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Fertility Rates Following Fixed-Time Artificial Insemination in Dairy Heifers in a Practical Progesterone-Based Protocol

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ABSTRACT

***Background*:** In bovines, more efficient management practices are important for maximizing profitability. In order to increase the pregnancy rates in artificial insemination (AI) programs, several hormonal protocols were developed to synchronize the follicular wave and the moment of ovulation in beef and dairy cattle. In dairy cattle, detection of estrus can be difficult due to a number of factors including the incidence of silent estrus. Hormonal treatments designed to control both luteal and follicular function has permitting efficient synchronizations of time of ovulation. Thus, the AI can be performed in a large number of animals on a fixed schedule without the need for detection of estrus. Using these management techniques, the fixed-time artificial insemination (TAI) can overcome the problem of accurate estrus detection and help in reducing the incidence of repeat breeding. In addition, with TAI in cattle operations, it is possible to facilitate management practices and commercialization, and to reduce the time and semen wasting with animals inseminated at incorrect times. The investigation of practical and efficient TAI protocols is important for reducing the labor and animal handling of TAI in dairy cattle, as well as for increasing the profitability of the cattle management system. This study was carried out in order to investigate the effectiveness of TAI in dairy heifers treated with a practical progesterone-based protocol.

***Materials, Methods & Results*:** This experiment was conducted at the university farm located in southwestern Brazil, during May 2009. Thirty-nine cycling crossbred dairy heifers were employed in this study. All animals received a single intramuscular injection of estradiol benzoate and intravaginal progesterone releasing device in a random stage of the estrous cycle (Day 0). On day 7 the animals were treated with PGF2á analogue and on day 9 the device was removed. Forty-eight hours after the device removal (day 11) a synthetic analogue of GnRH was administered and the animals were fixed-time artificially inseminated at the time of GnRH injection. The inseminations were performed using four different batches from the same Holstein bull. Among the heifers that were synchronized (87.2%), 30.8% ovulated until 24 h after TAI and 56.4% ovulated between 24 and 32 h after TAI. The conception rate was 61.5%. No effects of ovulation time in conception rates were detected. The conception rate from heifers that ovulated until 24 h after TAI was 58.3% and from heifers that ovulated between 24 and 32 h after TAI was 77.3%. The mean of ovulatory follicle in heifers that ovulated until 24 h was 14.3 mm and in heifers that ovulated between 24 and 32 h was 11.9 mm.

***Discussion*:** Taking together, the findings of the present study, along with those of others, emphasize the concept that development of practical methods for TAI offers significant advantages to dairy producers if conception rates are close or greater to those obtained after breeding at detected estrus. Thus, the results of the present study reinforce the possibility of making dairy cattle production more cost-effective using TAI. In conclusion, with the progesterone-based TAI protocol of the present experiment all synchronized animals ovulated up to 32 h after GnRH+TAI and no effects of ovulation time related to conception rate was detected. The exogenous control of luteal and follicular development facilitated the reproductive management and animal handling. Also, inseminating the heifers at the moment of GnRH injection in a progesterone-based TAI protocol is a practical strategy and provided satisfactory results regarding ovulation and conception rates in dairy heifers.

Keywords: conception rate, heifers, ovulation, progesterone.

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INTRODUCTION

In bovines, more efficient management practices are important for maximizing profitability [2]. In order to increase the pregnancy rates in artificial insemination (AI) programs, several hormonal protocols were developed to synchronize the follicular wave and the moment of ovulation in beef [2,3] and dairy cattle [1,12,17].

In dairy cattle, detection of estrus can be difficult due to a number of factors including the incidence of silent estrus [16]. Hormonal treatments designed to control both luteal and follicular function has permitting efficient synchronizations of time of ovulation [1-3,12,17]. Thus, the AI can be performed in a large number of animals on a fixed schedule without the need for detection of estrus [3].

Using these management techniques, the fixed-time artificial insemination (TAI) can overcome the problem of accurate estrus detection and help in reducing the incidence of repeat breeding [16]. In addition, with TAI in cattle operations, it is possible to facilitate management practices and commercialization, and to reduce the time and semen wasting with animals inseminated at incorrect times. The investigation of practical and efficient TAI protocols is important for reducing the labor and animal handling of TAI in dairy cattle, as well as for increasing the profitability of the cattle management system. This study was carried out to investigate the effectiveness of TAI in dairy heifers treated with a practical progesterone-based protocol.

MATERIALS AND METHODS

This experiment was conducted at the university farm located in southwestern Brazil (Fazenda do Glória, Uberlândia, MG), during May 2009. Thirty-nine cycling crossbred dairy heifers were employed in this study. All animals (n = 39) were kept on pasture and presented similar body condition score (body condition score between 2.5 and 3.5 on a point scale of 1 to 5).

The heifers received a single intramuscular injection (2 mg) of estradiol benzoate (EB[1]) and intravaginal progesterone releasing device (IPRD, 1 g progesterone[2]) in a random stage of the estrous cycle (Day 0). On day 7 the animals were treated with 500 μg (intramuscular) of prostaglandin F2α (PGF2α) analogue (d-cloprostenol[3]) and on day 9 the IPRD was removed. Forty-eight hours after the device removal (day 11) a synthetic analogue of Gonadotropin-releasing hormone (GnRH; 10 μg intramuscular of buserelin acetate[4]) was administered in order to synchronize the time of preovulatory gonadotropin surge. The animals were fixed-time artificially inseminated (TAI) at the time of GnRH injection. The inseminations were performed using four different batches from the same Holstein bull.

The follicular dynamics of all heifers were assessed by Transrectal ultrasonographic examinations (Shenzhen Emperor[5]) performed every 8 h from day 11 (application of GnRH + TAI) until ovulation has been detected. Ovulation was defined as the disappearance of a previously identified dominant follicle from one ultrasonographic examination to the next. The measurement of dominant follicle diameter was made on a frozen image of the apparent maximal area of the highest follicle in the ovary. Length (L) and width (W) of structures were used to calculate follicular diameter (D). Follicular diameter was calculated with the formula D = (L + W)/2. The diameter of the ovulatory follicle was considered as the diameter of dominant follicle in the last ultrasonographic examination before ovulation.

Ultrasonographic pregnancy diagnoses were performed 29 and 61 days after TAI in order to determine the conception rate and pregnancy losses. The conception rate on day 29 was calculated by dividing the number of pregnancies on day 29 by the total number of treated animals. The conception rate on day 61 was calculated by dividing the number of pregnancies on day 61 by the total number of treated animals.

Conception rate was analyzed using PROC LOGISTIC of the SAS program (SAS Institute Inc.,Cary, NC, USA), including in the model the effects of ovulation time after TAI (ovulation occurred until 24 h after TAI vs. ovulation occurred between 24 and 32 h after TAI). The ovulatory follicle diameter was analyzed using the unpaired *t*-test with Welch correction, in the GraphPad InStat program (GraphPad Software Inc, San Diego, CA, USA). The level of significance was set at 0.05.

RESULTS

Using the present TAI protocol, 34 heifers were synchronized and the ovulation rate obtained

was 87.2% (34/39). The conception rates at day 29 and at day 61 post-AI were 61.5% (24/39) and no pregnancy loss was observed. Among the heifers that were synchronized, 4 ovulated between 8 and 16 h after TAI (10.3%; 4/39), 8 ovulated between 16 and 24 h after TAI (20.5% ; 8/39) and 22 heifers ovulated between 24 and 32 h after TAI (56.4%; 22/39). No effects of ovulation time related to conception rate was detected (P = 0.25). The conception rate from heifers that ovulated until 24 h after TAI was 58.3% (7/12) and from heifers that ovulated between 24 and 32 h after TAI was 77.3% (17/22). The mean of ovulatory follicle in heifers that ovulated until 24 h after TAI (14.3 mm) was higher ($P < 0.05$) than in heifers that ovulated between 24 and 32 h after TAI (11.9 mm). The results are demonstrated in Table 1.

Table 1. Time to ovulation, conception rate and ovulatory follicle diameter following TAI in crossbred dairy heifers in a progesterone-based protocol.

Time to ovulation after TAI	Number of heifers	Conception rate	Ovulatory follicle diameter at TAI* (Mean ± SD)
Until 24 h	12	58.33% (7/12)[a]	14.32 ± 2.44 mm[a]
Between 24 and 32 h	22	77.27% (17/22)[a]	11.87 ± 1.38 mm[b]

[a,b]: Different superscript letters in the same column indicate significant differences ($P < 0.05$).
*TAI: fixed-time artificial insemination.

DISCUSSION

According to the results of the present work, it is possible to observe that AI at the moment of GnRH injection in the progesterone-based protocol utilized was a valuable strategy in the TAI protocol of dairy heifers.

The TAI is a management practice in dairy cattle that increases the number of inseminated animals and also can offer satisfactory fertility results [12,17]. The use of IPRD and intramuscular injection of EB is one of the most used treatments for TAI in beef and dairy cattle [2,3,16]. The concurrent treatment of EB and IPRD results in synchronized emergence of a new follicular wave. Also, an important effect of IPRD is avoiding that ovulation occurs until removal of device [3]. In cycling animals, the application of PGF2α induces the luteolysis and removes the endogenous source of progesterone (P_4). A second application of estradiol or GnRH after IPRD removal is crucial because it promotes a synchronization of preovulatory gonadotropin surge and a greater synchronization of ovulation [2,3]. Protocols such as Ovsynch and Cosynch are based on the initiation of follicular wave by GnRH before inducing regression of luteal tissue by PGF2α. After the PGF2α treatment, the emerging dominant follicle is induced to ovulate by a second GnRH treatment. The cows are either inseminated around 16 h after GnRH treatment (Ovsynch) or at the same time (Cosynch) that the GnRH [7]. The progesterone-based protocol of this experiment synchronized the growth of a new follicular wave (EB injection), the luteal function (PGF2α injection) and the time of ovulation (injection of GnRH) in 87% of cycling dairy heifers which is in agreement with previous works [12,14] using the Ovsynch protocol in cows.

The conception rate obtained in the present work (61.5%) was superior to previous results [13] obtained with Ovsynch protocol in dairy heifers (35.1%). It has been speculated that heifers may have more estrous cycles with three waves of follicular development [13], a fact contributing for better efficiency of progesterone-based protocols in this animal category. In this sense, other authors [1] utilized four different progesterone-based protocols to TAI in dairy heifers and obtained conception rates about 20% points higher than previously reported for dairy heifers bred to Ovsynch protocol in the absence of a CIDR. Other researchers [4] demonstrated that the conception rates in heifers receiving the Cosynch protocol were 51% suggesting that progesterone-based protocols seems to reach great TAI success rates under heifer management conditions [4].

In the present work, the ovulation was synchronized into an 8-hour period in 65% of heifers. In these animals the time of ovulation was between 24 to 32 h after the injection of GnRH, which is similar to the time from onset of estrus until ovulation

[12,18]. In the others heifers that ovulated (35%), the ovulation occurred up to 24 h after GnRH injection. Previous studies [9,11] have indicated that differences in the interval from insemination to ovulation may affect conception rates. Interestingly in the present study, the conception rate was not affected by the moment of ovulation. However, it is important to emphasize that a low number of animals were utilized in the present work. Thus, the differences between the results obtained in the present study and the studies reported above may perhaps be due to the low number of animals utilized in this work.

The viable sperm cells need of at least 6 h to pass through the oviduct [20]. Other researchers [5,15] demonstrated that the probability of successful fertilization decreases when AI is performed excessively close to the time of ovulation (less than 12 and 6 h before ovulation, respectively). In this sense, it was observed greater conception rates in the groups which the interval from insemination to ovulation was higher than 15 h compared to the group which the interval from insemination to ovulation was around 5 h [2]. In addition, greater conception rates were founded when the interval from insemination to ovulation was between 24 and 16 h [5], or between 24 and 12 h [15].

In the present study, heifers that ovulated between 24 and 32 h after TAI had numerically greater conception rate (77.3%) compared to the heifers that ovulated until 24 h after TAI (58.3%). The small number of animals utilized in this study could be an explanation why no effects of ovulation time in relation to conception rate were observed. However, it has been suggested that the sperm fertilizing ability is not reduced when AI is performed up to 36 h before ovulation [15]. Furthermore, results from that study demonstrated no differences in fertilization rates when AI was performed either between 36 and 24 h before ovulation or closer to ovulation, when semen of high quality is used [15], which may also have occurred in the present work.

It is important to mention that the present study utilized only one sire because pregnancy rate and embryonic loss can differ depending upon whether sires of lesser or greater fertility are used [2]. It was previous demonstrated [8] that highly fertile bulls presented similar pregnancy rates when TAI was performed 12 or 36 h before ovulation. However, when the animals were inseminated with lesser fertility bulls, the pregnancy rate were decreased when TAI occurred earlier (36 h before ovulation), showing that these sires necessitate a lesser interval from insemination to ovulation [8].

Moreover, no pregnancy loss was observed in the present experiment. It has been reported that a suboptimum P_4 concentration is correlated with embryo implantation failure and embryo loss [10]. Positive correlations among cross-sectional area of the ovulatory follicle one day before ovulation and area of the subsequently formed CL and P_4 concentration was demonstrated in a previous work [6]. However, in the present experiment the mean of ovulatory follicle in heifers that ovulated until 24 h was numerically higher (14.2 mm) and the conception rate numerically lower (58.3%) than in heifers that ovulated between 24 and 32 h (11.9 mm and 77.3%, respectively). In this sense, a previous work also observed that the higher cross-sectional area of the ovulatory follicle did not improve the pregnancy rate in Japanese black cows [6].

Taking together, the findings of the present and several other studies [12,14,15,17,19] demonstrated that development of practical methods for TAI offers significant advantages to dairy producers if conception rates are close or greater to those obtained after breeding at detected estrus [19]. Thus, the results of the present study reinforce the possibility of making dairy cattle production more cost-effective using TAI. Also, it is worth mentioning that development of resynchronization methods should help dairy producers improve reproductive performance [19].

CONCLUSION

In conclusion, the use of the progesterone-based TAI protocol proposed in the present experiment resulted with all synchronized animals ovulating up to 32 h after GnRH+TAI, with no effects of ovulation time related to conception rate being detected. The exogenous control of luteal and follicular development facilitated the reproductive management and animal handling. Also, inseminating the heifers at the moment of GnRH injection in a progesterone-based TAI protocol is a practical strategy and provided satisfactory results regarding ovulation and conception rates in dairy heifers.

SOURCES AND MANUFACTURERS

[1]Benzoato de Estradiol, Sincrodiol®. Ouro Fino, Cravinhos, SP, Brazil.

[2]Dispositivo intravaginal liberador de progesterona, 1 g progesterona; Sincrogest®. Ouro Fino, Cravinhos, SP, Brazil.

[3]D-cloprostenol; Sincrocio®. Ouro Fino, Cravinhos, SP, Brazil.

[4]Acetato de buserelina; Sincroforte®. Ouro Fino, Cravinhos, SP, Brazil.

[5]Shenzhen Emperor, 5 MHz. Nanshan, China.

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Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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