Effect of sperm kinematics and size of follicle at ovulation on pregnancy rate after timed AI of beef cows

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ABSTRACT

The objectives of this study were to evaluate whether the diameter of the preovulatory follicle and the post-thaw sperm kinematics are factors that affect successful establishment of a pregnancy after timed AI (TAI) of postpartum beef cows. Nelore cows ($n = 346$) were subjected to an estradiol-progesterone based TAI protocol. At TAI, cows were categorized according to the diameter of the preovulatory follicle (POF) in the Early Ovulation (EO group, $n = 184$), and Late Ovulation (LO group, $n = 162$) groups. Sperm were classified, according to CASA analysis, as Hyper-activated (H+), and Non-Hyper-activated (H-). The H + and H- semen was used to inseminate cows that were classified to be in the EO and LO groups. Thus, after distribution, the groups were: EOH+ ($n = 89$), EOH- ($n = 95$), LOH+ ($n = 96$), and LOH- ($n = 66$). Pregnancy per AI (P/AI) was greater ($P < 0.05$) for EOH+ (66.3%, 59/89), EOH- (65.3%, 62/95), and LOH- (72.7%, 48/66) groups than for LOH + group (52.1%, 50/96). The results of the study indicate that cows with a smaller POF are less likely to become pregnant when insemination is with semen considered as hyper-activated (H+). In contrast, when inseminations are performed with semen categorized to be non-hyper-activated (H-), cows of the EO and LO groups had acceptable and similar pregnancy rates as a result of timed AI.

1. Introduction

One of the goals of cattle reproductive management is to achieve pregnancy rates that result in profitability of beef production enterprises. The adoption of artificial insemination (AI) using protocols combining progesterone and estrogens to improve AI submission rates achieves consistent conception results (~50%) in postpartum beef cows (Sales et al., 2012). Several studies have been performed to evaluate the factors that affect the fertility of postpartum cows subjected to timed AI (TAI) protocols. Such factors include: hormone concentration; duration of progestagen treatment; time and dose of PGF2 alpha (PGF) injection; ovulation-induction agents (ECP, BE, LH, GnRH); estrous cyclicity status; parity; ovarian response; use and dose of equine chorionic gonadotropin (eCG); proportion of cows in estrus before AI; and body condition score (BCS; Meneghetti et al., 2009; Sa Filho et al., 2009, 2011; Ayres et al., 2014; Pfeifer et al., 2017). Cows with larger follicles are more likely to be in estrus, and have greater rates of ovulation and pregnancy per AI (P/AI) following TAI. The size of the follicle from which ovulation occurs influences P/AI in beef heifers (Perry et al., 2007) and suckled beef cows (Perry et al., 2005; Sa Filho et al., 2010; Pfeifer et al., 2012, 2015).

The pregnancy rate with use of TAI protocols, however, also depends on other factors that have not received appropriate attention (e.g., quality of semen samples used; Oliveira et al., 2012). Individual bulls differ in the production of sperm with the capacity to fertilize oocytes that can subsequently develop into blastocysts following *in vitro* fertilization (IVF; Zhang et al., 1997; Wei and Fukui, 1999; Sudano et al., 2011). Furthermore, there is a marked variability in field fertility among individual bulls (Correa et al., 1997; Ward et al., 2001; Andersson et al., 2004). Several noninvasive methods for examining bulls have been used to predict semen production potential and fertility (Thompson and Johnson, 1995; Lunstra and Coulter, 1997; Kastelic et al., 2001; Brito et al., 2004; Arteaga et al., 2005; Celeghini et al., 2007). *in vitro* methods have also been used to determine bull fertility (Correa et al., 1997; Verstegen et al., 2002; Januskauskas et al., 2003; Tartaglione and Ritta, 2004). The results of such approaches, however, do not always correlate with pregnancy outcomes in beef production enterprises (Sudano et al., 2011).

A more accurate estimation of the fertility potential of a TAI protocol that involves the more precisely controlled development and maturation of male and female gametes can be achieved when both semen characteristics and the ovarian response are considered. The study of kinematics in frozen–thawed bull semen, therefore, may provide further information about the viability of sperm in the female reproductive tract. Sperm require a period of maturation, defined as capacitation, subsequent to ejaculation and separation from the seminal plasma in the female reproductive tract before sperm attain the capacity to undergo an acrosome reaction and contribute to fertilization of an ova (Austin, 1951; Chang, 1951; Bedford, 1983). Sperm undergoing capacitation that includes hyper-activated motility which is characterized by asymmetrical flagellar beating - which results from an increase in the amplitude of the principal flagellar bend - prior to reaching the site of fertilization in the oviduct (Demott and Suarez, 1992; Suarez, 1987; Katz and Yanagimachi, 1980). Hyper-activation is an important aspect for sperm function because it affects the capacity of sperm to transit through the female reproductive tract and reach the site of fertilization (Suarez et al., 1991; Suarez and Dai, 1992; Suarez et al., 1992; Alasmari et al., 2013). Computer-aided semen analysis (CASA) has been used to characterize sperm motility and evaluate certain kinematic variables, such as curvilinear velocity (VCL), amplitude of lateral head displacement (ALH), and linearity (LIN). Such variables are associated with the hyper-activation characteristics of frozen–thawed semen (Mortimer and Mortimer, 1990; Shojaei et al., 2012), and may affect fertility in beef production enterprises.

Although several inherent semen quality factors can affect the results of a TAI program, to the best of our knowledge there have been no studies on the kinematic characteristics of sperm associated with ovarian responses from cows. Based on these considerations, the objective of the present study was to evaluate the P/AI rates of lactating beef cows as affected by size of the ovulatory follicle and kinematic characteristics of the semen used for AI. Thus, it was hypothesized that cows with lesser follicle responses, that consequently had a longer period subsequent to treatment before ovulation, would have an acceptable P/AI if insemination occurred with non-hyper-activated sperm. Furthermore, it was hypothesized that cows with larger preovulatory follicles (POFs) will have greater P/AI rates when there was insemination with hyper-activated sperm.

2. Materials and methods

The Committee for Ethics in Animal Experimentation from Embrapa approved all of the procedures performed in the experiment described in this manuscript (Protocol 04/2017).

2.1. Semen analysis

Batches ($n = 47$) of semen from 20 bulls were acquired from a semen-processing center (Seleon Biotechnology, Itatinga, SP, Brazil). Sperm motility was assessed by CASA (Ivos-UltiMate®; Hamilton Thorne Biosciences, Beverly, MA, USA). All samples used in the present study were processed by Seleon Biotechnology, and all semen analyses were performed by the same technician (JRP). The supplementary data file includes the results from the analysis of the 47 semen samples.

The following variables were analyzed using CASA: total motility (TM), progressive motility (PM), average path velocity (VAP), straight-line velocity (VSL), curvilinear velocity (VCL), amplitude of lateral head displacement (ALH), straightness (STR), and linearity (LIN). Approximately 100,000 cells were evaluated using CASA for each semen sample.

Because movement of the sperm head depends on flagellar bend and beat patterns, sperm cells with a relatively greater VCL and ALH, and lesser LIN are characteristic of hyper-activation (Mortimer and Mortimer, 1990; Marquez and Suarez, 2007; Shojaei et al., 2012). Thus, in comparison to the average of the group studied, batches with a relatively greater VCL and ALH, and lesser LIN were considered to be hyper-activated (H+). Furthermore, batches with a relatively lesser VCL and ALH, and greater LIN were considered to be non-hyper-activated (H-). Batches with at least one characteristic that did not fulfill the requirements were considered to be transitional (TR; Supplementary data file).

Of the 20 bulls evaluated in the present study, only samples from two bulls were selected for the fertility study (Appendix 1). Bulls B and K were chosen because there were semen collections from these bulls that contained hyper-activated (H+) or non-hyper-activated (H-) sperm. Two batches of semen with different hyper-activation characteristics were used in this study: H+ (bull B/batch 2 and bull K/batch 2), with LIN < 53%, ALH > 7.17 μm, and VCL > 164.3 μm/s; and H- (bull B/batch 1 and bull K/batch 1), with LIN > 53%, ALH < 7.2 μm, and VCL < 164.3 μm/s. All of the batches that were used were considered adequate for commercialization and use in AI, and sperm of the batches had similar motilities.

2.2. Assessment of field fertility

Multiparous lactating Nelore cows (*Bos indicus*; $n = 367$) from two commercial beef farms in Rondônia, Brazil were used in the

present study. All cows grazed *Brachiaria brizantha* pasture, were supplemented with mineralized salt, and were allowed free access to water. All cows were treated using the TAI protocol beginning 30 to 40 days postpartum. The cows were administered with 2 mg of estradiol benzoate (Bioestrogen®, Biogénesis-Bagó, Curitiba, Brazil) by intramuscular injection (i.m.), and received an intravaginal progesterone-releasing device (1.9 g progesterone, CIDR®, Pfizer Animal Health, São Paulo, Brazil) to synchronize stage of follicular wave emergence on Day 0. The CIDR was removed and the cows were given 150 µg of D-cloprostenol i.m. (PGF2α-analogue; Croniben®, Biogénesis-Bagó, Curitiba, Brazil, 1 mg of estradiol cypionate (ECP) i.m. (E.C.P.®, Pfizer, Cravinhos, Brazil), and 300 IU of eCG i.m. (Novormon®, Syntex, Buenos Aires, Argentina) on Day 8. All cows were subjected to TAI 48 h after CIDR removal.

On the morning of Day 10 (07:00 am), the diameter of the POF in each cow was assessed using ultrasonography (SIUI CTS-900, linear probe with 5 MHZ, Guangdong, China). There were some cows ($n = 21$) for which was not a follicle that was considered to be of an ovulatory size (≥ 8 mm) at TAI, and these cows were excluded from the experiment. As in a previous study (Pfeifer et al., 2015), the cows were categorized into one of two groups based on the diameter of the POF: an Early Ovulation (EO, $n = 184$) group, in which the cows had a ≥ 13-mm POF; and a Late Ovulation (LO group, $n = 162$) group, in which the cows had a < 13-mm POF.

Hyper-activated (H+) and non-hyper-activated (H-) semen batches from each of the bulls (B and K) that were selected for use in the study were assigned to be used for insemination of cows in both of the ovulatory groups (EO and LO). Thus, after these assignments, the groups were: EOH+ ($n = 89$), EOH- ($n = 95$), LOH+ ($n = 96$), and LOH- ($n = 66$).

Further ultrasonic examinations were performed 30 days post-TAI to assess pregnancy status. Visualization of the embryonic vesicle and detection of the embryo were the positive criteria for determining pregnancy.

2.3. Statistical analyses

All statistical analyses were performed using the SAS 9.0 software (SAS Institute Inc., Cary, NC, USA). The effects of ovulatory status (Early compared with Late), sperm hyper-activation (H+ compared with H-), and bull effect (Bull B compared with Bull K) on P/AI were analyzed using the Chi-square tests. The values for all variables from the laboratory analyses of semen were analyzed using the general linear models (GLM) procedure and Tukey's test was used to determine differences among the sperm kinematics groups (H-, TR, and H+). Similarly, the effect of diameter of the POF was evaluated using the GLM procedure, and the means were compared among the experimental groups using the Tukey's test. The BCS, lactation number, and days postpartum did not have a significant effect when included in the model and, therefore, were excluded from the final statistical model. Size of the ovulatory follicle was included in the final model for this analysis. Farm was included as a random effect in all analyses.

The relationship between the diameter of the POF at TAI on the probability of pregnancy was determined for each sperm hyper-activation classification group. A logistic regression curve was generated using the coefficients calculated from the interactive data analyses of SAS. The *P*-values that were less than 0.05 were considered to indicate significance, and *P*-values of less than 0.1 and greater than 0.05 were considered to indicate a tendency for differences.

3. Results

Of the 47 batches of semen evaluated, 21 were considered as H-, 14 as H+, and 12 as TR (Supplementary data file). Of the 20 bulls, four had exclusively H+ semen, six had exclusively H- semen, and only one bull had exclusively TR semen. Nine bulls had semen with more than one kinematics characteristic.

The results of the sperm kinematic assessments indicated that all the variables evaluated using CASA had an effect that was affected by extent of hyper-activation (Table 1). The sperm considered to be H+ had greater total motility, progressive motility, VAP, VSL, ALH, and VCL. In contrast, the H+ sperm had less LIN and STR than the H- sperm (Table 1).

The data for P/AI based on follicular ovulatory classification (Early or Late) at TAI, and sperm hyper-activation (H+ or H-) are included in Fig. 1. The ovulatory classification (EO and LO) and bull effect (Bull B and Bull K) had no effect ($P > 0.05$) on the P/AI (Table 1). Cows inseminated using semen containing H- sperm tended ($P = 0.06$) to have a greater P/AI than cows inseminated with semen containing H+ sperm (Table 2). The data for relationship between semen kinematics characteristics and the probability of

Table 1
CASA analyses of sperm with different kinematic characteristics.

	Motility pattern			
	Non-hyper-activated (H-)	Transitional Phase (TR)	Hyper-activated (H+)	*P*-Value
Total motility (%)	38.09 ± 1.84[A]	44.45 ± 2.04[AB]	48.47 ± 2.23[B]	0.001
Progressive motility (%)	29.11 ± 1.65[A]	34.28 ± 2.08[AB]	36.63 ± 1.7[B]	0.01
VAP (µm/s)	90.26 ± 2.08[A]	102.05 ± 3.57[B]	112.60 ± 2.45[C]	< 0.001
VSL (µm/s)	78.72 ± 1.97[A]	88.46 ± 3.80[A]	93.89 ± 2.50[B]	0.001
LIN (%)	55.47 ± 0.39[A]	53.42 ± 0.84[B]	48.84 ± 0.90[B]	< 0.001
STR (%)	85.14 ± 0.45[A]	84.55 ± 0.79[B]	81.16 ± 0.96[B]	< 0.001
ALH (µm)	6.10 ± 0.11[A]	7.16 ± 0.11[B]	8.78 ± 0.23[C]	< 0.001
VCL (µm/s)	143.73 ± 3.11[A]	164.73 ± 4.43[B]	194.40 ± 4.95[C]	< 0.001

VAP: Average Path Velocity; VSL: Straight-Line Velocity; LIN: Linearity; STR: Straightness; ALH: Amplitude of Lateral Head Displacement; VCL: Curvilinear Velocity; [AB]Within a row, means without a common superscript differed ($P < 0.05$).
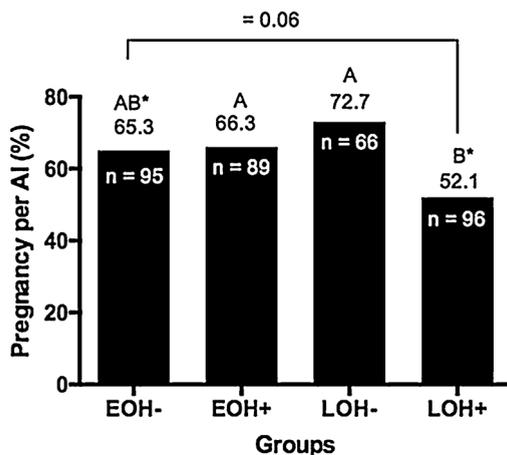
Fig. 1. Pregnancy per AI in suckled beef cows having relatively earlier or later ovulations relative to TAI when there was insemination with hyper-activated (H + or H-) sperm; Different letters indicate effect between groups ($P < 0.05$); EOH- and LOH + groups tended to differ ($P = 0.06$).

Table 2
Pregnancy per AI of cows with ovulations from different sized follicles (EO compared with LO) when inseminated with hyper-activated sperm (H + compared with H-), and sperm from different bulls (Bull B compared with Bull K).

	Pregnancy per AI, % (n/n)	P-value
Ovulatory Status		
Early	65.8 (121/184)	
Late	60.5 (98/162)	0.3
Sperm Hyper-activation		
H +	58.9 (109/185)	
H-	68.3 (110/161)	0.06
Bull effect		
B	66.2 (106/160)	
K	60.7 (113/186)	0.17

pregnancy are depicted in Fig. 2.

The values for diameters of the POFs at TAI for each treatment group are depicted in Fig. 3. As expected, cows classified as EO had larger POFs (14.8 ± 0.1 mm) than cows classified as having LO (11.0 ± 0.1 mm; $P < 0.0001$).

4. Discussion

Biological factors involved in the regulatory processes of male and female gamete maturation can have a considerable effect on the efficiency outcomes when using the assisted reproductive technologies. In that regard, as observed in the present study, the diameter of the preovulatory follicle and the post-thaw sperm kinematics are potential factors that affect successful establishment of a
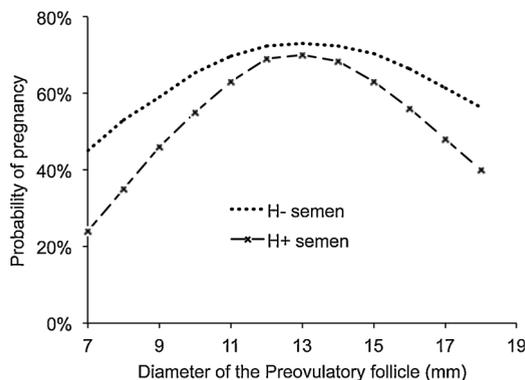

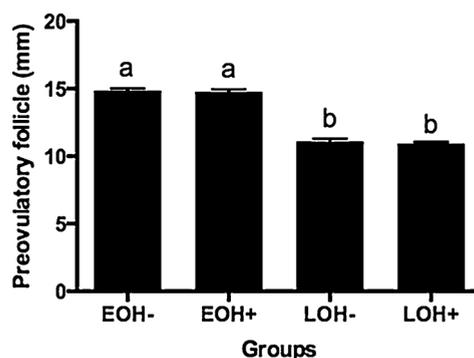**Fig. 2.** Probability of pregnancy on day 30 after fixed-time artificial insemination (FTAI) in beef cows inseminated using H + (••••••) or H- (–x–) sperm in cows with differing diameters of the pre-ovulatory follicle (POF) at TAI ($P = 0.001$).

Fig. 3. Diameter of preovulatory follicle at TAI in suckled beef cows having relatively earlier or later ovulations when cows were inseminated with hyper-activated (H + or H-) sperm; Different letters indicate differences among groups ($P < 0.05$).

pregnancy when there is use of TAI programs. Bulls undergoing semen collection procedures in commercial AI centers differ in fertility, even though there are minimum standards for semen quality. In the present study, the data from the post-thaw sperm analyses indicated there were differences in sperm kinematics among bulls and among semen batches collected from a single bull. There, however, were also different ovarian responses in cows, even though cows were subjected to the same TAI protocol. To the best of our knowledge, there are no published reports or associations between post-thaw sperm kinematics, ovarian responses, and fertility when there was use of TAI programs. The results of the current study support the hypothesis that cows with smaller POFs inseminated with non-hyper-activated (H-) semen and cows with larger POFs inseminated with hyper-activated (H+) semen have acceptable P/AI (> 65%). Surprisingly, the cows with larger POFs also had acceptable P/AI when cows were inseminated with non-hyper-activated semen.

The findings in the current study clearly indicate that the three classes of sperm motility - non-hyper-activated, transitional phase, and hyper-activated - represent differences in sperm kinematics among semen with the different classifications. The categorization of mammal spermatozoa into three different classes of motility has also previously occurred (Burkman, 1984). According to Mortimer and Mortimer (1990), hyper-activated spermatozoa can be defined as those that have a relatively lesser LIN, and greater ALH and VCL values. In the present study, as a variable to determine sperm hyper-activation in a single straw, the average values of VCL, LIN, and ALH were used in relation to the averages for all samples evaluated. Thus, semen was only considered to be hyper-activated if the VCL and ALH values were greater than the average values, and the LIN value was less than the average. In contrast, semen was only considered to be non-hyper-activated if the VCL and ALH values were less than average, and LIN was greater than the average. The batches for which there were Boolean data were considered to be in the transition phase. In the present study, when there was use of the criteria to classify sperm movement there were distinct classes of motility. From a statistical perspective, values for all the sperm kinematic variables studied (VAP, VSL, LIN, STR, ALH, and VCL) indicated there were differences among semen batches with different motion classifications. The variables evaluated in the present study to characterize sperm kinematics, therefore, can be considered reliable for the establishment of sperm hyper-activation in bull semen. Progressive non-hyper-activated sperm movement trajectories are generally very straight, with cells having markedly lesser ALH values and relatively lesser VCL values as compared with hyper-activated sperm. In contrast, sperm cells with progressively hyper-activated motility tracks have relatively greater VCL and ALH values, and lesser LIN values than those for non-hyper-activated sperm, indicating some net gain in distance of the motility track (Mortimer, 1997). Thus, the reference values for LIN, ALH, and VCL used in the present study to determine post-thaw hyper-activated batches in bull semen were very similar to those used by Mortimer (1998) to determine hyper-activated motility in human semen (VCL 159 μm/s, LIN 50%, and ALH 7 μm). In several studies, however, there has been use of different minimal cut-off values for VCL, LIN, and ALH variables that were considered to define sperm hyper-activation (Robertson et al., 1988; Griveau and Le Lannou, 1994; Green et al., 1995; Mortimer and Swan, 1995). The results probably depend on the calibration of the CASA equipment for conducting sperm motility assessments (e.g., frame rate used to characterize spermatozoa movement). Although different calibrations can be considered to determine bull sperm hyper-activation, there appears to be a consensus for the use VCL, LIN, and ALH variables from CASA analyses (Burkman, 1984; Mortimer, 1997; Mortimer and Mortimer, 1990; Marquez and Suarez, 2007). Although in some studies there has been use of sperm kinematics to determine hyper-activation, to the best of our knowledge there has been none of the studies where this information has been used to assess P/AI in cows when there was use of TAI programs.

Although there is a well-defined relationship between sperm kinematics of semen used for AI and size of ovulatory follicle on beef cow fertility, it is important to understand that the values for LIN, ALH, and VCL used in the present study should not be considered optimal for determining hyper-activation in post-thawed bull semen. Rather, these principles could be used to develop criteria to classify semen batches according to the kinematic characteristics, and these data may be used to customize AI procedures in timed AI beef cows.

The data from the present study clearly indicate the difference in the POF diameter between cows that have ovulations relatively earlier or later when there is use of TAI. Although the protocol for synchronization of time of estrus among cows that was used in the present study is a very effective and well-established method for inducing synchronized follicular wave emergence among cows, there is great variability in follicle size at the end of protocol application (Bo et al., 2003). Consequently, there is also great variability in

the fertility when a TAI protocol is used. This occurs because of the time range (3–6 days) during which the new wave of follicular development occurs (Bo et al., 2003; Kim et al., 2005; Martinez et al., 2005). At the time of TAI, the lifespan of the POF is 4 to 7 days (from wave emergence to TAI), which explains the range of follicle sizes at TAI. Ovulation from larger dominant follicles is accompanied with the development of a larger corpus luteum (CL), and consequently greater concentrations of progesterone are produced (Pfeifer et al., 2009; Sa Filho et al., 2010; Pfeifer 2012). Together, these factors have a positive effect on P/AI (Santos et al., 2001; Cutaia et al., 2003; Bo et al., 2004). In contrast, smaller follicles are associated with lesser ovulation rates (Vasconcelos et al., 2001; Meneghetti et al., 2009), smaller CL, and lesser progesterone production (Vasconcelos et al., 2001), and, consequently, an increased likelihood of pregnancy losses (Perry et al., 2007). Considering these negative effects of smaller follicles, it would be expected that cows with smaller POFs would have lesser P/AI. When the non-hyper-activated semen straws were used, however, ovulation from smaller follicles did not have a detrimental effect on P/AI, with rates similar to those for cows having ovulations from larger POFs. It is presumed that this was because cows with small follicles have later ovulation times and are more likely to become pregnant when there is insemination with non-hyper-activated sperm.

In other studies, the fertility when there is use of cryopreserved spermatozoa is also improved when the time from insemination to ovulation is reduced (Parrish and Foote, 1986; Chen et al., 1989; Maxwell et al., 1993). Unfortunately, uterine placement coinciding with ovulation is not yet a routine practice with AI (Thundathil et al., 1999). For that reason, the approach proposed with consideration of results from the present study - of conducting the AI procedure according to the ovarian response and sperm kinematics - may represent a practical alternative for improving fertility in timed AI of beef cows.

Cows with relatively earlier and later ovulations had similar fertility when semen with non-hyper-activated sperm was used. One possible reason why the use of such semen resulted in adequate fertility independent of the ovarian response is that non-hyper-activated sperm cells may hyper-activate rapidly when encountering an optimal hormonal environment in the oviduct when time of ovulation is approaching. Hamster spermatozoa observed in the ampullae of excised oviducts had a characteristic "whiplash" movement of hyper-activation (Yanagimachi, 1970; Katz and Yanagimachi, 1981). Furthermore, hyper-activation may be required for detachment of sperm from the oviduct epithelium in animals (Demott and Suarez, 1992; Gwathmey et al., 2003). Presumably, as a result of chemical factors in the oviduct there is signaling in the oviduct that initiates hyper-activation at the optimal time for fertilization to occur. Although hyper-activation often occurs during sperm capacitation, the two events are regulated by different pathways (Suarez and Ho, 2003). The presence of un-capacitated spermatozoa is positively correlated with fertility (Thundathil et al., 1999). Capacitated spermatozoa are less likely to survive until the transit to the site of fertilization is complete in the oviduct as compared with un-capacitated spermatozoa. Once capacitated, spermatozoa undergo an increase in metabolic rates, and increased membrane fluidity and permeability; if there is not transit to the oocyte, these cells undergo spontaneous acrosome exocytosis due to an uncontrolled influx of Ca^{2+} (Cormier et al., 1997). Hence, the fertilizing lifespan of capacitated spermatozoa is limited. Non-hyper-activated sperm may survive for a long time in the oviduct environment. Further studies, however, are required to address this question.

In contrast to the observations in other studies (Perry et al., 2005, 2007; Sa Filho et al., 2010), the cows with larger POFs at TAI in the present study did not have greater P/AI rates than the cows with smaller POFs. These results indicate that the later time of ovulation in the cows with smaller POFs was compensated for by the use of non-hyper-activated semen for AI of these cows. In contrast with what was observed using non-hyper-activated semen, hyper-activated semen only resulted in the relatively greater P/AI rates when it was used in cows that had relatively earlier ovulations in relation to time of AI. These data apparently differed from the results observed when semen from relatively greater and lesser fertility bulls were compared. The sperm motion characteristics of bulls with greater fertility are similar to those of hyper-activated sperm (i.e., greater ALH and VCL, and lesser LIN (Shojaei et al., 2012). In these studies, there was use of fertility records based on the percentage of cows having not returned to estrus within 56 days of conventional AI (56-day non-return rate; NRR) to characterize bull fertility. Thus, fertility of those bulls was probably not evaluated in TAI programs. This may explain why the relatively greater-fertility bulls were those that had hyper-activated semen characteristics. When a cow in estrus is inseminated, the interval between insemination and ovulation is more synchronous than that observed in TAI.

The P/AI results in the present study were considered to be very good. In the field, however, the ultrasonic examination of every single hormonally treated cow may be a barrier to uptake of use of this technique in beef production systems. In future studies, there should be use of other criteria to phenotypically estimate the ovulatory response of cows. The occurrence of estrus when using different P4 sources upon device removal and TAI has resulted in a greater P/AI. Cows in estrus have larger POFs and are more likely to become pregnant than those not in estrus (Sa Filho et al., 2010). Thus, as an alternative to ultrasonography, the identification of estrus after device removal could be useful for assessing the ovarian response of a cow at TAI. There is currently research being conducted to overcome these challenges, and new hypotheses are being tested in an attempt to answer these previously described research questions. Furthermore, an alternative that may be considered is the use of GnRH at TAI to improve synchronization of the time of ovulation. Injection of GnRH at TAI results in a greater synchrony in time when ovulations occur and subsequently an increase P/AI (Sa Filho et al., 2010). Thus, the use of GnRH could improve pregnancy, especially in cows inseminated with H + semen. The use of this alternative approach may lead to having a greater synchrony for having optimal sperm kinematic in the oviduct for fertilization at the time of ovulation.

The evaluation of the ovarian response of cows subjected to TAI by associating these observations with sperm movement characteristics represents a very important step towards understanding gamete maturation and interactions at the time of ovulation/conception. The insights obtained from the present study could lead to new approaches to AI, and the development of new protocols to improve fertility. Based on these findings, it should be possible to enhance fertility by considering the motility of sperm used for insemination to compensate for the loss in fertility that results for delays in ovulation.

The interesting results that we observed in the current study may encourage the semen industry to process and commercialize semen that is labeled for kinematic motion or to adopt techniques that allow for maintenance of sperm viability through a semen controlled release approach such as microencapsulation (Nebel et al., 1985; Vishwanath et al., 1997). Thus, the development of new technologies that prolong the viability of spermatozoa *in vivo* after AI will be beneficial to the industry because timing of AI relative to ovulation will be less important (Standerholen et al., 2015).

In summary, the results of this study indicate that VCL, ALH, and LIN are useful variables for the characterization of sperm hyper-activation in bulls used in TAI programs. Furthermore, the results of this study indicate that cows with smaller POFs are less likely to become pregnant when insemination occurs with hyper-activated sperm. In contrast, when there is insemination with non-hyper-activated sperm, cows with later and earlier times of ovulation can have similar and acceptable P/AI.

Acknowledgments

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.anireprosci.2018.12.009.

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