Global Science Research Journals

Vol. 3 (1), pp. 143-150, April, 2016

http://www.globalscienceresearchjournals.org/

International Journal of Clinical Biochemistry

Full Length Research Paper

Detection of embryonic mortality using progesterone and bovine pregnancy associated glycoprotein assays following artificial insemination of Gobra Zebu cattle in Senegal

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Accepted 13 March, 2016

Senegal has embarked in artificial insemination (AI) to improve the productivity of small traditional dairy farms. However the success rate of the overall AI programmes remained low. This study aimed at assessing the incidence of embryonic mortality occurring in cows inseminated artificially by using progesterone and pregnancy associated glycoprotein (PAGs) assays. For this purpose, 90 cows of local breed Gobra were sampled in 28 farms in the regions of Dakar and Thiès. Blood samples were collected from the cows on the day of insemination (D_0), then 21 days (D_{21}), 35 days (D_{35}) and 60 days (D_{60}) successively. Analyzes were performed by using by radioimmunological assays to determine plasma progesterone and PAGs levels. The pregnancy rate was 73.3% at D_{21} according to progesterone assays, and then, it dropped at 60% at D_{35} by using PAGs assays and finally, it was 47.8% at D_{60} with trans-rectal palpation. Non-pregnant (NP) and Early Embryonic Mortality (EEM) was estimated at 26.7% at day D_{21} and the Late Embryonic Mortality (LEM) was 25.5%. This study showed that the success of AI in Senegal is heavily influenced by embryonic mortality. Indeed, the high rate of LEM hampered the fertility of local cows breed and therefore, it might cause significant economic losses in small dairy farmers.

Keywords: Radioimmunological assays, Late and Early Embryonic mortality, Gobra zebu, Pregnancy diagnosis.

INTRODUCTION

In Senegal, the imports of milk and dairy products cost nearly a hundred million dollars per year (DIREL, 2010). These losses of currency severely affect the trade balance. In order to reduce these imports and to meet the demand of local milk and dairy products, the country is embarked in artificial insemination (AI) in the view to genetically improve local cattle breeds and increase the productivity of peri urban small dairy farms.

However, for more than two decades of practice of artificial insemination, the success rate obtained from various programmes remained less than expected i.e. 60%. Indeed, recent studies reported success rates of AI in various area of the country in programmes funded by the government. These rates were of 44.93%, 39.32% and 45.41% in the regions of Fatick, Kaolack and Louga respectively (Sawadogo, 2007; Kouamo et al., 2009).

The failure of AI has many causes in the context of Senegal, amongst of which malnutrition, poor healthcare and stressful environmental as reported by Sawadogo (2007), but embryonic mortality was recognized as a major cause of reproductive loss in cattle (Gabor et al., 2007; Lopez-Gatius and Garcia-Ispierto, 2010; Diskin and Morris, 2008). As stated by Bech-Sabat et al. (2010), embryo implantation in the uterus involves a close relationship established between the conceptus and maternal reproductive tissues. This step is very critical and is very sensitive to several forms of stress including genetic abnormalities. In the traditional breeding system, dairy cows are subjected to various forms of stress, such as malnutrition, high temperatures and intercurrent diseases which might account for the embryonic mortality and therefore the failure of the AI.

Embryonic mortalities are classified into two types: the Early Embryonic Mortality (EEM) and the Late Embryonic Mortality (LEM). The EEM occurs around the first 20 days after insemination. In this period, there is no effective technique of pregnancy diagnosis in cows. The LEM corresponds to embryo loss between the 16^{th} and the 42^{nd} day after insemination (Hanzen et al., 1999; Ledoux et al., 2006).

Recent research showed that cases of early embryonic mortalities (EEM) could be differentiated from late embryonic mortalities (LEM) through the combined use of progesterone and pregnancy-associated glycoprotein (PAGs) assays (Szenci et al., 2003). Indeed, plasma concentrations of pregnancy associated glycoprotein (PAGs) have been used both for pregnancy diagnosis and as a marker of foetal or placental viability by Lopez-Gatius et al. (2007). The PAGs assay is performed to monitor pregnancy failure during the late embryo and early foetal period (Whitlock and Maxwell, 2008). The combination of PAGs and progesterone assays predicts more precisely pregnancy loss than the use of plasma PAGs measurement alone (Szenci et al. 2000; Humblot 2001; Gabor et al. 2007). However, it remains very difficult to distinguish non-pregnancy (NP) from EEM because in both cases, the plasma progesterone level at D_{21-24} is low and pregnancy diagnosis at D_{30-35} is negative (Ponsart et al., 2007). In practice, high concentrations of progesterone observed 21 to 24 days after insemination and associated with low concentrations of PAGs at D_{30} indicate an embryo loss (Szenci et al., 2003, 2000; Pinto et al., 2000).

In high producing dairy cows, many studies were carried out on the causes of embryonic mortality occurring after artificial insemination (Lopez et al., 2007; Bech-Sabat et al., 2010; Inskeep and Dailey, 2010). In Senegalese local Gobra cow, very little is known about this subject. Hence, this study was initiated to investigate early and late embryonic mortalities in local inseminated cows by PAGs and progesterone essays. The objective of this study was to evaluate the incidence of embryonic mortality occurring between day 0 and day 60 post insemination through the combined use of the progesterone and PAGs assays.

MATERIAL AND METHODS

Study Area

The study was conducted from May 2007 to March 2008 in the regions of Dakar and Thiès (Figure 1). The study area is located in sahelian zone which is characterized by four months of rainy season (July-October) and 8 month dry season (November-June). The rainfall varies from one year to another with an average of 400 mm. The temperatures range from 20°C to 40°C with an average annual temperature of 30°C. The vegetation consists of woodland, shrub and herbaceous savannah. Natural pasture is abundant in the rainy season and consists of grasses and legumes. Livestock breeding system is the traditional system which is characterized by the transhumance around the few available pastures and water points.

The cattle population was estimated at 21,270 and 183,541 heads in the regions of Dakar and Thiès respectively (DIREL, 2010). Many cattle breeds and their crossbreeds are raised in the study area. In order of importance, there are two zebu breeds (*Bos indicus*) namely Gobra and Maure cattle and a taurine breed (*Bos taurus africanus*) represented by the N'Dama cattle, and the crossbreeds called Djakoré. Artificial insemination

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has been practiced since the 1990's. Cows are usually inseminated with semen collected from Montbeliards, Holstein, Jersey and Guzerat breeds (Kouamo et al., 2009).

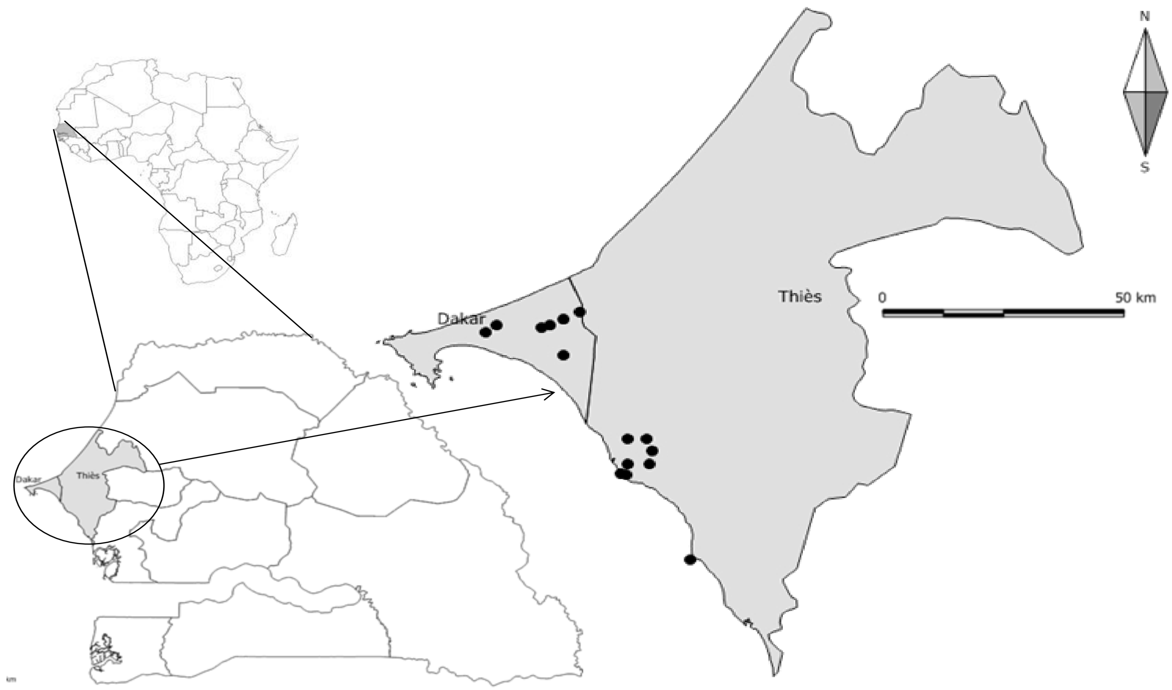


Figure 1: Study area and the location of sampling sites.

Sampling of the Inseminated Cows

Ninety (90) Gobra cows aged in average 6±2 years were selected from 28 farms based on specific criteria. The selection included heifers or cows with more than 3 months postpartum period and having a body condition score (BCS) ranked between 2.5 to 4 according a scale established by Vall and Bayala (2004). The selected cows were identified by ear tag and all the information about individual were recorded on data sheet. The cows were fed on natural pasture during the daytime and supplemented feed in the evening before milking.

From each cow, blood samples were collected in heparinized vacuum tubes, by jugular vein puncture, at D_0 (day of AI), D_{21}, D_{35} and D^{60} (21 days, 35 days and 60 days after AI respectively). The blood tubes centrifuged (1500 rpm for 15 minutes) and plasma samples were collected and stored at -20°C until the biochemical analyzes were performed.

Artificial Insemination Protocol

All selected cow benefited from feed supplementation and veterinary healthcare such as deworming and vaccination. Artificial inseminations were performed by induced heats using a protocol associating vaginal spiral PRID® and the injection of prostaglandin ($PGF_{2\alpha}$) and Pregnant Mare Serum Gonadotropin (PMSG).

Trans-rectal palpation was carried out 60 days after AI to confirm pregnancy status in the cows. The signs for confirmation were the asymmetrical uterus horns with thinness of the wall of the pregnant horn, the presence of a fluctuating fluid and an amniotic membrane.

PAGs and Progesterone Assays

The plasma concentrations of PAGs and progesterone were measured using the radioimmunoassays (RIA) as described by Sousa et al. (2003). The plasma PAGs and progesterone levels allowed to determine the physiological status of the inseminated cows (pregnant, not pregnant, or embryonic mortality). When the plasma levels are higher than 2ng/ml at D_{21}, the cows are suspected to be pregnant (Thimonier, 2000). The cut-off for plasma PAGs level is 0.8 ng/ml at D_{35} (Sousa et al., 2003).

Statistical Analysis

Data were computed and analyzed using the SPSS 12.0® software for the different calculations and graphic representations. The results were expressed as means ± standard deviation. The comparison of means between groups was done using the ANOVA test. Differences were considered to be statistically significant with values of $p<0.05$.

RESULTS

Pregnancy diagnosis and embryonic mortality

The pregnancy rates recorded by the three methods of pregnancy diagnosis were 73.3% at D_{21} using progesterone assay, 60% at D_{35} using PAGs assay and 47.8% at D_{60} with trans-rectal palpation (Figure 2).

The association of PAGs and progesterone assays and trans-rectal palpation leads to the detection of various physiological status 60 days after AI namely: NP or EEM, LEM and pregnant (Table 1).

From D_{21} to D_{35}, the pregnancy rate decreased by 13.3% (12 cows). These cows were diagnosed as pregnant by progesterone assay at D_{21}, but at D_{35} and D_{60}, there were non-pregnant by PAGs assay and trans-rectal palpation respectively. The pregnancy rate decreased by 12.2% (11 cows) between D_{35} to D_{60}. These 11 cows were diagnosed as pregnant at D_{21} and D_{35} according to the results of progesterone and PAGs assays respectively, but they were non-pregnant according to progesterone and PAGs assays and trans-rectal palpation at D_{60}. In total, out of the 90 inseminated cows, 43 (47.8%) became pregnant and sustained it, 23 (25.5%) aborted (LEM) and 24 (26.7%) remained non-pregnant or EEM at D_{60} (Figure 3).

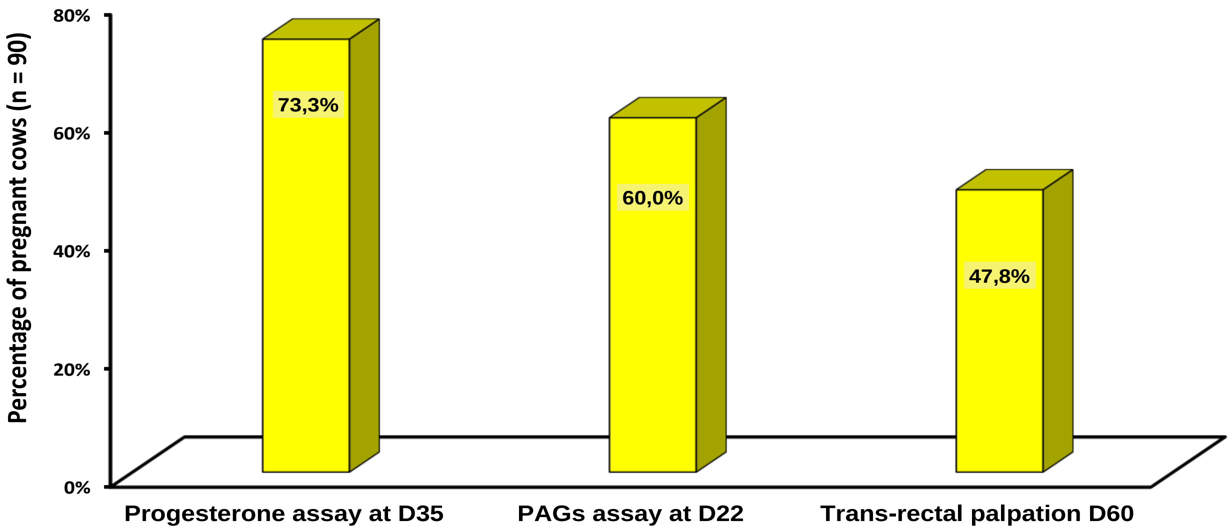


Figure 2: Proportion of pregnancy with each type of diagnosis

Table 1: Relationship between different situations after AI and the results of progesterone and PAGs assays and trans-rectal palpation

Progesterone (D_0)	Progesterone (D_{21})	PAGs (D_{35})	Trans-rectal palpation(D_{60})	Diagnosis
Low	Low	undetected	Undetected	EEM or NP
Low	High	undetected	Undetected	LAM
Low	High	Detected	Undetected	LAM
Low	High	Detected	Detectede	Pregnant

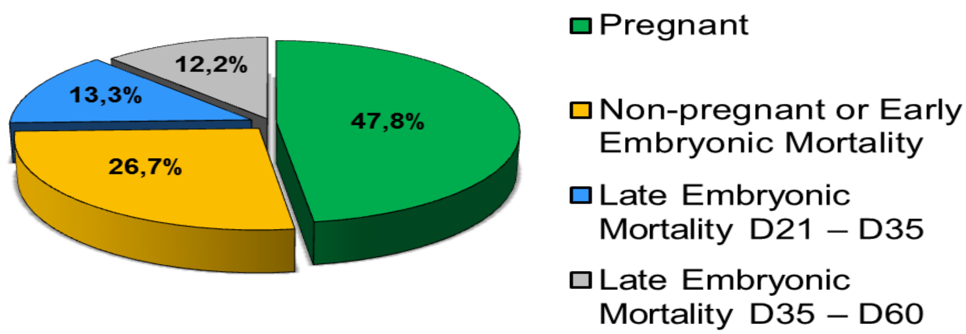


Figure 3: Proportion of cows according to the physiological status

Profiles of progesterone and PAGs according to the physiological status

Figures 4A and 4B show the profiles of the average plasma concentrations in progesterone and PAGs in pregnant cows, non-pregnant (or EEM) and in case of late embryonic mortality. In Pregnant cows the progesterone and PAGs concentrations in maternal blood continuously increased significantly ($p<0.05$) compared to the rest and reached a concentration of 5.06±2.33 ng/ml and 10.16±6.73 ng/ml at D_{60} of gestation respectively. In contrast, in case of the embryonic mortality, the concentration of PAGs and progesterone dropped sharply, though progesterone levels appear to rise later, albeit slightly.

Non-pregnant cows (or EEM) had fluctuating progesterone concentrations depending on the sexual cycle of the cows. They were 0.8± 0.24 ng/ml at D_0, 0.65±0.09 ng/ml at D_{21}, 1.33±0.54 ng/ml at D_{35} and 1.92±0.78 ng/ml at D_{60} (Figure 4A). On the other hand the concentrations of PAGs were very low in this category of cows ($P> 0.05$).

DISCUSSION

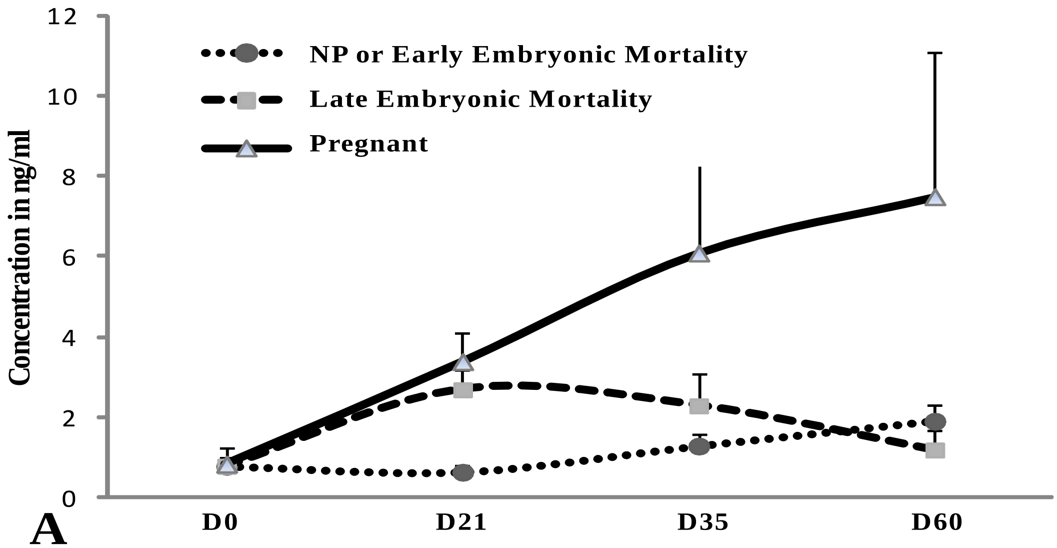
The rate of pregnancy/conception recorded in this study was 47.7%. Our results are in sync with those obtained in earlier genetic improvement programmes in Senegal (Sawadogo, 2007; Kouamo et al., 2009; Kouamo et al., 2010). However, this result is lower than the overall desirable goal of 60% for the National Artificial Insemination Programme of Senegal (Kouamo et al., 2009).

The use of both progesterone and PAGs assays in our study was aimed not only at establishing a reliable pregnancy diagnosis but also at assessing the occurrence of embryonic mortality after AI. This combination made it possible to differentiate between NP (or EEM) and LEM (Ponsart et al., 2007; Gabor et al. 2007).This study showed that cows diagnosed pregnant had progesterone concentrations >3ng/ml at D_{21}, PAGs detected at D_{35} and positive test for the trans-rectal palpation at D_{60}. High progesterone concentration is essential for the maintenance of pregnancy, increased progesterone levels being favorable to the development of the embryo (Geisert et al., 1992; Szenci et al., 2000; Humblot, 2001; Humblot, 2003).

During the pregnancy, the level of PAGs increases according to the development of the fetus. The PAGs concentration increased from 4.7±1.6 ng/ml (D_{35}) to 10.16±6.73 ng/ml (D_{60}). The values of PAGs in this study were higher compared to those found by other researchers: 3.6±1.73 ng/ml at D_{30} (Zoli et al., 1992), 6 ± 4.2 ng/ml at 8^{th} week in the Azawak zebu in Burkina Faso (Sousa et al., 2003). In the current study the animals were left in their natural environment and most of them were poorly fed. Poor diet can induce high concentrations of PAGs in cows (Sousa et al., 2003). Similar situations have been observed in other species like humans and sheep (Diskin and Morris, 2008). In fact, it seems that malnutrition is characterized by an increase in the surface of the placenta, which induced an increase of PAGs (Sousa et al., 2003). It was reported that excessive intake of energy (feed) in ewes during gestation reduced placental size (Wallace et al., 1996, 1997; Lumey, 1998). Under certain conditions of malnutrition, placental hypertrophy may occur in order to ensure the survival of the fetus, leading to high concentrations of PAGs (Sousa et al., 2003).

The high concentrations of progesterone observed 21 days after insemination was associated with low concentrations of PAGs at D_{35} of gestation and indicates an interruption of pregnancy in the embryonic period (Table 1). Pregnant cows are characterized by high concentrations of progesterone and PAGs throughout the pregnancy, a drop of the blood concentration in these hormones therefore permits the detection of embryonic deaths or abortions (Figures 4A and 4B).

Out of the 47 cows diagnosed as non-pregnant at D_{60}



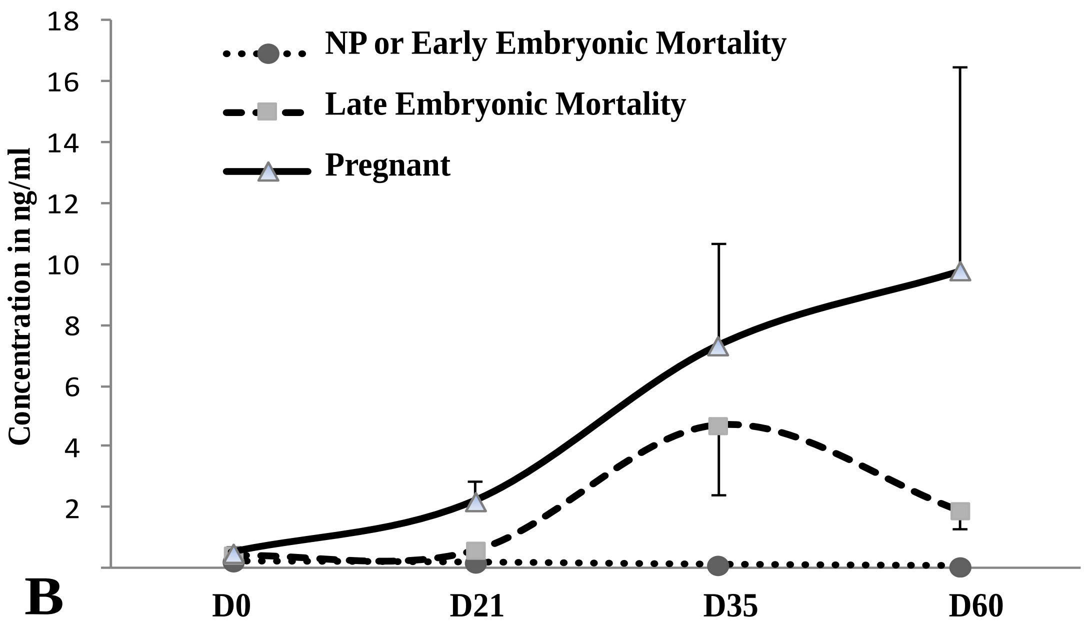


Figure 4A: Average concentrations of progesterone according to the physiological status of cows
Figure 4B: Average concentrations of PAGs according to the physiological status of the cow.

post AI, 23 were diagnosed positive to progesterone assays at D_{21} and 11 were positive to PAGs assays at D_{35}. This represents a LEM rate of 25.5% (Figure 3), and showed that the inseminator had succeeded with a performance of 73%. The non-pregnancy cases that were noted two months after AI were therefore not always due to the limited know-how of the inseminator, but also due to the occurrence of embryonic mortality.

However, it was not possible to distinguish non-pregnant cases from EEM, because in both cases, the concentration of progesterone is low at D_{21} of gestation and the PAGs assays at D_{35} is negative (Pinto et al., 2000). In our study the non-fecundation represented 26.7%. Inskeep and Dailey (2005) suggest that EEM accounts for 20-40% of insemination failure. Our results of 26.7% fall within this interval. Other reports on European cattle breed have revealed the following results: 20.5% for the Norman cows Humblot (2000) 30-35% in the Prim'Holstein (Pinto et al., 2000; Horan et al., 2005; Grimard et al., 2006) and 37-40% for the Prim'Holstein (Michel et al., 2003; Fréret et al., 2005, 2006).

When measured in livestock, the frequency of LEM appears lower than that of NP-EEM. The LEM affect, approximately 15% of inseminations and represents 30% of total embryonic losses (Inskeep, 2004). Estimates of the rate of LEM in dairy cows are between 5 and 12% according to some studies (Alexander et al., 1995; Santos et al., 2009). Silke et al. (2002) for instance reported embryo losses of 7.2% in cows and 6.1% in heifers between D_{28} and D_{84} of gestation. These percentages are lower than our results; that is 25.5% of LEM, which represent 49% of the total embryonic losses. The difference between our results and those of other authors could be explained by the fact that we worked with animals reared extensively by being fed mainly on natural pasture and receiving supplementation only occasionally. In contrast to previous studies (Silke et al., 2002) where animals were of European breed, reared in intensive systems and where the problem of poor feeding does not usually arise. Embryonic mortality can have several sources including genetic, endocrine, immunological, nutritional and environmental factors. It may also be due to chromosomal abnormalities, an infection or even lactation (Jainudeen and Hafez, 2000; Chebel et al., 2004; Bech-Sabat et al., 2010)).

The LEM contributes more to the degradation of fertility than the EEM due to delays attributed to rebreeding and the reforming risks (Fréret et al., 2006; Gabor et al., 2007; Whitlock and Maxwell, 2008; Inskeep and Dailey, 2010). The absence of pregnancy is detected at the next cycle, sometimes even after several cycles (Szenci et al., 2000; Humblot, 2001). Embryo mortality is generally recognized as the major cause of reproductive failure in breeding programmes (Inskeep and Dailey, 2005, 2010; Romano et al., 2007). It results in a smaller number of new calves, a loss in milk production, a slow genetic progress and a significant financial loss for the dairy farmers (Dunne et al., 2000; Lopez-Gatius and Garcia-Ispierto, 2010)). Improving fertility on farms is thus achieved by a recovery of the ovarian function, good heat detection, the establishment of pregnancy and its maintenance (Santos et al., 2009; Inskeep and Dailey, 2010).

CONCLUSION

In order to optimize the reproductive potential and consequently beef and milk production, fertility has to be improved. Embryonic mortalities constitute a real obstacle to the success of AI and affect significantly the genetic improvement in Senegal. Thus, it is necessary to continuously investigate the causes of embryonic mortalities in order to reduce their impact on dairy farms in Senegal. Indeed, embryonic mortalities account for the infertility in cattle and therefore, it is necessary to develop control strategies against the etiological factors of embryonic mortalities in order to reduce the economic impact of this scourge in dairy farms.

ACKNOWLEDGMENTS

We are very grateful to the “Agence Universitaire de la Francophonie” (AUF) and the National Artificial Insemination Program (PNIA) of Senegal for supporting this research work. We thank the livestock owners and the local veterinary services for their kind collaboration. We appreciated the devotion and availability of all the staff of the laboratory of Biochemistry of EISMV of Dakar.

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