Low levels of regulatory T-lymphocytes in blood of mares are associated with early pregnancy loss

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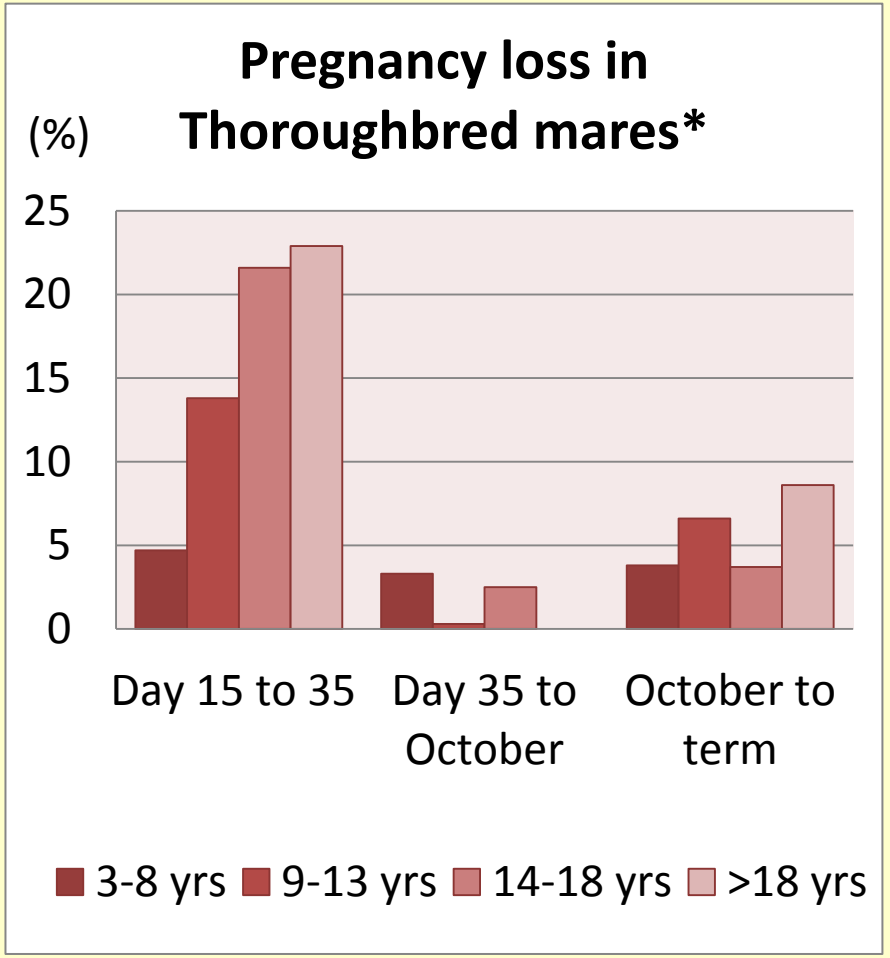
Pregnancy loss in horses

Early pregnancy loss

- morphologic defects or insufficient growth of conceptus
- failure of
  - maternal recognition of pregnancy
  - implantation
  - maternal tolerance of pregnancy



**Morris and Allen (2002)*

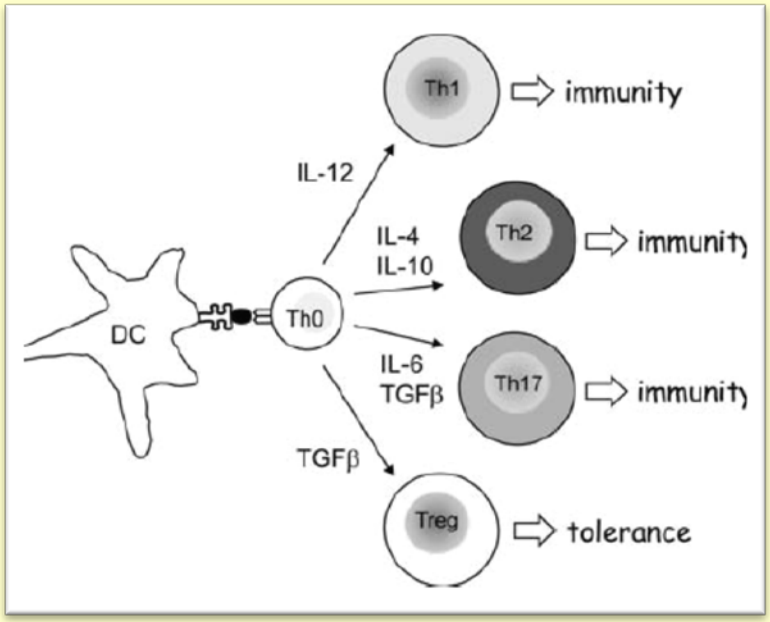
Maternal tolerance of pregnancy

- Fetal-placental unit presents paternally-derived alloantigen („allograft“)
- Exposure of mother to non-maternal antigen starts at mating (seminal plasma)
- Different regulatory mechanisms of the immune system interact to allow fertilization, implantation, and healthy gestation

⇨ **regulatory T cells (Treg cells)**

Regulatory T cells (Treg)

- suppress autoimmunity
- modulate immune responses to infection, tumors or organ transplantation
- implicated in immune tolerance of spermatozoa and conceptus tissue
  - ➢ in humans and mice, unexplained infertility and miscarriage are linked with numerical and functional Treg deficiency



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Aims and hypothesis

Hypothesis

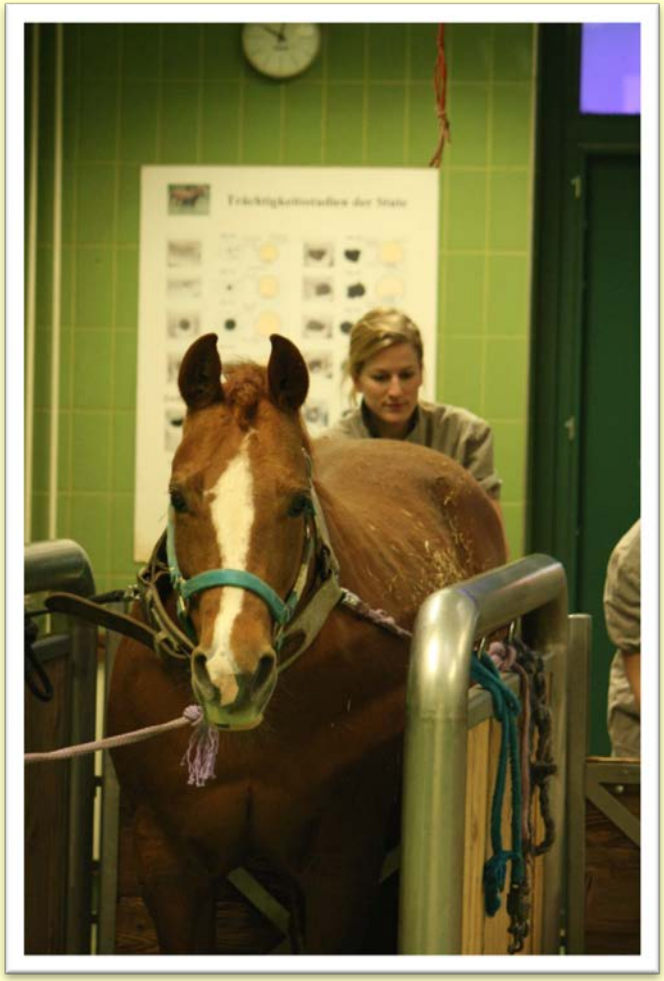
Low Treg levels contribute to pregnancy loss in mares

Aims of the study

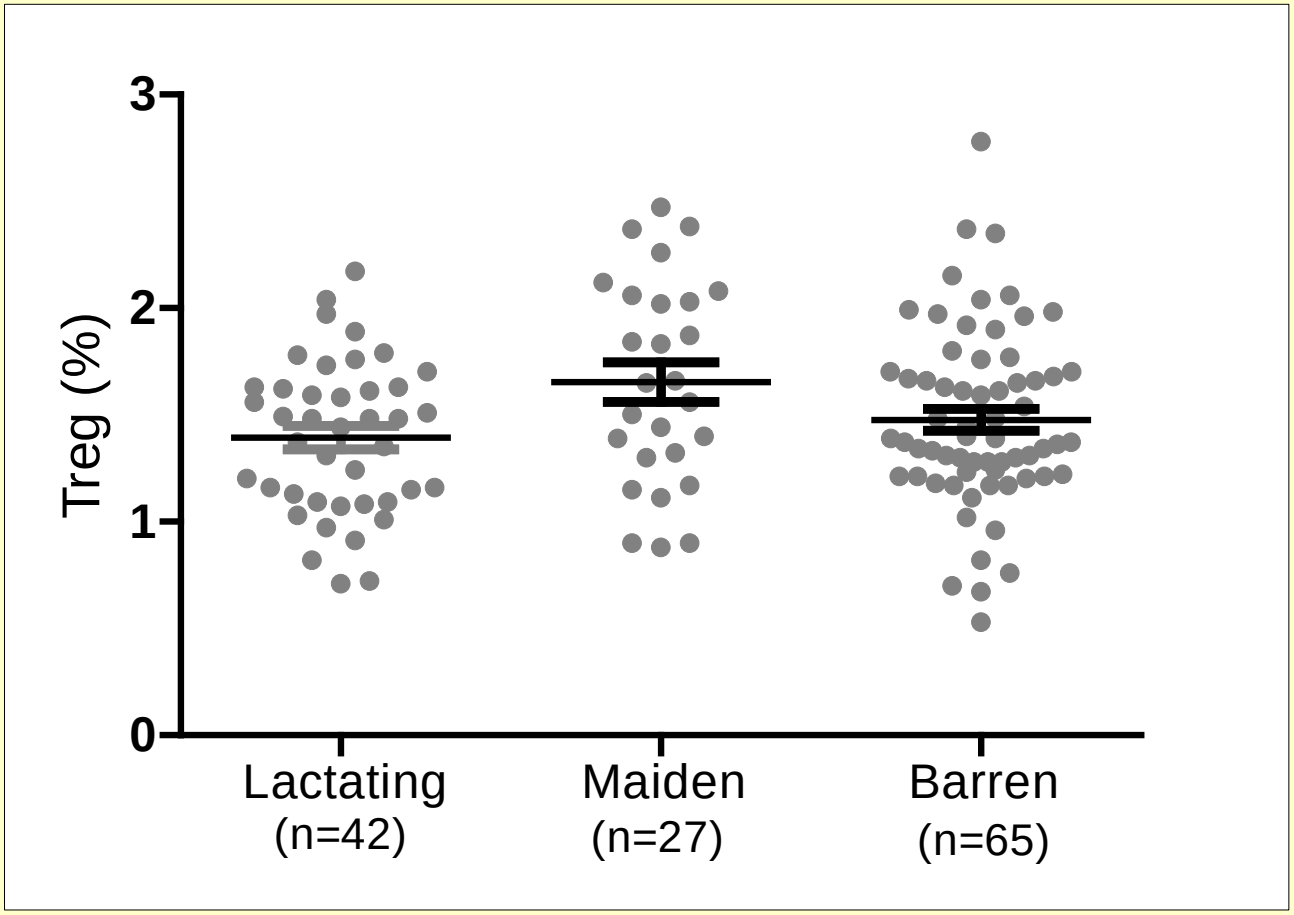
- Characterization of Treg levels in brood mares at the beginning of the breeding season
- Evaluation of pregnancy outcome in relation to Treg levels

Material and methods

- Healthy mares presented for breeding (n=156)
- Record of breeding history
- First oestrus -> collection of blood
- Determination of Treg level within the white blood cell population
  - ➢ quantitative real-time PCR based methylation assay targeting the demethylated equine FOXP3 TSDR DNA (-> naturally occurring Treg)
- Follow up of mares until term (n=110) -> foal born alive/pregnancy loss ≤day 45/pregnancy loss >day 45



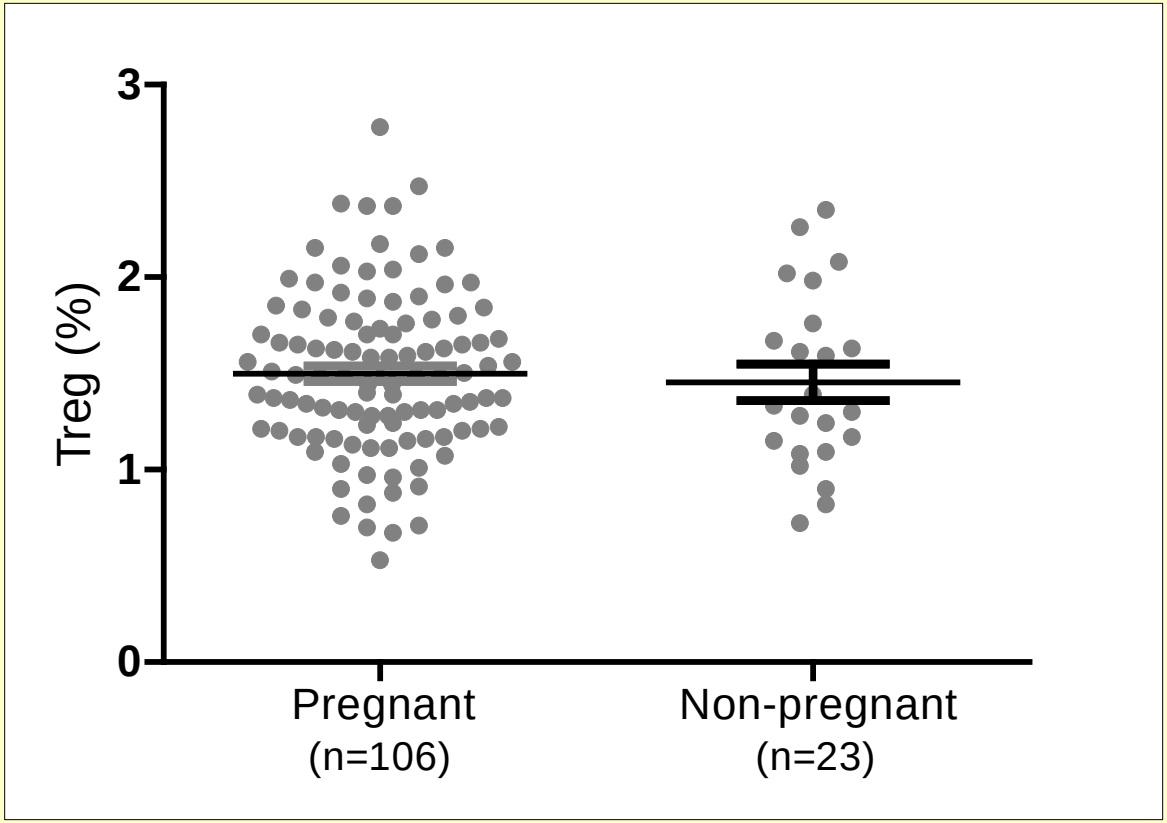
Distribution of Treg level in the mare population (n=156 mares)



No significant differences between groups

Treg levels and fertility – pregnancy rate

- N=129 mares
- Pregnancy diagnosis between days 14 and 20 of pregnancy
- Pregnancy rate 82%



No significant differences between groups

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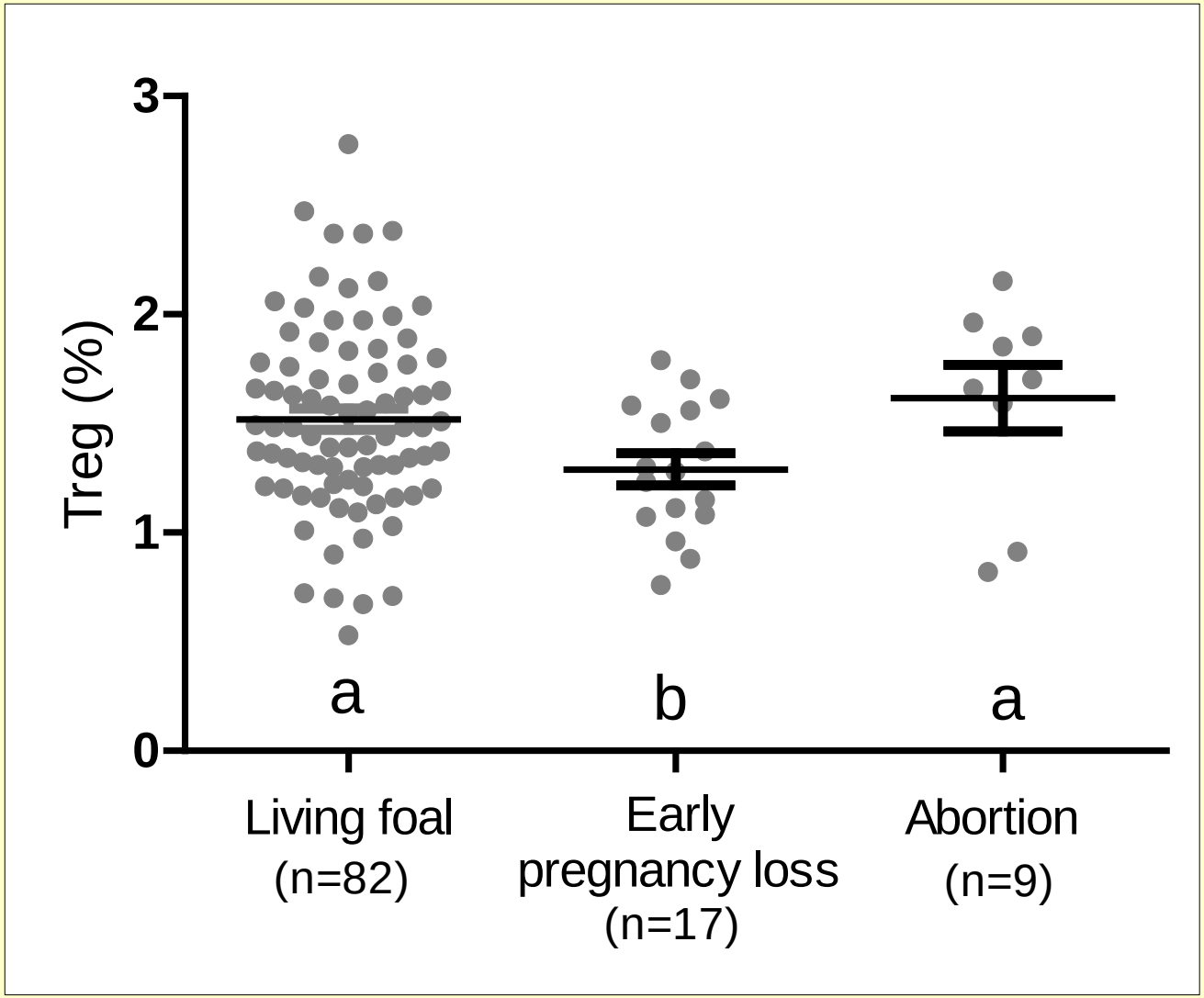
Treg levels and fertility – pregnancy loss



ab: significant differences between groups ($p<0.05$)

History of foaling and barren mares that lost their pregnancies ≤45 days

Mare	Age (years)	Fertility status	Production of living foals	History of early pregnancy loss	Treg level (%)
TA	9	Foaling	Yes	Yes	1.08
MR	4	Foaling	Yes	No	1.15
EM	11	Foaling	Yes	No information	1.58
FJ	6	Foaling	Yes	No information	1.61
FL	13	Foaling	Yes	Yes	1.79
CH	15	Foaling	Yes	Yes	1.07
DA	16	Barren	Yes	Yes	1.11
SO	14	Barren	Yes	Yes	1.23
NA	13	Barren	Yes	Yes	1.28
BL	14	Barren	Yes	Yes	1.30
FL	13	Barren	Yes	Yes	0.96
KE	16	Barren	Yes	Yes	1.70
RI	15	Barren	Yes	Yes	0.76
WA	4	Barren	No	Yes	1.48

Summary

- Treg levels in broodmares: 0.6 to 2.8% of the white blood cell population (mean 1.50 ± 0.4%)
- No association between Treg levels and…
  - mare`s status at the beginning of the season
  - per cycle pregnancy rate
  - maintenance of pregnancy >day 45
- Mean Treg level lower in mares that subsequently underwent early embryonic death (≤day 45)
- In this group high incidence of mares with history of former early pregnancy loss (11/17 mares)

Conclusion

- Results suggest an involvement of Tregs in maternal tolerance of pregnancy before or at implantation in the horse
- Low levels of naturally occurring Treg cells may explain the occurrence of repeated early pregnancy loss in some mares
- Small absolute differences in levels of Treg cells between groups -> prediction of early pregnancy failure in individual mares too inaccurate

Future perspectives

Stimulation of Treg levels for therapeutic purposes in horse mares?

- Exposure to paternal alloantigen occurs in two „waves“
  1. Transmission of seminal fluid at mating
  2. Invasion of maternal tissues by trophoblast cells at implantation
- In women and mice:
  seminal fluid -> endometrial inflammation
  -> stimulation of dentritic cells -> Treg ⇧

Treg levels in mares used for repeated embryo collection

- Experimental mares from the own research herd
- Repeated embryo collection, i.e. every oestrous cycle
- Most mares ovulate throughout the year
- Collection of blood samples for determination of Treg in March/April (i.e. beginning of „breeding season“)

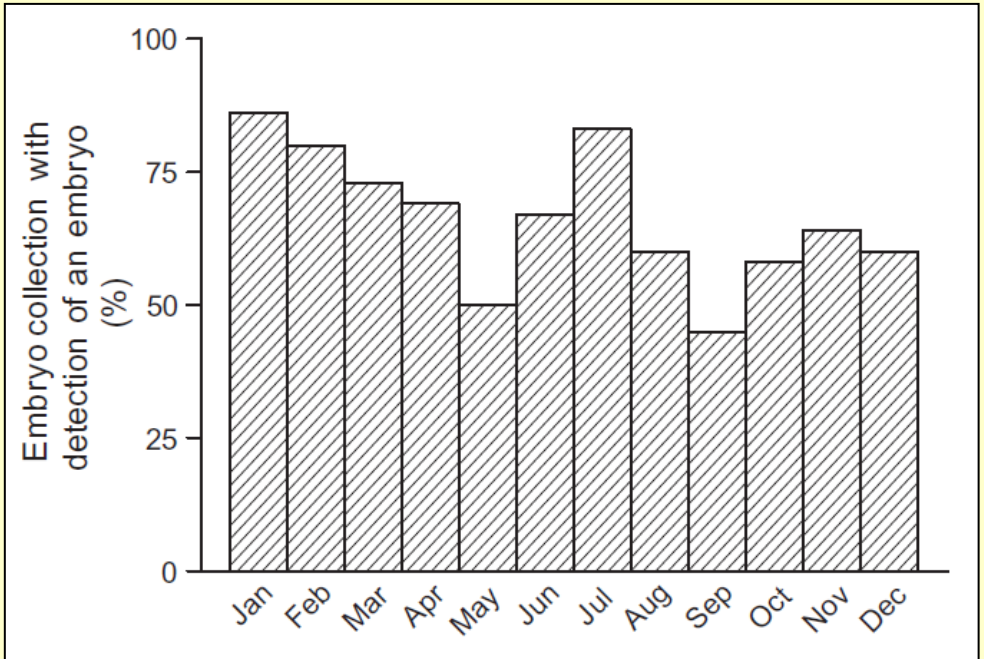


Fig. 1. Influence of the month of the year on the percentage of embryo collections with detection of an embryo (5–19 embryo collections per month). No significant differences between months (n.s.)

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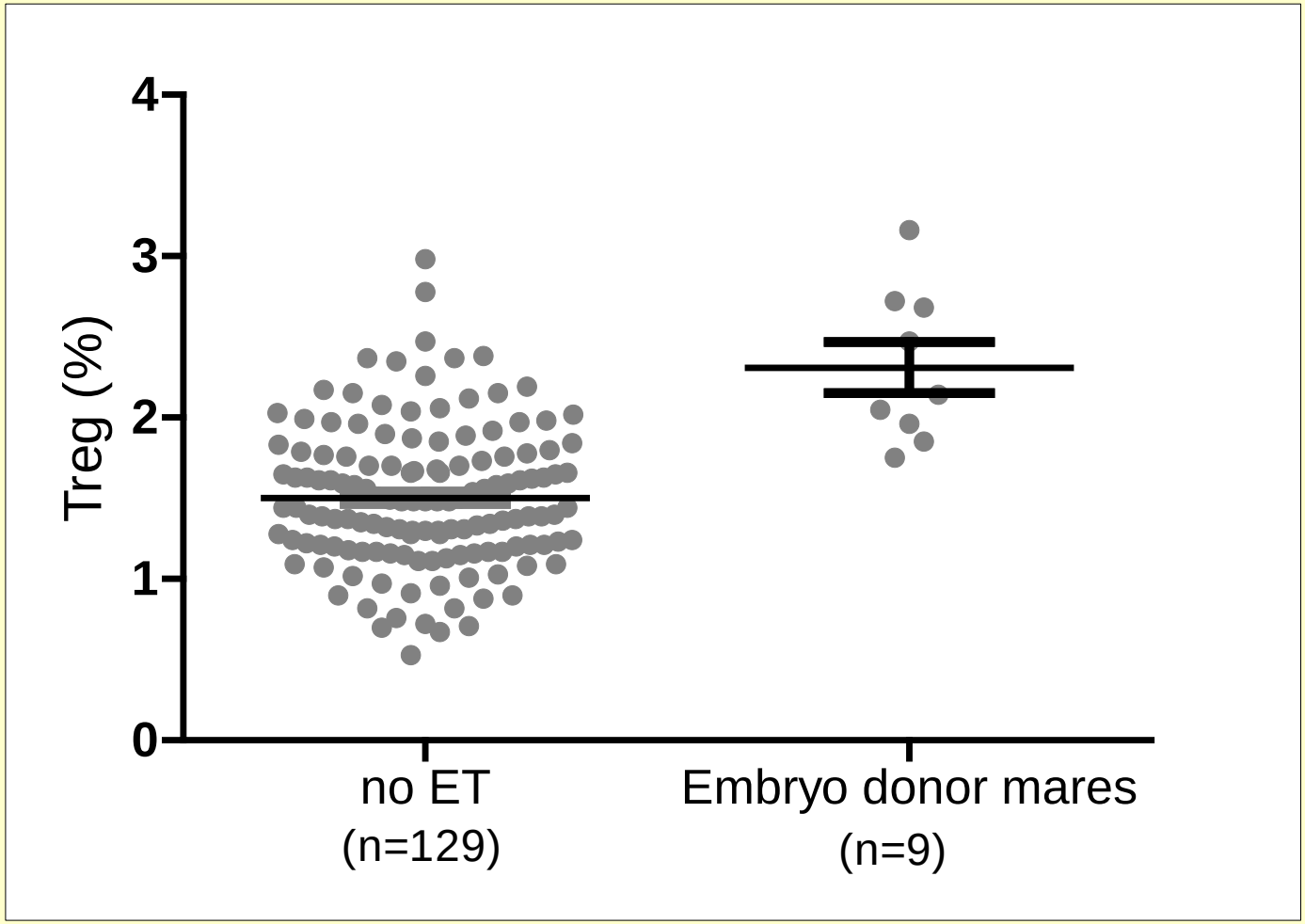
Treg levels in mares repeatedly used for embryo collection



P<0.001 between groups

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Future perspectives

- In mares used regularly for embryo production, repeated breeding may induce expansion of Treg levels
- Stimulation of Treg level before breeding (e.g. exposure to seminal plasma) could provide a „treatment“ promoting tolerance of paternal alloantigen at the time of embryo implantation also in horses

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