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Prenatal Sex Determination Methods in Veterinary Species: Advances, Efficacy and Practical Aspects

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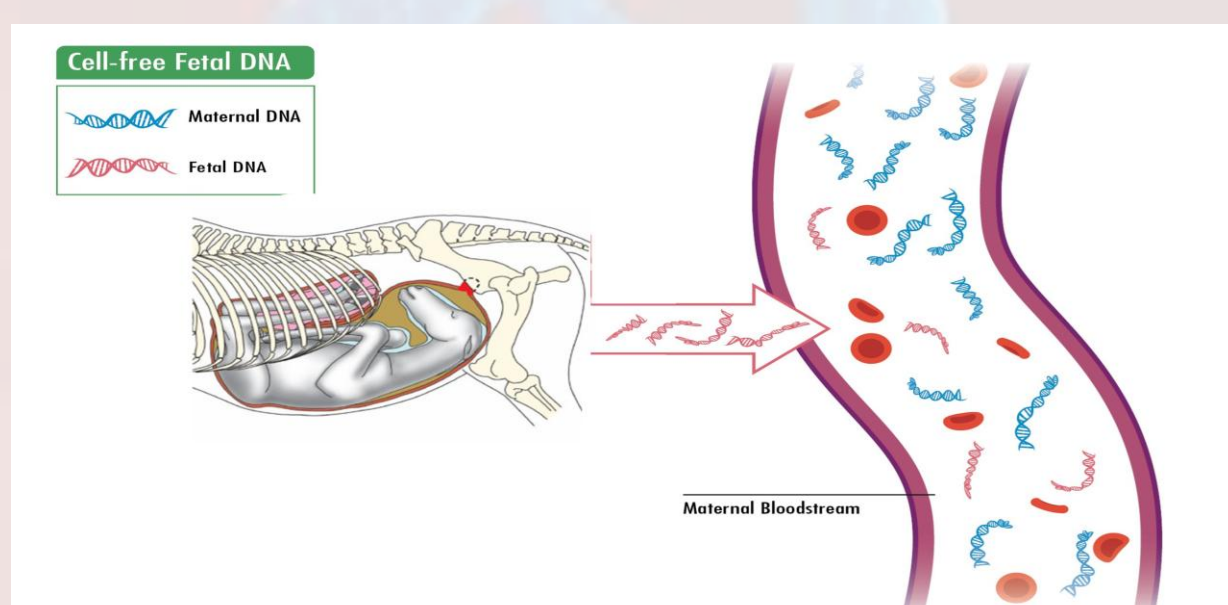
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Abstract: Prenatal sex determination represents a key element in veterinary reproductive management, supporting genetic improvement and economic efficiency in livestock and equine industries. This review summarizes progress in non-invasive diagnostic approaches using cell-free fetal nucleic acids (cfDNA and cfRNA) isolated from maternal plasma. Both classical and quantitative PCR (qPCR) targeting Y-specific genes (SRY, AMELY) and control genes (AMELX, β -actin, GAPDH) have shown increasing reliability. Detection success depends on placental type, gestational stage, and cfDNA concentration. Studies report >90% sensitivity for qPCR-based assays, compared with slightly lower sensitivity for endpoint PCR. Recent technological advances, including digital PCR and improved extraction kits, expand early and accurate fetal sexing potential in cattle, horses, and sheep, offering safe, non-invasive alternatives to ultrasonography or amniocentesis.

• Introduction

Early and precise identification of fetal sex supports selective breeding, disease prevention, and economic planning in animal production. The discovery of cell-free fetal DNA (cffDNA) in maternal plasma has enabled the adaptation of human non-invasive prenatal testing (NIPT) to veterinary species. The SRY gene on the Y chromosome initiates male differentiation, while genes such as SOX9, SF1, and AR act downstream in gonadal development.

Applications include: fetal sexing in bovine, ovine, and equine species, early pregnancy detection, and genetic disease screening.



• Material and method

Databases searched: PubMed, Web of Science, Scopus, ScienceDirect, CAB Abstracts, AGRIS (1990–2025).

Search terms: "prenatal sex determination," "SRY gene," "cfDNA," "maternal plasma," "qRT-PCR," "equine fetus," "livestock."

AI-assisted search optimisation: OpenAI and DeepSeek were used for semantic clustering of relevant studies.

Inclusion criteria: studies addressing prenatal or preimplantation sex determination in veterinary species using molecular or imaging techniques.

Data analysis: synthesis of methods, detection limits, and diagnostic accuracy by species and technique.

• Results and discussions

Main approaches identified:

Invasive techniques: embryo biopsy, amniocentesis, chorionic villus sampling — high accuracy, limited by risk.

Non-invasive molecular techniques:

- cfDNA detection from maternal plasma using PCR/qPCR for SRY, AMEL, or ZFY genes.
- Sensitivity 85–99%, depending on gestational age and plasma DNA yield.

Technological improvements:

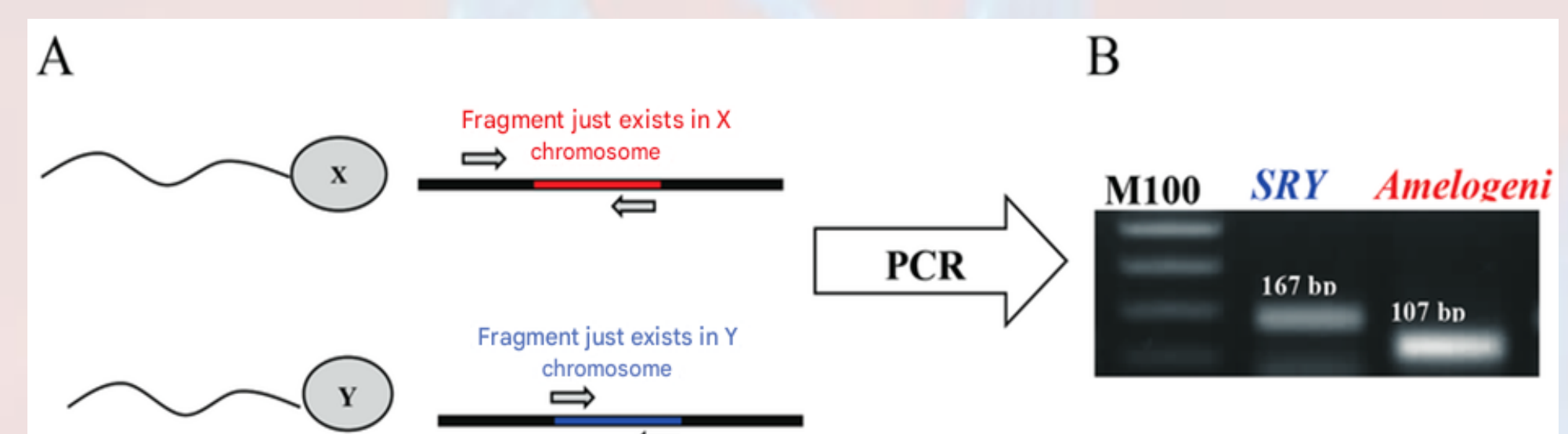
- Use of SYBR Green and TaqMan chemistries in qPCR.
- New extraction kits increasing recovery of fragmented cfDNA (150–200 bp).
- Real-time and digital PCR platforms improving quantification precision.

Species-specific considerations:

- Cattle: synepitheliochorial placenta – cfDNA detectable after day 45.
- Horses: epitheliochorial placenta – cfDNA detectable after day 50; NIPT feasible by ~8 weeks.
- Sheep: earliest reliable detection around day 40.

Advantages: rapid, accurate, non-invasive, applicable across gestation stages.

Limitations: need for standardization, low fetal DNA fraction in early pregnancy, and interspecies variation in placental permeability.



• Conclusions

Prenatal fetal sexing using cfDNA in maternal plasma is accurate, early, and non-invasive. qPCR-based detection of SRY/AMEL remains the most efficient molecular approach. Ongoing progress in sample processing and digital PCR technologies will further enhance sensitivity and reproducibility. Integration of molecular sexing into routine veterinary practice is expected to improve herd management and selective breeding programs.

