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SHORT COMMUNICATION

Revisiting the fate of the unfertilised sheep ovum

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Early researchers in reproduction hypothesised that unfertilised mammalian ova undergo degeneration and fragmentation after ovulation and are subsequently absorbed in the oviducts or uterus. Later experimental evidence was provided (Bindon 1969:183–184) showing that in ewes, 71% of unfertilised ova were retained in the uterus for up to 15 days after ovulation. He postulated that such ova would be expelled from the uterus when the cervix dilates at the next oestrus. In this report we highlight the incidental discovery of two ovum-like structures in the urine of a two-year-old Karakul ewe during a urinalysis practical session for first year Bachelor of Veterinary Medicine students. This finding aligns with the hypothesis that unfertilised ova in ewes may be retained within the uterus and is consistent with their subsequent release into the genitourinary tract without significant degeneration or fragmentation.

Urine was collected from the ewe by the midstream clean-catch method after induction of urine voiding by transient apnoea (Jones et al. 2012:325–360). Microscopic examination of urine was performed following spinning of the sample in a centrifuge at 450 × *g* for five minutes. After decanting the supernatant, a slide of the sediment was prepared, stained with Giemsa stain and shielded with a coverslip. The sediment was then viewed under a brightfield microscope at x100 and x 400 magnification.

Microscopic identification of ova involves both morphology and morphometry. The identification of the structures as ova in the present experiment was based on the following morphological and morphometric criteria:

1. Integrity of the corona radiata
2. Integrity of the zona pellucida
3. Integrity of the nucleus
4. Dimensions of ova and zona pellucida as measured using the software program ImageJ (National Institutes of Health 2026: https://imagej.nih.gov/).

The results are shown in Figure 1. In Figure 1a, the ovum still retained its round shape (116 µm in diameter) and the nucleus was clearly visible. Both the zona pellucida (32 µm) and corona radiata were intact. The ovum in Figure 1b was shrunken in volume (75 µm in diameter), had no clear nucleus and cumulus cells of the corona radiata were showing signs of dehiscence. Similarly, in the study of Bindon (1969), the sizes of the unfertilised ova (98–153 µm) and their zonae pellucidae (28–76 µm) agreed well with those of freshly ovulated ova. Likewise, minor changes occurring with ageing included a progressive reduction of cytoplasmic volume, karyorrhexis and irregularities in the vitelline membrane. Follicular dominance is weak in sheep (Driancourt et al. 1991). If an ovulatory luteinising hormone surge is triggered by the F1 follicle while F2 or F3 follicles are in slightly different stages of maturity, all can be ovulated simultaneously. Sheep therefore ovulate between one and three ova per oestrus cycle. The two ova observed in the present study were likely from the same ovulatory cycle but varying in ageing rates.

Other structures which may resemble ova in a urine sample collected by free-catch include pollen grains from environmental contamination and coccidial oocysts from faecal contamination. Coccidial oocysts in sheep belong predominantly to the genus Eimeria and are distinctly smaller to ova, ranging from 20–55 µm in size as illustrated in Figure 2a from our urinalysis archives. Urine collected from female animals is particularly susceptible to faecal contamination owing to the proximity of the anus to the vulva. Depending on plant species, pollen grains may have similar shape and size (5–200 µm) to mammalian ova. However, their outer shell is acellular, rigid and virtually indestructible as depicted in Figure 2b from our archives. In addition, pollen grains typically contain at least two nuclei; a larger vegetative nucleus and a smaller generative nucleus. It is also possible for non-ovum cells from the urogenital system to form clusters in urine. However, the identical architecture of the two structures observed in the present experiment is more suggestive of an organised biological morphogenesis compared to incidental aggregation.

Following ovulation, the time for entry of fertilised ova into the uterus in the ewe is between 66 and 72 hours (Holst 1974). The blastocyst secretes Interferon-tau (IFNT), also known as ovine trophoblast protein-1 (oTP-1), from its trophectoderm beginning around day 10 of gestation and peaking at day 16–18 (Martal et al. 1979; Bazer et al. 1991). IFNT acts as an anti-luteolytic factor by inhibiting the uterine endometrium from secreting Prostaglandin F2α. Beyond day 10, fertilised ova send the INFT signal to the ovaries to institute the corpus luteum verum. In contrast, unfertilised ova do not produce INFT therefore they would lose their histotrophic support with onset of luteolysis. These ova are likely to be flushed out of the uterus in the next oestrus when the cervix dilates, and uterine secretions increase under oestrogen dominance (Bindon 1969). The only phase of the Oestrus Cycle (Proestrus, Oestrus, Metestrus, Dioestrus) when the cervix opens in animals is during oestrus, to allow entry of sperm cells. Our hypothesis is that the ova observed in the present experiment were retained in the uterus. From there they were released into the vagina when the cervix became

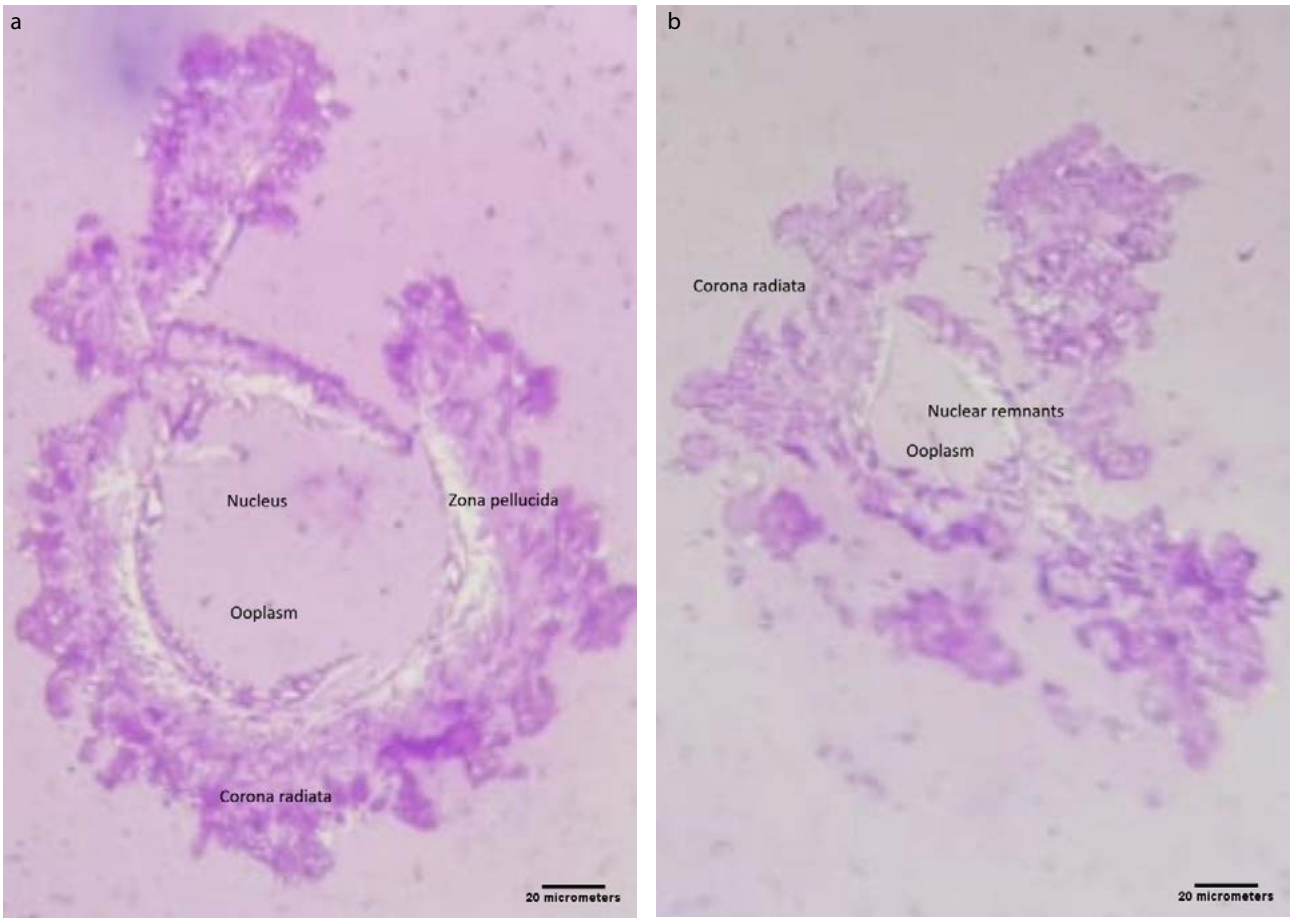

Figure 1: (a) Ovum 1 showing an oocyte still enveloped in a relatively intact vitelline membrane. The zona pellucida is visible as a clear halo surrounding the vitelline membrane. The last layer shows cumulus cells of the corona radiata enclosing the oocyte to form a cumulus-oocyte complex. (b) Ovum 2 with a pyknotic nucleus and shrunken ooplasm surrounded by a disintegrating zona pellucida and corona radiata. Giemsa stain.

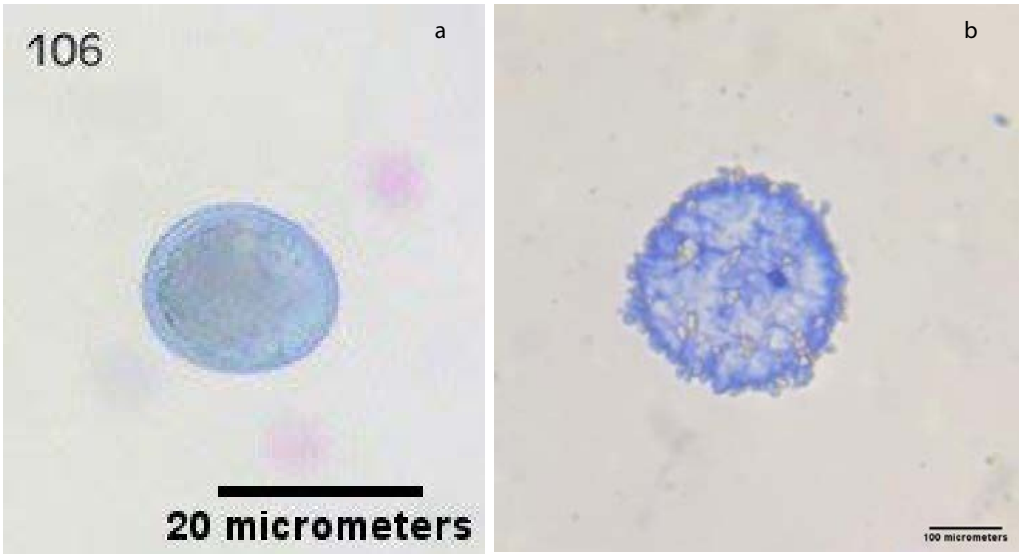

Figure 2: (a) Coccidial oocyst observed in the urine of a crossbreed female lamb (Tag No. 106) as a result of faecal contamination in free-catch urine. (b) Pollen grain observed in a smear from a vaginal epithelial swab due to environmental contamination of sample. Giemsa stain. (Source: Authors' archives)

patent during oestrus. Downstream inside the vestibule, the ova got mixed with incoming urine from the urinary bladder during micturition. Why ewes retain unfertilised ova in the uterus until the next ovulatory cycle, and whether the ova remain viable or not during this period, are intriguing questions still to be answered. Understanding the factors that prolong the viability of ova after ovulation is essential in assisted reproductive technologies (Smuts et al. 2025:39–48). If the retained ova in ewes are indeed viable, the present observations would find application in multiple ovulation and embryo transfer (MOET) and laparoscopic artificial insemination (LAI). The post-ovulatory lifespan of the ovum is critical in both technologies.

Conflict of interest

The authors declare they have no conflicts of interest that are directly or indirectly related to the research.

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Ethical approval

The author/s declare that this submission is in accordance with the principles laid down by the Responsible Research Publication Position Statements as developed at the 2nd World Conference on Research Integrity in Singapore, 2010.

Prior to the commencement of the study, ethical approval was obtained from the following ethical review board: *University of Namibia Ethics Committee, Ethical Clearance Reference Number NEC0021*.

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