

# Breeding Management

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A 2.5-year old, intact female Scottish terrier was presented on a Friday morning for breeding management. The dog had not been bred previously.

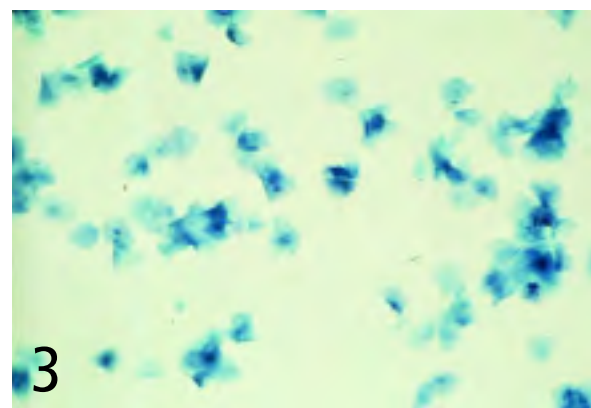
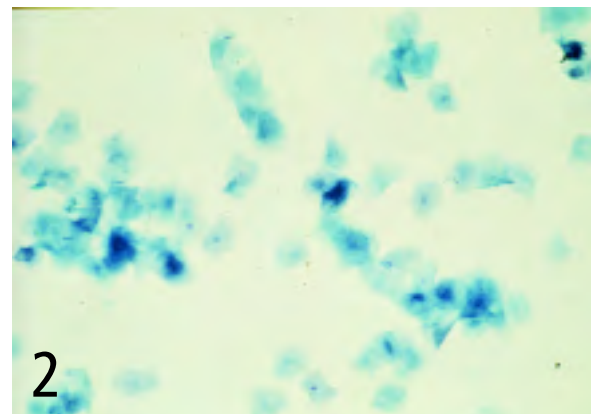
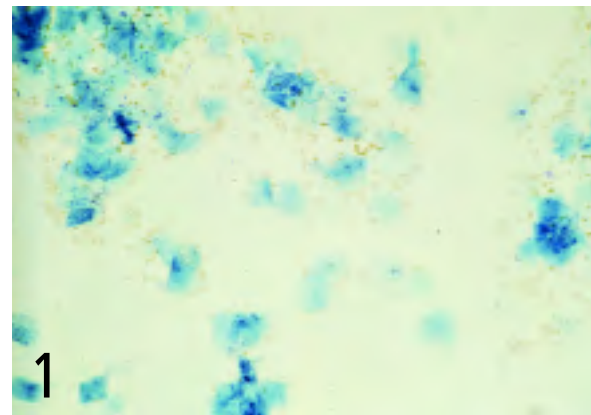
**History.** According to the owner, estrous cycles had been normal. The owner had first noticed a bloody vaginal discharge 2 days earlier and presumed the bitch was in early proestrus. He wished to breed the dog with semen he had frozen from a champion sire, now deceased; he had two doses of semen from that sire. The semen was packaged in 0.5-ml straws at a concentration of 200 million/ml, and postthaw motility was approximately 20%.

**Diagnostic Evaluation.** On the day of presentation, a swab was taken of the bitch's vaginal epithelium to obtain a sample for cytologic evaluation. Approximately 90% of the vaginal cells were cornified superficial cells (**Figure 1**). A blood sample was also obtained to determine the progesterone concentration, which was found to be 2.04 ng/ml (6.5 nmol/ml). The bitch could not be brought back to the clinic until 3 days later (Monday), at which time 100% of the vaginal cells were superficial, and the slide had a clear background (**Figure 2**); progesterone was 4.06 ng/ml (12.9 nmol/ml). Two days later (Wednesday, 5 days after initial presentation), cytologic evaluation still showed 100% superficial cells with a clear background (**Figure 3**); progesterone concentration was 6.07 ng/ml (19.3 nmol/ml).

**ASK YOURSELF...**

What would be the best day for insemination with the frozen semen?

- A. Wednesday
- B. Thursday
- C. Friday
- D. Saturday
- E. Sunday



continues

## INSIGHTS FROM CLINICAL CASES . DISCUSSION

### Correct Answer: D Insemination was performed on Saturday.

Proestrus averages 9 days in duration, although variation is common. A range of 3 to 17 days is considered normal. During proestrus, a serosanguineous vaginal discharge is commonly observed. The percentage of superficial cornified epithelial cells observed on a vaginal cytologic evaluation usually increases approximately 10% per day, reaching 100% superficial cells by the beginning of estrus. A common practice is to obtain vaginal cytologic samples until they contain 60% to 70% superficial cells, at which time plasma progesterone samples are obtained every other day. In the present case, the owner thought the dog had just begun to exhibit signs of proestrus, but cytologic evaluation indicated that she was in late proestrus/early estrus and progesterone was 2 ng/ml, indicative of the initial rise. It is not uncommon for clients to misjudge the stage of their bitch's cycle.

Progesterone values during proestrus are less than 2 ng/ml. An initial increase in serum progesterone concentration to 2 to 3 ng/ml coincides with the LH surge in early estrus. A subsequent progesterone sample can confirm that it is truly the initial rise. On the day of presentation, the bitch had a progesterone value of 2.04 ng/ml, seemingly indicative of the initial increase in progesterone associated with the LH surge. Additional samples could not be obtained until 3 days later; obtaining them sooner would have been useful to determine whether the 2.04 ng/ml observed on Friday was indeed the initial increase in progesterone.

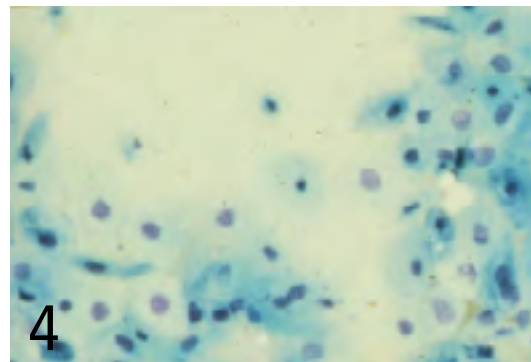
Estrus averages 9 days in length but can range from 3 to 21 days. The appearance of a vaginal cytologic specimen remains constant during estrus, with a clear background and 100% superficial epithelial cells. Additional information on the stage of the estrous cycle can be obtained by the use of vaginoscopy. Visual changes in the vaginal mucosa can be used to stage the estrous cycle, estimate the fertile period, and assist in timing breeding. Ovulation is usually associated with a serum

progesterone concentration of 5 to 8 ng/ml and is expected 2 days after the LH surge. An additional 2 to 3 days is then required to complete maturation of the oocytes before fertilization can take place. Therefore, the fertile period is 4 to 7 days after the LH surge. In the present case, 3 days after the presumed initial rise, progesterone was 4.06 ng/ml and progesterone did not reach a concentration of 6.07 ng/ml, suggestive of ovulation, until 2 days later (5 days after progesterone had reached 2.04 ng/ml).

While fresh semen can be inseminated earlier in estrus and still achieve acceptable results, frozen semen has a diminished "lifespan," normally considered to be about 24 hours. Therefore, insemination with frozen semen should be withheld until the oocytes have matured and are capable of being fertilized. Insemination with frozen semen is recommended 3 days after progesterone reaches 5 to 8 ng/ml. Progesterone values during the fertile period, at the time of insemination with frozen semen, are usually expected to be in the 12- to 20-ng/ml range.

Vaginal cells abruptly return to being noncornified at the end of estrus. The day that the vaginal cytology is observed to be 40% to 60% noncornified is considered day 1 of diestrus. Day 1 usually occurs about 8 days after the LH surge, or approximately 6 days after ovulation. Vaginal cells in this bitch continued to be fully cornified until 15 days after the initial increase in progesterone (2 ng/ml), or 12 days after assumed ovulation (progesterone concentration of 6 ng/ml), not decreasing to 40% superficial cells until more than 2 weeks after the initial visit (**Figure 4**).

Unfortunately, the bitch did not appear to have a normal estrous cycle. Beginning progesterone testing earlier in the cycle and obtaining samples at least every other day until ovulation would have helped to map her cycle. Testing of LH (either mail-out or in-house, if available) would have provided additional information on the time of ovulation. Vaginoscopy may have been useful to stage the cycle and to time breeding. Quantitative progesterone obtained on the day insemination with frozen semen was



planned may also have been useful to confirm ovulation, but was declined by the owner. The interval from the initial increase in progesterone to ovulation in this dog was 5 days instead of 2, the time from initial rise in progesterone to day 1 of diestrus was 15 days instead of 8, and the time from assumed ovulation to day 1 was 12 days instead of 6. When the prospective estimation of the fertile period does not correspond to retrospective estimation based on day 1 of diestrus, a poor prognosis for fertility is expected. In this case, the owner was advised to use an alternate source of semen in this bitch of unproven fertility because of the finite number of available doses from the desired male, although the suggestion was declined. Fresh semen with a greater longevity would have been preferable to frozen semen in this case. ■

See Aids & Resources, back page, for references, contacts, and appendices.

#### TAKE-HOME MESSAGES

- The fertile period is from 4 to 7 days after the LH surge or the initial increase in progesterone.
- If only a single breeding can be done, the bitch should be inseminated 3 days after progesterone reaches 5 to 6 ng/ml.
- It is not uncommon for clients to misjudge the stage of their bitch's cycle.
- A series of progesterone tests provides more valuable information than a single sample.