Available online at www.sciencedirect.com
ScienceDirect
Theriogenology 72 (2009) 1120–1131
Theriogenology
www.theriojournal.com

Follicle population, cumulus mucification, and oocyte chromatin configuration during the periovulatory period in the female dog

K. Reynaud [a,*], C. Viaris de Lesegno [a], M. Chebrout [a], S. Thoumire [a], S. Chastant-Maillard [a,b]

[a] *UMR 1198 INRA/ENVA/CNRS Biologie du Développement et Reproduction, Ecole Nationale Vétérinaire d'Alfort, 7 Avenue du Général de Gaulle, 94704 Maisons-Alfort Cedex, France*

[b] *Reproduction, Ecole Nationale Vétérinaire d'Alfort, 7 Avenue du Général de Gaulle, 94704 Maisons-Alfort Cedex, France*

Received 6 August 2007; received in revised form 5 June 2009; accepted 12 July 2009

Abstract

This study was designed to describe the follicular population present on the canine ovary (*Canis familiaris*) during the preovulatory period and essentially the changes in oocyte size, mucification, and chromatin configuration occurring from before the luteinizing hormone (LH) surge up to postovulation. In a first experiment, ovaries of beagle bitches were collected before (n = 21) or after LH surge but before ovulation (post-LH surge/preovulation stage, n = 24) as determined using hormone (LH, estradiol, progesterone) assays and ultrasonography. All large (>2 mm) follicles were measured and punctured. The numbers of oocytes collected per follicle and the degree of cumulus mucification were recorded. In a second experiment, ovaries were similarly collected before (n = 13) and after the LH surge but before ovulation (n = 11) as well as after ovulation as determined by ultrasonography (n = 9). Chromatin configuration of the oocytes was observed by DNA staining and confocal microscopy. In Experiment 1, before the LH peak, an average of 13.5 ± 0.7 follicles per bitch (total 284 follicles) were detected, and the maximal follicle diameter reached 6.5 mm. Large follicles were observed already in this period of the cycle and as early as when progesterone was still below 0.5 ng/mL. After the LH peak but before ovulation, 11.0 ± 0.7 follicles were present (total 264 follicles). Fully mucified cumulus cells were observed only in follicles larger than 4 mm. Multi-oocytic follicles represented 7% (before LH peak) and 4% (after LH peak) of the follicular population. In Experiment 2, all the oocytes were at the germinal vesicle (GV) stage, but three chromatin configurations could be distinguished: diffuse, partly grouped, and fully grouped chromatin. The proportion of oocytes with fully grouped chromatin increased with the follicular diameter and the time in estrus, the maximum being observed after the LH peak. These results suggest that (1) before LH peak, follicles are already of large diameter, similar to the ones at ovulation; (2) the ability for cumulus mucification is acquired during the late steps of follicular growth; (3) three GV patterns may be observed during the periovulatory period.

Keywords: Bitch; Chromatin; Dog; Folliculogenesis; Oocyte; Periovulatory

1. Introduction

The bitch differs from other mammalian females by a specific folliculogenesis and by a specific endocrine pattern associated with the periovulatory period. The canine folliculogenesis is characterized by a long interestrus period (6 mo) and a high proportion of

* Corresponding author. Tel.: +33 1 43 96 70 97; fax: +33 1 43 96 71 30.
E-mail address: kreynaud@vet-alfort.fr (K. Reynaud).

0093-691X/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2009.07.006

multi-oocytic follicles (5% to 14% [1]), one follicle containing up to 17 oocytes [2]. Even though the ability of the multi-oocytic follicles to reach the final stages of growth and to ovulate has been suggested [3,4], it has never been quantified. More generally, little is known about the follicular population in the proestrous/estrous ovaries.

The endocrinology of the periovulatory period is also unusual in the bitch. After a rise and decrease of estradiol, luteinizing hormone (LH) peak is followed 36 to 50 h later by ovulation, but these two events occur in presence of an already significantly increased progesterone serum concentration due to a preovulatory luteinization of the follicles [5,6]. The physiologic role of this preovulatory rise in progesterone is still unknown, as well as the factor responsible for its induction. In vitro studies have demonstrated that progesterone increases acrosomal reaction rates in the dog [7], but the importance of progesterone for the oocyte, intrafollicular or oviductal, remains unknown. If the preovulatory life of the oocyte takes place in a singular endocrine pattern, the postovulatory progression of the canine oocyte is also very different from the classic pattern. Ovulation releases oocytes at the germinal vesicle (GV) stage, meiotic completion occurring in the oviduct 56 to 72 h after ovulation [4,8]. In other mammalian species, the relationship between the oocyte and the surrounding granulosa cells is of key importance for the control of meiosis resumption. This relationship is strongly modified after LH peak by the mucification of the cumulus cells.

Even if metaphase II stages appear only during the oviductal transport in the bitch, it is questionable whether the final follicular growth is associated with cumulus-oocyte complex (COC) modifications, especially granulosa mucification and oocyte nuclear maturation. In fact, one of our previous studies suggested that the GV is modified during late follicular growth [9].

In this study, our objectives were to describe the follicular population present in the canine ovary during pre-LH and post-LH/preovulatory phases and to characterize the association between follicular size and cumulus expansion (Experiment 1) and to study the link between the follicular size and the chromatin configuration of the GV (Experiment 2).

2. Materials and methods

Unless otherwise indicated, all chemicals were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France).

2.1. Monitoring of ovarian cycles

Ovarian cycles of bitches (*Canis familiaris*) were followed weekly by vaginal smears coupled to Harris-Shorr staining. Heat initiation (“proestrus” smears) was considered when percentage of superficial (= cornified) and large intermediate cells started to increase [10]. Eosinophilic index was also determined. During proestrus, serum progesterone was assayed every other day after blood collection performed at the cephalic vein or jugular vein (Elecsys enhanced chemiluminescence kit; Roche Diagnostics, Meylan, France; intra-assay and interassay coefficients of variation $<2\%$ [4]). When more than 80% of the vaginal cells were superficial, serum progesterone was assayed daily. When progesterone levels reached 0.5 ng/mL, blood was collected three times a day, and serum was stored at $-20\,^{\circ}C$ until assayed, a posteriori, for LH concentrations (ELISA method; LH Detect kit; INRA, Nouzilly, France [11]). On average, dogs were collected 7 d before the LH surge.

When progesterone concentration increased above 2 ng/mL, ovarian transabdominal ultrasonography was performed two to three times a day to follow follicular growth and to monitor the occurrence of ovulation [4] (HDI 3500 ultrasonograph, probe 7.5 MHz, resolution 0.19 mm; ATL, Philips Systèmes Médicaux, Suresnes, France).

Serum estradiol levels were assayed at the day of surgery (Elecsys kit; Roche Diagnostics; inter-assay and intraassay coefficients of variation were 1.2% and 6.5%, respectively).

2.2. Definition of follicular phases

Bitches at the pre-LH stage had an estrus vaginal smear, no LH rise was detectable on serum samples, and follicles larger than 2 mm were visible at ultrasonography. At post-LH/preovulatory stage, bitches displayed an estrus vaginal smear, LH had at least begun to rise (>1 ng/mL), and antral follicles were still visible at ultrasonography (ovulation did not start). For bitches at the postovulation stage, ovulation was followed as previously described [4]: ultrasonography was performed three times per day, and ovulation was characterized by the disappearance of anechogenic structures and the appearance of periovarian liquid. Ovulation time was defined as the mean time between the last ultrasonography with all follicles visible and the one where there was a significant change or complete disappearance of follicles. Bitches were neutered at different time after ovulation, between 6 h and 44 h after ovulation.