Proceedings of the Society for Theriogenology 2013 Annual Conference

Aug. 7-10, 2013 – Louisville, KY, USA

Next SFT Meeting:

Aug. 6-9, 2014 – Portland, OR, USA

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Retrospective evaluation of breeding management data and breeding type and correlation with successful breeding of bitches
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Abstract
Data were retrieved from medical records for breeding management of 576 dogs of 88 breeds. Pregnancy rate was highest with natural service and with surgical insemination with either chilled or frozen semen. Pregnancy rate was lower for vaginal insemination with any type of semen. Age of the bitch at the time of breeding was associated overall with success, with younger bitches (4.4 ± 1.7 years) more likely to be successfully bred than older bitches (5.2 ± 1.7 years) (p=0.04). For all semen types, litter size was significantly greater with surgical insemination than with vaginal insemination (p=0.0004). Serum progesterone concentration on the day of the luteinizing hormone (LH) surge was 3.1 ± 1.0 ng/ml, with a range of 1.3 to 6.2 ng/ml (n = 63). In general, a lower progesterone concentration was associated with success when using fresh semen and a higher progesterone concentration was associated with success when using frozen semen. This was a directed study project for a senior veterinary student. Collecting these data and talking through how the cases had been managed and significance of the findings were excellent learning opportunities for this student.

Keywords: Breeding management, progesterone, LH, litter size, artificial insemination

Introduction
Several factors unique to bitches make breeding management more challenging than in other species. Because we have limited access to the ovaries physically or visually, we must infer what is happening on the ovaries by diagnostic testing. Bitches ovulate an immature oocyte, which must undergo one more meiotic division before it can be fertilized. For this reason, optimal breeding is offset from ovulation day. Many of the diagnostic tests used approximate the day of ovulation but only endocrine assays provide the general practitioner with any accuracy in prospectively determining ovulation day.

Luteinizing hormone is the stimulus for ovulation in bitches. It is secreted pulsatilely, with a large, single peak. Ovulation occurs 36 to 50 hours later.1 Direct measurement of LH is the most definitive diagnostic test available. Unfortunately, commercial LH assays are not readily available and turn-around time makes their use impractical for clinical cases. In-house LH assays are intermittently available. In-house LH assays do not provide quantitative measurement of serum LH concentration but only differentiate low from high (less than or greater than 1 ng/ml [3.1 nmol/L]).

Progesterone is the hormone assay most commonly used for assessment of ovulation date in bitches. Because bitches undergo preovulatory luteinization, rise in serum progesterone concentrations can be used to infer date of the LH peak and prospectively predict ovulation day. In general, progesterone concentration on the day of the LH peak will be about 2.0 ng/ml and on ovulation day from 4 to 10 ng/ml.2 Some veterinarians prefer not to look at individual values but instead watch for a sudden increase in progesterone concentration by 3 ng/ml or more from one day to the next, denoting that as ovulation day. Some denote the first day progesterone concentration is 5 ng/ml or greater as ovulation day.3 Finally, some veterinarians look for an absolute value of progesterone at the time of breeding, with anecdotal reports of breeding optimized when progesterone is greater than 10 ng/ml or when progesterone is 15 ng/ml. The authors are unaware of scientific studies documenting clinical significance of the latter method.

Type of semen (fresh, chilled, or frozen) and insemination technique (natural service, vaginal insemination, or transcervical or surgical intrauterine insemination) impact pregnancy rate and litter size in bitches, as does age of the bitch. Breeding management with hormone assay is required to optimize timing of breeding, especially as more sophisticated breeding techniques are used. Commonly recommended timing of insemination for a single breeding is two days post-ovulation for fresh or chilled
semen and three to four days post-ovulation for frozen semen.\(^4\) Fertilization has been reported to occur 3.5 to 7.5 days after the LH peak in bitches.\(^5\)

Vaginal insemination most commonly is done using an infusion pipette. More specialized equipment has been described but has not been demonstrated to increase pregnancy rate or litter size.\(^6,7\) Transcervical insemination can be done as a blind technique, using a rigid catheter, or may be done with assistance of a long, narrow endoscope used to see the cervix and aid passage of a polypropylene urinary catheter through the cervix.\(^8\) There is one report of laparoscopic intrauterine insemination in bitches.\(^9\) Surgical insemination is done via laparotomy with exteriorization of the uterus and direct injection of semen into the uterine body.

Besides timing of breeding, number of times bred has a significant effect on pregnancy rate, with a greater number of breedings associated with increased pregnancy rate and litter size.\(^10-13\) For the purposes of this review, the authors will not attempt to pull out details of breeding timing from the information presented, as it is variably reported. Similarly, pregnancy rate will be the term used in this discussion. Some papers report conception rate, although that terminology is inappropriate considering inability of determining actual number of conceptuses in bitches. Some papers report whelping rate and in this review, those are described as pregnancy rate, recognizing some inaccuracy due to possible pregnancy loss when considering litter size.

This study is an evaluation of success in providing breeding services for one veterinary clinic in the United States, using data drawn from the medical records.

**Materials and methods**

Banked serum samples were donated by a local practitioner who does extensive reproductive work. Progesterone had been assayed using chemiluminescence, either at a commercial laboratory or in-house. Biweekly quality control sample measurements were performed using both internal and external controls. Complete medical records were maintained for all bitches. For about one-fourth of these bitches, samples were collected daily from some point in proestrus through insemination.

Data were retrieved from all records for each breeding management episode including breed; age; dates of progesterone sampling and breeding; LH assays performed, if any; type of breeding performed, number of breedings/cycle; semen quality; success of breeding; and litter size.

Statistical analysis was performed using descriptive statistics, analysis of variance (ANOVA), Student’s t-testing for non-categorical data, and calculation of the Pearson’s correlation coefficient. Significance was set at p<0.05.

**Results**

Data were available for 576 dogs of 88 breeds. The Labrador retriever was best represented (27.1%). The next most common breeds, in order, were the German shepherd dog, Australian shepherd, golden retriever, border collie, Bernese mountain dog, Rottweiler, bichon frise, German wirehaired pointer, American Staffordshire terrier, Cavalier King Charles spaniel, and English setter. Mean age of bitches bred was 4.5 ± 1.8 years (n=311).

Overall pregnancy rate varied with type of breeding (Table 1). Pregnancy rate was highest with natural service and with surgical insemination with either chilled or frozen semen. Pregnancy rate was lower for vaginal insemination with any type of semen. Pregnancy rate for transcervical insemination with chilled semen was low, as was sample size.

Age of the bitch at the time of breeding was associated overall with success, with younger bitches (4.4 ± 1.7 years) more likely to be successfully bred than older bitches (5.2 ± 1.7 years) (p=0.04). There were no specific breeding types that were more or less successful relative to age of the bitch (p=0.72). Litter size varied with age of the bitch and with type of breeding. Age of the bitch was not associated with litter size overall but for the most common breed, the Labrador retriever, there was a positive association of age with decreasing litter size (r=0.19), with the effect enhanced specifically for surgical insemination with frozen semen (r=0.34). Association with type of breeding and litter size was
significant (p=0.02; Table 2). For all semen types, litter size was significantly greater with surgical insemination than with vaginal insemination (p=0.0004).

Serum progesterone concentration on the day of the LH surge was 3.1 ± 1.0 ng/ml, with a range of 1.3 to 6.2 ng/ml (n = 63). Concentration of progesterone on the first breeding day was not different between successful (13.7 ± 8.5, n=192) and unsuccessful attempts (12.5 ± 7.4, n=56) for all types of breeding together (p=0.35). Concentration of progesterone on the first breeding day was different between successful and unsuccessful attempts for some types of breeding (Table 3). In general, a lower progesterone concentration was associated with success when using fresh semen and a higher progesterone concentration was associated with success when using frozen semen.

Discussion

This was a directed study project for a senior veterinary student. Collecting these data and talking through how the cases had been managed and significance of the findings were excellent learning opportunities for this student. Specific competencies demonstrated by the student included ability to describe use of measurement of LH or progesterone in serum for breeding management, pros and cons of various types of semen and insemination techniques, and how to evaluate a given bitch to optimize pregnancy rate and litter size. She also was able to provide the clinic with the information that their success rate with surgical insemination of chilled and frozen semen approximated natural service, suggesting they are doing a good job providing reproductive services. A fair number of bitches were lost to follow-up and the student is considering recommendations for record keeping at the clinic to better capture these data.

Age of the bitch is associated with pregnancy rate. In one study, bitches over six years of age had lower whelping rates than did younger bitches.11 This trend of greater likelihood of success when breeding bitches at a younger age was upheld in our study.

Reported pregnancy rate for natural service in dogs ranges from 84.5% to 100%.9,14-17 Fresh semen usually is introduced vaginally; reported pregnancy rates are 47.8 to 100%.15,18 There was one report of pregnancy rate of 25% with vaginal insemination of fresh semen but this low value was attributed to significant backflow of semen along the catheter used, with subsequent loss through the vulva.16 Pregnancy rate for transcervical insemination with fresh semen is 65.2% to 90%.15,16,19 There are reports of surgical insemination with fresh semen. In those studies, pregnancy rate was 83.7 to 100%.20,21 Because spermatozoa that are used immediately after collection are viable for a prolonged period, and because the goal when determining insemination technique for a given type of semen is to use the safest and least invasive method with the lowest risk of side-effects that is still likely to achieve the desired results, surgical insemination would only be used if there was proven subfertility with vaginal insemination.20,22

For chilled semen, pregnancy rate with vaginal insemination is 33 to 89% and with transcervical insemination is 65.6%.15,23

Frozen semen is viable for only a short time after thawing, so intrauterine insemination is recommended. Pregnancy rate for vaginal insemination with frozen semen is generally low, 10 to 60%, with one report of 80% success rate in a group of five dogs.10,11,15,18,24,25 Pregnancy rate may be increased by inseminating multiple times and by introducing prostatic fluid vaginally after the semen is deposited.26 Reported pregnancy rate for transcervical insemination with frozen semen is 52.0 to 100%.10-12,15,16,24,25,27 Reported pregnancy rate for surgical insemination with frozen semen is 70.8 to 89.4%.10,21,28

Pregnancy rates in this group of bitches agreed with what has been demonstrated previously in the literature. The small number of transcervical inseminations with chilled semen precluded meaningful interpretation of that data. In general, there is less information about success rate with chilled semen in the veterinary literature.

Litter size is associated with size of the breed, with larger breed bitches having larger litters.21,29-31 Correlation between litter size and weight of the dam is 0.83.32 Another breed-specific component that may impact litter size is inbreeding. Inbreeding coefficient is negatively correlated with litter size in bitches, suggesting that choosing for specific desirable characteristics may unintentionally be associated
with choosing for decreased reproductive success. Pedigree analysis to determine inbreeding coefficient was not performed in this study.

Age of bitch has been associated with litter size in bitches. In one study in which age was not associated with litter size, bitches were not bred beyond about four years of age. In a study evaluating litters registered with the American Kennel Club, 10% of bitches showed a decline in litter size after their second litter or three years of age. In another study, litter size decreased after the fifth parity or about six years of age. As might be expected, age-related decline in litter size occurred at a younger age in larger breed bitches. Data from the best-represented breed in this study, the Labrador retriever, agree with these trends from the literature.

Litter size is impacted by timing of breeding, by type of breeding, and by number of breedings. Litter size is optimized by breeding two to three days post-ovulation. In one study evaluating longevity of canine ova post-ovulation, it was determined that litter size declined significantly by eight days post-ovulation. Cervical closure occurs at about six days post-ovulation but even introduction of semen directly into the uterus does not promote normal litter size, suggesting that aging of the ova or asynchrony with the intrauterine environment play a role.

In one study evaluating birth of 10,810 litters from 224 bitches, litter size was greater for those bitches bred by natural service than with any form of artificial insemination (AI). In other studies, litter size was demonstrated to be greater with fresh semen than with frozen semen. In that study, litter size decreased by 0.4 pups with fresh semen AI and by 1.3 pups with frozen semen AI. Average litter size with vaginal insemination with fresh semen is 5.8 and with transcervical insemination is 6.5. There is one report of transcervical insemination with fresh semen, with litter size of 5.0 pups. Average litter size with vaginal insemination of chilled semen is 5.8 and with transcervical insemination is 6.4. Average litter size with vaginal insemination of frozen semen is 4.7 and with transcervical insemination is 5.0 to 6.9 pups. Finally, it has been demonstrated that litter size is positively associated with number of inseminations. In this study, we demonstrated greater litter size with surgical insemination than with vaginal insemination, and greater litter size with surgical insemination than with natural service. This was an uncontrolled population, with many dogs that bred by natural service in this community not available for analysis, suggesting that these data are skewed by the population seen.

Assays available for measurement of progesterone in serum or plasma include radioimmunoassay (RIA), chemiluminescence (CLA) assay, and enzyme-linked immunosorbent assay (ELISA). Enzyme-linked immunosorbent assay has been demonstrated to be about 90% accurate, compared to RIA. Comparison of RIA to CLA demonstrated correlation of 0.98, with some studies suggesting consistently higher values with RIA than with CLA measurement. There is value in handling the sample consistently and in using the same kind of assay throughout a given breeding management episode. Use of evacuated serum separator tubes has been associated with higher concentrations measured by CLA, some studies suggest that letting the serum sample sit on the clot may be associated with artificially low serum progesterone as the hormone molecule adheres to red blood cells in the clot, some studies suggest generally higher values using RIA compared to CLA, and finally, some studies have demonstrated diurnal variation in progesterone secretion in bitches. Sample handling was consistent within the facility and CLA was used for assay of all samples in this study.

Serum progesterone concentration is reported to vary from 2 to 4 ng/ml or 1 to 1.9 ng/ml on the day of the LH peak. Data from this study agree with data from the first study cited. Ovulation occurs two days after the LH peak, with reported values of 38.0 ± 4.4 hrs with ultrasound observation and a range of 24 to 72 hours with laparoscopic observation. Serum progesterone concentration on ovulation day has been reported to vary from 3 to 10 ng/ml. Because spermatozoa that have been manipulated are less likely to be viable long-term than are spermatozoa that have not been chilled or frozen, it is not surprising that success in this study was associated with relatively lower progesterone concentrations for fresh semen, presumably associated with breeding a bit earlier and relying on long-term viability of spermatozoa, and with relatively higher concentrations for frozen-thawed semen.
Conclusion

Retrospective evaluation of data permitted this veterinary clinic to assess its success rate relative to the veterinary literature and to fine-tune breeding management and record keeping. Data such as these provide insights to guide client decision making.

Acknowledgment

Source of funding: The Theriogenology Foundation

References

42. Hegstad-Davies RL: A review of sample handling considerations for reproductive and thyroid hormone measurement in serum or plasma. Theriogenology 2006;66:592-598.

Table 1: Pregnancy rate by breeding type

<table>
<thead>
<tr>
<th>TYPE</th>
<th>PREGNANCY RATE (%)</th>
<th>SAMPLE SIZE</th>
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</thead>
<tbody>
<tr>
<td>NATURAL</td>
<td>85.2</td>
<td>27</td>
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<tr>
<td>FRESH VAG</td>
<td>61.3</td>
<td>31</td>
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<tr>
<td>CHILLED VAG</td>
<td>66.7</td>
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<tr>
<td>CHILLED TCI</td>
<td>33.3</td>
<td>3</td>
</tr>
<tr>
<td>CHILLED SX</td>
<td>81.8</td>
<td>11</td>
</tr>
<tr>
<td>FROZEN SX</td>
<td>84.9</td>
<td>86</td>
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Table 2: Litter size by breeding type

<table>
<thead>
<tr>
<th>TYPE</th>
<th>OVERALL N</th>
<th>OVERAL LITTER SIZE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NATURAL</td>
<td>21</td>
<td>6.0 (2.3)</td>
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<tr>
<td>FRESH VAG</td>
<td>19</td>
<td>4.9 (2.1)</td>
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<tr>
<td>CHILLED VAG</td>
<td>8</td>
<td>4.4 (1.6)</td>
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<tr>
<td>CHILLED TCI</td>
<td>2</td>
<td>5.0</td>
</tr>
<tr>
<td>CHILLED SX</td>
<td>6</td>
<td>7.0 (3.9)</td>
</tr>
<tr>
<td>FROZEN SX</td>
<td>56</td>
<td>6.8 (2.6)</td>
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</table>
Table 3: Progesterone concentration in serum at time of first breeding by breeding type

<table>
<thead>
<tr>
<th>TYPE</th>
<th>OVERALL N</th>
<th>OVERALL P4</th>
<th>SUCC N</th>
<th>SUCC P4</th>
<th>UNSUCC N</th>
<th>UNSUCC P4</th>
<th>P VALUE</th>
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<tbody>
<tr>
<td>NATURAL</td>
<td>41</td>
<td>7.0 ± 4.8</td>
<td>23</td>
<td>6.5 ± 4.7</td>
<td>4</td>
<td>6.2 ± 1.1</td>
<td>0.9</td>
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<tr>
<td>FRESH VAG</td>
<td>61</td>
<td>10.8 ± 6.7</td>
<td>19</td>
<td>8.1 ± 4.3</td>
<td>12</td>
<td>13.9 ± 8.9</td>
<td>0.02</td>
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<tr>
<td>CHILLED VAG</td>
<td>27</td>
<td>10.3 ± 5.6</td>
<td>8</td>
<td>9.6 ± 6.4</td>
<td>4</td>
<td>10.8 ± 5.7</td>
<td>0.76</td>
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<tr>
<td>CHILLED TCI</td>
<td>6</td>
<td>18.4 ± 10.9</td>
<td>1</td>
<td>12.6</td>
<td>2</td>
<td>12.0</td>
<td>NA</td>
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<tr>
<td>CHILLED SX</td>
<td>14</td>
<td>16.5 ± 5.3</td>
<td>9</td>
<td>18.3 ± 4.4</td>
<td>2</td>
<td>13.5</td>
<td>NA</td>
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<tr>
<td>FROZEN SX</td>
<td>120</td>
<td>21.6 ± 4.6</td>
<td>73</td>
<td>21.6 ± 4.2</td>
<td>13</td>
<td>19.1 ± 4.1</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Succ = successfully bred, Unsucc = not successfully bred