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Relationship between follicle size after FSH treatment and efficiency of oocyte recovery

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Abstract

The goal of this experiment was to evaluate the effect of two FSH protocols on follicle diameter and the number of oocytes obtained by transvaginal oocyte aspiration. Forty-six, healthy donor cows were randomly assigned to one of two treatments. To synchronize ovarian follicular growth, follicles > 2 mm were aspirated from all animals (D0 = Day 0 of treatment) using a 7.5 MHz convex ultrasound transducer (Scanner 200 Vet, Pie Medical). Experimental treatments were based on the day of follicular aspiration after animals received a single, 250 IU i.m. injection of FSH (Pluset, Serono). In Group 1 (G1; n = 19), animals received FSH on Day 1, and follicles were aspirated on Day 2 in order to have small follicles at the time of follicular aspiration. Animals from Group 2 (G2; n = 27) received FSH on Day 2, and the oocyte recovery was performed on Day 5 in order to aspirate large follicles at the time of follicle aspiration. Follicle number and diameter and the number and quality of oocytes were evaluated. A total of 716 follicles were aspirated (239 from G1 and 477 from G2). In G1, an average of 12.6 ± 1.4 follicles were aspirated per cow and all had a diameter of < 5 mm. In G2, 17.7 ± 1.1 follicles were aspirated per cow and all had a diameter of > 5 mm. The number of follicles and proportion of follicles for each diameter class (< 5, 5-10, and > 10 mm) were different between groups ($P < 0.05$). Four hundred and forty-eight oocytes (192 from G1 and 256 from G2) were obtained. Efficiency of oocyte recovery (number of oocytes divided by the number of follicles) was greater in G1 than in G2 although the average number of oocytes obtained per cow for each group did not differ (10.1 ± 1.27 and 9.4 ± 1.06 for G1 and G2, respectively). The FSH protocol affected follicular development and the efficiency of oocyte recovery.

Keywords: FSH, oocyte recovery, follicles, cattle.

Introduction

Since the first report of ultrasound-guided transvaginal ovum pick up (Pieterse *et al.*, 1988), many aspects of this procedure were studied to increase the efficiency of oocyte recovery. This procedure has been performed with convex and linear transducers (Seneda *et al.*, 2003; Bols *et al.*, 2004), different kinds of needles (Bols *et al.*, 1997), and vacuum pressure (Bols *et al.*, 1996). Oocyte recovery has been evaluated in problem cows (Looney *et al.*, 1994; Bols *et al.*, 1996), at different frequencies of follicle aspiration (Goodhand *et al.*, 1999), in prepubertal calves (Brogliatti and Adams, 1996), and at different stages of the follicular wave (Hendriksen *et al.*, 2004). One of the most studied aspects is the stimulation of follicle growth prior to oocyte recovery. Renowned research teams have worked with eCG (Pieterse *et al.*, 1992), BST (Bols *et al.*, 1998), and FSH (De Roover *et al.*, 2005) to stimulate follicular growth and obtain oocytes. The advantages of follicular stimulation seem obvious, more follicles result in more oocytes (De Roover *et al.*, 2005). However, there are conflicting data about this issue. Even though protocols, designed to simulate follicular growth, performed before follicular aspiration have increased numbers of follicles available for aspiration, the efficiency of oocyte recovery decreased with use of FSH or BST (Pieterse *et al.*, 1991; Walton *et al.*, 1993; Bols *et al.*, 1998) despite the fact ovaries were larger and easily manipulated during follicular aspiration after the hormonal stimulation (Techakumphu *et al.*, 2004). To explain these results, it was considered that FSH induced asynchrony between the oocyte and its follicle (De Roover *et al.*, 2005). Another aspect could be the follicle size. The larger diameter follicles produced by these protocols could explain the lower efficiency of oocyte recovery. A better efficiency of oocyte recovery from small follicles, in comparison with large follicles, is due to three aspects: a lower intrafollicular pressure, less viscous follicular fluid, and a smaller amount of intrafollicular fluid (Seneda *et al.*, 2001). Thus, the lower efficiency of oocyte recovery after FSH treatment could mostly be explained by the larger size of the follicles at the time of follicular aspiration.

The objective of the present study was to investigate the effect of FSH treatment on follicle size and on bovine oocyte recovery by transvaginal ultrasound-guided follicle aspiration.

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Materials and Methods

Equipment

A real-time, B-mode ultrasound scanner (Scanner 200 Vet, Pie Medical, Netherlands) equipped with a 7.5 MHz convex array transducer was fitted to an intravaginal device (Pie Medical, Netherlands) with a stainless steel needle guide. A disposable 19-gauge hypodermic needle (40 mm long; Becton Dickinson, Brazil), connected to a 50 ml conical tube (Corning, USA) via silicon tubing (0.8 m; 2 mm i.d.), was used for follicular puncture. The aspiration was performed at a vacuum pressure corresponding to 13 ml of H_2O/min generated by a vaccum pump (Rocket Medical, England). Aspiration medium was PBS (Nutricell, Brazil) supplemented with 10% fetal bovine serum (Nutricell, Brazil) and heparin (17 UI/ml). The same operator performed all procedures.

Animals

Non-pregnant Blond d'Aquitane and Caracu (both *Bos taurus*) embryo donor cows, 7 to 9 years old, were used in this experiment. Healthy animals (n = 46) were kept on an *ad libitum* grazing regime with mineral supplementation during a 4-week period (March to April, summer and fall in the Southern Hemisphere). Cyclicity was verified by ultrasonographic examination followed by observation of estrous behavior for 60 days prior to beginning of the experiment.

Donor preparation

Before each aspiration session, feces were removed from the rectum and the perineal area was cleaned with tap water and 70% ethanol. Prior to follicle aspiration, each cow received caudal epidural anesthesia using 7 ml of 2% lidocaine to decrease peristalsis and discomfort. The transducer was inserted through the vagina, advanced to the fornix, and positioned so that the best ultrasound images of individual ovaries were obtained.

Treatments

To synchronize follicle growth, every week, 11 to 12 animals at random stages of the estrous cycle were subjected to aspiration of all follicles > 2 mm on Day 0 of treatment (D0). Animals were randomly assigned to one of two treatment groups. All animals received a single, i.m. injection of 250 IU FSH (Pluset, Serono, Italy) on either D1 or D2. Animals from Group 1 (G1, n = 19) received FSH on D1 and were subjected to follicular aspiration on D2, and animals from Group 2 (G2, n = 27) received FSH on D2 and were subjected to follicular aspiration on D5. All animals received treatment only once. The follicles and oocytes recovered and considered for analysis were only from aspiration after FSH treatment.

Follicle aspirations

Follicles from both ovaries were measured and classified according to diameter: < 5mm, 5 to 10 mm, and > 10mm. After that, they were counted and subsequently aspirated. All follicles from each diameter category were aspirated together. The needle was pressed to pierce the vaginal wall, introduced into the ovary, and inserted into individual follicles at the same time that the aspiration pump was activated. After aspiration of all follicles, an extra 20 ml of medium was aspirated to recover oocytes from the needle and silicone tubing. All procedures were performed by the same operator. Immediately following aspiration, the recovered follicular fluid was passed through an Emcon embryo filter that was subsequently washed with a phosphate buffer solution (PBS-Nutricell, Brazil) supplemented with 5 % fetal calf serum (FCS), and recovered oocytes were counted.

Recovery and evaluation of oocytes

The measurement of efficiency of oocyte recovery, or recovery rate, was expressed as the total number of oocytes recovered divided by the total number of aspirated follicles. Immediately after recovery, cumulus-oocyte complexes were classified according to the presence of cumulus cells around the oocyte as follows: *good* - more than 3 layers of cumulus cells; *regular* – at least one layer; *denuded* – partly covered by cumulus cells or not at all; or *atretic* – dark cumulus oophorus and signs of cytoplasmic degeneration (Seneda et al., 2001).

Statistical analyses

Data were analyzed by ANOVA. Each aspiration event was considered as an experimental unit. Independent variables were treatment, class of follicles (< 5 mm, 5 to 10 mm, > 10 mm), class of oocytes (good, regular, denuded, or atretic), and the treatment by class interaction. Dependent variables were numbers and percentages of follicles and recovered oocytes. When treatment by class interactions were significant ($P < 0.05$), effects of treatment were evaluated separately within each class. Differences between means were determined using Tukey's test. Means are expressed with the standard error of the mean.

Results

A total of 716 follicles were aspirated. Two hundred thirty-nine bovine follicles were punctured in animals that were given FSH on Day 1 (G1) and 477 follicles from those given FSH on Day 2 (G2). On average, 12.6 ± 1.4 follicles were obtained per cow from G1, all of which were < 5 mm. From G2, the percentage

of observed follicles of diameters from < 5 mm, 5 to 10 mm, and > 10 mm were 4.8, 25.4, and 70%, respectively. Number and proportions of follicles in each class of follicular diameter were different (P < 0.05) between G1 and G2 (Fig. 1 and Table 1).

Four hundred forty-eight oocytes were obtained, 192 from G1 and 256 from G2. An average of 10.1 ± 1.3 oocytes were obtained from 12.6 ± 1.4 follicles per cow in G1 and 9.4 ± 1.1 oocytes from 17.7 ± 1.1 follicles per cow in G2. Mean number of oocytes collected per cow did not differ between groups (P > 0.05; Table 1).

The quality of oocytes was estimated after evaluation of the number of granulosa cell layers around the oocyte and the morphology of the cytoplasm (Seneda *et al.,* 2001). There was no correlation between follicular diameter, treatment, or quality of recovered oocytes (P > 0.05); however, there was a greater proportion of atretic oocytes in G2 (P < 0.05; Fig. 2).

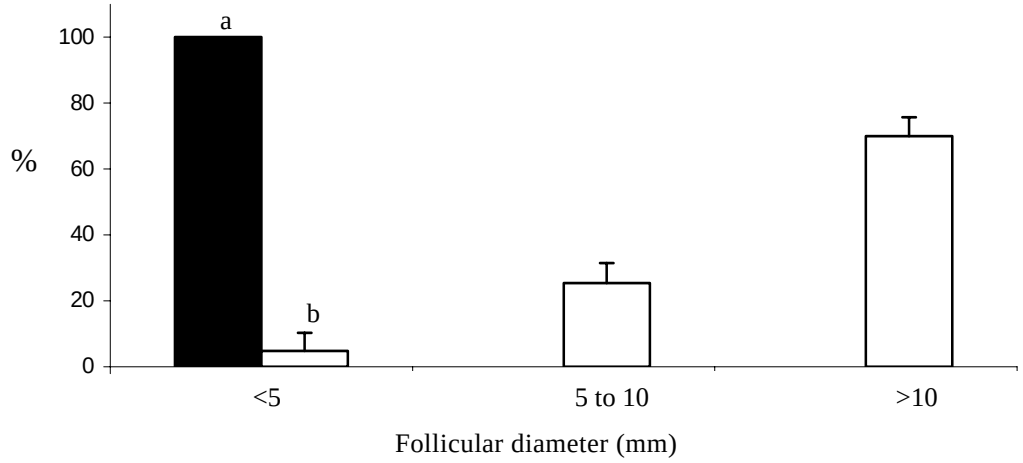

Figure 1. Effect of FSH treatment on Day 1 and follicle aspiration on Day 2 (solid bars) or FSH treatment on Day 2 and aspiration on Day 5 (open bars) on the proportion of follicles in each follicle diameter class at the time of follicle aspiration. Within each class of follicle diameter, means with different letters are different (P < 0.05).

Table 1. Effect of FSH treatment on Day 1 and follicle aspiration on Day 2 (G1) or FSH treatment on Day 2 and aspiration on Day 5 (G2) on the mean number of aspirated bovine follicles and recovered oocytes per cow.

Treatments	Aspirated cows	Total follicles per cow (SEM)	Total oocytes per cow (SEM)
G1	19	12.6 (± 1.4) [a]	10.1 (± 1.3) [a]
G2	27	17.7 (± 1.1) [b]	9.4 (± 1.1) [a]

Means in the same column with different superscripts are different (P < 0.05).

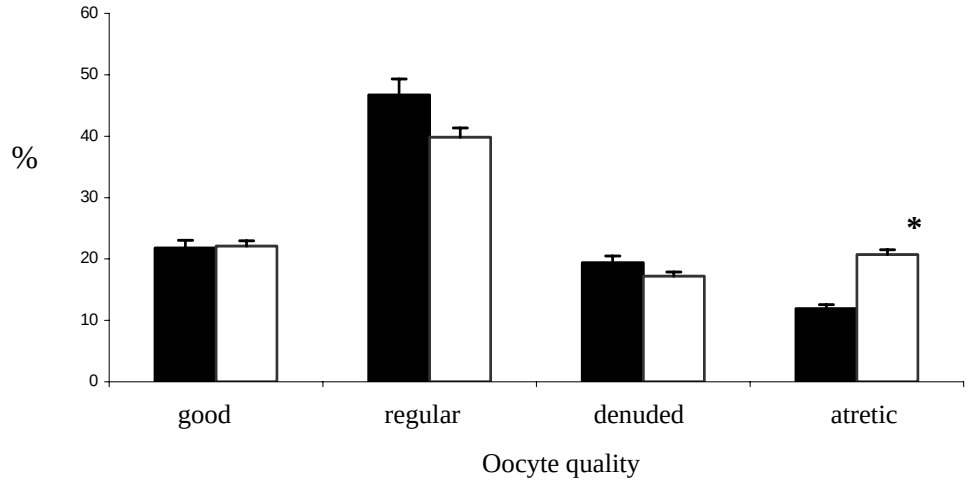

Figure 2. Effect of FSH treatment on Day 1 and follicle aspiration on Day 2 (black bars) or FSH treatment on Day 2 and aspiration on Day 5 (white bars) on the percentage of oocytes recovered after follicle aspiration for each oocyte quality classification. There were no differences in the percentage of good, regular, or denuded oocytes between groups, except for a larger (* P < 0.05) proportion of atretic oocytes in the Day 2-treated group.

Discussion

Despite information that FSH has limited efficacy for follicle aspiration (Gibbons *et al.*, 1994), utilization of a gonadotropic stimulation has been recently reported (Goodhand *et al.*, 2000; Reis *et al.*, 2002; Blondin *et al.*, 2002; Merton *et al.*, 2003; De Roover *et al.*, 2005). In the present study, the efficiency of FSH protocols were related to follicle diameter at the moment of oocyte recovery. The treatments G1 and G2 were applied to yield follicles of a standardized diameter at the time of follicular aspiration. This result allowed us to evaluate effects regarding follicle diameter on the number of oocytes recovered after FSH treatment.

The number of oocytes after FSH treatment in both groups was similar. However, a lower number of follicles were aspirated per cow in G1 (12.4 ± 1.4) in comparison with G2 (17.7 ± 1.1). Considering that all follicles from G1 cows were < 5mm, the present results were similar to our previous data that was obtained without FSH stimulation (Seneda *et al.*, 2001). Smaller intrafollicular fluid volume, pressure, and viscosity in small follicles could be related to the greater efficiency of oocyte recovery (Edwards *et al.*, 1980). Moreover, the time required to aspirate all follicles from each cow was approximately 50% less in G1 than in G2 because we aspirated a lower number of follicles on average per cow in G1. Another important observation was the smaller amount of blood during puncture of follicles in G1 cows. This was probably due to a smaller vascular perfusion in small follicles as well as smaller damage to the ovarian stroma.

The quality of aspirated oocytes was similar between G1 and G2 except for a greater proportion of atretic follicles in G2. Because animals in G2 presented a greater proportion of larger-sized follicles, these findings refute previous reports in which there was no relationship between follicle diameter and oocyte quality (Rhodes *et al.*, 1997; Seneda *et al.*, 2001). On the other hand, this finding confirms the results of Blondin and Sirard (1995) who showed an influence of follicle diameter on oocyte quality. This contrast could be associated with the use of FSH in the present experiment compared to no FSH treatment in previous reports. The effects of FSH can considerably influence aspects of follicle development such as the time necessary for follicles to achieve maturity, the rate of cell division, and LH receptor expression in the granulosa cells (Lawson *et al.*, 2003). Another possible explanation could be the accelerated luteinization of some follicles after FSH treatment (Luborsky *et al.*, 2002). Perhaps some of these aspects could be related to the atretic oocytes recovered from the larger follicles.

The results of this experiment could contribute to enhance the efficiency of oocyte recovery after FSH treatments. Considering follicle diameter at the moment of ovum pick up, it is possible to obtain a high efficiency of oocyte recovery. In addition, the lower number of aspirated follicles and the smaller amount of blood found during puncture could be an important subject of study for future experiments regarding damage to the ovary after puncture.

In conclusion, the FSH treatment followed by aspiration on D2 and D5 was an appropriate approach to yield follicles of standardized diameters at the time of follicular aspiration. The best efficiency of oocyte recovery was obtained when all ovarian follicles were less than 5 mm in diameter. Therefore, the diameter of follicles at the moment of oocyte recovery must be considered, and this should be considered when selecting an FSH protocol to use prior follicular aspiration.

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