Theriogenology 79 (2013) 267–273

Contents lists available at SciVerse ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Occurrence and characteristics of residual follicles formed after transvaginal ultrasound-guided follicle aspiration in cattle

J.H.M. Viana [a,*], M.D. Dorea [b], L.G.B. Siqueira [a], E.K.N. Arashiro [c], L.S.A. Camargo [a], C.A.C. Fernandes [d], M.P. Palhão [d]

[a] Embrapa, Juiz de Fora, MG, Brazil
[b] Federal University of Espirito Santo, Alegre, ES, Brazil
[c] Federal University of Minas Gerais, Belo Horizonte, MG, Brazil
[d] University of Jose do Rosario Vellano, Alfenas, MG, Brazil

ARTICLE INFO

Article history:
Received 14 April 2012
Received in revised form 16 August 2012
Accepted 17 August 2012

Keywords:
Follicle ablation
Ovarian follicular atresia
OPU
Ovary
Steroidogenesis
Bovine

ABSTRACT

Ultrasound-guided transvaginal follicle aspiration is used to recover cumulus-oocyte complexes (for IVF) and to synchronize follicular wave emergence (ablation of dominant follicle). Although aspirated follicles are generally supposed to undergo immediate atresia, there are indications that they may remain active. The objective was to evaluate the occurrence and characteristics of residual follicles (RF) after transvaginal follicle aspiration in cattle. Ovarian follicular wave emergence was synchronized in Holstein cows (N = 13) in the presence (groups 1 and 3) or absence (groups 2 and 4) of norgestomet implants. The largest follicle was aspirated at a diameter of 8 mm (groups 1 and 2) or 12 mm (groups 3 and 4). Ovarian follicles were visualized (transrectal ultrasonography) every 12 h after wave emergence. Follicular fluid samples were collected from the largest follicle and from the ensuing RF and concentrations of estradiol and progesterone were determined. After aspiration, 73.2% (52/71) of the follicles refilled with fluid, and a new antrum was detected 12 to 24 h later. Norgestomet did not affect ($P > 0.05$) RF occurrence or diameter, but in RF from group 4, concentrations of estradiol decreased (-530.7 ± 133.9 ng/mL; $P < 0.01$) whereas progesterone increased ($+429.6 \pm 171.7$ ng/mL; $P < 0.05$) relative to preaspiration. In RF, there were three steroidogenesis patterns: (1) high estradiol concentration and high estradiol:progesterone ratio (estradiol-active RF); (2) low estradiol, but high progesterone concentrations (luteinized RF); and (3) low estradiol and low progesterone concentrations (inactive RF). Estradiol-active RF were more likely ($P < 0.05$) from follicles with high estradiol concentrations (regardless of diameter). In conclusion, fluid-filled structures (RF) with variable steroid production patterns are frequently formed after ultrasound-guided follicle aspiration. The occurrence and features of these RF depended on the diameter and status of these follicles before aspiration.

1. Introduction

Ultrasound-guided transvaginal follicle aspiration, also known as ovum pick-up (OPU), has become the technique of choice for recovering cumulus oocyte complexes from live donors for IVF, both in cattle [1] and humans [2]. In essence, OPU is an adapted biopsy procedure in which ovarian follicles are visualized with real-time ultrasonography, and their content is aspirated using a needle connected to a vacuum system. The ultrasound-guided needle is a reliable and practical way to access follicles within the ovary; it has been used to evaluate the intrafollicular environment [3], to biopsy preantral follicles [4], and to perform intrafollicular injections [5].

* Corresponding author. Tel.: +55 32 3311 7438; fax: +55 32 3311 7401.
E-mail address: jhmviana@cnpgl.embrapa.br (J.H.M. Viana).

0093-691X/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2012.08.015

Follicular dominance is a key mechanism in ovarian follicular dynamics for establishment of the species-specific ovulation rate (reviewed by Ginther et al. [6]). In cattle, the appearance of a dominant follicle causes atresia of the subordinate follicles within the same follicular wave (reviewed by Ginther et al. [7]); negative effects of follicular dominance on superovulation are well documented [8–10]. Ultrasound-guided follicle aspiration became, consequently, an alternative to selectively remove large antral follicles and to control follicular dynamics [11], and was therefore used by various research groups to ablate the dominant follicle and thereby synchronize follicular wave emergence [12–14] or to improve superovulatory response [9,15–19]. Follicular dominance has also been demonstrated to negatively affect oocyte developmental potential [20–22]; therefore, short OPU intervals were proposed to avoid establishment of dominant follicles and improve IVF outcome [23–25].

Follicular aspiration causes follicle collapse and lumen disappearance, based on the ultrasound image [9]. The remaining follicular wall cells are generally supposed to undergo atresia after aspiration, but in most studies, the fate of the aspirated follicles was not recorded thereafter, and this expected immediate atresia remained a presumption. Ginther et al. [26] were apparently the first to describe refilling of follicle contents after aspiration, and hypothesized that active components of the follicle might not have been destroyed or removed. Subsequently, our group described this phenomenon as fluid-filled structures, larger than 6 mm in diameter, and with irregular fibrin clumps visible ultransonographically 12 to 24 h after initial collapse of aspirated follicles [27]. We also highlighted the possibility of the maintenance of steroidogenic activity by these residual follicles, after measuring estradiol and progesterone concentrations in fluid samples collected from these structures [27]. Interestingly, not much attention has been given to these refilled follicles. Such structures were reported only in a few other studies, and usually as a secondary observation [28]. Hayashi et al. [29] monitored dominant follicles aspirated at various intervals before or after GnRH treatment, but the focus was on CL development. To our knowledge, no specific study has been reported that addressed steroidogenic activity or the biological relevance of residual follicles formed after aspiration.

The aim of the present study was to evaluate the occurrence and steroidogenic activity of residual follicles formed after ultrasound-guided aspiration of the largest follicle in various diameters from cows, with or without exogenous norgestomet.

2. Materials and methods

2.1. Animals and location

This study was performed at Santa Monica Experimental Station, located in Valença, RJ, Brazil. Thirteen pluriparous, nonlactating, Holstein cows with regular cyclic-luteal activity were used. The cows were kept in pasture (*Brachiaria sp*) supplemented with corn silage, with ad libitum access to minerals and water.

2.2. Experimental design

At the first ultrasound examination all cows were in diestrus; therefore they were given sodium cloprostenol (500 μg im; Sincrocio; Ourofino Agronegocio, Sao Paulo, SP, Brazil) and received an ear implant containing norgestomet (3 mg; Crestar; Merck Animal Health, Boxmeer, The Netherlands). The emergence of a new follicular wave was synchronized by an estradiol benzoate treatment (2 mg im; Sincrodiol, Ourofino Agronegocio) immediately after implant insertion. Transrectal ultrasonography of the ovaries (Aquila Vet; Esaote, Genova, Italy) with an 8.0 MHz linear-array transducer was performed every 12 h and the follicles were individually monitored from emergence until aspiration, and further residual follicles were also monitored. The experimental design had two main factors: diameter of the follicle before aspiration and norgestomet treatment. Cows were randomly assembled to four treatment groups and aspiration was performed when the largest follicle reached the expected diameter of deviation (8 mm) or dominance (12 mm), in the presence or not (implant removed at wave emergence) of norgestomet treatment.

Follicle aspiration was performed with the same ultrasound device (Aquila Vet; Esaote), but with a microconvex 7.5 MHz transducer. The ultrasound image was used to guide a 20 ga disposable needle coupled to a Teflon circuit. The ovarian follicle aspiration procedure was performed as previously described [25]. A conventional OPU system was adapted to individually recover follicular fluid from the targeted follicles into 1.5 mL tubes. Follicular fluid was then centrifuged at 600 X *g* for 10 min to remove cells and cumulus-oocyte complexes, and the supernatant stored at −20 °C until radioimmunoassay analysis. After aspiration, collapsed follicular walls were monitored every 12 h and the presence of a fluid-filled antrum was referred to as a residual follicle (RF). The content of RF was collected 36 to 48 h after the first follicle aspiration, using the same procedures.

After follicle aspiration, each cow was assembled into a different group, in a crossover distribution. A total of 18 follicles were sampled in each group, for a total of 72 follicles (6.0 ± 0.9 per cow, ranging from 3 to 12).

2.3. Hormonal assays

Intrafollicular concentrations of estradiol and progesterone were determined by solid-phase I^{125} radioimmunoassay (RIA), using commercial RIA kits (TKE22 Coat-A-Count Estradiol and TKPG5 Coat-A-Count Progesterone, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) at the Endocrinology Laboratory of the College of Veterinary Medicine and Animal Science, São Paulo State University, São Paulo, SP, Brazil. If necessary, samples were diluted 1:10 to 1:1000 to fit the standard curves. Assay sensitivity was 0.02 ng/mL and 10 pg/mL for progesterone and estradiol, respectively. Inter- and intra-assay coefficients of variation were 2.75% and 1.87%, respectively, for progesterone; and 6.0% and 12.7% for estradiol. Quality control was performed according the manufacturer's instructions, using samples of known concentrations of