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Insemination of four cows per dose of frozen semen with a fixed-time artificial insemination protocol

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Abstract

The aim of the study was to evaluate the practicality of a 1:4 dilution of doses of semen in fixed-time artificial insemination (FTAI) programs. Nelore cows (n = 803) were synchronized by a conventional FTAI protocol. For AI in the diluted group (n = 392), 0.5 ml semen straws were thawed and 0.5 ml of extender containing amino acids and methylxanthine derivatives was added. The straws were fractionated into four straws of 0.25 ml each (dilution 1:4) with approximately 2.5 x 10^6 spermatozoa each and the insemination was performed in the ipsilateral horn to the ovary containing the dominant follicle. In the control group (n = 411), AI was performed in the uterine body with one straw of 0.5 ml (10 x 10^6 spermatozoa) containing semen from the same bull and batch. The conception rate was 49.2% (193/392) for the diluted group and 50.1% (206/411) for the control group, with 1.97 and 0.50 pregnancies per dose of semen, respectively. The dilution of semen provided a pregnancy index similar to the control group; however, the technique increased the number of pregnancies per dose, allowing for the best use of semen of high genetic value and reducing the cost per pregnancy.

Keywords: bull, fractionation, FTAI, pregnancy, sperm dose.

Introduction

Artificial insemination (AI) is a reproductive technique that is widely used to achieve improvements in production and accelerate the selection and genetic breeding of a herd. Estrus detection, however, may be problematic in tropical areas because *Bos indicus* cattle have a short length of estrus, with a high incidence of estrus occurring during the dark hours (Sá Filho and Vasconcelos, 2011). Thus, fixed-time artificial insemination (FTAI) has been presented as an alternative to overcome these barriers. There are numerous protocols for the synchronization of ovulation for fixed-time insemination, and the most appropriate choice of protocol depends on the technical evaluation of the condition of the farm facilities and the animals to be inseminated.

The effect of low sperm concentration on conception rates in dairy cattle has been investigated in several studies (Foote and Kaproth, 1997; Den Daas *et al*., 1998; Andersson *et al*., 2004; Bodmer *et al*., 2005; Verberckmoes *et al*., 2005; Haugan *et al*., 2007; Seidel and Schenk, 2008), primarily due to the increased use of sexed semen in AI programs in cattle, in which the number of spermatozoa is reduced compared to a conventional dose of semen.

The aim of this study was to evaluate the practicality of a 1:4 dilution of doses of semen in FTAI programs using Nelore cows. The fractionation of the dose of semen aimed to optimize the number of offspring per AI dose.

Materials and Methods

The study was conducted with multiparous Nelore cows (n = 803) which were randomly synchronized by a conventional FTAI protocol on a farm located in the city of Londrina, PR, Brazil. On day 0, an intravaginal progesterone device (CIDR®, Pfizer, Brazil) was inserted and 2 mg estradiol benzoate (Estrogin®, Farmavet, Brazil) was administered intramuscularly. Eight days later (day 8), the device was removed and 0.15 mg D-cloprostenol (PGF2α, Preloban®, Intervet, Brazil), 200 IU eCG (Folligon®, Intervet, Brazil) and 0.5 mg estradiol cypionate (ECP®, Pfizer, Brazil) were administered intramuscularly. On the morning of day 10, 392 cows underwent transrectal ultrasonography (2 operators; Shimadzu - SDU 350 XL 8 MHz, Kyoto, Japan) to determine the diameter of the dominant follicle. In this group, the cows were inseminated on the afternoon of day 10, 50 to 54 h after removal of the device. The sperm was deposited in the middle of the uterine horn ipsilateral to the ovary containing the dominant follicle. For AI, 0.5 ml straws containing a sperm concentration of 2.5 x 10^6 from the same bull and batch with proven fertility were thawed in water at 35°C for up to 20 sec. Before dilution, 60% motility and a vigor of 3 were found. Then, 0.5 ml of semen extender (5 mM pentoxifylline and 5 mM

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caffeine), 10 IU/ml heparin and NaCl 0.9% was added (Numabe *et al*., 2001). After the new evaluation (75% motility and 4 for vigor), the diluted semen was separated into four straws of 0.25 ml (dilution 1:4) each containing approximately 2.5 x 10^6 spermatozoa each and stored at 4-5°C until the time of insemination. The other cows (n = 411) were considered as the control group and received the same FTAI protocol one day later. For the control group, AI was performed by the same inseminator using a full dose of 10 x 10^6 sperm in a 0.5 ml straw containing semen from the same bull used in the other group. This group of cows was inseminated with a conventional dose of one straw per cow with semen deposited in the uterine body. Pregnancy diagnosis was performed by transrectal ultrasonography 45 days after AI. Statistical analysis was performed by Chi-square test using the Statistical Software program MNITAB15.

Results and Discussion

In the present work, the conception rate using diluted doses of semen was 49.2%, with 1.97 pregnancies per dose of semen (Table 1). The conception rate was similar to the control group (50.1%) and to those found by Verberckmoes *et al*. (2005) when they evaluated conventional AI and AI in the uterine tubal junction in combination with decreasing doses of cryopreserved semen (12, 8, 4 and 2 x 10^6 sperm). In that study, the authors observed that neither the different doses of semen used nor the site of semen deposition influenced the conception rate in Holstein cows (47.1%) or heifers (64.3%).

Table 1. Conception rate of Nelore cows inseminated with a diluted (1:4) or full dose of semen.

Groups	Cow numbers	Conception rate (%)	Total doses/group	Dose/pregnancy
Diluted dose	392	193 (49.2)[a]	98	1.97
Full dose	411	206 (50.1)[a]	411	0.50

[a]P > 0.05.

In recent decades, issues such as the minimum number of sperm required to achieve acceptable conception rates have motivated new research projects involving AI in cattle (Crespilho *et al*., 2007). One of the greatest obstacles in determining the most optimal insemination dose with the best cost/benefit relationship and good fertility rates is the wide variation in semen quality of individual bulls. Several studies have reported marked variability between fertility rates taken individually ("bull effect"), observing significant differences between animals and conception rate in relation to the number of spermatozoa used for insemination (Nehring and Rothe, 2003; Andersson *et al*., 2004; Crespilho *et al*., 2007; Schenk, 2008), the fertilization rate and the number of embryos recovered after embryo transfer (Saacke *et al*., 2000) and the maintenance of pregnancy after AI (Lima *et al*., 2004). Some bulls may show individual differences in fertility; therefore, to obtain good conception rates using a low-dose insemination, it is important to choose bulls that have good fertility results when using low concentrations of sperm per dose (Baruselli *et al*., 2007). In our study, the dilution and fractionation of the dose of conventional semen provided a pregnancy rate of approximately 50%.

Although some studies have not observed an influence of the location of semen deposition in the reproductive tract on conception rates (Andersson *et al*., 2004; Verberckmoes *et al*., 2005; Kurykin *et al*., 2007), we chose to deposit the semen in the middle of uterine horn ipsilateral to the ovary containing the dominant follicle, thus enhancing the access of the sperm to the oocyte.

The temperature for the conventional method of thawing semen is 35-37°C. However, since we had to perform all steps described in the Material and Methods, we progressively provided the diluted sperm at the temperature of 4-5°C to maintain low sperm metabolism for up to 2 h. Immediately before the time of insemination, we carefully warmed the sperm to the conventional temperature of insemination, i.e., 35-37°C.

Despite the increase in work with the semen dilution and evaluation of females, the use of a 1:4 semen dilution provided a pregnancy rate similar to the control group, allowing a better use of semen with high genetic value and reducing the cost per pregnancy.

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