Chapter 1

The Importance of Semen Quality in AI Programs and Advances in Laboratory Analyses for Semen Characteristics Assessment

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1. Introduction

In the last decades, livestock sector has undergone a process of biotechnology incorporation with the main goal of enhancing productivity and improving the genetic makeup. In this sense, artificial insemination (AI) is considered as the most important biotechnology incorporated into livestock production systems because it implies the use and/or globalization of proven bulls, which represent a key tool in obtaining animals with higher genetic merit [1].

The wide use of bovine AI was mainly attributed to the development of methods that ensured cell viability after storage for long periods by reducing sperm metabolism, due to important progresses in studies involving cryoprotectants [2].

Nowadays, AI is considered as the most worldwide used reproductive biotechnology [3] with an extremely interesting benefit-cost relationship. Despite the unquestionable role of this biotechnology in improving productivity, many causes have accounted for the range in results and/or some unsatisfactory indices of bovine AI programs, highlighting several factors inherent to female physiology and/or farm management [4-9]. Nevertheless, another factor positively correlated with the AI outcomes that require appropriate attention, correspond to quality of semen samples used in the programs [10]. Therefore, the aim of this chapter is to review the importance of the quality of semen used in reproductive programs as well as the use of laboratory tests for predicting bull fertility.

2. The importance of semen quality for AI programs

Regarding the quality of semen used in AI programs, it has been reported that differences in fertility level could be attributed to variations in sperm qualitative characteristics [11].The success of bovine AI programs largely depends on the use of good quality semen. When only high fertility bulls are used, better conception rates are achieved, which reduces costs of reproductive programs [12].

Individual bulls may differ in their ability to fertilize oocytes and/or to develop to blastocyst stages after *in vitro* fertilization (IVF) procedures [12-18]. In addition, different sires and/or batches may differ in the individual response to induction of *in vitro* sperm capacitation methods, [14] and in the response to acrosomal mantainence after *in vitro* incubation [19].

Moreover, the bull influence is an important factor affecting *in vivo* reproductive outcomes [8,11, 20,21]. Ward et al. [20] demonstrated that kinetics of embryo development post insemination may vary between bulls. Andersson et al. [21] observed a high variability in fertility among bulls using different sperm concentrations per dose at AI. Sá Filho et al. [8] reported a high variation in conception rates depending on the bull utilized in a Timed-AI program. Moreover, Oliveira et al. [10] observed that the sire with numerically lower field fertility also presented inferior semen quality based on the several *in vitro* sperm characteristics assessed.

Furthermore, semen handling (and/or semen thawing protocol) might also be an important factor influencing in semen quality and, therefore, in AI results. Hence, it is deemed necessary to alert to the practice of simultaneous thawing of multiple semen straws at the moment of AI.

For instance, the Brazilian Association of Artificial Insemination recommends, for bovine AI, the thawing procedure of a single frozen semen straw (0.5 mL) in water bath unit at a temperature of 35 to 37°C for 30 seconds [22]. However, the large size of breeding herds using Timed-AI protocols in Brazil have resulted in the routine practice of thawing multiple straws simultaneously in the same water-bath unit to increase the convenience of semen handling and the number of inseminations in a short period.

Because the size of breeding herds continues to increase and the use of estrus synchronization (as well as the fixed-time artificial insemination protocols) becomes more frequent worldwide, there are increasing probabilities that several cows will be inseminated on the same day. Hence, several inseminators have used the practice of thawing, simultaneously, more than one straw of semen in the same thawing-bath unit to increase the convenience of semen handling. However, under these conditions, some straws remain in the thawing bath while insemination occurs. Consequently, the thermal environment of the water bath could have some influence in sperm viability and fertility.

With this concern Brown et al. [19] demonstrated, in a laboratory study, that semen straws must be agitated immediately after plunging to prevent direct contact among semen doses and refreezing during the thaw process. In this case, the simultaneous thawing of multiple straws had no effect on percentage of motile spermatozoa and acrosomal integrity when up to ten 0.5-mL semen straws were simultaneously thawed in a thermostatically controlled thawing

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bath of 36°C [19]. Later, several other studies were performed regarding this thawing practice, in order to evaluate the effect of simultaneous thawing of semen straws on *in vivo* fertility following AI [23-28].

Goodell [24], in a study with only 180 reproductive outcomes, reported a decrease in conception rates of the third and fourth insemination in the sequence, when more than two straws were thawed at once. However, Kaproth et al. [26] and Dalton et al. [27] demonstrated that experienced AI technicians can simultaneously thaw multiple semen straws and inseminate up to four cows within a 20 min interval, without adverse effects on field fertility. Sprenger et al. [25] observed that an interaction of herd by sequential insemination tended to influence field fertility outcomes. In one herd, conception rates of straws number 6 and ≥ 7 were lower than conception rates of straws 1 to 5. However, in the further eleven herds evaluated, sequential insemination had no effect on conception rate. The authors concluded that, given that recommended semen handling procedures are followed, more than two straws can be thawed at once without compromising semen fertility.

DeJarnette et al. [29,30] reviewed several studies regarding the effects of sequence of insemination after simultaneous thawing on conception rates with data collected from about 19,000 inseminations. The combined data from several studies suggested that several straws can be thawed at once with no significant fertility concern, provided that inseminators strictly adhere to recommended semen handling procedures. The authors recommended thawing (at 35°C for a minimum of 45 seconds) only the number of straws that can be deposited within 10 to 15 minutes in the female reproductive tract (always maintaining the thermal homeostasis during this interval) to avoid semen fertility impairment. In addition, it was stated that the more important issues regarding semen handling is time, temperature, hygiene and inseminator proficiency. Technicians that fail to abide by the standard recommendations will likely realize less than optimal conception rates irrespective of the number of straws thawed [29].

Hence, in general, the standard recommendations for cryopreserved bovine semen are (unless otherwise specified by the manufacturer): 1) to thaw no more straws than can be deposited in the female within 15 minutes between thawing and insemination, in a water-bath at 35°C for a minimum of 45 seconds, always maintaining thermal homeostasis during this interval; 2) Prevent direct straw to straw contact during the thaw process; 3) Implement appropriate thermal and hygienic protection procedures to maintain thermal homeostasis and cleanliness during gun assembly and transport to the cow [29].

Still, in a recent study, Oliveira et al. [28] observed that pregnancy rate was affected by sequence of insemination, depending on which bull was utilized in a timed AI program. In this experiment, groups of ten semen straws (0.5 mL) were simultaneously thawed at 36°C. After 30 seconds, semen straws were removed (one straw at a time) from water-bath and subsequently deposited in the cows for AI. One semen straw was used for each cow, in the same sequence that they were removed from water-bath. All animals utilized in the study were Nelore cows (n = 944). The inseminations were performed with semen from three Angus bulls, during Brazilian summer season (breeding season for beef cattle). Timed AI procedures were performed in a covered and protected area. The results demonstrated that one of the three sires had reduced fertility for inseminations performed with the group of straws associated with

the longest interval from thawing to AI. However, semen from the other two bulls was not significantly different with respect to field fertility for any straw group (Straw Group 1: inseminations with 1st, 2nd and 3rd straws of the sequence; Straw Group 2: inseminations with 4th, 5th and 6th straws of the sequence; Straw Group 3: inseminations with 7th, 8th, 9th and 10th straws of the sequence). The mean time (±SD) of straws remaining in the thawing bath were 01:30 ± 00:51 for Straw Group 1, 03:36 ± 01:10 for Straw Group 2 and 06:13 ± 01:44 min for Straw Group 3. There was an interaction between sire and Straw Group (Conception rate of Sire 1: Straw Group 1 = 58.1%, Straw Group 2 = 60.2% and Straw Group 3 = 35.3%, $P < 0.05$; Conception rate of Sire 2: Straw Group 1 = 40.2%, Straw Group 2 = 50.5% and Straw Group 3 = 51.7%, $P > 0.05$; Conception rate of Sire 3: Straw Group 1 = 59.8%, Straw Group 2 = 51.0% and Straw Group 3 = 48.6%, $P > 0.05$). Overall conception rate of cows inseminated with first straw in the sequence (Straw 1) was 58% and of cows inseminated with tenth straw in the sequence (Straw 10) was 44% ($P > 0.05$). According to the results, semen fertility of some sires appeared to be more negatively affected by sequence of insemination than others. However, because the high environmental temperature during the field experiment may have potentiated the effects of incubation time on semen quality, the possibility that the thermal environment of thawing bath could have interfered on sperm fertility (mainly of bull that presented reduced conception rate associated to sequence of insemination), was considered. In summary, it was stated that the number of straws that can be simultaneously thawed without compromising semen fertility seems to vary for each bull. Unfortunately, the laboratory analyses did not clarified the effect of interaction between sire and straw group observed in field experiment of this respective study [28]. Thus, the reason why semen from some bulls seems to be more susceptible to specific thawing environments and/or procedures remained to be elucidated. The authors concluded that sequence of insemination after simultaneous thawing of multiple semen straws might affect fertility outcomes, depending on the sire utilized in the reproductive program. Hence, under similar environmental conditions, 10 semen straws should not be simultaneously thawed, because it could affect conception rates, according to the semen that is being used. Therefore, in similar routine procedures of timed AI programs consisting of large herds, it seems more cautious to not exceed the number of six semen straws for simultaneous thawing [28].

Similarly, Lee et al. [23] had previously reported that sequence of insemination may influence conception rates when up to four straws were thawed at once. Although all inseminations (n = 89) occurred within recommended time constraints (i.e., within the limit of 15 minutes between thawing to AI), the loaded AI guns were exposed to direct solar radiation (in a tropical environment; Hawaii) during transport from thawing-bath to cow. The data suggested that the thermal insult might had reflected in a linear reduction in conception rates from the first (48%) through the forth (25%) gun used in sequence [23].

Thus, an important consideration to be made is the possibility of a significant interaction between ambient temperature and interval to semen deposition. According to Shepard (unpublished; cited by [29]), an interaction of ambient temperature and interval to semen deposition might occur due to extended thaw duration (>10 min) when ambient temperatures are above 17°C, suggesting that higher environmental temperatures may be problematic to

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post-thaw fertility maintenance. In view of the fact that the studies of Lee et al. [23] and Oliveira et al. [28] were performed during warm seasons of tropical (or subtropical) environments, it can be suggested that greater sequences of insemination might compromise conception rates when associated with the effects of higher ambient temperatures and/or solar exposure.

Given the above observations, even though that many factors related to semen quality might influence AI outcomes, it is noteworthy that the use of high fertility bulls reduces the chances of field fertility impairment. Hence, an adequate evaluation of semen quality may reduce the effect of sire on reproductive outcomes, which is commonly observed in field trials. Thus, since a proper prediction of bull fertility is increasingly required, we consider appropriate to review the correlations between field fertility and *in vitro* sperm characteristics assessed by classical and modern semen analyses.

3. Correlation between *in vitro* sperm characteristics and *in vivo* bull fertility

Nowadays, many classical and modern methods have been used for laboratory assessment of *in vitro* semen characteristics following cryopreservation with the main purpose of predicting the fertility potential of a semen sample [11, 31-39].

Among the several sperm characteristics evaluated by laboratory techniques, sperm motility [33,40,41], morphology [42,43] and plasma membrane integrity [11,35,36,38] are the most used laboratory tests for assessing *in vitro* semen quality. However, the results of such assays do not always correlate with the real fertility of a semen sample [12,44].

In this sense, the relationship of *in vitro* semen characteristics and *in vivo* sire fertility has been the subject of much study [12,41,44,45-47]. Nevertheless, substantial variations are commonly observed in different experiments and low correlations are usually detected when single *in vitro* sperm characteristics are isolated compared to the field fertility [12,44]. Until now, the most efficient and accurate method to estimate the fertility of a particular bull is to accomplish the field fertility tests [44], which is very laborious, expensive and time consuming [46].

Alternatively, embryo culture techniques allow exploring *in vitro* bull fertility. The employment of such techniques has provided interesting but contradictory results regarding correlations between embryo *in vitro* embryo production (IVP) and *in vivo* bull fertility. Although positive correlations between IVP results and field fertility has been reported for some authors [12,14,16, 17, 20,46,48], other studies did not confirm the positive high correlations between *in vitro* fertilization (IVF) outcomes and *in vivo* fertility of evaluated sires [49,50,51]. However, Sudano et al. [12] recently demonstrated that it is possible to estimate bull fertility based on IVF outcomes, using a Bayesian statistical inference model.

Although interesting, it is still precipitated to ensure that the individual ability of fertilizing oocytes *in vitro* is a useful parameter for predicting *in vivo* bull fertility following AI. Hence, according to Ward et al. [20], a range of protocol variations among different IVP laboratories, the low repeatability in the results, as well as the various factors that may affect IVP outcomes,

adds even more uncertainty if the *in vitro* ability for oocytes fertilization of a semen sample is sufficient accurate for predicting the sire field fertility. Additionally, it is noteworthy that more practical and/or simple laboratory techniques for assessing semen quality would be more advantageous for AI industry than the employment of IVP procedures.

Correa et al.[11] observed that the total number of motile spermatozoa tended to be higher in high fertility bulls. Farrell et al. [41] demonstrated that multiple combinations of motility sperm variables obtained by Computer Assisted Semen Analysis (CASA) had higher correlations with bull field fertility than single parameters evaluated separately. The authors observed that the combination of Progressive Motility, ALH (amplitude of lateral sperm head displacement), BCF (sperm beat cross frequency), and VAP (Average Path Velocity) presented high correlation value ($r^2 = 0.87$) and that the combination of ALH, BCF, linearity, VAP and VSL (Straight-Line Velocity) presented even higher correlation value ($r^2 = 0.98$). Hence, it has been demonstrated that sperm motility evaluations are important for the assessment of semen quality, mainly when CASA is used for assessing semen motility patterns. This non-subjective sperm analysis provides an opportunity to assess multiple characteristics on a large sample of spermatozoa, which allows assessing several sperm motility parameters with high repeatability [33,41].

Even though that computer-based analysis provides high accuracy of *in vitro* motility evaluation [33,41], the assessment of different aspects related to sperm physiology may guarantee better investigation of semen quality [38,52]. Changes in membrane architecture and sperm compartment functionality may interfere with cellular competence and with the process of fertilization. These changes can be monitored using fluorescent probes that are able to bind and stain specific structures of the cell permitting a direct diagnosis [38]. Celeghini et al. [38,53] reported an efficient and high-repeatability technique for simultaneous evaluation of the integrity of plasma and acrosomal membranes, as well as mitochondrial function, using a combination of the following probes: propidium iodide (PI), fluorescein isothiocyanate–Pisum sativum agglutinin (FITC-PSA) and tetrachloro-tetraethylbenzimidazolcarbocyanine iodide (JC-1) respectively.

Januskauskas et al. [35] found significant correlations between field fertility and plasma membrane integrity assessed by PI. Conversely, Brito et al. [54] reported no significant correlation between bovine *in vitro* fertilization (IVF) and plasma membrane integrity, measured by Eosin/Negrosin staining, CFDA/PI, SYBR-14/PI and HOST (hypo-osmotic swelling test). Nevertheless, Tartaglione and Ritta [36] demonstrated that the combination of plasma membrane integrity and functional laboratory tests presented high correlation coefficient with *in vitro* bull fertility. The authors demonstrated that combination of Eosin/Negrosin staining test with HOST presented high correlation coefficient with *in vitro* fertility outcomes. When sperm plasma and acrosomal membrane integrity results (assessed by Trypam/Blue Giemsa staining) were included in the regression model, a higher correlation coefficient was obtained. The authors emphasized that higher is the capacity for predicting semen fertility when higher number of sperm evaluations is performed [36].

Another concern of semen fertility studies is the occurrence of sperm oxidative stress. Spermatozoa are susceptible to oxidation of their plasma membranes due to the presence of

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polyunsaturated fatty acids [37]. Reactive oxygen species (ROS) may become cytotoxic through damage to proteins, nucleic acids and membrane lipids, if ROS concentrations overcome the natural defense mechanisms of the cell and extending medium [55]. Hence, since the high production of ROS might cause damages to plasma membrane structure, it can impair sperm function and motility [34,37]. A high degree of membrane lipid destabilization may lead to functional capacitation, reducing the sperm lifespan and fertilizing capacity [56]. In this sense, Hallap et al. [57] demonstrated that the amount of uncapacitated spermatozoa may provide valuable information about frozen–thawed semen quality.

Although the molecular basis involving the whole process of sperm capacitation has not yet been fully elucidated, it is recognized that sperm capacitation is a sequential event of biochemical alterations that involve numerous physiological changes. Some events related to the beginning of capacitation process include the removal of peripheral membrane factors, changes in membrane fluidity and in lipid composition [58,59]. Thus, the mammalian sperm capacitation is associated with reorganization of plasma membrane due to phospholipids redistribution of cholesterol removal [57]. Hence, the lipophilic probe Merocianina 540 may be used to monitor the level of phospholipid bilayer disorder of plasma membrane. Using this probe, the fluorescence intensity is increased with increasing membrane bilayer disorder, which can be an indicative of initial sperm capacitation process. In laboratory studies, this probe is commonly associated with the use of the probe Yo-Pro-1, which allows the simultaneous analysis of plasma membrane integrity. This is due to the fact that Yo-Pro-1 is a specific DNA probe with excitation and emission of fluorescence similar to the Merocianina 540 (around 540 nm) [57,58].

As stated above, oxidative stress is a recognized contributor to defective sperm function [34,37,39,60]. Spermatozoa is very susceptible to peroxidative damage because of their high cellular content of polyunsaturated fatty acids that are particularly vulnerable to this form of stress [37]. Recently, a fluorescence assay using the fluorophore 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid (C11-BODIPY$^{581/591}$) has been successfully applied for detecting lipid peroxide formation in living bovine sperm cells [34]. This assay relies on the sensitivity of C11-BODIPY$^{581/591}$, a fluorescent fatty acid conjugate, which readily incorporates into biological membranes [60]. Upon exposure to ROS, the C11-BODIPY$^{581/591}$ responds to free radical attack with an irreversible shift in spectral emission from red to green that can be quantified by flow cytometry [37,60]. Still, it is noteworthy that the negative effect of some ROS-generating systems does not require lipid peroxidation to induce cytotoxic changes in spermatozoa. In this sense, Guthrie and Welch [61] observed that Menadione and H_2O_2 decreased the percentage of motile sperm but had no effect on BODIPY oxidation.

In an interesting study, Kasimanickam et al. [39] reported that bull fertility was positively correlated to plasma membrane integrity and progressive motility. According to the authors, plasma membrane integrity significantly influenced the fertilizing capacity of a sire. Moreover, the authors demonstrated that plasma membrane integrity and progressive motility were negatively correlated to sperm lipid peroxidation and that lipid peroxidation and bull fertility was also high negatively correlated. Bulls with higher sperm lipid peroxidation were more

likely to have a high DNA fragmentation and low plasma membrane integrity. Also, these bulls presented lower chances of siring calves [39]. These results are in accordance with Zabludovsky et al. [62] which also had demonstrated negative correlations between lipid peroxidation and IVF fertilization outcomes in humans.

It has frequently been reported that low-fertility bulls generally had high seminal content of morphologically abnormal cells [63]. Sperm with classically misshapen heads did not access the egg following AI since they do not traverse the female reproductive tract and/or participate in fertilization [43]. Some geometrical alterations of head morphology can cause differences in sperm hydrodynamics. According to [63], abnormal-shaped heads should be of primary concern regarding male fertility. The recognition of uncompensable cells in the ejaculate is currently best based on abnormal levels of sperm with misshapen heads [63].

Ostermeier et al. [32,64] also observed that some sperm morphometric variables were able to detect small differences in sperm nuclear shape which seems to be related to sire fertility. According to Beletti et al. [65], the application of computational image analysis for morphological characterization allows the identification of minor morphometric alterations of sperm head. However, little is known about the influence of such abnormalities on bull fertility. Because mammalian sperm heads consist almost entirely of chromatin, even minor changes in chromatin organization might affect sperm head shape. Nonetheless, morphological alterations in sperm head are not always caused by alterations in chromatin condensation. In the same way, chromatin abnormalities are not always followed by evident morphological irregularities [32,65,66].

A number of methods are available for identifying alterations in the stability of sperm chromatin. Sperm chromatin structure analysis (SCSA), currently the most used of these methods, is based on a flow cytometric evaluation of the fluorescence of spermatozoa stained with acridine orange [32,67]. Another method for chromatin evaluation uses a cationic dye, toluidine blue (at pH 4.0) that exhibits metachromasy. This dye binds to ionized phosphates in the DNA. In normal sperm chromatin, few dye molecules bind to DNA; this result in staining that varies from green to light blue. Spermatozoa with less compacted chromatin have more binding sites for the dye molecules, resulting in staining that varies from dark blue to magenta [65].

Whereas human-based methods for assessing sperm parameters involve a high degree of subjectivity in the visual analysis, computer-based methods for image processing and analysis are currently available. It can provide a more objective evaluation of cell motility and sperm morphological abnormalities, in addition to greater sensitivity, accuracy, speed and reproducibility. Computational morphometric analysis of spermatozoa usually considers basic measurements like the area, perimeter, length and width, as well as features derived from the measurements, such as the width:length ratio, shape factor and others [68]. An interesting approach is to use image analysis to characterize the sperm chromatin in smears stained with toluidine blue which also allows a morphometric analysis to be done concomitantly with the investigation of chromatin [65,69].

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An interesting study of [32] demonstrated that the average of sperm head shape identified to be from high fertility bulls was more tapered and elongated (more elliptical) than the average shape of sperm identified to be from low fertility bulls. In addition, the authors observed that quantifying changes in sperm shape can be detected by Fourier parameters, which characterize the curvilinear perimeter of sperm head using harmonic amplitudes to describe the sperm nuclear shape. The relationship between sire fertility and Fourier parameters of sperm morphometric analysis was investigated. It was observed that Fourier descriptors were able to detect small differences in sperm nuclear shape from bulls with different fertility [32;64]. According to [63], the most promising method of quantifying changes in sperm head shape is utilizing the Fourier harmonic amplitude analysis.

Acevedo et al. [70] reported that spermatogenic disturbance resulted in production of abnormal sperm and that sperm DNA vulnerability to acid denaturation was positively associated with sperm having misshapen heads. This provided more support for the assertion that occurrence of sperm with misshapen heads can signal chromatin abnormalities and potential incompetence for fertilization of a semen sample [63]. Kasimanickam et al. [39] reported that some deleterious effects of sperm lipid peroxidation are also related to impairment in sperm DNA, which may also reduce bull fertilizing potential. The sires with high sperm DNA fragmentation index presented lower sperm fertilization potential; whereas sires with lower DNA fragmentation index presented higher chance of siring calves [39].

Besides the intense efforts from worldwide researchers, until now, no single laboratory test has accurately predicted the real fertilizing capacity of a semen sample [52, 71]. Hence, in spite of some interesting results of *in vitro* sperm characteristics, a notable consideration is the importance of field trials when definitive conclusions are taken regarding semen fertility.

4. Conclusions and implications

Individual bulls may differ in their ability to fertilize oocytes and/or to develop to blastocyst stages after *in vitro* and *in vivo* fertilization procedures. Hence, the success of bovine reproductive programs largely depends on the use of good quality semen. When only high fertility bulls are used, better fertilization rates and reproductives outcomes are achieved, increasing the reproductive efficiency and thus, reducing the costs of the programs.

The sequence of insemination after simultaneous thawing of multiple semen straws may present different effect and/or relevance on fertility outcomes, depending on the sire that is being used in the reproductive program. However, the reason why semen from some bulls seems to be more susceptible and/or differently affected to specific procedures, semen handling protocols, and/or environments remains to be further investigated. It is noteworthy, though, that the use of different sires, semen extenders, thawing bath volumes, semen straw volumes, AI technicians, semen handling procedures, number of AI guns utilized, ambient conditions, farm management and cow categories, as well as the use of different laboratory analyses, might generally influence the results obtained.

Worth mentioning though, that when the correct semen handling recommendation is provided, as well as the adequate cautious and/or proficiency of AI technician is assured, the sequence of insemination is not likely to severely impact semen quality and reproductive performance in AI programs. Thus, it is deemed reasonable to attempt to the fact that the care and concern with semen storage and handling is essential to obtain satisfactory reproductive outcomes after AI. In addition, greater attention should be directed to the simultaneous thawing of multiple semen straws, especially when the thawing procedures do not include a thermostatically controlled water-bath unit.

Even though that an *in vitro* semen assay for determining bull fertility would be of great benefit to AI programs, it is unlikely that the evaluation of a single sperm characteristic may reflect the real sperm fertilization capacity of a semen sample, considering the complexity of the reproductive process.

In spite of the promising results reported above, until now, no single laboratory test was able to accurately predict, with the required repeatability, the real fertilizing capacity of a sire. Hence, potential bull fertility can be estimated from laboratory semen assessment with higher accuracy when a combination of several *in vitro* sperm analysis is performed.

Still, further studies contributing to the understanding of seminal differences among bulls that might be related to differences in fertility rates commonly observed in AI programs must be encouraged.

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