Stiftung Tierärztliche Hochschule Hannover
University of Veterinary Medicine Hannover, Foundation

Pregnancy-associated glycoprotein ELISA as sensitive and specific laboratory based pregnancy test in cattle

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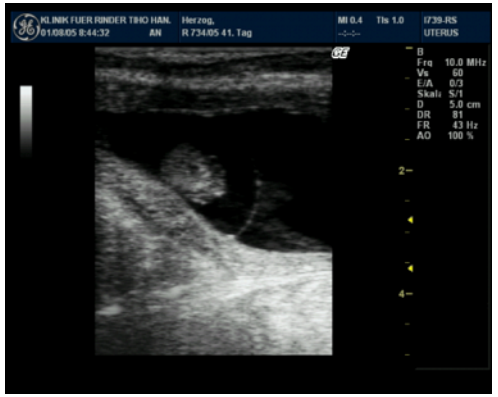
- detection of pregnancy in dairy cows is **one economic key parameter** on commercial dairy farming

- **early** and **confident** detection of nonpregnant cows may improve reproductive efficiency by minimizing the interval between detection and AI

- **pregnancy detection**
  - manual transrektal palpation
  - transrektal ultrasonography
  - laboratory based methods
    - progesterone
    - pregnancy associated glycoproteins (PAG)

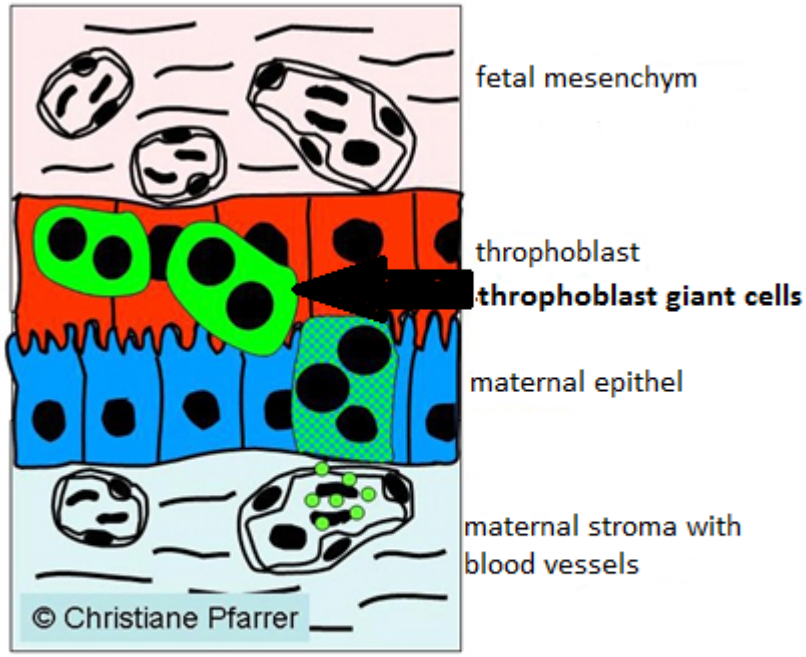
Abbildung 2.67 Prüfung der Doppelwandigkeit (Eihautgriff) zur Feststellung einer Frühgravidität. (Zürich)

Grunert und Berchtold 2000



Pregnancy-associated glycoproteins (PAGs)

- belong to aspartatic peptidases
- decribed by Butler et al. 1982 and Beckers et al. 1986
- 22 PAG molecules and more than 100 PAG genes were already described
- produced in trophoblast cells and trophoblast giant cells (TGC)
- PAG molecules **reach maternal blood** and used as parameter to **detect pregancy** in cattle



bovine Placenta, modified by M. Piechotta

Objective I

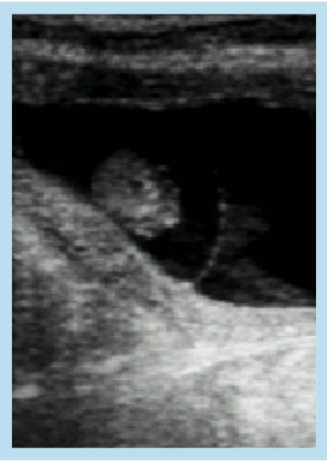
Determine test characteristics of a semi-quantitative PAG ELISA in pluriparous cows under field conditions.

Objective II

Test stability of PAG proteins for pregnancy diagnose in uncentrifuged whole EDTA blood.

Material and Methods I

Ultrasonography was defined as gold standard.



Pluriparous cows (n=297) from 5 farms
were examined 25-46 days after AI.

All cows were > 90 days after calving.

Concurrently, blood samples (coccygeal vessels)
were collected for semiquantitative determination of blood PAG.
(pregnant – repeat – not pregnant)
(IDEXX, Westbrook, ME, USA).

Calculations

	ultrasonography -pregnant-	ultrasonography -not pregnant-
PAG positive	correct positive (cp)	falsely positive **(fp)**
PAG negative	falsely negative **(fn)**	correct negative (cn)

Contingency table used for calculations

Tests	Formula
Sensitivity	cp/(cp+**fn**)
Specificity	cn/(cn+**fp**)
PPV	cp/(cp+fp)
NPV	cn/(cn+fn)
Accuracy	(cp+cn)/all

Results I

	PAG Test
Sensitivity	99.0%
Specificity	91.4%
PPV	96.7 %
NPV	97.4%
Accuracy	96.2%

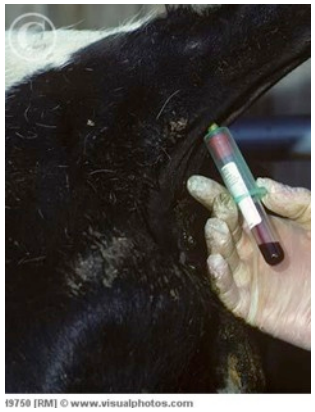
n=290

Seven cows (2.4%) were classified in a repeat category

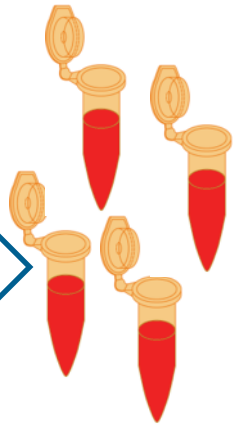
Falsely negative n=2

Falsely positive n=7

Stability of PAG molecules was tested in 10 whole blood samples containing EDTA.



aliquots



centrifuged

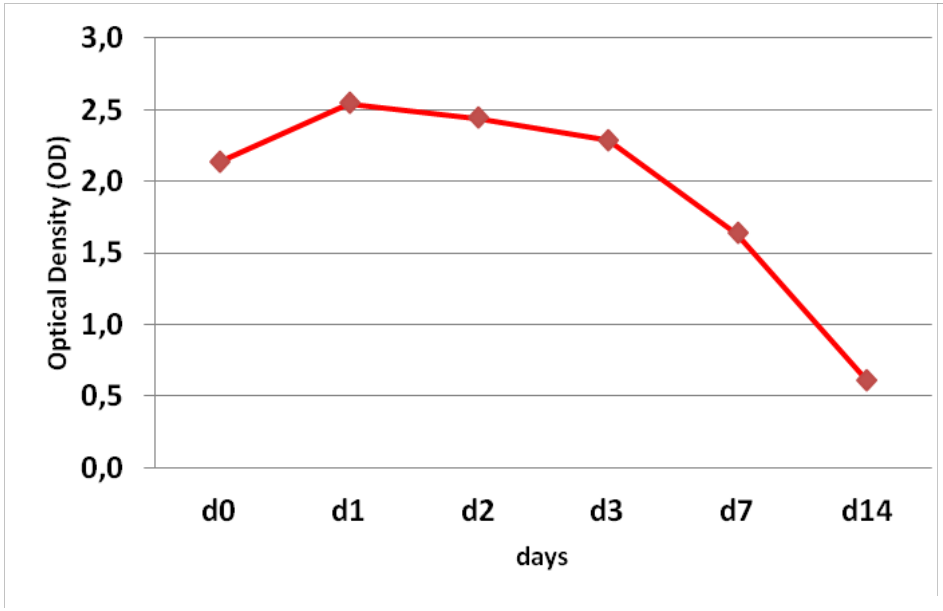
PAG ELISA

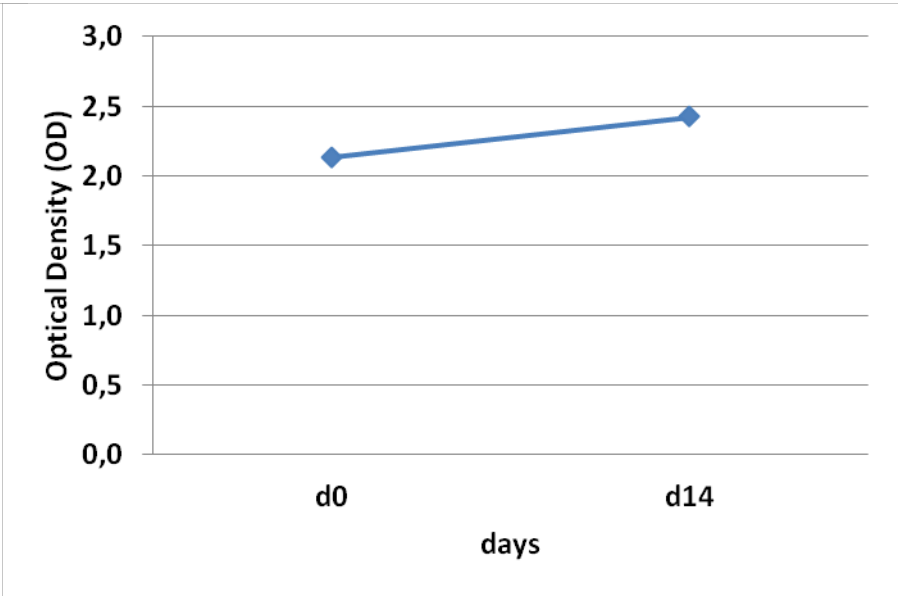
- 1,2,3,7 and 14 days — 37°C
- 14 days — 4°C

Results II

	day 0	day 1 37°C	day 2 37°C	day 3 37°C	day 7 37°C	day 14 37°C	day 14 4°C
1	pregnant	pregnant	pregnant	pregnant	repeat	not pregn.	pregnant
2	pregnant	pregnant	pregnant	pregnant	pregnant	repeat	pregnant
3	pregnant	pregnant	pregnant	pregnant	pregnant	repeat	pregnant
4	pregnant	pregnant	pregnant	pregnant	pregnant	not pregn.	pregnant
5	pregnant	pregnant	pregnant	pregnant	pregnant	pregnant	pregnant
6	pregnant	pregnant	pregnant	pregnant	pregnant	repeat	pregnant
7	pregnant	pregnant	pregnant	pregnant	pregnant	pregnant	pregnant
8	pregnant	pregnant	pregnant	pregnant	repeat	not pregn.	pregnant
9	pregnant	pregnant	pregnant	pregnant	pregnant	repeat	pregnant
10	repeat	repeat	repeat	repeat	repeat	not pregn.	repeat

Results II





Mean optical density in PAG ELISA 37°C and 4°C

PAG was detectable and showed comparable pregnancy diagnose after 3 days (37°C) and 14 days (4°C) in whole EDTA blood.

In conclusion

- PAG blood pregnancy assay was a sensitive and specific method to detect pregnancy in cattle.
- Lower specificity due to more falsely positive results which may due to embryonic losses (PAG is still measurable but the embryo could not detected by ultrasonography)
- PAG was stable in whole EDTA blood for at least three days at 37°C and 14 days at 4°C. Therefore, uncentrifuged whole blood samples can be send by post services to laboratories measuring PAG.

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