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Male Fertility Assessment:

From Charolais to Chihuahuas

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What is fertility?

The answer to this question differs between livestock, where numbers and timing are important, and companion animals (and humans), where the lines are more blurred.

Even within livestock, differences can occur in the definition of "acceptable" fertility. In swine, for example, pregnancy rate and timing are both important, as well as litter size. This might contrast with a champion "club calf" sire, where quality of the calf is more important than "strike rate".

For beef cattle, fertility concerns the ***rate*** at which females get pregnant, (i.e the time frame in which they get pregnant), and is not necessarily about ***pregnancy rate*** (i.e. percent pregnant). This is because sub-fertile bulls can often achieve the same pregnancy rate as high-fertility bulls, if given enough time to do it.

In this discussion, we are going to focus upon practical methods of evaluating potential male fertility, with the emphasis on reducing diagnostic error. The presentation will feature a number of problems and cases encountered in different species. Here, similar principles apply across species, with the major differences applying more to methods of semen collection, than to its subsequent handling and evaluation. As semen collection methodology is a major topic in itself, we will discuss it only in reference to its effect upon sperm numbers and semen quality.

What is the male's function?

The job-description of the male is to deposit fertile sperm at the appropriate female site at the best time for subsequent fertilization to occur. Thus the initial physical exam should take particular note of anything that might interfere with any part of this process.

For a natural breeding bull, "red flags" might include such factors as lameness, heritable faults (e.g. umbilical or scrotal hernia, testicular hypoplasia), penile problems (e.g. intact frenulum, hematoma) and significant loss of vision. Such a list might differ for animals used in A.I., and for companion animals (although, I would submit that heritable faults should be on *all* lists).

Why Poor Semen Quality?

Poor semen quality can occur due to different problems, not all of which are the fault of the male. Here it can be handy to refer to a decision tree, such as in Figure 1. This discussion will include ways to differentiate between different causes, to help the diagnostician determine the origin and significance of problems encountered.

Semen Collection:

As before, this will not be covered in depth. However, it is relevant to discuss methods of collection in relation to quality of the ejaculate. Here the first important question is: ***Did we obtain a representative semen sample?***

This question is most easily answered if the male completes a normal, complete ejaculation. However, this is not always easily apparent. Completion of an ejaculatory thrust (bulls, rams) or palpation of normal urethral contractions (stallion), can provide reasonable evidence. However, this becomes more difficult when semen is collected using methods that do not require normal ejaculation. Examples include:

- Trans-rectal massage in bulls.
    - This process usually results in an ejaculate derived from the more distal areas of the male tract. Collection of a truly representative sample is difficult to assess, and "quality" can vary with time of semen storage in those areas. In other words, a "good" sample is self-evident, but a "poor" sample may not reflect the animal's true capabilities.
- Use of an electro-ejaculator (EEJ)
    - Ejaculates obtained via EEJ tend to contain more accessory genital fluids than do "natural" ejaculates (which are more likely to be "representative"). Here, it is a human foible to award lower scores for motility when observed samples are more dilute. Conversely, over-concentrated semen samples can also result in errors in motility estimation.
- Drug-induced ejaculation (e.g. in horses).
    - Drug-induced ejaculation has been employed with stallions which are incapable of natural service.

Other problems occurring with the semen collection process can adversely affect findings. Some of the more common problems encountered include:

- High/low Temperature
    - It is important that all surfaces (collection tube, diluent/extender, slides, stain) coming into contact with fresh semen should be at body temperature.
- Spermatoxic collection environment
    - Easily overlooked, and include water, disinfectants, plastics, UV light.
- Contamination

- Dirt, feces, blood, pus.

Semen Handling:

Obtaining a representative sample is only the start of a process in which it is important to ensure that the representative nature of the ejaculate is maintained through each evaluation step. Here a number of traps lurk for the unwary, including:

- Temperature (again)
- Timing (i.e. time from collection to assessment)
- Spermatoxic action (again)
- Poor staining (as below)
- Non-physiologic diluent/extender

Unless care is taken to avoid these traps, errors in diagnosis and prognosis can occur. Problems such as low motility, and increased secondary sperm abnormalities can be caused by poor handling. Fortunately, if in doubt, we can double-check some of these aspects as with the following situations:

1. "Cold shock".
    a. Cold shock may be suspected if any/all of the following occur:
        i. Characteristic sperm movements (backwards/circling, "shimmering")
        ii. Large difference between motility and live/dead assessments (especially if sperm morphology is good).
        iii. Possibly in some circumstances – increased numbers of distal midpiece reflexes (DMRs) without accompanying retained droplets.
2. Poor handling/extender
        i. Low percent intact acrosomes (PIA)
        ii. Presence of numerous bacteria
        iii. Xs clumping
        iv. Simple tail abnormalities
        v. Loose sperm heads
        vi. Crystal formation
3. Poor preparation/staining/microscopy
        i. Sperm too dark, light, concentrated or sparse.
        ii. "Halo" effect
        iii. Undetected subtle sperm defects (especially if consistent).
        iv. Curiously increased numbers of narrow heads.
4. "Other"

Some remedies:

- Routinely place a few drops of fresh semen into an appropriate fixative (isotonic formal saline or PBS gluteraldehyde). This will keep indefinitely. However, this preparation needs to be assessed under phase contrast (or DIC) microscopy.
- Practice making a "good" stained semen smear.
- Check stain quality (and replace if necessary); mix before use.
- Use 1000x for morphology reading
- Have microscope re-aligned and cleaned regularly

- Count at least 100 sperm.
- Re-check yourself, and update regularly.

Identifying Defective Sperm.

Current concepts can improve (and simplify) how we recognize and categorize sperm abnormalities. These include:

1. Abnormal sperm head morphology is related to damaged DNA (Erenpreiss 2006).
2. Oxidative stress is the major cause of DNA damage in the male gamete (Aitken 2002: Lewis and Aitken 2005)
3. DNA abnormalities in sperm cause male-factor sub-fertility.
4. Routine sperm assessment parameters are only partially successful in identifying such damage.

Oxidative stress, in turn, affects the following (Aitken and Krausz 2001):

- Disulfide bonds (chromatin, midpiece)
- Integrity of DNA of sperm nucleus and mitochondria
- Lipid peroxidation (membranes, motility)
- Influenced by antioxidants (seminal plasma, female tract)
- Associated with residual cytoplasm; i.e. proximal and distal droplets (Aitkin 2002).

Indeed, a human study (Aziz et al 2000) found significant positive relationships between levels of sperm ROS production and the proportion of sperm with abnormal head shape, acrosome abnormalities, midpiece defects, cytoplasmic droplets and tail defects.

In turn, DNA damage in the male gamete has been associated with poor semen quality, low fertilisation rates, impaired pre-implantation development, increased abortion and elevated disease levels (including cancer) in offspring. (Lewis and Aitken 2005).

We can use these concepts to simplify sperm abnormality recognition and classification based upon the following principles:

1. Compromised sperm often have multiple "problems", some of which are not easily observed with routine techniques.
2. Although many sperm abnormalities are recognized, *they mostly result from a small number of possible pathogenic pathways.*
3. These pathogenic pathways are predictable, providing detectable ***patterns or clues***

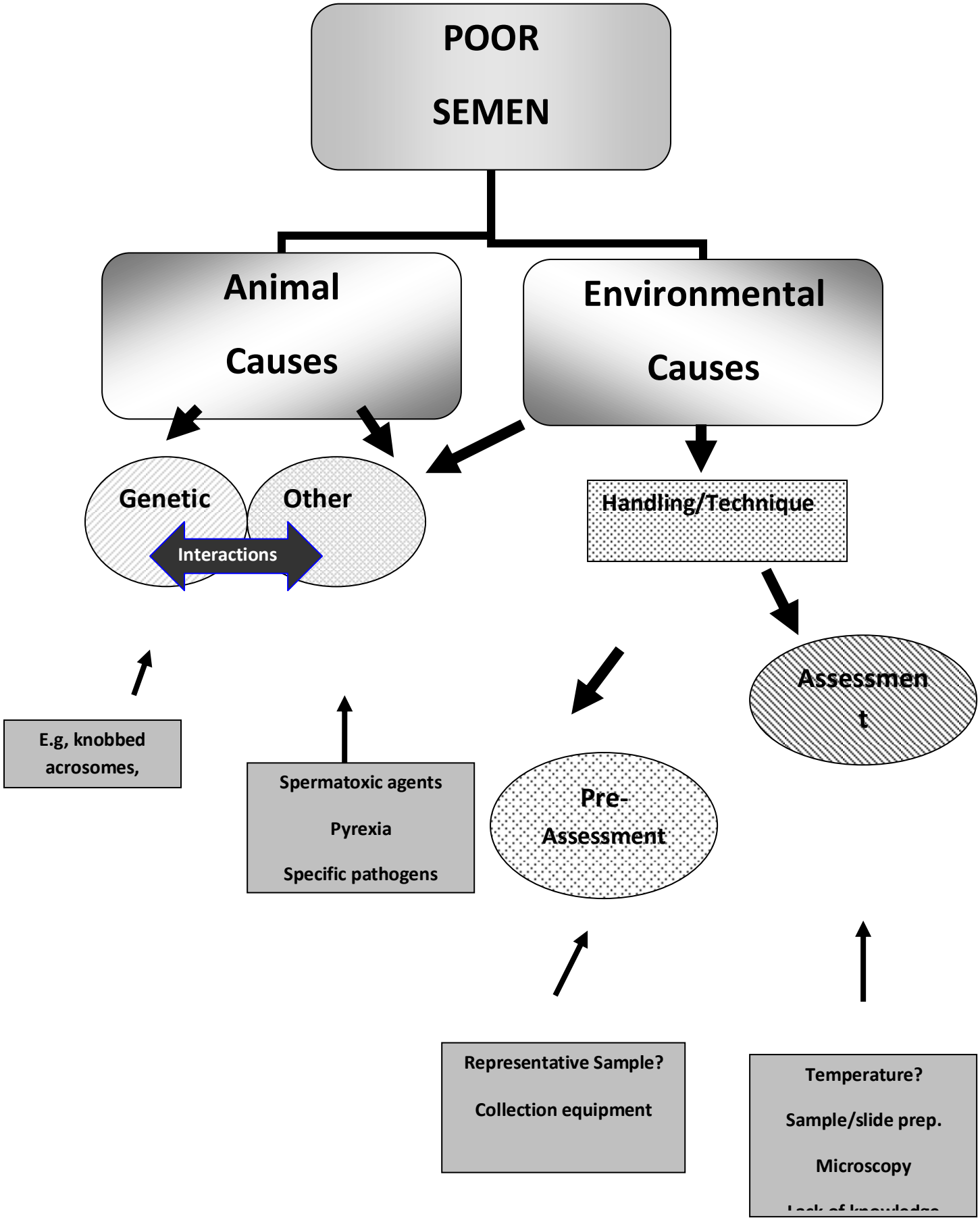
The Bottom Line:

1. Identify the "best" markers (or clues) of the different causes of sperm damage (e.g. the diadem/crater defect and its sequelae).
2. Use best diagnostic techniques and equipment available (within reason). Here it is highly recommended to use 1000X + microsocpy
1. Identify total "abnormal sperm" (irregardless of types or numbers of abnormalities represented)*.

** although it is advantageous to count the specific defects for collation and monitoring purposes*

In the presentation, a number of examples will be provided of problems encountered in both fresh and preserved semen, including those due to handling and diagnostic error.

References available on request


POOR SEMEN
Animal Causes
Environmental Causes
Genetic
Other
Interactions
Handling/Technique
Assessmen t
E.g, knobbed acrosomes,
Spermatoxic agents
Pyrexia
Specific pathogens
Pre-Assessment
Representative Sample?
Collection equipment
Temperature?
Sample/slide prep.
Microscopy