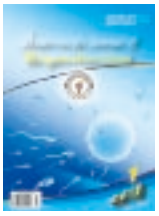

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Sperm dosage and site of insemination in relation to fertility in bovines

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ABSTRACT

Low sperm numbers in artificial insemination (AI)-doses are being used widely to make the best use of high genetic value bulls as well as sex-sorted semen. Sperm concentration needed for AI to obtain reasonable fertility, taking genetic value of bull and numerous others components into consideration is one of the essential constituents for successful AI breeding program. However, low sperm concentrations in AI-doses lead to reducing post-thaw viability. The reduction in viability of low sperm doses may be affected by fresh semen volume, sperm number and seminal plasma level at final dilution. Reduction in quality and fertility of low sperm doses is one of the limitations for their use in successful AI programme. Sperm number per AI required to achieve optimum fertility is one of the main crucial things to AI industry, and numerous efforts have been made in this regard. Due to great variability among bulls, sperm number per AI could be a limiting factor in achieving acceptable fertility values. Fertility of low sperm doses may vary among bulls, and non-return rates (NRRs) with low sperm doses may be determined by fertility level of bull. On the basis of individual bulls, sperm numbers in AI doses needed to be adjusted to reduce the variations in NRRs among bulls. Utilizing high fertile bulls for low sperm doses with acceptable non-return rates (NRRs) may be a way to cover a large number of bovines under AI in countries like India. Deposition site within the uterine horn may alter non return rates following inseminations with low sperm doses. Following deep-uterine inseminations, acceptable pregnancies may be achieved with low sperm doses and even if ovulation side is unknown.

1. Introduction

The most commonly used biotechnology used in livestock farms in various developed and developing countries is Artificial insemination (AI). The advantages of AI include the use of high genetic merit progeny tested sires that are having some desired traits to an ample number of females. Use of frozen semen technology has allowed worldwide dissemination of genetic progress[1]. In AI industry, the genetic impact of sires is limited due to the production efficiencies and decreased sperm functions during cryopreservation[2,3]. The packaging of less than 20-30 million sperm in French midi straws has nowadays been used as a standard for dairy industry[4–6]. Ten to fifteen million progressive motile sperm in French midi straws were essential for achieving desirable fertility level[6]. Due to increased

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demand of high fertile bull semen and need to implement packaging of less sperm in sex sorted semen, semen straws are produced with lesser number of sperm[7]. The lesser sperm numbers is essential due to loss of a huge time period in sperm sorting for sex[8]. Thus, the packaging of low sperm doses in the French mini or French midi straws will make more practical use of sex-sorted frozen sperm.

AI with low sperm numbers is increasing widely to enhance the benefit from elite bulls and to provide sufficient scope for broader application of sex-sorted semen[9]. Milk and meat production efficiency is thought to increase with the commercial application of sex-sorted semen[10]. By use of sex-sorted sperm, female progeny can be obtained from elite dairy cows, and acceptable meat quality and good growth calves can be produced from lower genetic value cows. On an average in cattle, an insemination dose of 1-2 million frozen sperm are present in sex-sorted semen which is about 10% of conventional sperm dose (10–20 million spermatozoa). Sorting process and the low dose reduced non-return rates (NRRs) to two-thirds and one-third, respectively, in sexed semen and effect of both these varied among bulls[11].

Sperm dose required for AI for obtaining adequate fertility, considering bull's genetic merit and other factors is one of the most critical elements in an AI breeding program. A number of factors which compromise sperm fertility level include bull's inherent fertility, semen collection and processing techniques, sperm packaging, shipping and ageing before use, the skill of inseminator and managemental factors related to cow and herd[12,13]. During storage, changes in the spermatozoa were reduced with the use of frozen-thawed semen. But differences in resistance of spermatozoa from individual bulls to freezing, use of various extending media and new packaging and freezing procedures added new variables[14].

2. Sperm dosage in relation to fertility

In bovines, the primary aim of AI is the broader use of proven sire's. Salisbury and Demark first reported the relationship of number of sperms inseminated and fertility level of bull[15]. Sperm number has been related to conception based on Poisson distribution model; which was demonstrated by Schwartz *et al*[16]. Sperm number per AI dose for achieving optimum fertility is one of the main attributes that determines the number of sperm doses produced from the bulls ejaculate[17]. The number of spermatozoa per AI that is needed to obtain acceptable fertility results is crucial to the AI industry. The goal is to optimise the use of top bulls by maximising the total output of sperm and the number of spermatozoa per AI, thus inseminating as many cows as possible without lowering the pregnancy rate. The reduced fertility associated with decreased sperm number may be mainly due to harmful effects of higher dilution on sperm survival than the effect of the lower number of sperms.

The study on effect of two different sperm doses *viz.* 20 and 100 million (packaged in French midi straws) on fertility revealed that fertilization rates were improved with both fresh and frozen sperm doses, however, the percentages of motile and viable sperm remained unaffected with increased sperm concentration[18].

Sperm numbers ranging from five to fifteen million per insemination dose have been found optimum for fertility[19]. Another study revealed that eight and twelve million sperm per dose lead to lower NRRs as compared to sixteen million sperm, and a conclusion was drawn that fourteen million sperm in French midi straw were the threshold for optimum NRRs[20], however, an another study reported 8.4 million sperm was a minimal number for acceptable fertility[21]. Schenk *et al*[22] determined that 11×10^6 spermatozoa/0.5 mL was a critical insemination dose level affecting fertility and that this level differed from the other three levels of 15, 17, and 22×10^6 spermatozoa/dose. At University of Colorado State, with the use of two, five and ten, million frozen sperm/inseminate, the percent pregnancy rates of fifty-three, seventy and seventy-one, respectively were achieved[4]. In this experiment around 100 heifers under field conditions per group were inseminated with modified uterine horn insemination technique.

It is generally accepted that 15 million total frozen spermatozoa in a French mini straw have been found to achieve acceptable conception rates provided that post-thaw sperm survival is equal to or greater than fifty percent[23,24]. It is not yet well known that sperm parameters are involved in the decrease of fertility when sub-optimal sperm concentrations are used. Vishwanath and Shannon[24] observed adverse effects on fertility with reduced sperm numbers in extended, liquid or frozen semen. These changes were shown as variations in NRR, which is defined as the number of bovines that were inseminated and did not return to service within a specific period. The study revealed 2.5 and 20 million bull spermatozoa per straw were optimum for liquid and cryopreserved semen, respectively. Sperm numbers of 0.5 million spermatozoa/straw and 5 million spermatozoa/straw were thus considered sub-optimal for liquid and frozen semen, respectively. By reducing the sperm number per AI dose, fertility was reduced by an average of 7% and 7.9% for liquid and by frozen semen. AI with low-sperm numbers (2 million/straw) deposited intra-uterine using a conventional AI protocol lead to significant reduction of pregnancy rates compared with standard doses of 15 million sperm/straw, with apparent differences among individual bulls; this may be responsible for the compromised fertility of sexed-semen[25]. The results confirmed when attempting the use of inter-bull differences have previously been observed by other authors[26–28], but which were considered not large enough to discourage attempts to reduce the sperm numbers per straw (for instance 10 million total spermatozoa/straw) without significant negative influence on either post-thaw sperm viability or its level of fertility[27,28]. When sperm doses were reduced from 10 to 2.1 million, there was a reduction in conception rates by 8.3% and 13.6%, in frozen conventional and frozen sex-sorted semen[29]. When accompanied by sire effect, numerous studies revealed that storing suboptimum sperm numbers (5 million per dose) resulted in significant reductions in NRR[24,29]. Following two days storage of liquid low sperm doses (3 and 4 million sperm/dose), NRR of dairy cows was significantly reduced in liquid semen doses as compared to frozen-thawed sperm doses (20 million sperm per straw). The reason may be an altered balance between antioxidants of diluents and reactive oxygen species generation, leading to elevated oxidative stress status following two days of storage of liquid semen doses (3 and 4 million sperm/dose) as compared to frozen-thawed sperm

doses[30]. Karakaya *et al*[31] compared pregnancy with artificial insemination with sex-sorted and conventional semen in lactating dairy cows and concluded that fertility was significantly reduced with sex-sorted sperm than with conventional semen even after using it in high fertile cows.

It has been described that maximum conception rates can be achieved within a vast range of viable sperm numbers (ranging from one to eleven million)[32]. If handling process affects are taken into consideration, two million doses of frozen sperm per straw might not be sufficient to achieve desirable pregnancy rates[9]. Studies revealed that bull factor and dilution effects have more pronounced affect on conception rates as compared to the site of semen deposition inside the uterus[25]. This was further confirmed by various other studies including the studies on the use of sex-sorted semen[33,34]. Ballester *et al*[9] reported that there were no significant differences in pregnancy results between 2 and 15 million doses (43% *vs* 47%) under routine AI-conditions (semen deposition in the uterine body) in a large number of commercial dairy herds (even when the total number of cows was lower than optimum). The lower pregnancy rates (32.1%) reported by Andersson *et al*[25] were achieved by 2 million spermatozoa within the uterine body than those achieved with 15 million spermatozoa (46.4%).

The determination of minimum sperm dose that is required for achieving optimum fertility is still a great challenge to the industry of bovine AI[17]. It is revealed that the number of inseminated sperm could be a limiting factor in fertility, mainly due to the considerable variability among bulls[32]. A number of attempts have been made to find the optimal sperm number per insemination dose without compromising the fertility level of bull[35]. Kommisrud *et al*[36] used 12, 15 and 18 million doses for artificial insemination and found no significant differences in fertility between 12 and 18 million sperm doses of high fertile bulls. The fertility of frozen buffalo semen was not compromised when sperm doses were decreased from 30 to 15 million[37]. In case of beef cattle, at fixed time insemination with non-sorted and sex sorted sperm revealed that the pregnancy rates were higher with non-sorted sperm as compared to sex-sorted sperm[38,39]. Obtaining acceptable pregnancies (closer to those with non-sex sorted sperm) with sex sorted sperm may be possible by AI upon estrus detection in case of heifers[40].

3. Site of semen deposition in relation to fertility

Pregnancy rate appears to be influenced by sperm number per AI dose and deposition site in the uterine horn. In dairy bovines, intracornual insemination with reduced sperm doses has resulted in variation among the results[41], and certainty remained questionable for such techniques. In beef cattle, the results of comparing deep intracornual artificial insemination (DIAI) with conventional AI using low sperm numbers (4 million cryopreserved sperm) revealed that in DIAI, the pregnancy rates were higher (67.4%) as compared to AI (48.8%)[42]. In bilateral DIAI, the semen deposition in middle part of uterine horn enhanced fertilisation of oocytes[43]. In estrus-synchronized heifers, a study was carried out to determine the efficiency of single fixed time deep intracornual insemination with 2 million sperm with deep single standard dose, single and dual standard dose (40 million) intrauterine insemination in heifers[44]. No significant effects on pregnancy rates were observed after depositing semen at different insemination sites *viz.* intracorneal, near the utero-tubal junction or in the middle of uterine horn. When compared to the insemination in the uterine body with conventional sperm doses, NRRs were not reduced significantly, as the sperm numbers were decreased from 20–25 million to 12–15 million[27,45], whereas other study revealed the reduced NRRs after use of 8 million and 12 million compared to 16 million spermatozoa[20]. The total pregnancy rates after intracornual insemination with 2 million spermatozoa at a fixed period as well as at a spontaneous estrus differed non-significantly from that obtained after insemination in the body of the uterus[25]. Others have reported either significantly increased[46,47] or reduced[48] pregnancy proportions from intracornual inseminations. Achieving acceptable pregnancies with low sperm numbers would potentially enhance the broader use of sex-sorted spermatozoa[49,50]. The rate of sorting and the dilution requirements for sorting are responsible for the limited application of sexed sperm number available with current technology for insemination[51]. With the use of deep intracornual insemination with as few as 3×10^5 non-frozen sexed spermatozoa, around 54% pregnancy rate has been reported[52].

In synchronised heifers, the primary benefit of deep intracornual insemination with single low sperm doses is the use of valuable semen efficiently as compared to conventional insemination with the double standard doses, and in comparison with conventional inseminations with the single standard doses, higher pregnancy rates were achieved[44]. No significant effects of semen deposition in the curvature or the uterine body on pregnancies rates were reported by Hawk and Tanabe[50]. However, there is a report of 11%–20% increase in pregnancy rates from the deposition of semen in the curvature[44] or the cranial half[51] of the horn compared to deposition in the uterine body. Similar non-return rates have been achieved by the deposition of semen in the middle part of the uterine horn, limits the need of depositing semen close to the utero-tubal junction, however, in case of low sperm doses, effects of deposition site may be remarkable[44].

Pregnancy may be achieved even if the side of ovulation is not fully known by the use of deep intracornual insemination. Depositing liquid semen ipsilaterally or contralaterally deep in the uterus in relation to the side of ovulation did not significantly affect pregnancy rates[52]. Greater care needed to be taken while semen depositions inside the uterine horn due to possibility of follicle rupture and uterine wall perforation due to uterine tonicity during estrus. As per the Hunter[53], deep-uterine inseminations may be used to improve pregnancy rates in cattle by using low dosages or low fertility semen. Decreasing frozen sperm number from 12 to 2 million in bilateral utero-tubal junction insemination yielded no acceptable beneficial effect on pregnancy rates[54]. Thus, it seems that neither sperm number nor semen deposition site influences rate of pregnancy. Even though extensive studies have been done on deep-uterine inseminations, the beneficial effects of this technique are not consistent across various studies. Considering the fact that a number of sperms inseminated may not be a factor responsible for limiting

fertilization, in numerous studies, no differences in non-return rates have been reported after deep and conventional insemination. Only the inseminations with less than threshold sperm numbers can result in reduced NRRs[34]. The utero-tubal junction insemination may be more useful than the conventional insemination, when sperm concentration is a limiting factor for acceptable fertility. Two independent studies[25,44] in cattle demonstrated the effect of low dose insemination (2 million) in combination with deep and conventional insemination. In both of these investigations, no acceptable difference in NRRs were found between deep and conventional AI.

4. Conclusion

Fertility level of bull and breed of the bull may be responsible for determining NRRs with low sperm doses. Adjusting sperm number on individual bull basis may help to reduce variations in NRRs among bulls. Sperm number per AI dose may be reduced in case of high fertile bulls to cover a large number of animals. Inseminating females with low sperm doses of high fertile bulls with reasonable NRRs may help in future to cover a vast population of bovines which are not yet under AI coverage in countries like India. The NRRs variations following AI with low sperm numbers may also be due to their deposition site within the uterine horn. The acceptable pregnancies may be achieved even if the side of ovulation is not known in case of deep-uterine inseminations. A productive means for achieving reasonable pregnancies with low sperm doses may be by deep-uterine inseminations.

Conflict of interest statement

The authors declare that they don't have any conflict of interest.

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