

Recovery of *In Vivo* Matured Oocytes from a Bitch with Hydrometra

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Abstract : One year old mixed-breed bitch was examined to retrieve *in vivo* matured oocytes. Laparotomy was performed 72 hr after ovulation determined by serum progesterone concentration, and abnormally enlarged left uterus horn was found. Both ovaries had eight corpus lutea, and a total 16 *in vivo* matured oocytes having perivitelline space within 25 μ m, polar body, and metaphase II nucleus were recovered by flushing oviducts. This is the first study to confirm *in vivo* maturation of oocytes from a bitch with hydrometra, which suggests that oocytes recovered from canids with reproductive disease could be valuable sources for assisted reproductive technologies.

Key words : dog, hydrometra, oocyte maturation, oocyte recovery.

Introduction

Dogs are one of the most important experimental animals for understanding human diseases due to the genetic and physiological similarities (11). However, assisted reproductive technologies (ART) including *in vitro* oocyte maturation, *in vitro* embryo culture, and intracytoplasmic sperm injection for application of dogs as human disease model is not yet established compared to other domestic animals such as cows and pigs (1). A key obstacle is the limitation to obtain dog oocytes due to unique reproductive characteristics including monoestrus, ovulation at germinal vesicle stage, and preovulatory luteinization (2). Thus, studies on oocytes recovery from female dogs are necessary for the advance of canine ART.

Canine oocytes have been commonly obtained from dogs undergoing ovariohysterectomy at animal hospitals. Surgical removal of ovaries and uterus is indicated for prevention or treatment of reproductive disease that accumulate abnormal fluid within uterus lumen. Although hydrometra, accumulation of a sterile and watery secretion in the uterus, is often observed incidentally (5), there has been no report about *in vivo* oocyte maturation in a bitch with hydrometra. Therefore, we aimed to report the first case of recover *in vivo* matured oocytes from a bitch having unilateral hydrometra.

Case

One year old mixed large breed, nulliparous female dog was cared in indoor facilities to retrieve *in vivo* matured oocytes following a standard protocol by the Committee for Accreditation of Laboratory Animal Care and according to

the Guideline for the Care and Use of Laboratory Animals of Seoul National University (approval number is SNU-141121-6). All chemicals were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA) unless otherwise specified.

From the first day of spontaneous proestrus determined by vaginal bleeding, blood was collected from cephalic vein to analyze serum progesterone concentrations using an Immulite 1000 (Siemens Healthcare Diagnostics Inc., Flanders, NJ) (6). During the late estrus to early diestrus, serum progesterone concentration was spontaneously increased by 3.04, 5.24, 5.01, 15.0 and 20.5 ng/ml. Ovulation day was determined when the day serum progesterone was at 5.01 ng/ml (6). About 72 hr after the ovulation, the dog was anesthetized with 4.0 mg/kg of ketamine (Yuhan, Seoul, Korea) and 0.9 mg/kg of xylazine (Rompun[®], Bayer Korea, Seoul, Korea), and the anesthesia was maintained with isoflurane (Hana Pharm. Co., Ltd., Seoul, Korea). After an aseptic laparotomy, both ovaries and uteruses were exteriorized from the abdomen. Although both ovaries had each eight corpus lutea with similar morphology (Fig 1B, C), abnormally enlarged left uterus horn was found while the other was normal (Fig 1A). A total 16 *in vivo* oocytes were flushed out from oviducts using Hepes buffered tissue culture medium-199 (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum, 2 mM NaHCO₃ and 5 mg/ml bovine serum albumin (13), and maturation status was assessed by their perivitelline space (PVS) and polar body (PB) extrusion under a microscope (Nikon-Narishige, Tokyo, Japan). Oocyte without first PB was regarded as immature, and among the others, oocytes with PVS < 25 μ m and > 25 μ m were classified as mature and aging, respectively. It was confirmed under microscope that all oocytes had normal round shape with one PB, PVS within 25 μ m and similar diameter within normal range which was referred to matured status (Fig 1D) (4,8). Nucleus at metaphase II stage was also confirmed

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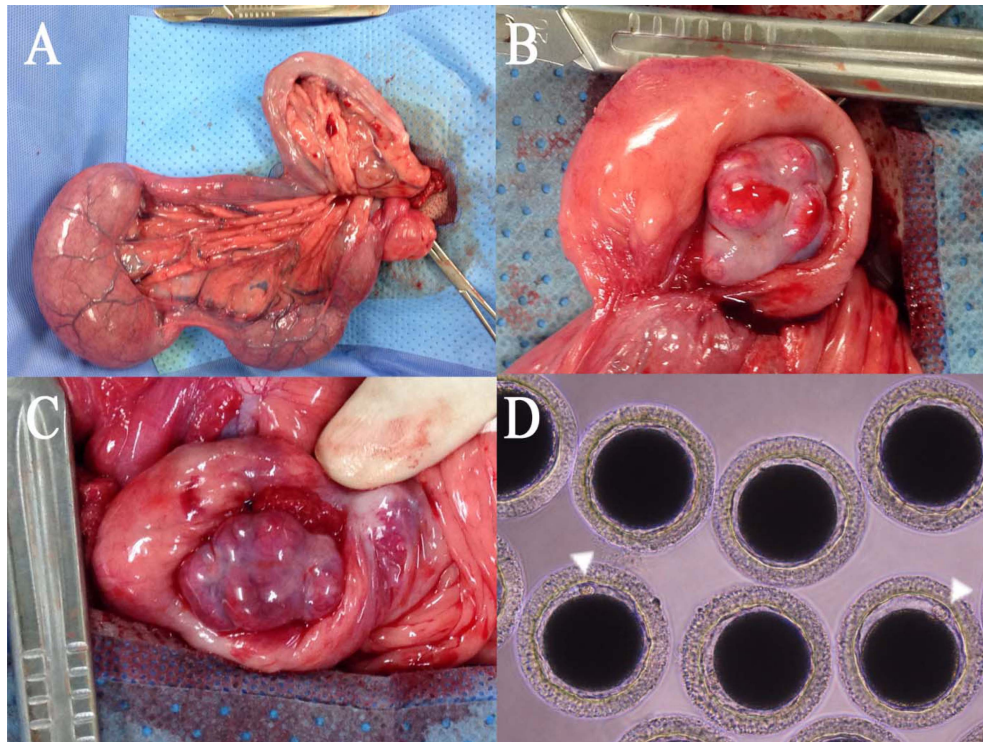


Fig 1. Uterine horns, ovaries and oocytes of a 1-year-old mixed breed bitch with unilateral hydrometra. (A) Abnormally enlarged left uterus horn was found while the other was normal. (B, C) Normal ovarian morphology having corpus lutea in the left (B), and right (C) ovarian bursa. (D) All oocytes recovered from both ovaries had a polar body (white arrow heads) and perivitelline space within 25 μm (original magnification $\times 200$).

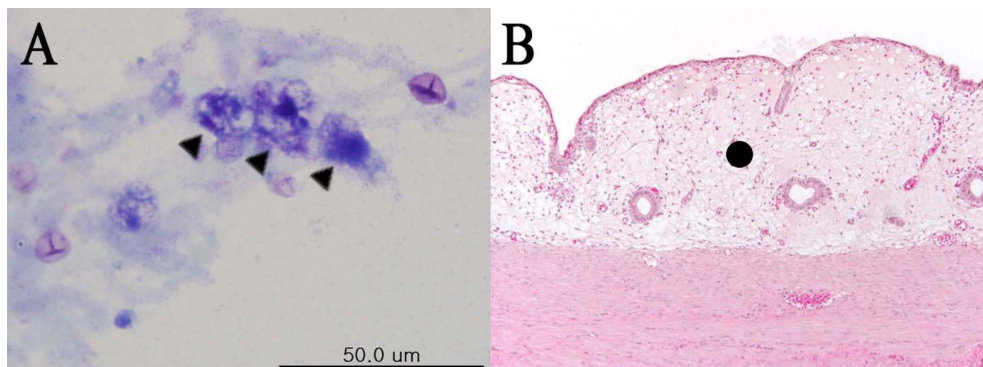


Fig 2. Cytological examinations of the fluid contents and histopathological examinations of the uterine tissue derived from uterus with hydrometra. (A) Amorphous basophilic proteinaceous materials and several macrophages (black arrow heads) were observed by cytological examination of the clear fluid contents derived from the enlarged left uterus horn. (B) Accumulation of eosinophilic amorphous materials (black round mark) in the endometrium was seen by the histological examination.

under ultraviolet light after bisbenzimidazole (Hoechst 33342) staining (10). After ovariectomy, cytological examination using the clear fluid content derived from the enlarged left uterus horn and histopathological examination of the formalin-fixed enlarged uterus tissue were performed. Amorphous basophilic proteinaceous materials and several macrophages were observed by cytological examination (Fig 2A), and partial simple hemorrhage or accumulation of eosinophilic amorphous materials in the endometrium was revealed by histopathological examination (Fig 2B). As antimicrobial therapy, 10,000 IU/kg Benzathine penicillin G and 15,000 IU/kg procaine penicillin G (IM, PPS[®], Daesung Micro-

biological Labs Co. Ltd.) was given to the bitch.

Discussion

The incidence of hydrometra has not been obvious because it is generally found incidentally during ovariectomy or examinations for unrelated conditions (5). No systemic clinical signs typically are observed in a bitch with hydrometra, which is comparable with pyometra causing varying degrees of systemic illness (14). Fransson *et al.* (3) investigated various uterine conditions of 60 female dogs regardless of estrus cycle, and revealed that only 3 dogs (5.0%) had

hydrometra/mucometra. Due to the difficulty of observing clinical signs of hydrometra and insufficient number of cases, a relation between hydrometra and estrus cycle has not yet been analyzed. Retrospective analysis of experimental dogs performed laparotomy in our laboratory revealed that this was the only case of a total 515 bitches in estrus stage. Therefore, incidence rate of hydrometra among bitches in estrus stage was less than 0.2%.

There have been reports of concurrent diseases such as inguinal herniation (15), persistent Mullerian duct syndrome and sertoli cell tumor (9), and cystic endometrial hyperplasia (CEH) (5) with hydrometra. Although the accurate mechanisms that cause hydrometra have not been elucidated, it is suggested that it might result from CEH, cysts that develop from endometrium (14). Pathogenesis of CEH includes secretion of progesterone during diestrus which results in increase of secretory activity of endometrial glands, decrease of myometrial contractility, and functional closure of the cervix (5). These changes allow fluid accumulation within the uterine lumen (5). However, the dog in our case was in estrus and there was no histopathological evidence of CEH. The occurrence of hydrometra without other pathological changes of uterus is not a frequent case (12).

Average number of corpora lutea of a mixed breed dog aged 1-7 yr and weighing 20-35 kg was reported 12.4 ± 0.8 (8), and there was no significant difference between nullipara (11.6 ± 10.8) and multipara (12.5 ± 0.2) (7). A total 16 corpora lutea was observed from the dog with hydrometra, and this implies that hydrometra during estrus stage did not influence ovarian follicle development. In addition, it has been reported that average $93.8 \pm 1.4\%$ of oocytes were recovered from a large mixed breed dog (7). The oocyte recovery rate was not influenced by parity of oocyte donor dog (7). The 100% of *in vivo* matured oocytes recovery in our case may indicate that pathological status of the uterus does not influence both hormonal regulation in the oviduct to mature the oocytes and anatomical structure within the oviduct.

Conclusions

In conclusion, this is the first study to report *in vivo* matured oocytes retrieved from a bitch with hydrometra. Also, successful recovery of *in vivo* matured oocytes from a bitch with hydrometra suggested that oocytes recovered from canids with reproductive diseases could be valuable sources for ARTs such as somatic cell nuclear transfer or intracytoplasmic sperm injection.

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자궁수종이 있는 개에서 체내 성숙 난자의 회수

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서울대학교 수의학과 수의산과 및 생명공학, *충청북도 청주시 (주)옵티팜

요 약 : 한살령의 잡종 암개에서 체내 성숙된 난자를 회수하기 위한 실험이 진행되었다. 혈청 프로게스테론 농도를 이용하여 배란 후 약 72시간 뒤에 개복술을 시행하였고, 비정상적으로 확장된 왼쪽 자궁각이 발견되었다. 양쪽 난소는 각각 여덟 개의 황체를 가지고 있었고, 배지를 난관에 관류시켜서 총 16개의 난자를 회수하였다. 모든 난자는 극체가 돌출되어 있었고, 25 μm 미만의 난황주위 공간과 제 2중기의 핵을 가지고 있었다. 본 증례는 자궁수종을 가진 암개에서 체내 성숙된 난자를 확인한 최초의 연구이며, 생식기 관련 질병을 가진 개과 동물에서 회수된 난자가 보조 생식 기술에 사용될 수 있음을 의미한다.

주요어 : 개, 자궁수종, 난자 성숙, 난자 회수