EFFECT OF FLUNIXIN MEGLUMINE ADMINISTRATION BEFORE THE MATERNAL RECOGNITION OF PREGNANCY ON THE REPRODUCTIVE PERFORMANCE OF SENEPOL HEIFERS

by

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ABSTRACT

The inability to inhibit the luteolytic hormone prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) during the maternal recognition of pregnancy is one major cause of embryonic mortality in cattle, negatively affecting reproductive performance. Previous research has related higher pregnancy rates (PR) to the use of non-steroidal antiinflammatory drugs, in both, humans and cattle by inhibiting the $PGF_{2\alpha}$ synthesis. Nevertheless, to our knowledge, this treatment has not been evaluated in Puerto Rican beef cattle. This study aimed to compare PR after fixed TAI in purebred Senepol heifers who received flunixin meglumine (FM; 1.1mg/kg of body weight (BW), intravenous; n = 19 and 23; for Trials 1 and 2, respectively) or the equivalent volume of saline solution (Control; n = 19 and 24 for Trials 1 and 2, respectively) as a placebo. For pregnancy detection, blood samples were collected from coccygeal venipuncture 29 or 30 d post-insemination and evaluated in a commercial laboratory (BioPRYN; Saint Cloud, FL). For Trial 1, more than one AI bull and day of treatment administration were used. Additionally, air temperature (AT) and relative humidity (RH) were recorded every five minutes during both trials and later combined to calculate the temperature humidity index (THI). Environmental conditions were average by hour and used in a descriptive manner. The PR related variables were analyzed by the Pearson's chi square test (proc FREQ) of SAS. In Trials 1 and 2, the AT, RH, and THI were affected by the hour of the day; with AT and THI exceeding their literature established critical values for optimum reproductive performance (16.7°C and 68, respectively) during 100% of the day. In Trial 1 the PR values were not affected by AI bull (P = 0.5402) or treatment day (P = 0.5359); therefore data were combined for further

analysis. No differences in PR were observed between the FM and Control groups in Trial 1 (P = 0.5158), Trial 2 (P = 0.4741), or in the pooled data (P = 0.9223). The reduced number of observations, the negative effects that heat stress exerts over reproduction, and the animal parity related reproductive performance, may have limited any possible reproductive advantage associated with the FM administration. Future studies should address these hypotheses.

RESUMEN

La incapacidad de inhibir la secreción de la hormona luteolítica prostaglandina F2α (PGF2α) durante el periodo de reconocimiento maternal de la preñez es una de las principales causas de muerte embrionaria en bovinos, afectando negativamente su eficiencia reproductiva. Estudios previos han asociado el uso de antiinflamatorios no-esteroidales con mayores tasas de preñez (TP) en humanos así como en bovinos, debido a la inhibición de la síntesis de PGF2α. Sin embargo, según nuestro conocimiento, este tipo de tratamiento no ha sido evaluado en ganado de carne puertorriqueño. El presente estudio comparó las TP en novillas Senepol inseminadas a tiempo fijo a las cuales se les administró flunixin meglumine (FM; 1.1mg/kg de peso vivo; intravenoso; n = 19 y 23 para las pruebas 1 y 2, respectivamente) o el respectivo volumen de solución salina (Control; n = 19 y 24 para las pruebas 1 y 2, respectivamente) como placebo. Para la determinación de la TP, se tomaron muestras de sangre coccigeal de cada novilla 29 ó 30 d post-inseminación, que fueron evaluadas por un laboratorio comercial (BioPRYN; Saint Cloud, FL). En la prueba 1, se utilizó más de un toro de inseminación (TI) y día de administración del tratamiento. Adicionalmente, la temperatura del aire (TA) y humedad relativa (HR) se recopilaron cada 5 minutos durante ambas pruebas y se combinaron para calcular el índice de temperatura y humedad (ITH). Las condiciones ambientales fueron promediadas por hora y utilizadas de manera descriptiva. Las TP se analizaron mediante la prueba de chi-cuadrado de Pearson (proc FREQ) en SAS. En las pruebas 1 y 2, la TA, la HR y el ITH fueron afectados por el tiempo; con TA e ITH excediendo sus valores críticos establecidos en la literatura para reproducción óptima (16.7°C and 68, respectivamente) durante el 100% del día. La TP no fue afectada por el TI (P = 0.5402) o día del tratamiento (P = 0.5359); por lo que los datos se combinaron para su análisis. No se observaron diferencias en TP entre el grupo FM y el control en la prueba 1 (P = 0.5158), la prueba 2 (P = 0.4741) o en los datos combinados (P = 0.9223). La cantidad reducida de observaciones, los efectos negativos que el estrés por calor ejerce sobre la reproducción, así como la edad reproductiva de los animales evaluados pudieron haber

limitado la posibilidad de observar diferencias en TP asociadas con la administración de FM. Futuros estudios deben evaluar estas hipótesis.

DEDICATION

To my parents Carmen Correa López and Rafael Contreras Montaña for teaching me the meaning of hard work and sacrifices; for being in every stage of my life and for their unconditional love. To my family-in-law, Gloria Arroyo García, Ángel R. Jiménez Padilla and Glorianne Jiménez Arroyo for their guidance and support. To my grandmother mamá Gloria who takes care of us from heaven and is responsible for the many blessings we received from God. Last, but not less important, I would like to dedicate this work to my husband Ángel Luis Jiménez Arroyo, who showed me the real meaning of life, to seek happiness in the simplest things, and to love the agriculture.

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LIST OF ABREVIATIONS

AI = artificial insemination

AT = air temperature

BW = body weight

CL= corpus luteum

Control = placebo treated group

COX-1 = cyclooxygenase 1 enzyme

COX-2 = cyclooxygenase 2 enzyme

EM = embryonic mortality

FM = flunixin meglumine group

FSH = follicle stimulating hormone

GnRH = gonadotropin releasing hormone

IFNT = interferon tau

LH = luteinizing hormone

MRP = Maternal recognition of pregnancy

NSAIDs = non-steroidal antiinflammatory drugs

$PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$

PR = pregnancy rates

RH = relative humidity

TAI = timed artificial insemination

THI = temperature humidity index

1. INTRODUCTION

Reproductive efficiency in dairy and beef cattle is essential for maintaining a steady food supply and preventing economic losses. In livestock, embryonic mortality (EM) accounts for considerable economical losses (Sreenan and Diskin, 1986); occurring mostly between days 8 and 16 post- artificial insemination (Ayalon, 1978; Diskin and Sreenan, 1980). The inability of the embryo to inhibit the secretion of the luteolytic hormone prostaglandin F2α ($PGF_{2\alpha}$) during the maternal recognition of pregnancy (MPR) is the major cause of this problem (Thatcher et al., 2001). Therefore, the evaluation of strategies that may help to decrease the rate of EM during such a susceptible stage is imperative.

The non-steroidal anti-inflammatory drugs inhibit the synthesis of the COX enzymes, which catalyze the reaction where prostaglandins are produced (Anderson et al., 1996). Therefore, several drugs in this group, including aspirin, have been evaluated as an attempt to inhibit $PGF_{2\alpha}$ synthesis, decreasing luteolysis, and subsequently maintaining early pregnancy in several species including humans (Schisterman et al., 2015; Mumford et al., 2016) and equine (Bollwein et al., 2003). Flunixin meglumine (FM), another non-steroidal anti-inflammatory drug, has also been associated with the suppression of $PGF_{2\alpha}$ synthesis in cattle, resulting in the maintenance of the corpus luteum with a subsequent constant production of progesterone (Geary, 2012). Such progesterone secretion is essential for pregnancy maintenance, embryonic growth and uterine function (Bridges et al., 2013). However, to our knowledge, such an attempt has not been evaluated in the Senepol cattle population of Puerto Rico. Thus, the purpose of

this study was to evaluate the effect of FM administration on early pregnancy rates in Senepol heifers.

2. LITERATURE REVIEW

2.1. Economic Impact

The United States Department of Agriculture reported in 2016 an U.S.A. beef cow inventory of approximately 30.3 million heads (NASS, 2016); while in 2015 the total respective beef consumption was 11.27 billion kg, meaning a retail equivalent value of $105 billion (ERC, 2015). For Puerto Rico, during 2014, beef consumption was 22.35 kg per capita, producing about 7.27 million kg of beef, and generating $29.2 million in meat sales (FCR, 2014). It is estimated that embryonic loss in U.S. beef farms exceeds $1.2 billion in annual losses (Geary, 2005). Due to the importance of the beef industry in economical and in food supply terms, it is essential to maintain reproductive efficiency in these animals.

2.2 Reproductive Physiology

2.2.1. Hypothalamic-Pituitary-Gonadal Axis

The neurons within the hypothalamus are responsible for the production of gonadotropin releasing hormone (GnRH), which is essential for the reproductive function in all vertebrates (Yao et al., 2011). The hypothalamic secretion of GnRH into the portal pituitary circulation controls the follicle stimulating hormone (FSH) and luteinizing hormone (LH) release from the anterior pituitary (Beshay and Carr, 2013). The pituitary gland is divided in two major lobes: the adenohypophysis and the neurohypophysis, also known as the anterior and posterior pituitary, respectively (Beshay and Carr, 2013). The

main difference between these two pituitary lobes is their communication with the hypothalamus; while the anterior pituitary is vascular, the posterior pituitary has a neuronal connection (Beshay and Carr, 2013).

Puberty is initiated when GnRH is released from the hypothalamus, stimulating the secretion of FSH and LH from the anterior pituitary (Nguyen et al., 2017). In mammals, FSH performs an essential role in the control of follicular growth (Fortune, 1994). The FSH surge causes a follicular wave, which is the recruitment of a group of small follicles to grow on the ovaries (Wiltbank et al., 2002). Using ultrasound scanner, Ginther et al. (1989) described that most estrous cycles in heifers consist of two or three follicular waves. The dominant follicle of the first follicular wave is unable to cause the LH surge, necessary for ovulation, due to the existence of a functional corpus luteum (CL) and high progesterone concentrations (Wiltbank et al., 2002). In cattle, most ovulations occur in the second or third follicular wave when the larger follicles, also known as Graafian, produce a greater amount of inhibin and estradiol, which are the two primary FSH inhibitors (Wiltbank et al., 2002). The FSH receptors will remain the same in the granulosa cells of the dominant follicle, but the LH receptors increase by the secretion of estradiol (Ginther et al., 1996), which also induce the LH surge (Nanda et al., 1989). The LH surge unchain a series of biochemical reactions that results in the rupture of the Graafian follicle, followed by the oocyte expulsion and the formation of the CL (Acosta and Miyamoto, 2004). The CL is a reproductive gland that develops from a rearrangement in the granulosa and thecal cells of the post-ovulatory follicle (Niswender et al., 2000). Its main function is the production of progesterone, indispensable for pregnancy maintenance (Shams and Berisha, 2004). During pregnancy progesterone acts on the hypothalamic-

pituitary-gonadal axis interfering with the GnRH surge from the hypothalamus and reducing GnRH receptors in the pituitary (Niswender et al., 2000), while causing a negative feedback by suppressing the FSH and LH secretion (Goodman et al., 2004).

2.2.2. Maternal Recognition of Pregnancy and Embryonic Mortality

Maternal recognition of pregnancy (MRP) consists on a biochemical communication between the conceptus and its mother to synthesize and release a constant amount of progesterone (Niswender et al., 2000). The MRP in cows occurs in days 14-17 post-estrus (Inskeep, 2004). In ruminants, the conceptus emits a pregnancy recognition signal necessary for the CL maintenance called interferon tau (IFNT; Bazer, 2013). The IFNT prolongs the lifespan of the CL by preventing the synthesis of prostaglandin F2 α (PGF2α) from the endometrium (Lucy et al., 1995). Thereby, progesterone production supports uterine functions, early embryonic development, implantation, and placentation (Spencer and Bazer, 2004). About 29-39% of the reproductive failures in livestock are caused by EM during days 8-16 post-insemination (Dunne et al., 2000). The major cause of EM is the inability of the embryo to inhibit the luteolytic hormone PGF2 α during the MRP stage (Thatcher et al., 2001). The constant secretion of progesterone by the CL is essential for the normal development of the embryo (Thatcher et al., 1994). Therefore, if we can extend the existence of a functional CL, it may be possible to reduce embryonic mortality and enhance pregnancy rates (PR).

2.2.3. Corpus Luteum Regression

At the end of the luteal phase and in the absence of interferon tau, progesterone secretion is reduced and its receptors downregulated in the hypothalamus and endometrium; at that moment, estrogen sensitivity is returned to these tissues (McCraken et al. 1999). The return of estrogen action stimulates the hypothalamic secretion of oxytocin and upregulates the endometrial oxytocin receptors (McCraken et al. 1999). Silvia et al. (1991) proposed that the oxytocin pulses released from the posterior pituitary gland induce the secretion of endometrial $PGF_{2\alpha}$. In ruminants, such prostaglandins are transported through the utero-ovarian plexus to regress the CL (Banu et al. 2003). The levels of $PGF_{2\alpha}$ secreted in the uterus triggers additional release of oxytocin from the CL (Silvia et al. 1991). Luteal oxytocin stimulates a greater secretion of $PGF_{2\alpha}$, which is required for luteolysis (Silvia et al. 1991). These responses create a positive feedback loop until uterine refractoriness to oxytocin and luteal refractoriness to $PGF_{2\alpha}$ are reached (Silvia et al. 1991). Two main theories attempt to explain the regression of the bovine CL, which is induced by the $PGF_{2\alpha}$ luteolytic cascade. On the first one, $PGF_{2\alpha}$ exposes the CL to hypoxic conditions by decreasing its blood supply (Skarzynski et al., 2008) and depriving it from nutrients (Pharriss et al., 1970). Although a more recently study differ from the theory established, suggesting that $PGF_{2\alpha}$ stimulates nitric oxide secretion causing an acute increase in local blood flow to the CL, which is essential to deliver another substance required for luteolysis in the cow (Miyamoto et al., 2005).

2.3. Non-steroidal Anti-inflamatory Drugs and Prostaglandin F2α Inhibition

Non-steroidal anti-inflamatory drugs (NSAID's) are commonly used to treat pain, inflammation, swelling, and fever (Vane and Blotting, 2003). Its beneficial effects have been related to its ability to prevent the formation of prostanoids by the inhibition of the cyclooxygenase 1 and 2 enzymes (COX-1 and COX-2; Elli et al., 2001). The Cox-1 and Cox-2 are rate-limiting enzymes that catalyze the production of prostaglandins from arachidonic acid (Sun et al., 2004); including $PGF_{2\alpha}$ (Okuda et al., 2002) during an inflammatory response (Anderson et al., 1996) or reproduction (Fortier et al., 2008). $PGF_{2\alpha}$ is the primary luteolytic factor in the cow (Miyamoto et al., 2005). Therefore, NSAID's including aspirin, have been evaluated as an attempt to inhibit the $PGF_{2\alpha}$ synthesis, subsequently decreasing luteolysis and maintaining early pregnancy in several species including humans (Schisterman et al., 2015; Mumford et al., 2016), equine (Bollwein et al., 2003), and bovine (Spencer et al., 2016). Flunixin meglumine, another member of the NSAID's family, has also been previously associated in other countries with the suppression of $PGF_{2\alpha}$ in cattle (Geary et al., 2012).

2.3.1. Flunixin Meglumine

As described by Smith et al. (2008), the therapeutic dose of FM is 1.1 to 2.2 mg/kg of body weight (BW) by the intravenous route. It can be used to treat pyrexia associated with bovine respiratory disease and mastitis, as well as inflammation associated with metritis and peritonitis (Smith et al., 2008). In fact, multiple studies have evaluated the effect of FM on the reproductive performance in beef and dairy cattle. For instance, Aiumlamai et al. (1990) reported a decrease in PGF2α metabolite concentration in blood

and a delay in luteolysis after FM administration in bovines. However, there is considerably variation in the literature in terms of the effects this drug may exert on the PR values in this species and, although it is not clear yet, the parity of the animal may be an influencing factor. Merrill et al. (2004) and Geary et al. (2010) did not observe differences in PR ($P > 0.05$) between beef heifers receiving FM and a non-placebo control group. However, when Merrill et al. (2004) analyzed data from multiparous beef animals, the FM group tended to have a higher PR compared to their non-placebo control group ($P < 0.08$), which suggest that the effect of such a treatment may be dependent on the animal's parity. Moreover, when Merrill et al. (2007) analyzed a pooled dataset combining the 66 beef heifers from their previous study (where they did not find treatment differences in PR; Merrill et al. (2004)) with the data of 127 multiparous beef cows, they observed a higher ($P < 0.05$) combined PR for the FM treated females compared to the non-treated ones. In fact, Guzeloglu et al. (2007) reported that in dairy heifers, FM reduces the synthesis of $PGF_{2\alpha}$ during early pregnancy, resulting in a greater PR ($P < 0.04$) in comparison to a non-treated group.

Moreover, there is no clear evidence in the literature of the best time to administer this drug, suggesting that the treatment day may affect the results. Geary et al. (2010) did not find differences ($P = 0.80$) in beef cows receiving a similar treatment on days 11-13.5 post- artificial insemination. Nevertheless, Merrill et al. (2007) and Guzeloglu et al. (2007) found differences in PR between treatments ($P < 0.05$) by applying the FM treatment on day 14 and 15-16, respectively. Also, to our understanding, the efficiency of such a treatment has not been evaluated in animals with impaired reproduction in comparison with normal ones.

3. MATERIALS AND METHODS

3.1. Animals and treatment

Two trials were conducted in Finca Montaña at the University of Puerto Rico's Agricultural Research Station in Aguadilla, PR. Trial 1 was performed from February 12, 2016, through March 23, 2016, whereas Trial 2 was conducted from February 27, 2017, through April 7, 2017. A total of 38 and 47 purebred Senepol heifers were used for Trials 1 and 2, respectively. Animals were balanced by age and body weight for each trial (Table 1). The heifers were synchronized using a modification of the Co-Synch + CIDR & TAI protocol described by Larson et al. (2006) (Figure 1). The protocol consisted of heifers receiving a controlled internal drug release device (CIDR; Pfizer Animal Health, New York, NY) containing 1.38g of progesterone for 7 days. At CIDR insertion and removal days, 100 µg of GnRH (Cystorelin; Merial, Athens, GA) and 25 mg of $PGF_{2\alpha}$ (prostaglandin $F_{2\alpha}$; Lutalyse; Pfizer Animal Health, New York, NY) were administered intramuscularly, respectively. In this study no estrous detection was performed and all heifers were inseminated at a fixed timed artificial insemination (TAI) 72 h post CIDR removal with commercially available semen from one of three purebred Senepol sires for Trial 1 and one Red Angus sire for Trial 2. On the insemination day, heifers received a second dose of GnRH. In Trial 1 Senepol sires were distributed in a representative manner between the treatment and control group. Heifers were randomly assigned to receive 1.1 mg/kg of BW of FM (FM group; n=19 for Trial 1 and n=23 for Trial 2; Banamine, Intervet Schering Plough Animal Health, Millsboro, DE) or the equivalent volume of saline solution (Control group; n=19 for Trial 1 and n=24 for Trial 2) by the intravenous route on

day 13 or 14 after AI in Trial 1, while for Trial 2 the treatment administration was performed only on the day 14 post AI. The anti-inflammatory doses used in this study were determined based on previous literature (Merrill et al., 2004; Merrill et al., 2007; Geary et al., 2010). Heifers were maintained in a large paddock with *ad libitum* access to tropical grasses and water. Natural shade was provided close to the fences of the paddock.

Table 1. Descriptive statistics (means ± standard errors of the mean) for the purebred Senepol heifers evaluated in Trials 1 and 2.

	Trial 1		
	C	**FM**	**P-Value**
Age, days	710 ± 22.3	720 ± 23.3	0.1853
BW, kg	462.6 ± 29.0	462.4 ± 30.6	1.0000
	Trial 2		
	C	**FM**	**P value**
Age, days	727 ± 21.6	730 ± 17.7	0.6569
BW, kg	439.2 ± 30.9	442.2 ± 30.3	0.7578

Notes:
C= Control group; heifers received intravenous saline solution as a placebo. The volume of saline solution was adjusted to the respective dose of flunixin meglumine based on body weight.
FM=Treatment group; heifers received flunixine meglumine (1.1 mg/kg of body weight, intravenous).

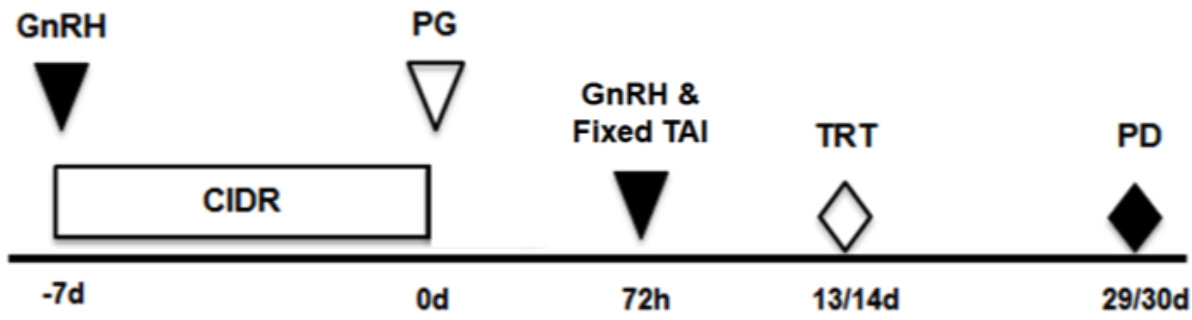

Figure 1. Experimental protocol used for ovulation synchronization, fixed time artificial insemination (TAI) and treatments application (TRT) in the present study. The protocol consisted of the insertion of a controlled internal drug release (CIDR; Pfizer Animal Health, New York, NY) containing 1.38 g of progesterone for 7 days. At CIDR insertion and removal days, 100 µg of GnRH (Cystorelin; Merial, Athens, GA) and 25 mg of PGF2α (prostaglandin $F_{2\alpha}$, Lutalyse; Pfizer Animal Health, New York, NY) were administered intramuscularly, respectively. Heifers were artificially inseminated at fixed time 72 h post CIDR removal and immediately after received a second dose of GnRH. For the TRT, heifers were randomly assigned to receive 1.1 mg/kg of BW of flunixin meglumine (Banamine, Intervet Schering Plough Animal Health, Millsboro, DE) or the equivalent volume of saline solution as a placebo by the intravenous route on days 13 or 14 after TAI for Trial 1 and only on the day 14 for Trial 2. The pregnancy detection (PD) was performed by blood samples taken on day 29 or 30 after AI for Trial 1 and only on the day 30 for Trial 2. Blood samples were evaluated at a commercial lab (BioPRYN, Central Florida Large Animal Veterinary Services, Saint Cloud, FL). Due to handling limitations in Trial 1, heifers were divided in two similar groups (in two continuous days and balanced by treatment and AI bull) for synchronization and insemination. However, in this trial the treatment administration and the pregnancy test blood samples were performed the same day for both groups.

3.2. Pregnancy Diagnosis

Heifers were managed through the farm's animal handling facility and individually restrained for blood collection from coccygeal venipuncture in 10 mL tubes (BD Vacutainer, Serum Blood Collection tubes, Franklin Lakes, NJ, USA) on day 29 or 30 after TAI for Trial 1 and only on the day 30 for Trial 2. Due to handling limitations in Trial 1, heifers were divided in two similar groups (treatments and AI bulls homogeneously represented) and inseminated in two continuous different days; however, the treatment admistration and blood sample collection were performed the same day for both groups. Blood samples were sent to a commercial lab were an Enzyme-linked immunosorbent assay (ELISA) was performed to determine the pregnancy status of each heifer by

measuring placental Pregnancy-Specific Protein B (BioPRYN, Central Florida Large Animal Veterinary Services, Saint Cloud, FL).

3.3. Environmental Conditions

Air temperature (AT) and relative humidity (RH) were continuously recorded in the experimental paddock every five minutes from the beginning of the estrous synchronization protocol until the pregnancy detection day. In Trials 1 and 2, two data loggers (HOBO U23 Pro v2, Onset Computer Corporation, Bourne, MA, USA) were used in direct solar radiation exposure; and in Trial 2, two additional data loggers were used under artificial shade. The obtained AT and RH values were combined to calculate the temperature humidity index (THI) as previously established by Vitali et al. (2009) by means of the formula:

$$THI = (1.8 \times AT + 32) – (0.55 – 0.55 \times RH) \times [(1.8 \times AT + 32) – 58], \quad \text{(Eq.1)}$$

where AT and RH are expressed in ºC and as a decimal, respectively.

3.4. Statistical Analyses

Statistical analysis was performed using SAS (SAS 9.3 Inst. Inc., Cary, NC). The Pearson's chi-square test of the FREQ Procedure was used to evaluate the relationship between two categorical variables. For Trial 1, possible effects of AI bull and treatment day on the heifer's PR were evaluated. For Trials 1, 2, and for all data combined, the treatment effects over the PR values were also evaluated with the aforementioned test. A significance level of 0.05 was established. Environmental conditions are presented in a descriptive manner.

4. RESULTS AND DISCUSSION

In Trial 1, no differences in PR were observed between AI bulls (33.3, 62.5, and 54.2% for bulls A, B, and C, respectively; P = 0.5402); therefore data were combined for further analysis. For Trial 2, only one AI bull was used. Flunixin meglumine administration did not affect the PR values in Trial 1 (P = 0.5158), Trial 2 (P = 0.4741), or in the pooled data (P = 0.9223) when compared to the control (Figure 2). Table 2 summarizes the reproductive results previously published by others using a similar approach. Similar to our study, Merrill et al. (2004) and Geary et al. (2010) did not observe differences in PR (P > 0.05) between beef heifers in the FM and the non-placebo Control groups. Moreover, working with dairy cattle, Rabaglino et al. (2010) and von Krueger and Heuweiser (2010) did not find differences (P > 0.05) in PR between the FM and the non-treated heifers. However, when Merrill et al. (2004) analyzed data from beef cows (multiparous animals), the FM group tended to have a higher PR compared to their non-placebo Control group (P < 0.08), which suggest that the effect of such a treatment may be dependent on the animal's parity. Multiple publications in dairy cattle (Pursley et al., 1997; Sartori et al., 2002; Pryce et al., 2004) and beef cattle (Hanzen et al., 1994; Colazo et al., 2004) have reported superior fertility in heifers than in cows. In fact, diverse factors including lactation related energy demands, parity, age, and nutrition may produce differences in oocyte quality and uterine environment that reduce fertility in cows in comparison with heifers (Pursley et al., 1997). Therefore, the optimum fertility normally observe in heifers may not have allowed to observed any possible advantage associated with the administration of an anti-inflammatory drug on early pregnancy maintenance. In fact, a similar approach (with other non-steroidal anti-inflammatory drugs as aspirin) has proved to improve

pregnancy rates (PR) in women, always in patients with impaired reproductive performance (Wada et al., 1994; Revelli et al., 2008; Dentali et al., 2012). Moreover, when Merrill et al. (2007) analyzed a pooled dataset combining the 66 beef heifers from their previous study (where they did not find treatment differences in PR; Merrill et al., 2004) with the data from 127 multiparous beef cows, they observed a higher ($P < 0.05$) combined PR for the FM females in comparison with the non-treated ones. These results further suggest that such an approach may only be advantageous in animals with impaired fertility. However, it is important to notice that even with the aforementioned superior fertility in heifers, there is also in the literature a considerable amount of contradictory evidence in favor of higher PR values later in life in beef cows (Mickelsen et al., 1986; Shorten et al., 2015), thus limiting the strength of this hypothesis. Moreover, Guzeloglu et al. (2007) reported that in dairy heifers, flunixin meglumine reduces the synthesis of $PGF_{2\alpha}$ during early pregnancy, resulting in greater PR values ($P < 0.04$) in comparison to a non-treated group. Therefore, future work on this area is required to achieve a better understanding of the effects of using FM as an attempt to improve PR in cattle.

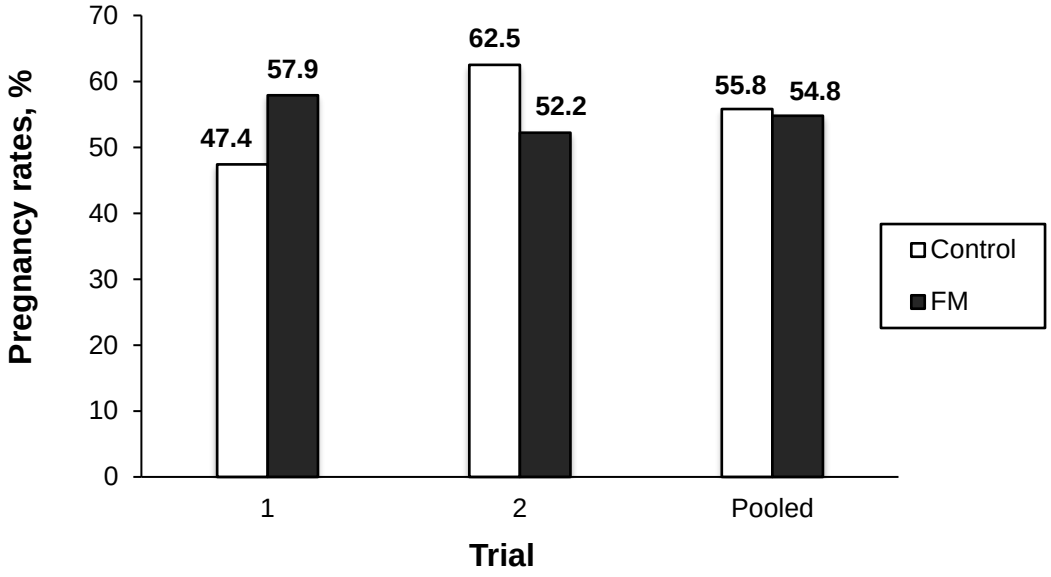

Figure 2. Pregnancy rates for time artificially inseminated purebred Senepol heifers that received flunixin meglumine (FM) or saline solution (control) in Trial 1 (P=0.5158), Trial 2 (P=0.4741), and Pooled data (P=0.9223).

The day the treatment is administered may potentially affect the PR values, as it is established that in the cow, the maternal recognition of pregnancy occurs between the days 14 – 17 post-estrous (Roberts et al., 1996; Inskeep, 2004), which suggest that the treatment may need to be administered at a specific time in order to avoid luteolysis. However, in Trial 1 there were no differences in PR between the heifers that received the treatment on day 13 and those that received it on day 14 post artificial insemination (47.1 and 57.1, respectively; P = 0.5359). In Trial 2, treatment was administered on the day 14 post artificial insemination for all the heifers. Likewise, when administering a similar one-single dose treatment on days ranging from 11 to 13.5 post- artificial insemination, Geary et al. (2010) did not find reproductive differences (P = 0.80) in beef cows. Rabaglino et al. (2010), in Holstein heifers, obtained similar reproductive results (P = 0.83) between FM and non-treated groups when applying a two-dose treatment on days 15.5 and 16.

Also, von Krueger and Heuweiser (2010) applied higher doses of flunixin meglumine on days 14, 15 or 16 post- artificial insemination; obtaining no differences ($P > 0.05$) in PR between treatment days. Nevertheless, Merrill et al. (2007) and Guzeloglu et al. (2007) found greater PR ($P < 0.05$) in the FM treated animals when applying a single dose on day 14 or a double dose on days 15-16 post- artificial insemination, respectively. Therefore, there is no clear evidence in the literature on the best time to administer such a treatment and the time window that a physiologically active dose of FM for this purpose remains on the animals' body needs to be determined.

Opposite to the present study, the Control group animals in the Merrill et al. (2004) and (2007), Guzeloglu et al. (2007), Rabaglino et al. (2010), and von Krueger and Heuwieser (2010) studies did not receive placebo. Also, in the study of Geary et al. (2010), only the FM group was gathered and processed through an animal handling facility while the Control group remained on pasture. Handling the animals and applying an intramuscular injection only in the FM group could have potentially added bias in to their results, because of the effects that stress exerts on fertility. For instance, in domestic animals, stressors interfered with the precise timing of reproductive hormones (Dobson and Smith, 2000). In fact, management stressors in female farm animals result in reproductive problems through mechanisms acting on the hypothalamic, pituitary, and ovarian axis, thus negatively affecting the uterine function (von Borell et al., 2007). Additionally, Morimoto et al. (1991) associated the presence of stress with physiological events that may impair reproduction in rats that were cage-switched and exposed to a new environment. .

Furthermore, in the study of Geary et al. (2010), there were 2 different locations and each of them used 2 different AI protocols. Although they did not observe a locations x treatment interaction on PR (P = 0.75), using different locations and AI protocols could be another source of variation affecting their results. In fact, differences in pasture and diet, breed, climate, and geographic location have been reported to affect the effectiveness of TAI protocols (Lamb et al., 2010). Moreover, several authors have demonstrated that the success of estrous and ovulation synchronization directly depends on the protocol evaluated (Busch et al., 2007, Leitman et al., 2008, Lamb et al., 2010). Therefore, the implications of such results may not help to better understand the trends observed in our study.

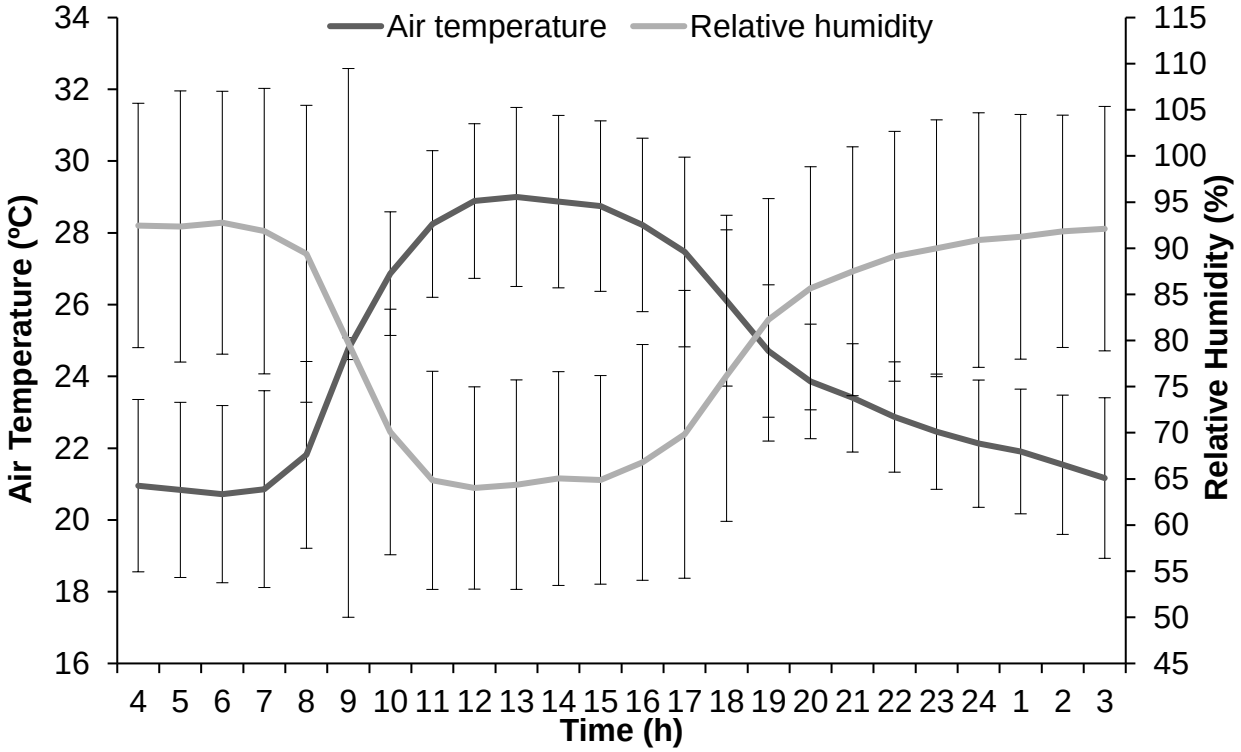

Figure 3. Mean air temperature and relative humidity values through the 24 hours of the day in Trial 1.

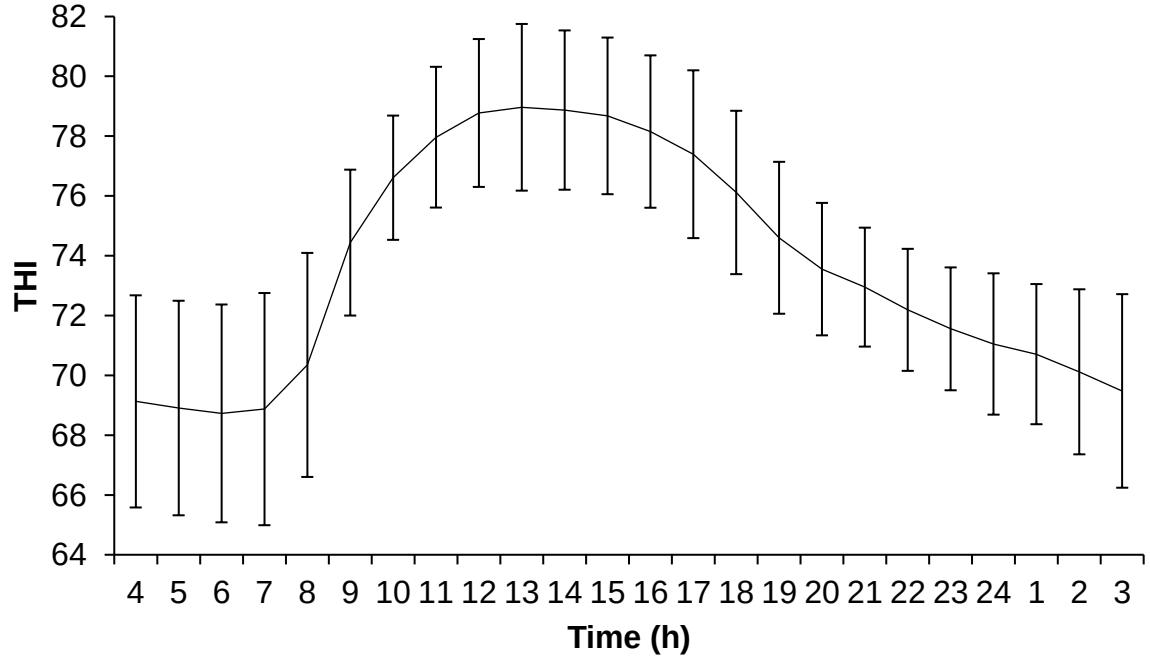

Figure 4. Mean temperature humidity index values through the 24 hours of the day in Trial 1.

Figures 3 and 4 present the mean hourly values of AT and RH, and THI observed during Trial 1 for the 24 hours of the day, respectively. During the day, time affected the AT, RH, and THI. Minimum mean daily AT values of 20.84 ± 0.07ºC were observed from 0400 to 0700h. Later, AT linearly increased 8.02ºC from 0700 to 1200h, reaching a mean maximum daily plateau of 28.88 ± 0.07ºC from 1200 to 1500h. Then, a decrease in AT of 7.79ºC, on average, was observed from 1500 to 0400h. The opposite trend was observed for RH. The RH presented its mean maximum daily value of 92.36 ± 0.44% from 0400 to 0700h, and decreased 27.83% from 0700 to 1200h. The lowest mean daily plateau in RH (65.02 ± 0.44%) was observed during the 1200 to 1600h time period, followed by an increase of 27.57% from 1500 to 0400h. The THI followed a very similar trend to the one observed for AT. It showed its minimum mean daily plateau of 68.84 ± 0.09 during the 0500 to 0700h period. Then, the THI values increased 9.90 units from 0700 to 1200h, until its highest mean daily value of 78.82 ± 0.09 was attained from 1200 to 1500h. Then, the THI decreased 9.55 units from 1500 to 0400h.

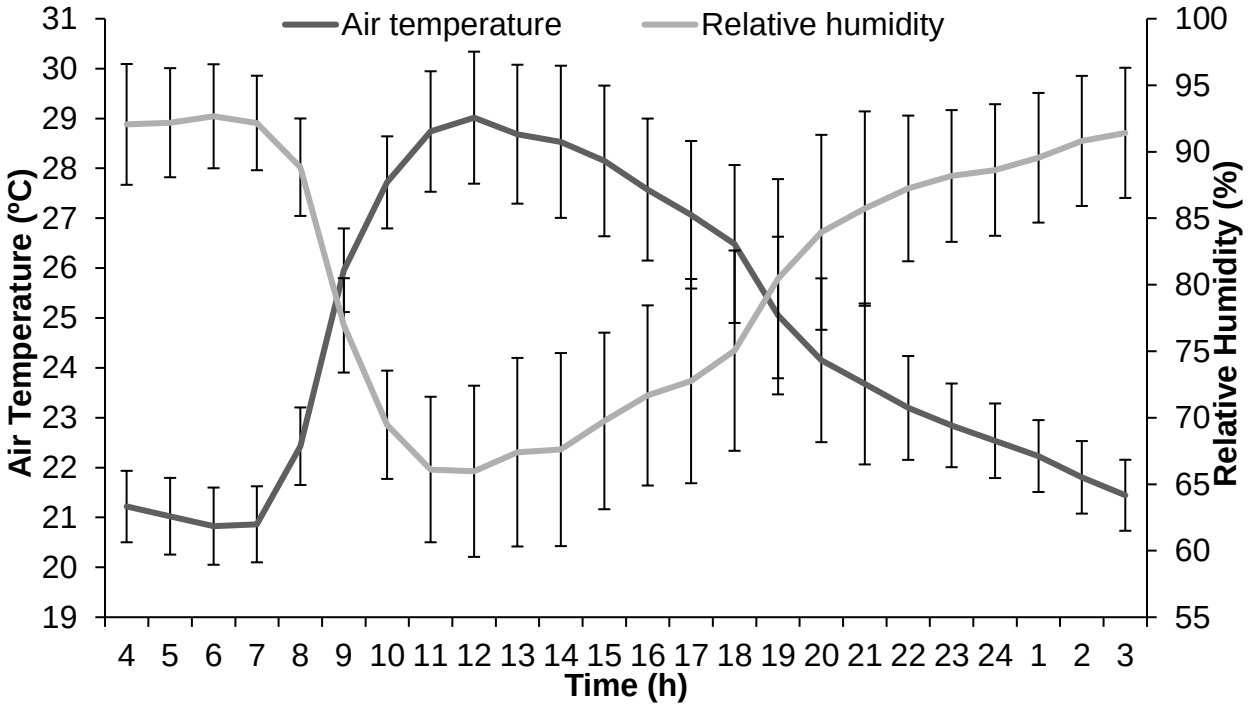

Figure 5. Mean air temperature and relative humidity values through the 24 hours of the day in Trial 2.

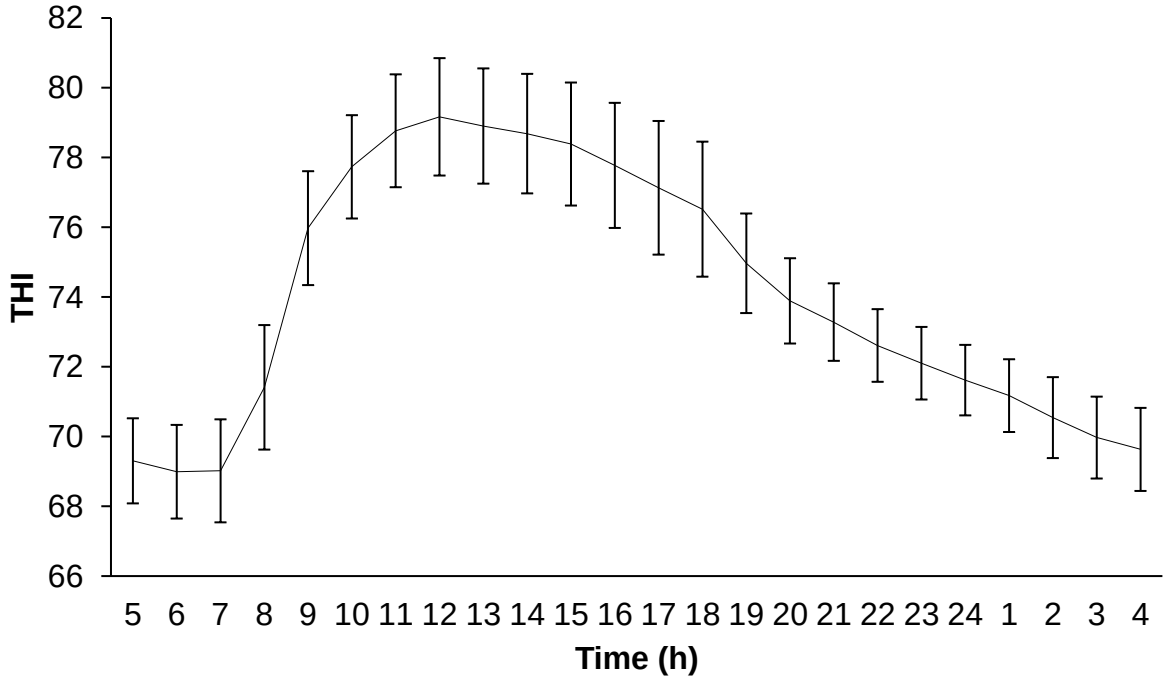

Figure 6. Mean temperature humidity index values through the 24 hours of the day in Trial 2.

Figures 5 and 6 show the mean values of the environmental conditions (AT and RH, and THI, respectively) in Trial 2 during the 24 hours of the day. An effect of time was observed for the AT, RH, and THI. Similar to Trial 1, a minimum mean daily AT value of 20.84 ± 0.03ºC was found from 0600 to 0700h; increasing 8.15ºC from 0700 to 1200h, reaching a mean maximum value of 29.02 ± 0.04ºC at 1200h, and decreasing an average of 8.19ºC from 1200 to 0600h. Also in this trial, the opposite trend was observed for RH, showing a daily maximum mean of 92.27 ± 0.13% from 0400 to 0700h, and decreasing 24.56% from 0700 to 1200h, until reaching its lowest mean daily value in RH of 65.97 ± 0.21% at 1200h, followed by an increment of 26.08% from 1200 to 0400h. As for Trial 1, in Trial 2 the THI followed a similar trend to the one observed in the AT values. From the 0600 to 0700h period, THI showed its minimum mean daily values of 69.00 ± 0.05. Then, its values increased 10.15 units from 0700 to 1200h, until its highest mean daily value of 79.17 ± 0.06 was reached at 1200h. Then, the THI decreased 10.17 units from 1200 to 0600h.

Amundson et al. (2006) stated that minimum daily AT values exceeding 16.7ºC around the reproductive season negatively affect the reproductive performance in *Bos taurus* beef cows. Moreover, they also established that mean daily THI values under 69 during the breeding period resulted in optimum PR values, while reaching or exceeding a mean daily THI value of 72.9 caused a significant reduction in this variable. Based on this literature, our animals were continually exposed to a hostile environment. During Trials 1 and 2, the mean AT exceeded this critical value during 100% of the day. Moreover, our minimum mean THI values in Trials 1 and 2 were never below 69 and the

mean THI was above 72.9 the 54.9% of the study for Trial 1 and 54.2% for Trial 2. Therefore, heifers in the present study were exposed to conditions normally associated with heat stress in the literature. Heat stress refers to when the environmental conditions are so adverse that the cow's heat dissipation mechanisms are not efficient any more, resulting in an increase in body temperature and negatively affecting the animal's physiology (West, 2003). Heat stress strongly impairs multiple female reproductive functions by redirecting blood flow from inner organs to the body surface in order to facilitate the heat dissipation (Sprott et al., 2001), but decreasing uterine perfusion (Roman-Ponce et al., 1978). Also, in mammals, heat stress interrupts oocyte development and maturation, early embryonic development, and fetal growth (Hansen, 2009). Cows exposed to heat stress decrease estrus duration and show an affected embryo development (Jordan, 2003). Therefore, these factors may have influenced the obtained results, probably limiting the possibility of observing any FM treatment related differences in PR.

However, it is important to mention that our study evaluated purebred Senepol heifers, a breed highly adapted to hot and humid weathers, known for their considerably high productive and reproductive efficiency under such environmental conditions (Cianzio, 2002). Moreover, to our understanding, the critical environmental thresholds for reproductive performance have only been evaluated in beef cattle breeds originated in temperate regions, which limit their extrapolation to our conditions. Also, in general, the PR values observed in our study fall inside the range of those previously published by others using the same or similar AI protocols in beef heifers not exposed to heat stress (Larson et al., 2006). Therefore, although not directly accounted for, the impact that heat

stress exerts over the reproductive performance of our heifers should be limited. It is plausible that such an outstanding reproductive performance normally observed in this breed may limit any possible benefits related to any reproductive therapy, thus decreasing the possibility of observing differences in the present study.

Table 2. Summary of results from previous studies evaluating the effect of flunixin meglumine treatment on pregnancy rate in cattle.

Animals, n	Pregnancy detection, d[a]	Pregnancy rate[b]		P-Value	Treatment day[d]	Dosis, mg/kg, im	Placebo[e]	References
		Control, % (n)	FM[c], % (n)					
130 Beef Heifers	33-35	54 (35/65)	63 (41/65)	0.15	14	1.1	No	Merrill et al., 2004
63 Beef Cows	55-57	66 (21/32)	80 (24/31)	<0.08	14	1.1	No	Merrill et al., 2004
193 Beef Cows and Heifers	30-55	64 (62/97)	73 (70/96)	<0.05	14	1.1	No	Merrill et al., 2007
52 Dairy Heifers	29	50(13/26)	76.9 (20/26)	<0.04	15-16	1.1	No	Guzeloglu et al.,2007
323 Dairy Heifers	32	60.0 (99/165)	60.8 (96/158)	0.83	15.5-16	1.1	No	Rabaglino et al.,2010
247 Beef Heifers	29 and 75	55 (-)	56 (-)	0.37	13	1.1	No	Geary et al., 2010
705 Beef Cows	47	58 (-)	57 (-)	0.80	11-13.5	1.1	No	Geary et al., 2010
307 Dairy Heifers	39-40	58.7 (88/150)	58.6 (92/157)	0.99	14/15 and15/16	2.2	No	von Krueger and Heuwieser, 2010
246 Beef Cows and Heifers	40	57.3 (71/124)	47.5 (58/122)	>0.05	7 and 16	2.2	Yes	Rossetti et al., 2011

Note:
[a]Pregnancy detection is expressed as days post- artificial insemination and was assessed by means of ultrasonography or transrectal palpation.
[b]Pregnancy rates are described in parenthesis as the number of cows that result pregnant over the total amount of cows evaluated in the respective treatment.
[c]FM = flunixin meglumine treated group. i.m. = intramuscularly.
[d]Treatment day refers to the number of days after AI when treatment was administered.
[e]Indicates if the control group received as placebo as part of its treatment.
(-) Number of animals could not be determined from the article.

5. CONCLUSIONS

In the present study the flunixin meglumine administration did not enhance reproductive performance in Senepol heifers. Possible explanations for this trend include the great environmental adaptation of this breed to the tropics and the reproductive superiority of this parity group, as well as the limited observation in this study may have not allowed to achieve a further significant improvement in pregnancy rates. However, although there are results in the literature that may help to support these hypotheses, there is also a considerable amount of contradictory evidence. Therefore, future studies should be directed to achieve a better understanding of these trends, including the evaluation of this approach in cattle with impaired reproduction such as stressed multiparous or lactating cows.

LITERATURE CITED

Acosta, T. J., and A. Miyamoto. 2004. Vascular control of ovarian function: ovulation, corpus luteum formation and regression. Anim. Reprod. Sci. 82:127-140.

Aiumlamai, S., K. Odensvik, G. Stabenfeldt, and H. Kindahl. 1990. Regulation of prostaglandin biosynthesis with flunixin meglumine in the bovine species. Transbound. Emer. Dis. 37:16-22.

Amundson, J. L., T. L. Mader, R. J. Rasby, and Q. S. Hu. 2006. Environmental effects on pregnancy rate in beef cattle. J. Anim. Sci. 84:3415-3420.

Anderson, G. D., S. D. Hauser, K. L. McGarity, M. E. Bremer, P. C. Isakson, and S. A. Gregory. 1996. Selective inhibition of cyclooxygenase (cox)-2 reverses inflammation and expression of cox-2 and interleukin 6 in rat adjuvant arthritis. J. Clin. Invest. 97:2672-2679.

Ayalon, N. 1978. A review of embryonic mortality in cattle. J. Reprod. Fertil. 54:483-493.

Banu, S. K., J. A. Arosh, P. Chapdelaine, and M. A. Fortier. 2003. Molecular cloning and spatio-temporal expression of the prostaglandin transporter: A basis for the action of prostaglandins in the bovine reproductive system. Natl. Acad. Sci. USA. 100:11747-11752.

Beshay V. E. and B. R.Carr .2013. Hypothalamic-pituitary-ovarian axis and control of the menstrual cycle. In: T. Falcone and W. W. Hurd, editors, Clinical reproductive medicine and surgery: a practical guide. Springer, NY. p. 31-42.

Bollwein, H., F. Weber, B. Kolberg, and R. Stolla. 2002. Uterine and ovarian blood flow during the estrous cycle in mares. Theriogenology. 57:2129-2138.

Bollwein, H., F. Weber, S. Steffen, and R. Stolla. 2003. The effect of acetylsalicylic acid and captopril on uterine and ovarian blood flow during the estrous cycle in mares. Theriogenology. 61:301-309.

Bridges, G. A., M. L. Day, T. W. Geary, and L. H. Cruppe. 2013. Triennial reproduction symposium: Deficiencies in the uterine environment and failure to support embryonic development. J. Anim. Sci. 91:3002-3013.

Busch, D. C., D. J. Wilson, D. J. Schafer, N. R. Leitman, J. K. Haden, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2007. Comparison of progestin-based estrus synchronization protocols before fixed-time artificial insemination on pregnancy rate in beef heifers. J. Anim. Sci. 85:1933-1939.

Cianzio, D. 2002. Historia breve del ganado Senepol en Puerto Rico: un informe en colaboración. Simposio Senepol, Santa Cruz, USVI. p.1-7.

Colazo, M. G., J. P. Kastelic, P. R. Whittaker, Q. A. Gavaga, R. Wilde, and R. J. Mapletoft. 2004. Fertility in beef cattle given a new or previously used CIDR insert and estradiol, with or without progesterone. Anim. Reprod. Sci. 81:25-34.

Dentali, F., W. Ageno, E. Rezoagli, E. Rancan, A. Squizzato, S. Middeldorp, M. Margaglione, and E. Grandones. 2012. Low-dose aspirin for in vitro fertilization or intracytoplasmic sperm injection: a systematic review and a meta-analysis of the literature. J. Thromb. Haemost. 10:2075-2085.

Departamento de Agricultura del ELA, División de estadísticas Agrícolas. 2014. Fondo para el fomento de la industria de Carne de Res de Puerto Rico. http://www.fondocarnederes.com/ (Accessed 7 February 2017.)

Diskin M. G. and J. M. Sreenan. 1980. Fertilization and embryonic mortality rates in beef heifers after artificial insemination. J. Reprod. Fertil. 2:463-468.

Dobson, H. and R. F. Smith. 2000 What is stress, and how does it affect reproduction? Anim. Reprod. Sci. 60-61:743-752.

Dunne, L. D., M. G. Diskin, and J. M. Sreenan. 2000. Embryo and foetal loss in beef heifers between day 14 of gestation and full term. Anim. Reprod. Sci. 58:39-44.

Elli, M., B. Gaffuri, A. Frigerio, M. Zanardelli, D. Covini, M. Candiani, and M. Vignali. 2001. Effect of a single dose of ibuprofen lysinate before embryo transfer on pregnancy rates in cows. Reproduction. 121:151-154.

Fortier M. A., K. Krishnaswamy, G. Danyod, S. Boucher-kovalik, and P. Chapdelaine. 2008. A postgenomic integrated view of prostaglandins in reproduction: implications for other body systems. J. Physiol. Pharmacol. 59(Suppl 1):65-89.

Fortune, J.E. 1994. Ovarian follicular growth development in mammals. Biol. Reprod. 50:225-232.

Geary, T. 2005. Management Strategies to Reduce Embryonic Loss. Proc. Range Beef Cow Symposium XIX. Rapid City, SD. p. 69-78.

Geary, T. W. 2012. Effects of adrenocorticotropic hormone and flunixin meglumine on pregnancy retention in beef cows. J. Anim. Sci. 90:207-211.

Geary, T. W., R. P. Ansotegui, M. D. MacNeil, A. J. Roberts, and R. C. Waterman. 2010. Effects of flunixin meglumine on pregnancy establishment in beef cattle. J. Anim. Sci. 88:943-949.

Ginther O. J., M. C. Wiltbank, P. M. Fricke, J. R. Gibbons, and K. Kot. 1996. Selection of the dominant follicle in cattle. Biol. Reprod. 55:1187-1194.

Ginther, O. J., L. Knopf, and J. P. Kastelic.1989. Temporal associations among ovarian events in cattle during oestrous cycles with two and three follicular waves. J. Reprod. Fertil. 87:223-230.

Goodman, R. L., L. M. Coolen, G. M. Anderson, S. L. Hardy, M. Valent, J. M. Connors, M. E. Fitzgerald, and M. N. Lehman. 2004. Evidence that dynorphin plays a major role in mediating progesterone negative feedback on gonadotropin-releasing hormone neurons in sheep. Endocrinology. 145:2959-2967.

Guzeloglu, A., H. Erdem, M. K. Saribay, W. W. Thatcher, and T. Tekeli. 2007. Effect of the administration of flunixin meglumine on pregnancy rates in Holstein heifers. Vet. Rec.160:404–406.

Hansen, P. J., 2009. Effects of heat stress on mammalian reproduction. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 364:3341-3350.

Hanzen, C., Y. Laurent, and W. R. Ward. 1994. Comparison of reproductive performance in Belgian dairy and beef cattle. Theriogenology. 41:1099-1114.

Inskeep, E. K. 2004. Preovulatory, postovulatory, and postmaternal recognition effects of concentrations of progesterone on embryonic survival in the cow. J. Anim. Sci. 82:E24-E39.

Jordan, E. R. 2003. Effects of heat stress on reproduction. J. Dairy Sci. 86(E. Suppl.):E104-E114.

Lamb, G. C., C. R. Dahlen, J. E Larson, G. Marquezini, and J. S. Stevenson. 2010. Control of the estrous cycle to improve fertility for fixed-time artificial insemination in beef cattle: a review. J. Anim. Sci. 88(E. Suppl.):E181-E192.

Larson, J. E., G. C. Lamb, J. S. Stevenson, S. K. Johnson, M. K. Day, T. W. Geary, D. J. Kesler, J. M. DeJarnette, F. N. Schrick, A. DiConstanzo, and J. D. Arseneau. 2006. Synchronization of estrus in suckled beef cows for detected estrus and artificial insemination and timed artificial insemination using gonadotropin-releasing hormone, prostaglandin F2α, and progesterone. J. Anim. Sci. 84:332-342.

Leitman, N. R., D. C. Busch, J. F. Bader, D. A. Mallory, D. J. Wilson, M. C. Lucy, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2008. Comparison of protocols to synchronize estrus and ovulation in estrous-cycling and prepubertal beef heifers. J. Anim. Sci. 86:1808-1818.

McCracken, J. A., E. E. Custer, and J. C. Lamsa. 1999. Luteolysis: a neuroendocrine-mediated event. Am. Physiol. Soc. 79:263-324.

Merrill, M. L., R. P. Ansotegui, J. A. Paterson, and T. W. Geary. 2004. Effect of flunixin meglumine on early embryonic mortality in stressed beef females. Proc. West. Sec. Amer. Soc. Anim. Sci. 55:304-307.

Merrill, M. L., R. P. Ansotegui, P. D. Burns, M. D. MacNeil, and T. W. Geary. 2007. Effects of flunixin meglumine and transportation stress on pregnancy establishment in beef cows. J. Anim. Sci. 85:1547-1554.

Mickelsen, W. D., L. G. Paisley, and P. B. Anderson. 1986. Survey of the prevalence and types of infertility in beef cows and heifers. J. Am. Vet. Med. Assoc. 189:51-54.

Morimoto A., T. Watanabe, K. Morimoto, T. Nakamori, and N. Murakami. 1991. Possible involvement of prostaglandins in psychological stress–induced responses in rats. J. Physiol. 443:421-429.

Miyamoto, A., K. Shirasuna, M. P. B. Wijayagunawardane, S. Watanabe, M. Hayashi, D. Yamamoto, M. Matsui, and T. J. Acosta. 2005. Blood flow: a key regulatory component or corpus luetum function in the cow. Domest. Anim. Endocrinol. 29:329-339.

Mumford, S. L., R. M. Silver, L. A. Sjaarda, J. Wactawski-Wende, J. M. Townsend, A. M. Lynch, N. Galai, L. L. Lesher, D. Faraggi, N. J. Perkins, K. C. Schliep, S. M. Zarek, and E. F. Schisterman. 2016.Expanded findings from a randomized controlled trial of preconception low-dose aspirin and pregnancy loss. Hum. Reprod. 3:657-665.

Nanda, A. S., W. R. Ward, and H. Dobson. 1989. Effects of naloxone on the oestradiol-induced LH surge and cortisol release in transported cows. J. Reprod. Fertil. 87:803-807.

Nguyen, L. T., A. Reverter, A. Cánovas, B. Venus, A. Islas-Trejo, L. R. Porto-Neto, S. A. Lehnert, J. F. Medrano, S. S. Moore, and M. R. S. Fortes. 2017. Global differential gene expression in the pituitary gland and the ovaries of pre-and postpubertal Brahman heifers. J. Anim. Sci. 95:599-615.

Niswender, G.D., J.L. Juengel, P.J. Silva, M.K. Rollyson, and E.W. McIntush. 2000. Mechanisms controlling the function and life span of the corpus luteum. Physiol. Rev. 80:1-29.

Okuda, K., Y. Miyamoto, and D. J. Skarzynski. 2002. Regulation of endometrial prostaglandin F2α synthesis during luteolysis and early pregnancy in cattle. Domest. Anim. Endocrinol. 23:255-264.

Pharriss, B. B., J. C. Cornette, and G. D. Gutknecht. 1970. Vascular control of luteal steroidogenesis. J. Reprod. Fertil. Suppl. 10:97-103.

Pryce, J. E., M. D. Royal, P. C. Garnsworthy, and I. L. Mao. 2004. Fertility in high-producing dairy cow. Livest. Prod. Sci. 86:125-135.

Pursley, J. R., M. C. Wiltbank, J. S. Stevenson, J. S. Ottobre, H. A. Garverick, and L. L. Anderson. 1997. Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. J. Dairy Sci. 80:295-300.

Rabaglino, M. B., C. A. Risco, M. J. Thatcher, F. Lima, J. E. P. Santos, and W. W. Thatcher. 2010. Use of a five-day progesterone-based timed AI protocol to determine if flunixin meglumine improves pregnancy per timed AI in dairy heifers. Theriogenology. 73:1311-1318.

Revelli, A., E. Dolfin, G. Gennarelli, T. Lantieri, M. Massobrio, J. G. Holte, and I. Tur-Kaspa. 2008. Low-dose acetylsalicylic acid plus prednisolone as an adjuvant treatment in IVF: a prospective, randomized study. Fertil. Steril. 5:1685-1691.

Roberts, R. M., S. Xie, and N. Mathialagan. 1996. Maternal recognition of pregnancy. Biol. Reprod. 54:294-302.

Roman-Ponce, H., W. W. Thatcher, D. Caton, D. H. Barron, and C. J. Wilcox. 1978. Thermal stress effects on uterine blood flow in dairy cows. J. Anim. Sci. 46:175-180.

Rossetti, R. C., A. Perdigao, F. S. Mesquita, M. Sa Filho, G. P. Nogueira, R. Machado, C. M. Membrive, and M. Binelli. 2011. Effects of flunixin meglumine, recombinant bovine somatotropin and/or human chorionic gonadotropin on pregnancy rates in Nelore cows. Theriogenology. 76:751-758.

Sartori, R., R. Sartor-Bergfelt, S. A. Mertens, J. N. Guenther, J. J. Parrish, and M. C. Wiltbank. 2002. Fertilization and early embryonic development in heifers and lactating cows in summer and lactating and dry cows in winter. J. Dairy. Sci. 85:2803-2812.

Schams, D., and B. Berisha. 2004. Regulation of corpus luteum function in cattle– an overview. Reprod. Domest. Anim. 39:241-251.

Schisterman, E. F., S. L. Mumford, K. C. Schliep, L. A. Sjaarda, J. B. Stanford, L. L. Lesher, J. Wactawski-Wende, A. M. Lynch, J. M. Townsend, N. J. Perkins, S. M. Zarek, M. Y. Tsai, Z. Chen, D. Faraggi, N. Galai, and R. M. Silver. 2015. Preconception low dose aspirin and time to pregnancy: findings from the effects of aspirin in gestation and reproduction randomized trial. J. Clin. Endocrinol. Metab.100:1785-1791.

Shorten, P. R., C. A. Morris, and N. G. Cullen. 2015. The effects of age, weight, and sire on pregnancy rate in cattle. J. Anim. Sci. 93:1535-1545.

Silvia, W. J., G. S. Lewis, J. A. McCraken, W. W. Thatcher, and L. Wilson, JR. 1991. Review: hormonal regulation of uterine secretion of prostaglandin F2α during luteolysis in ruminants. Biol. Reprod. 45:655-663.

Skarzynski, D. J., G. Ferreira- Dias, and K. Okuda. 2008. Regulation of luteal function and corpus luteum regression in cows: hormonal control, immune mechanisms and intercellular communication. Reprod. Domest. Anim. 43:57-65.

Smith, G. W., J. L. Davis, L. A. Tell, A. I. Webb, and J. E. Riviere. 2008. Extralabel use of nonsteroidal anti-inflammatory drugs in cattle. J. Am. Vet. Med. Assoc. 232:697-701.

Spencer, J. A., K. Steinkamp, B. Shafii, A. Ahmadzadeh. 2016. The effect of aspirin on prostaglandin $F_{2\alpha}$ secretion in lactating dairy cows. PAS. 32:681-686.

Spencer, T. E., and F. W. Bazer. 2004. Conceptus signals for establishment and maintenance of pregnancy. Reprod. Biol. Endocrinol. 2:49.

Sprott, L. R., G. E. Selk, and D. C. Adams. 2001. Factors affecting decisions on when to calve beef females. Prof. Anim. Sci. 17:238-246.

Sreenan, J. M. and M. G. Diskin.1986. Embryonic mortality in farm animals. Martinus Nijhoff publishers, Brussels-Luxembourg. p.VII.

Sun, T., L. Shi-Jie, D. Hong-Lu, T. Chun-Bo, W. Hong-Bin, and Y. Zeng-Ming. 2004. Cyclooxygenases and prostaglandin E synthases in the endometrium of the rhesus monkey during the menstrual cycle. Reproduction. 127:465-473.

Thatcher, W. W., A. Guzeloglu, R. Mattos, M. Binelli, T. R., Hansen and J. K. Pru. 2001. Uterine-conceptus interactions and reproductive failure in cattle. Theriogenology. 56:1435-1450.

Thatcher, W. W., C. R. Staples, G. Danet-Desnoyers, B. Oldick, and E. P. Schmitt. 1994. Embryo health and mortality in sheep and cattle. J. Anim. Sci. 72:16-30.

USDA, Economic Research Service. 2015. Statistics and information, U.S. beef industry. https://www.ers.usda.gov/topics/animal-products/cattle-beef/statistics-information/ (Accessed 7 March 2017).

USDA, National Agricultural Statistics Service. 2016. Beef cows: inventory on January by year, US.https://www.nass.usda.gov/Charts_and_Maps/Cattle/bcow.php (Accessed 7 March 2017).

Vane, J. R., and R. M. Botting. 2003.The mechanism of action of aspirin. Thromb. Res. 110:255-258.

Vitali, A., M. Segnalini, L. Bertocchi, U. Bernabucci, A. Nardone, and N. Lacetera. 2009. Seasonal pattern of mortality and relationships between mortality and temperature-humidity index in dairy cows. J. Dairy Sci. 92:3781-3790.

von Borell, E., H. Dobson, and A. Prunier. 2007. Stress, behavior and reproductive performance in female cattle and pigs. Horm. Behav. 52:130-138.

von Krueger, X. and W. Heuwieser. 2010. Effect of flunixin meglumine and carprofen on pregnancy rates in dairy cattle. J. Dairy Sci. 93:5140-5146.

Wada, I., C. C. Hsu, G. Williams, M. C. Macnamee, and P. R. Brindsen. 1994. The benefits of low-dose aspirin therapy in women with impaired uterine perfusion during assisted reproduction. Hum. Reprod. 9:1954-1957.

West, J. W., B. G. Mullinix, and J. K. Bernard. 2003. Effects of hot, humid weather on milk temperature, dry matter intake, and milk yield of lactating dairy cows. J. Dairy Sci. 86:232-242.

Wiltbank, M. C., A. Gümen, and R. Sartori. 2002. Physiological classification of anovulatory conditions in cattle. Theriogenology. 57:21-52.

Yao, B., L. Hai-Yan, G. Yu-Chun, S. Shan-Shan, T. Xiao-Qian, L. Xiao-Jun, G. Yi-Feng, C. Ying-Xia, and Y. Guo-Bin. 2011. Gonadotropin-releasing hormone positively regulates steroidogenesis via extracellular signal-regulated kinase in rat Leydig cells. Asian J. Androl. 13:438-445.