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Effect of the interval between estrus onset and artificial insemination on sex ratio and fertility in cattle: a field study

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Abstract

We have carried out a field trial in cattle to study the effect of the interval between the onset of estrus and AI on sex ratio and fertility. Data were obtained from 716 cows that had been inseminated at different times between 8 and 44 h from the visual detection of estrus. Before analyzing the data, it was grouped in three intervals considering the time between estrus onset and AI (8–18, 18–30, and ≥30 h). Our results show that the percentage of calved females (73.05%) is significantly superior for early inseminations (8–18 h), and it decreases 1.85% per hour from the onset of estrus. Delayed AIs (≥30 h) produce a significant deviation of the sex ratio towards the males (72.06%); nevertheless, fertility (percentage of successful pregnancies) diminishes significantly, from 66.19% (8–18 h) to 45.35% (≥30 h). In conclusion, variations in the interval between the onset of estrus and AI modify sex ratio. However, we must consider its effect on fertility.

Keywords: Cattle; Artificial insemination; Estrus detection; Estrus onset; Sex ratio; Field trial

1. Introduction

In animal production systems, the possibility of modify sex ratio can result in a substantial increase of the production in intensive cattle farms. Also, sex ratio manipulation can sensibly enhance the effectiveness of selection and genetic improvement programs, through the differential increment of males or females born after AI [1].

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Sexing technology takes advantage of the physiological differences between X and Y spermatozoa, favoring the presence of the desired kind of spermatozoa in the moment of fertilization and deviating the physiological sex ratio. The most accurate techniques include the use of flow cytometry to separate the X and Y sperm populations, and using the corresponding type to perform artificial insemination [2–5]. However, a great drawback of this approach is the high cost of a flow cytometer.

On the other hand, some researchers have studied the variation of the sex ratio depending on the time of the mating relative to ovulation [1,6,7]. Nevertheless, results have been very variable. In white-tailed deer (*Odoicoleus virginianus*) Verme and Ozoga [8] indicated that matings occurring near the onset of estrus produced a higher proportion of females, whereas late matings produced more males. This effect has been observed in other species (human: 6, 9, 10; hamster: 11, 12; mouse: 13; sheep: 14).

In cattle, results are controversial, since some authors have not observed such a relation [7,15,16], whereas other indicated that sex ratio can be altered varying the moment of AI relative to the onset of estrus [17,18]. Due to this lack of consensus, further research has been recommended [19]. Besides, it has also been remarked that delaying the insemination can negatively affect fertility results, which should be seriously considered in this kind of studies [20,21].

In the present study, we have taken into account that the modification of sex ratio in cattle can have a great economic impact, and that there is no agreement among the studies carried out to date. We carried out a field trial, analyzing the variation of sex ratio in cattle offspring considering the time interval between the onset of the estrus and the moment of the insemination. Besides, we assessed the effect of this interval on the effectiveness of the AI (% fertility).

2. Material and methods

2.1. Data collection

The data used in this experiment were collected in dairy farms from the region of Asturias, in the North of Spain (mean size of the explotations: 50 cows, European Holstein–Frisian; average milk production: 5750 l per cow per year). Total number of inseminated cows were 716. The animals were inseminated at different times from the detection of the onset of estrus (between 8 and 44 h). The insemination was carried out using standard methodology for cattle, using frozen/thawed semen, which was deposited in the uterus. Estrus onset was detected and noted down by an experienced person, who carried out continue observations throughout the day, watching behavior and clinical and gynecological symptoms characteristic of the estrus in females of this species [22]. The percentage of successful pregnancies (fertility) and the percentage of calved females relative to total calved animals (percentage heifers) were registered, in order to determine the variation of fertility and sex ratio regarding the moment of the artificial insemination. To sum up, we get the following data for each cow: time of AI relative to the detection of estrus (estrus-insemination, EI), pregnancy outcome (resulting in a live calf or not), and the sex of the calf. The usual routine in the studied farms consists in inseminating the cows 12–24 h from

the onset of estrus. This routine was respected as far as possible during data collection, in order not to impair the fertility results of the farms. Consequently, there is a smaller number of animals in the group corresponding to delayed inseminations (EI $\geq$ 30 h).

2.2. Statistical analysis

Collected data were grouped in three time intervals according to EI (8 h $\leq$ EI $<$ 18 h; 18 h $\leq$ EI $<$ 30 h, and EI $\geq$ 30 h). The SAS^{TM} v. 8 package was used to perform statistical analysis.

Fertility is a binary variable (each cow either calved or did not). Thus, it was analyzed in a categoric model (PROC CATMOD), considering farm and EI as factors of variation. After that, EI classes were compared using the χ^2-test (PROC FREQ).

Sex ratio was studied by means of two statistical analyses. Firstly, calf sex frequencies in each EI intervals were compared by means of χ^2 (PROC FREQ), using a binary variable for the study (calved heifer: YES/NO). Secondly, we performed a regression study between the percentage of calved heifers and EI time in hours, using lineal regression (PROC REG).

3. Results

Table 1 summarizes the results of sex ratio and fertility studies. Sex ratio study showed that there are significant differences among the three EI groups regarding the percentage of calved heifers, which consistently decreases from 73.05% in early inseminations (8 h $\leq$ EI $<$ 18 h) to 27.94% in late inseminations (EI $\geq$ 30 h). Analysis of the categoric model for the study of fertility excluded farms as factor of variation, but EI affected fertility significantly. Comparison of the three EI groups showed that there is a highly significant reduction of fertility in the EI $\geq$ 30 group respects to the other two. EI interval between 8 and 18 h displays both the highest fertility and the highest percentage of calved females.

Regression study evinces a direct relationship between sex ratio and EI, which agrees with the results of the analysis of frequencies (Table 1). The regression model (Fig. 1) shows that the percentage of calved heifers decreases significantly as interval EI increases, at a rate of -1.85% per hour from the onset of estrus ($r = -0.66$; $P < 0.001$).

Table 1
Variation of the fertility and the proportion of calved heifers according to the interval onset of the estrus—moment of AI (EI)

EI (h)	Cows calved/total inseminated cows	Calved heifers/total calved animals
8 h $\leq$ EI $<$ 18 h	141/213 (66.20%)^{a}	103/141 (73.05%)^{A}
18 h $\leq$ EI $<$ 30 h	214/353 (60.62%)^{a}	128/214 (59.81%)^{B}
EI $\geq$ 30 h	68/150 (45.33%)^{b}	19/68 (27.94%)^{C}
Total	423/716 (59.08%)	250/423 (59.10%)

Rows with different superscripts (a, b) differ $P < 0.01$.
Rows with different superscripts (A, B, C) differ $P < 0.05$.

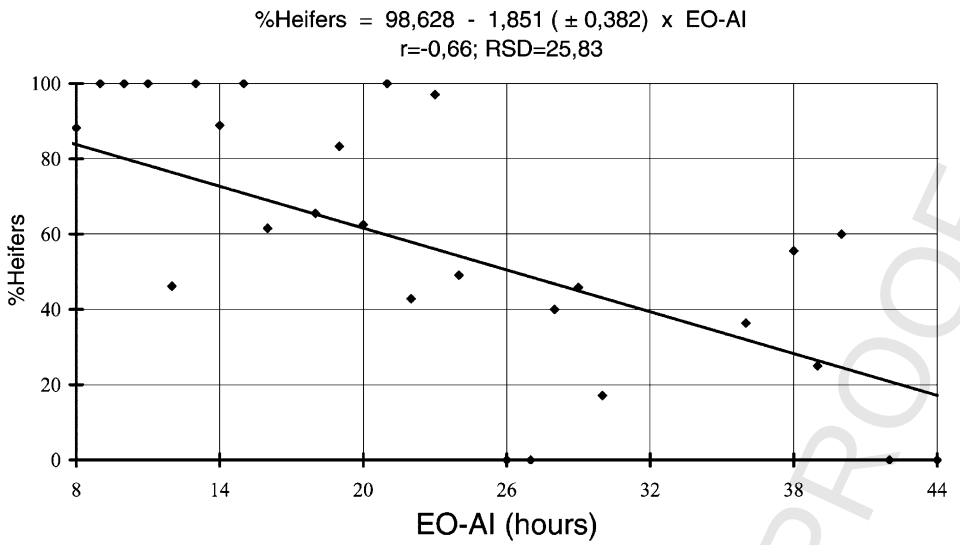

Fig. 1. Regression study of the percentage of calved heifers, based on the interval between the onset of the estrus and the AI in cows.

4. Discussion

The control of sex ratio in farms conveys many advantages, since it allows to sensibly improve the yield of the operations based on the type of production (milk or meat). At this moment, there are many techniques of proven effectiveness that allow to alter the normal sex ratio, such as flow cytometry sorted sperm. However, they are of little practical use, because the cost of preparation of purified samples of X or Y sperm is still excessively high [1]. An alternative, the variation of the time of AI with respect to the onset of the estrus, can be an effective technique to vary sex ratio without increasing the costs.

In cattle, estrus lasts an average time of 18 h, and ovulation follows 10–12 h later, making 30–32 h from the onset of estrus to the ovulation [23,24]. Our results indicate that the percentage of females in the offspring can be increased performing the AI within the first 18 h from the onset of the estrus, whereas delaying the AI significantly increases the percentage of males. When insemination is carried out beyond 30 h from the onset of the estrus, the percentage of males increases significantly. According to the regression analysis (Fig. 1), the percentage of males increases 1.85% per hour from the onset of estrus.

The results of the present work can be explained considering that there are many physiological differences between Y and X spermatozoa. For instance, Rohde et al. [25] found that Y spermatozoa progress more quickly through cervical mucus than those carrying an X chromosome. Although this is not applicable to our study, since the semen was deposited in the uterus, differences between X and Y spermatozoa could be expressed in other places of the female genital tract. Indeed, there is a process of sperm selection in the oviduct, in which spermatozoa interact with the oviductal epithelium, forming a

reservoir at the uterotubal-isthmus junction, and undergo capacitation. Those sperm that reach an adequate capacitation state are released and can move to the fertilization place [26,27]. The high percentage of calved heifers in our study when cows are inseminated within the first 18 h from the onset of estrus can be explained by the fact that Y sperm in the isthmus would achieve capacitation earlier than X sperm, release from the oviductal epithelium, and reach the fertilization place long before the ovulation. Having undergone capacitation, most of these cells would die. However, X sperm, which would have undergone capacitation later and have longer lifespan, would reach the fertilization place at the adequate moment. Thus, in these conditions, it is more likely that X spermatozoa would fertilize the ovum [17]. On the other hand, if insemination were delayed (EI $\geq$ 30 h), Y spermatozoa would have more chances of fertilizing the ovum, since they would arrive at the fertilization place around the moment of the ovulation and before X sperm.

Although some authors have not found a relationship between the moment of the IA and sex ratio in cattle [7,15,16], other studies agree with our results [17,18,28]. The disparity of results between authors could be due to methodological differences, specially the detection of estrus, the use of different AI protocols and the variability between males and ejaculates [29,30]. In a review on this subject, Rorie [19] concluded that results of different works are conflicting and insufficient, and that this area merits more investigation in livestock species.

Wehner et al. [17], using an electronic device that measures the conductivity of the cervical mucus to determine the state of the estral cycle in cows, indicated above 90% of effectiveness regarding sex selection, only varying the time of insemination. These authors obtained 93% of females inseminating approximately 20 h before the ovulation (12 h from the onset of estrus), and 92% of males inseminating 10 h before the ovulation (22 h from the onset of estrus), which is comparable to the use of flow cytometry to obtain sorted semen. Although we agree with these results, percentages are higher than those of our study. Wehner et al. [17] also reported 22 h as the interval EI in which more males were produced, whereas we obtained the higher proportion of bulls after 30 h EI. These discrepancies could be explained by methodological differences both in the detection of the estrus and in the AI protocol. Indeed, they used a more precise method in order to determinate the estral state of the cows relative to the moment of ovulation, and the inseminations were carried out at fixed times, whereas we used ampler intervals in our work.

Other studies have reported similar observations in diverse species, as Krackow [31] pointed out in a revision on this subject. In white-tailed deer, Verme and Ozoga [8] indicated that matings within 36 h after the onset of estrus rendered a higher proportion of females (72.9%), whereas late matings rendered a higher proportion of males (69.7%). In sheep, Gutierrez-Adan et al. [14] obtained 60% of females inseminating the sheeps 5 h before ovulation and 75% of males inseminating 5 h after the ovulation. There are many studies in other species indicating some influence of the insemination timing and the sex ratio (human: 6, 9, 10; hamster: 11, 12; mouse: 13).

Our results show that the percentage of calved females can be increased acting on the time interval between the onset of estrus and insemination (8 h $\leq$ EI $<$ 18 h), with no loss of fertility. Nevertheless, increasing the proportion of males by means of delaying the

moment of the AI (EI $\geq$ 30 h), significantly diminish fertility ($P < 0.01$) from 66.19 to 45.35%.

Many studies in cattle support our fertility results. Pursley et al. [18] carried out AI at hour 0, 8, 16, 24, and 32 after inducing the ovulation, finding that fertility diminishes significantly when cows were inseminated after the ovulation time (AI at hour 32). There is an interval of time in which ovum reaches an adequate maturational state and position to be fertilized. Therefore, insemination must be carried out at the corresponding time. Dransfield et al. [21] reported that delaying AI lowered fertility, and recommended to perform AI between 4 and 12 h from the detection of the onset of estrus. In general, it is recommended to inseminate the animals between 12 and 18 h after estrus onset [20,32,33], in order to achieve good fertility.

In conclusion, sex ratio in cattle can be modified simply varying the moment of the AI with respect to estrus onset. Early inseminations favor heifer calving, while not impairing fertility, whereas delaying AI favors bull calving, but with a significant diminution in fertility. The use of this methodology to vary sex ratio must consider its effect on fertility, that could jeopardize its practical application.

Uncited references

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