Two example cases have been reported here to show that Pooled Analysis is a useful tool in reviewing existing published and unpublished data. Pooled Analysis, as well other meta-analysis techniques, supports decisions on future experiment-designs and eventually avoid to carry out new dedicated experiments.

Introduction

- Optimal allocation of limited resources such as animals is both a scientific and an ethical issue
- Reduction of animal numbers can be achieved by improved experimental design, statistical analysis and literature reviews
- A good experimental design aims to balance the power of the study with the sparing of animals, money and time
- Good literature reviews contribute to the understanding of how procedures can be optimised and refined and avoid unnecessary duplication of animal studies

Pooled Analysis

- Is a peculiar type of meta-analysis that combines individual-level data (i.e. raw data) from previous studies producing a new pooled result
- The advantage of a Pooled Analysis is that having data on individual subjects allows the researcher to aggregate data into common categories rather than having to rely on the data summaries reported in published manuscripts or reports.
- Like any meta-analysis, a secondary purpose of Pooled Analysis is to evaluate the sources of heterogeneity to determine whether a meta-analysis is feasible

Table 1. Results of a Pooled Analysis: MUN, mg/dl

<table>
<thead>
<tr>
<th>CORN</th>
<th>LowCP</th>
<th>11.0±0.3a</th>
</tr>
</thead>
<tbody>
<tr>
<td>HighCP</td>
<td>14.5±0.9c</td>
<td></td>
</tr>
<tr>
<td>ALFALFA</td>
<td>LowCP</td>
<td>12.7±0.3b</td>
</tr>
<tr>
<td>HighCP</td>
<td>12.4±0.3b</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Results of a Pooled Analysis: Excreted N, g/d

<table>
<thead>
<tr>
<th>CORN</th>
<th>LowCP</th>
<th>335±12</th>
</tr>
</thead>
<tbody>
<tr>
<td>HighCP</td>
<td>328±12</td>
<td></td>
</tr>
<tr>
<td>ALFALFA</td>
<td>LowCP</td>
<td>346±12</td>
</tr>
<tr>
<td>HighCP</td>
<td>316±11</td>
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The case of progestrone pattern in the bitch.

Progestrone: key hormone for endometrial development, uterine secretions, endometrial glandular secretion, inhibition of uterine motility, elimination of leukocyte responsiveness in the uterus and development of mammary glands

- Measurement of serum progesterone concentrations (P4) during pregnancy: useful to manage risk pregnancies in the bitch and avoid abuse of progesterone
- Guidelines in the decision as to whether to supplement progesterone/progestagen or not in cases of impending abortion due to luteal insufficiency are lacking

AIM: systematic design to draw up a general P4 pattern for canine pregnancy

M aterials and methods:

- 136 studies reporting data of P4 in bitches with normal pregnancy
- Among 19 studies involving a total of 148 bitches selected according to the pre-defined criteria
- Beagle, Labrador Retriever, German Shepherd, cross-bred, unknown breed
- method for P4 measurement: CLIA or RIA
- time of blood sampling: morning or afternoon
- weeks from the luteinizing hormone peak (W LH)
- data were not weighted for intra-study variability
- raw data were used when available (requested to the authors); otherwise the reported means were considered as referred to an individual bitch (37 bitch)
- so that studies with raw data were available weighted (depending on the number of animals) than other studies.

A forth-order regression on W-LH was tested to depict a general P4 pattern and its standard deviation calculated by the balance and as milk yield function

ANOV A was used to analyse the effect of age, breed and study on P4 concentration at each of the 9 weeks from LH peak to parturition

RESULTS and CONCLUSIONS:

Regression (figure 1, 2): results didn’t provide clear-cut reference values on P4 during the pregnancy; confidence limits of the pooled values were huge, especially after 3W LH

Most experiments used a small number of animals this being sufficient to make reliable estimates and comparisons under a given single set of assumptions and conditions; however the limited sampling and large variability among studies did not allow to infer general conclusions about the entire pregnancy of canine population

ANOVA: the observations were unbalanced among different factors; sometime there was confusion between factors and not all the level of the factor were present for all the studies; thus unbiased effects of factors were hardly estimable. Testing different models and subset (introducing and deleting studies and factors), produced different results; meaning that the system was not well described by the current dataset and models; however, looking at what was remained, more or less true across different analysis we were able to say that Study, Breed and Method of analysis had significant effect on the P4

The meta-analysis failed in producing a modeled pattern, but provided information to standardize protocols for further studies needed to establish data which could be considered normal at each week of pregnancy and to assess the probability that individual deviations from an average curve may be caused by affiliation to a specific breed of method of analysis. However, the canine population is composed of hundreds of different breeds, and it is unlikely that a single pattern of P4 concentration may work for all. Thus, a complete physical exam, ultrasonographic assessment of foetal viability and blood workup remain fundamental steps in deciding whether or not a pregnant bitch should be supplemented with progesterone or a progestagen, at least until more data is available on P4 concentrations during pregnancy on each specific breed