curve from which concentrations of progesterone were estimated. Seven (07) assays were done for both

protocols which corresponded to: day 0 (CIDR insertion), day 3 and day 5 (after CIDR insertion), day 7 (removal of CIDR), days 8 and 9 (day of artificial insemination), days 15 and 21 after artificial insemination. The concentration of progesterone was assayed using ClinPro International® kit test. Non-pregnancy was determined for the progesterone concentration values less than 3 ng/ml at the end of all assays (21 days after artificial insemination).

Oxidative stress parameters estimations

Malondialdehyde (MDA) was assayed by the method of Satoh and the activity of Superoxide dismutase (SOD) by the method of Misra and Friedovich. These two parameters were

assayed at the same seven-period intervals as in the progesterone concentration assay.

Statistical analysis

Data were subjected to descriptive statistics and analysis of variance using the multifactor analysis of variance (ANOVA). Chi-square test was used to determine the interactions between the studied parameters. Results were expressed as mean \pm standard deviation. Significant differences between means were separated using the Tukey's test at the probability of $P < 0.05$ using the software SPSS version 23.0.

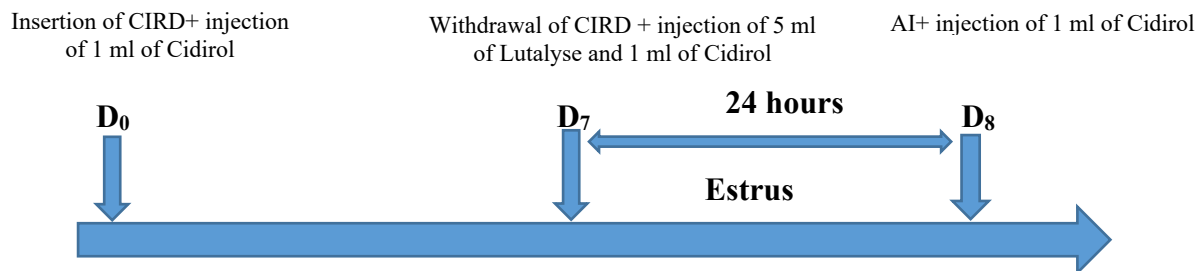


Figure 1. Protocol 1 of induction and synchronization of estrus and artificial insemination

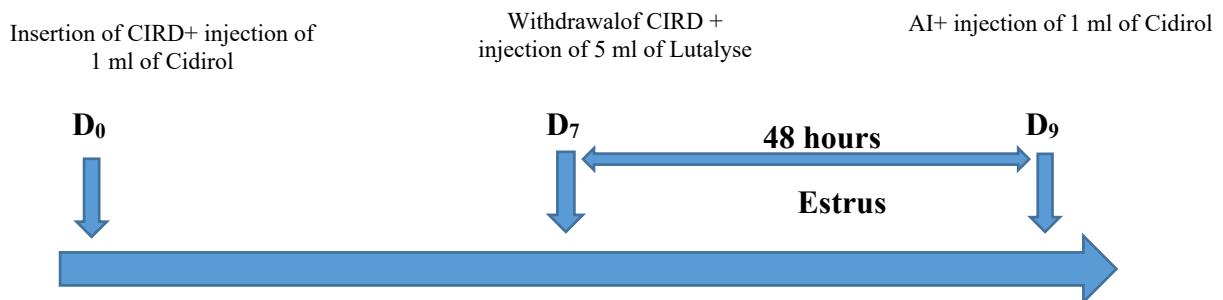


Figure 2. Protocol 2 of induction and synchronization of estrus and artificial insemination

Results

Synchronization, gestation rates and mean progesterone concentrations relative to different protocols and ages

Table 1 shows the rates of synchronization and gestation respectively and the concentration of the progesterone for both pregnant and non-pregnant cows, obtained along the synchronization process and after artificial insemination. According to this table, the best outcomes were obtained with protocol 1 for synchronization and gestation rate (97.47% and 89.47% respectively) compared to protocol 2 ($p < 0.05$). Independently of protocols, adult cows gave the best rate of synchronization and gestation than heifers. Cows of protocol 1 also showed higher means of progesterone concentrations relative to those of protocol 2 ($p < 0.05$).

Synchronization, gestation rates, and mean progesterone concentrations relative to different protocols and breeds

Table 2 represents the success rates of synchronization and artificial insemination and the means of progesterone concentration relative to the different protocols and breeds.

This result shows that the lowest rates of synchronization and gestation were obtained in Borane cows of protocol 2 and relatively higher rates of synchronization and gestation in Gudali cows of the same protocol. The performance of protocol 1 with respect to the breed was comparatively better than that of protocol 2.

Synchronization, gestation rates and mean progesterone concentrations relative to different protocols and body condition score (BCS)

Table 3 presents the success rates of the reproduction techniques with respect to the body condition score and different protocols. Independently of protocols, all cows with BCS of 4-5 recorded the highest success rates of synchronization and gestation unlike the cows with BCS of 3.5. This result also reveals that, independently of BCS, mean progesterone concentrations were significantly lower in protocol 2 than the concentrations recorded with the Cows of protocol 1 ($p < 0.05$).

Figures 3 and 4 summarize the variation of progesterone concentrations during the synchronization protocols, the days before, during, and after insemination. The figure does not include the endogenous factors stated previously. This figure

illustrates that among the cows of the two protocols, concentrations of progesterone increased during the days following the introduction of vaginal spirals. Then oscillated along the period of estrus induction and remained higher after

insemination for pregnant cows and along the gestation period to maintain the fetus. This figure permits to note that, between the two protocols, the higher concentrations were registered with cows of protocol 1.

Table 1. Synchronization, gestation rates and mean progesterone concentrations relative to different protocols and ages

		Protocol 1			Protocol 2		
		Adults (4-6 years)	Heifers (2-3 years)	M	Adults (4-6 years)	Heifers (2-3 years)	M
N		9	10	19	10	10	20
Synchronization rate %		100.00 ^a	90.00 ^a	94.47 ^A	90.00 ^{ab}	50.00 ^b	69.23 ^B
Progesterone (ng/ml)	P	17.73±12.31 ^a	21.80±10.07 ^a	19.77±11.19 ^A	11.25±7.75 ^{ab}	4.54±3.25 ^b	7.90±5.50 ^B
	NP		1.67±0.90	1.78±0.98	1.20±0.75	1.17±0.58	1.19±0.81
Gestation rate %		100.00 ^a	80.00 ^a	89.47 ^A	60.00 ^{ab}	40.00 ^b	50.00 ^B

(N): number of cows; (P): pregnant; (NP): non-pregnant. Within lines, means with the different superscript differ significantly, i.e. $p \leq 0.05$.

Table 2. Synchronization rate, gestation rate and mean progesterone concentration between the protocols and breeds

Protocol 1			Protocol 2		
	Borane	Simmental	Borane	Cross- breed (S*G)	Gudali
N	8	11	5	9	6
Synchronization rate %	87.25 ^a	100.00 ^a	40.00 ^b	77.78 ^{ab}	100.00 ^a
Progesterone (ng/ml)	P	19.91±10.98 ^a	4.25±2.50 ^b	6.67±2.70 ^{ab}	15.00±10.67 ^a
	NP	1.35±0.25	1.75±1.15	1.25±1.00	1.82±0.79
Gestation rate %	91.66 ^a	87.50 ^a	20.00 ^b	77.70 ^a	33.30 ^b

(N): number of cows; (P): pregnant; (NP): non-pregnant; (M): means. Within lines, means with the different superscript differ significantly, i.e. $p \leq 0.05$.

Table 3. Synchronization and insemination rates, means concentration of progesterone according to protocols and body condition score (BCS)

Protocol 1			Protocol 2		
Body condition score	3.5	4-5	3.5	4-5	
N	7	12	7	13	
Synchronization rate %	85.72 ^a	100.00 ^a	43.00 ^b	85.00 ^a	
Progesterone (ng/ml)	P	19.77±15.21 ^a	4.48±3.30 ^b	9.15±7.58 ^a	
	NP	2.05±1.58	1.25±0.26	0.45±0.21	1.76±0.74
gestation rate %	85.72 ^a	92.30 ^a	28.75 ^b	77.53 ^a	

(N): number of cows; (P): pregnant; (NP): non-pregnant. Within lines, means with the different superscript differ significantly, i.e. $p \leq 0.05$.

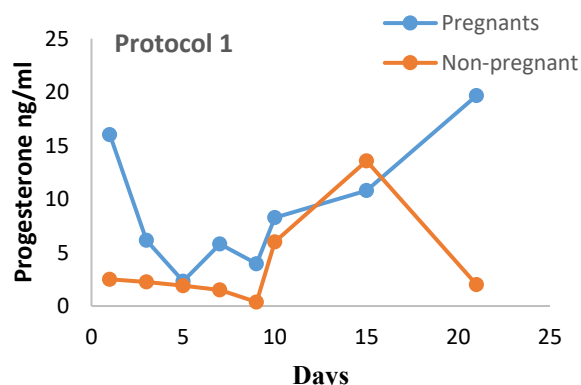


Figure 3. Variation of progesterone concentration for protocol 1

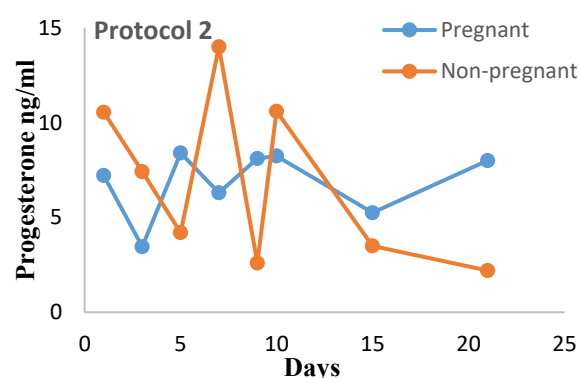


Figure 4. Variation of progesterone concentration for protocol 2

Oxidative stress parameters

With the two protocols, there was no significant difference between concentrations of MDA and activity of SOD in pregnant and non-pregnant cows. However, non-pregnant cows showed an increase of both parameters during days 9 and 10 after the introduction of the progesterone spiral. But analysis revealed positive and non-significant correlations between MDA and SOD.

Table 4 shows the values of MDA and SOD according to the breed, physiological status, and synchronization protocols. From this table, it can be observed that a negative significant interaction only occurs with Borane of the second group. Also, the variation of parameters is most observed in the Cows of the second group.

Table 5 gives the values of MDA and activity of SOD

according to the Cow body condition score (BCS), physiological status, and synchronization protocols. This table shows that the activity of SOD increased with the rank of BCS. This SOD activity is significant in Cows with a BSC of 4-5.

The variation of MDA and activity of SOD with the age of Cows, different protocols, and physiological status were also evaluated. In this case, just the results obtained with pregnant cows were used to facilitate the comparison. The concentration of MDA varied significantly between adult Cows of the two groups. But a non-significant correlation was observed between MDA and the activity of SOD. Also in heifer, a non-significant correlation was obtained. However, the concentration of MDA and activity of SOD recorded were highest in the heifer of the first group to those of the second group.

Table 4. Values of MDA and SOD according to the breed, physiological status, and synchronization protocols

Parameters		Protocol 1		Protocol 2		
		Borane	Simmental	Borane	Cross-breed (S*G)	Gudali
Malondialdehyde ($\mu\text{M/ml}$)	P	4.19 ± 1.41	1.92 ± 0.82	0.78 ± 0.12	1.08 ± 0.71	1.27 ± 0.61
	NP	0.86 ± 0.26	0.82 ± 0.31	0.66 ± 0.18	1.04 ± 0.77	1.30 ± 0.97
Superoxide Dismutase (Mm/ml)	P	3.80 ± 3.23	4.32 ± 2.22	2.96 ± 1.15	3.94 ± 3.25	5.18 ± 2.55
	N	4.25 ± 2.45	7.66 ± 4.95	3.68 ± 1.38	3.68 ± 2.68	7.12 ± 3.5
R		0.484	0.664*	-0.494*	0.538	0.582

(N): number of cows; (P): pregnant; (NP): non-pregnant. (r) degree of correlation between MDA and SOD. (*) Significant at $p \leq 0.05$

Table 5. Values of MDA and activity of SOD according to the Cow body condition score (BCS), physiological status and synchronization protocols

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

(N): the number of cows; (P): pregnant; (NP): non-pregnant. (r) degree of correlation between MDA and SOD. (*) Significant at 0.05, (**) significant at 0.005

Discussion

This study revealed that the best rates of synchronization and artificial insemination were recorded with Cows of protocol 1. This observation is similar to the synchronization rate obtained by (10) using the same application in protocol 1. This study also revealed that Cidriol used in the two protocols that were injected in protocol 1 at day 0 and day 7 after the introduction of spiral corresponded to the right moment of injection unlike in protocol 2 where injections were realized at day 0 and day 9 after spiral introduction. Cidriol is one of the products used in synchronization processes that permit to induce estrus and ovulation. Therefore, this product administered at the right time will permit us to obtain the best results like is the case with protocol 1. In protocol 2, the ninth day when Cidriol was injected was also corresponding to the same time that AI was carried out.

This did not allow for enough time for the synchronization

process to effectively take place before insemination.

In general, the best rate of synchronization was obtained in adult cows. This is explained by the fact that fertility increases with the age of a cow and then reduces at some age-dependent on the species and other factors. Tada *et al.* (11) who reported the best fertility rate with cows within 3-6 years old also confirm this observation. These results also showed that Gudali cows highly responded to the synchronization protocol but a lower rate of gestation was recorded after AI. This is explained by the low ovulation rates caused by the later injection of Cidriol in protocol 2 and also due to its low reproductive performance according to Marichatou *et al.* (5). Concerning the body condition score, the lowest rates were recorded among cows with BCS of 3.5. (12) observed that the best gestation rate after AI was obtained in cows with BCS of 4 to 5. The reason is that, fertility decreases with the energetic deficit in mammals.

Progesterone profiles were assayed in cows of the two protocols. Results showed that higher concentrations of progesterone were obtained with protocol 1. Generally, progesterone concentration increased after day 0 (introduction of spirals), reduced during the days around estrus manifestation, and increased 2-3 weeks after AI for pregnant cows and drastically reduced at the same periods for non-pregnant cows. These observations are confirmed in a study reported by (13) where progesterone concentrations were lower the day of estrus, higher 10 days after AI due to the presence of corpus luteum, and lower again 21 days after AI for non-pregnant cows.

Concerning the relationship between synchronization, OS, and internal factors, correlations have revealed an association between the concentration of MDA and the activity of SOD. However, non-significant effects have been observed on the rate of synchronization, because of low variation in the concentration of MDA during the synchronization steps. This observation is similar to that reported by Aydilek *et al.* (14) who found no significant modification of MDA and SOD along the period of estrus synchronization. This observation could be due to the concentrated supplements in the cows' ratio which prevented the high variability of MDA concentration. In this study, we found that the increasing level of MDA was local (in ovaries) and could not be significantly detected in the serum. This result is confirmed by (15) who reported that the oxidative status of ovaries didn't reflect their general status. In adults cows, we recorded an intensive activity of SOD on the day of spirals removal. This observation is due to the highest level of progesterone released by the spirals which contribute to increase the activity of SOD and in parallel, reduce the level of MDA. According to Hasan *et al.* (16) it is clear that SOD is involved in the improvement of the cows' reproductive performance by promoting the formation of gametes, their motility and the mechanisms of oocyte fertilization. It is also involved in the remodeling of female reproductive tract. In this study, we recorded non-significant correlation between concentration of MDA and the activity of SOD with respect to adult Cows and Heifers respectively. But we noted a pronounced imbalance between these two oxidative parameters both in Gudali and in Heifer of the second group. According to a study carried out by Celi *et al.* (14) this situation provoked reproductive failures and early embryonic losses as shown by the gestation rate of these animals. These reproductive failures are also due to the negative effects of by-products released after estrus synchronization application hormones. However analysis didn't show significant interaction between OS parameters, fertility and endogenous parameters. Like stated previously, this is explained by the fact that, the oxidative status of females don't reflect the oxidative status of their reproductive tract or their embryo. We found that OS increased with the diminution of rank of BCS. The reason is that oxidative stress is more important with energy imbalances due to physiological processes which tend to compensate the energy deficit and this activity leads to the release of free radicals. This observation joins the investigation carried by (15) where oxidative stress increased with negative energy balance in animals.

Conclusion

This study suggests that products used in estrus synchronization protocols should be applied early after the introduction of progesterone spiral to promote estrus manifestations and

ovulation at right time for the best results of artificial insemination. It is also clear that the improvement of local productivity will pass through the monitoring of the effects of endogenous and exogenous factors on animal reproductive performances. It is now evident that there is a relationship between variations of oxidative stress (OS) parameters, endogenous factors, and methods of control of reproduction (estrus synchronization techniques, AI, and so on). Results in the present study suggest that estrus synchronization methods and AI contributes to the variation of OS parameters such as Malondialdehyde and superoxide dismutase which leads to an imbalance resulting in OS. This OS in return affects an animal's fertility according to endogenous factors such as physiological status, breed, body condition score, and age. Prospective studies should be done to assess the overall OS parameters to make a more concrete conclusion on the relationship between these parameters and animal fertility.

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Conflict of interests

The authors declare no conflict of interest in the realization and publication of this piece of work

Authors contributions

Hakoueu flora designed and carried out the work of synchronization, insemination, blood samples collection, and data collection, Ndzi Elvis Ndukong was in charge of laboratory analyzes, Nsadzetsen Gilbert Adzemye was the veterinarian in charge of health, Mbiba Hassanu Fanadzenyuy and Kinso Ambrose Limnyuy were the field technicians, Kouamo, Ngoula Ferdinand and Bayemi Henri were the supervisors and readers.

Ethical issue

This study is in accordance to ethical guidelines of the Institute of Agricultural Research for Development (IRAD), Cameroon

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