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Short-term storage of canine preantral ovarian follicles using a powdered coconut water (ACP®)-based medium

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Abstract

The objective was to investigate the use of powdered coconut water (ACP®)-based medium for short-term preservation of canine preantral follicles. Pairs of ovaries from mongrel bitches (n = 9) were divided into fragments. One ovarian fragment, treated as a fresh control, was immediately fixed for histological analysis, whereas the other six ovarian fragments were stored either in phosphate-buffered saline (PBS; control group) or ACP medium in isothermal Styrofoam boxes containing biological ice packs. The boxes were sealed and opened only after 12, 24, or 36 h. After opening each box, the ovarian fragments were submitted to histological analysis. In total, 12,302 preantral follicles were evaluated, with 64.5% primordial, 33.3% primary, and 2.3% secondary follicles. There were multiple oocytes in 1.3% of the follicles analyzed. At 24 h, ACP was more efficient in preserving follicular morphology than PBS ($P < 0.05$). Compared with the fresh control group, a significant reduction in the percentage of morphologically normal ovarian follicles was observed for PBS, starting at 24 h; however, the decline started only at 36 h for the ACP medium. During the experiment, the temperature inside the isothermal boxes increased from 3 to 9 °C ($P < 0.05$), despite a constant room temperature. In conclusion, powdered coconut water (ACP) was an appropriate medium for short-term storage of canine preantral ovarian follicles.

Keywords: Dog; Ovary; Ovarian follicle; Preservation; *Cocos nucifera*

1. Introduction

The preservation of canine female gametes has been neglected for a long time. The development of assisted reproductive techniques using canine ovarian follicles is important, as it allows oocytes from genetically valuable bitches, including those with reproductive pathology, to be preserved [1]. Oocyte retrieval is important not only for the breeding of domestic dogs, but also as a rich source of genetic material that can be stored in genetic banks, aiding in preservation of endangered species [2].

Since the interval from collection of ovaries to the beginning of *in vitro* culture may be prolonged, maintenance of viability is a critical issue [3]. Preservation of female gametes can be better achieved by storing pieces of ovarian tissue containing numerous immature small oocytes enclosed within preantral follicles (PFs) [4]. The PFs represent more than 90% of the ovarian follicular population in dogs [5], and include: primordial follicles that contain small oocytes (approximately 25 μm in diameter), with a single

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granulosa cell layer but no zona pellucida (ZP); primary or early PFs that contain small, pale oocytes (78 ± 15 μm) with a distinctive ZP; and secondary or advanced PFs that contain fully grown oocytes (>100 μm), with dark cytoplasmic lipid [6].

There are very few studies on the general aspects of manipulation of canine PFs. Durrant et al. [7] reported the isolation and characterization of canine PFs and suggested the use of enzymatic digestion in association with mechanical isolation. Bolamba et al. [8] reported that after using collagenase and DNase for isolation, PFs could be cultured in vitro, and the oocytes obtained were capable of reinitiating meiotic divisions. Regarding preservation, Lopes et al [3] recently reported that preservation of canine PFs might be better accomplished through low-temperature (4 or 20 °C) storage in minimal essential medium (MEM), which ensured maintenance of their morphology and viability for up to 12 h. In other studies, although not focused specifically on PFs, canine ovaries were successfully stored in physiological saline (0.9% NaCl) [9] or phosphate-buffered saline (PBS) [10–12] for short intervals, and the oocytes subsequently used for IVM.

For goats [13], sheep [14], and cattle [15], a coconut (*Cocos nucifera*) water-based medium has been successfully used for preserving PFs. Since coconuts are not universally available, powdered coconut water (ACP®, ACP Biotecnologia®, Fortaleza, CE, Brazil) has been developed. This powder can be easily stored and transported, and after reconstitution, its biochemical characteristics were very similar to those of fresh coconut water. Although ACP was a suitable alternative for preserving canine semen [16], the effects of ACP solution on canine female gametes are not known. The objective of the present study was to investigate the use of PBS- and ACP-based media for short-term storage of canine PFs.

2. Materials and methods

2.1. Animal care and use

Experimental protocols and animal care were approved by the research committee of the Universidade Federal Rural do Semi-Árido (UFERSA), Mossoró, Brazil. Nine nonpregnant mongrel bitches, aged 8 to 36 mo and weighing 10 to 15 kg, with no abnormalities detected on physical, clinical, and laboratory examinations (blood count), and no history of reproductive problems, were used. These bitches were obtained from private owners.

2.2. Collection and preparation of the ovaries

Pairs of ovaries from mongrel bitches (n = 9) were removed aseptically following ovariectomy at the UFERSA veterinary hospital. The reproductive system was completely examined to rule out any pathology. The ovaries were trimmed for removal of the *bursa ovarica* and the corpora lutea were excised with a scalpel.

2.3. Experimental design

The experimental design is shown (Fig. 1). Each pair of ovaries was washed twice in sterile saline

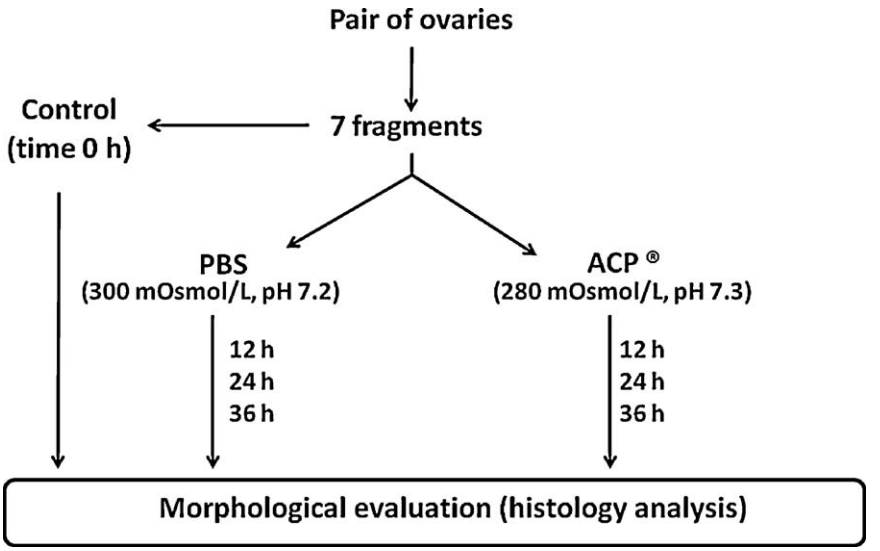

Fig. 1. Experimental designs: pairs of canine ovaries (n = 9) were divided into seven fragments, with one selected as fresh control (time 0 h), whereas the others were incubated in PBS or ACP® for 12, 24, or 36 h. Fragments were subsequently subjected to morphological analysis.