

# Oocyte Recovery and Maturation Rates in Estonian Sport Horses

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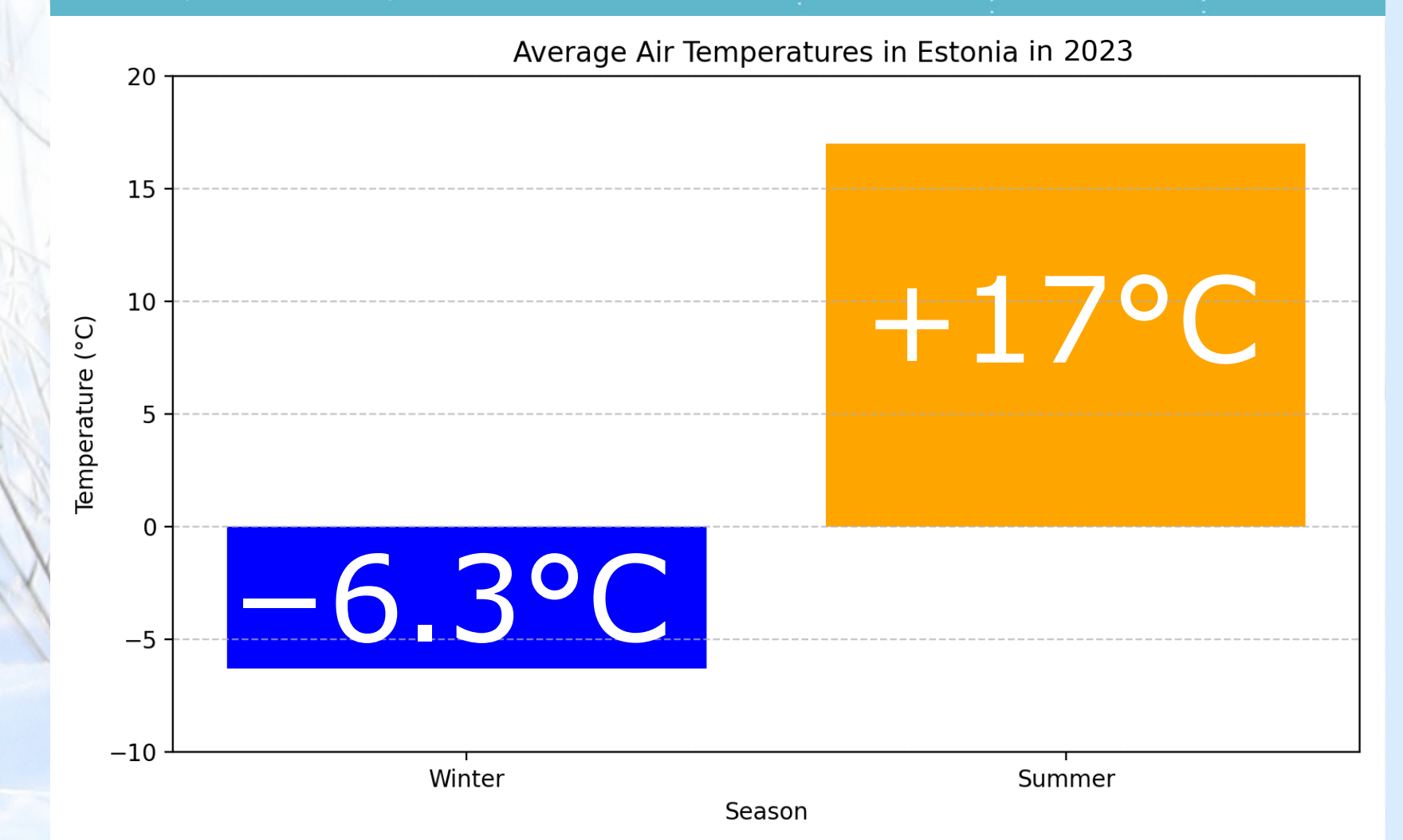
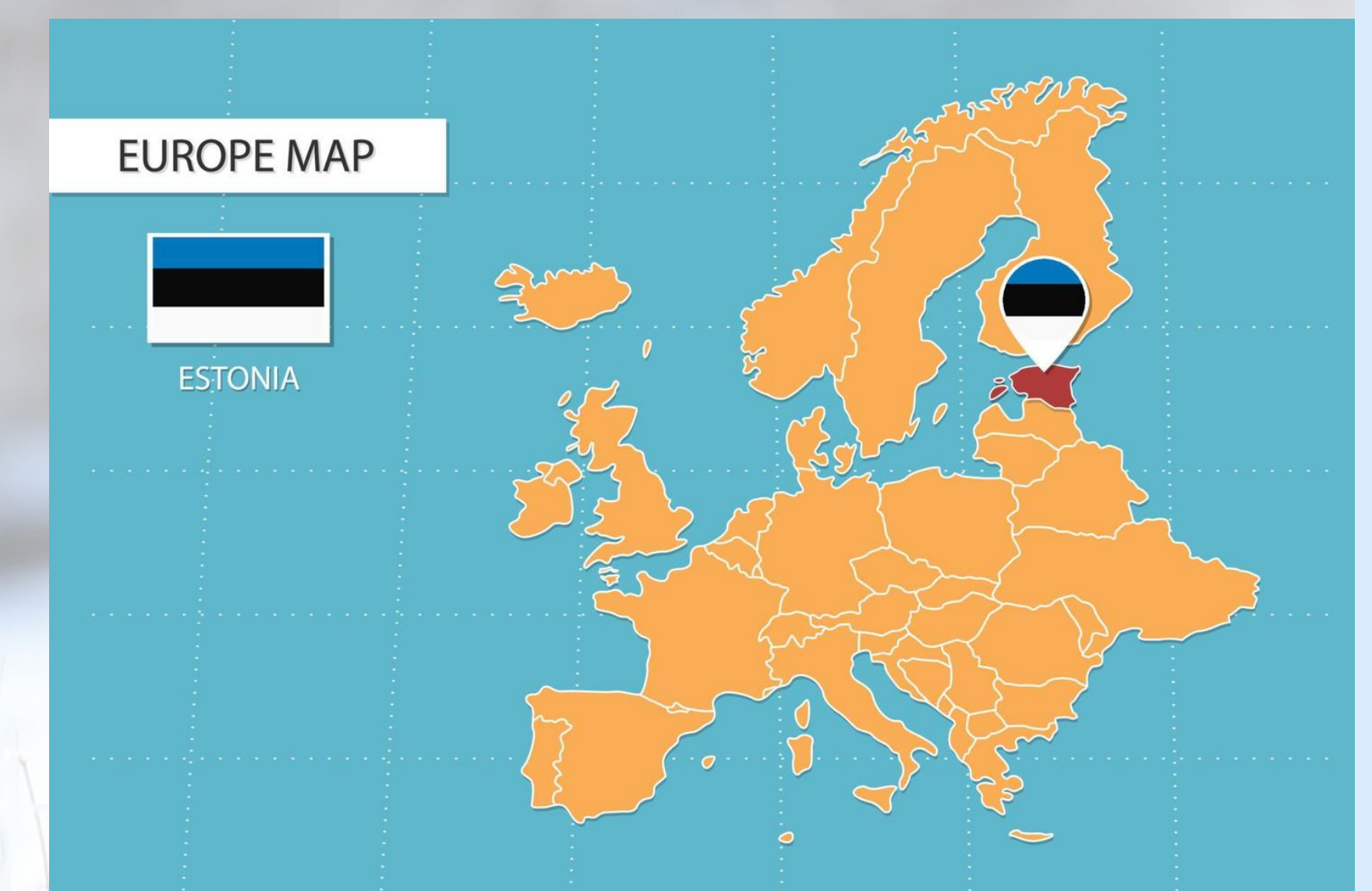
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## INTRODUCTION

Ovum pick-up (OPU) and intracytoplasmic sperm injection (ICSI) are rapidly gaining interest in the horse industry. The ability to preserve the genetics, efficiently use scarce or expensive semen, and produce embryos out of the breeding season from competing mares has proven attractive to sport horse breeders. Recently, our research group introduced the commercial equine *in vitro* embryo production system in Estonia, achieving the first commercial ICSI pregnancy. **The Estonian Sport Horse breed is a mix of European warm-bloods whose success in show jumping and dressage disciplines has gained international interest.** Breed has been shown to significantly affect the efficiency of an OPU-ICSI program (Fonte et al. 2024 Theriogenology 223, 47–52). Thus, **the aim of our study was to to evaluate the effect of the seasonality on oocyte recovery rates and the effect of different oocyte holding media on maturation rates in ESH.**

## METHODS

A total of 15 ESH mares, aged between 3 and 23 years, were subjected to OPU over the course of a year. The OPU procedure was performed as previously described by Papas *et al.* (2021 Animals 11, 2004), with prewarmed commercial equine OPU medium (Stroebech Media ApS). Recovered cumulus-oocyte complexes (COCs) were held at 22°C for 20 h in one of three holding media: an in-house made medium (IH) consisting of 40% M199 with Earle's salts, 40% M199 with Hanks' salts, 25 mM HEPES, and 20% FBS; a commercial equine holding medium (ST, Stroebech Media ApS); or EmCare embryo holding medium (EM, ICPBio). Then, oocytes were matured in groups of 5–20 COCs in 300 µL of maturation medium (Equine IVM Medium; Stroebech Media ApS) under heavy oil (Stroebech Media ApS) for 28–32 h at 38.2°C under 5% CO<sub>2</sub> in air. Analysis of variance test was used to assess statistical differences.

## RESULTS

**Table 1. OPU sessions, recovery rates and total maturation rate in Estonian Sport Horse mares**

	N°(%)
Total OPU sessions	47
Follicles aspirated	649
COCs recovered	425 (65.49)
COCs retrieved per mare	9.04 ± 4.38
Total nuclear maturation rate	305 (71.76)

The nuclear maturation rate was significantly higher in oocytes held in IH medium compared with those held in ST medium. No significant differences were found between ST and EM holding medium, nor between IH and EM medium (Table 2).

**Table 2. The effect of holding medium on nuclear maturation of oocytes**

Comparison	Maturation rate, %	p-value
IH vs ST	76.74 vs 64.94	<b>0.029</b>
ST vs EM	64.94 vs 73.74	0.123
IH vs EM	76.74 vs 73.74	0.321

No statistical differences were found in oocyte recovery rates based on the time of year in Estonia (Figure 1).

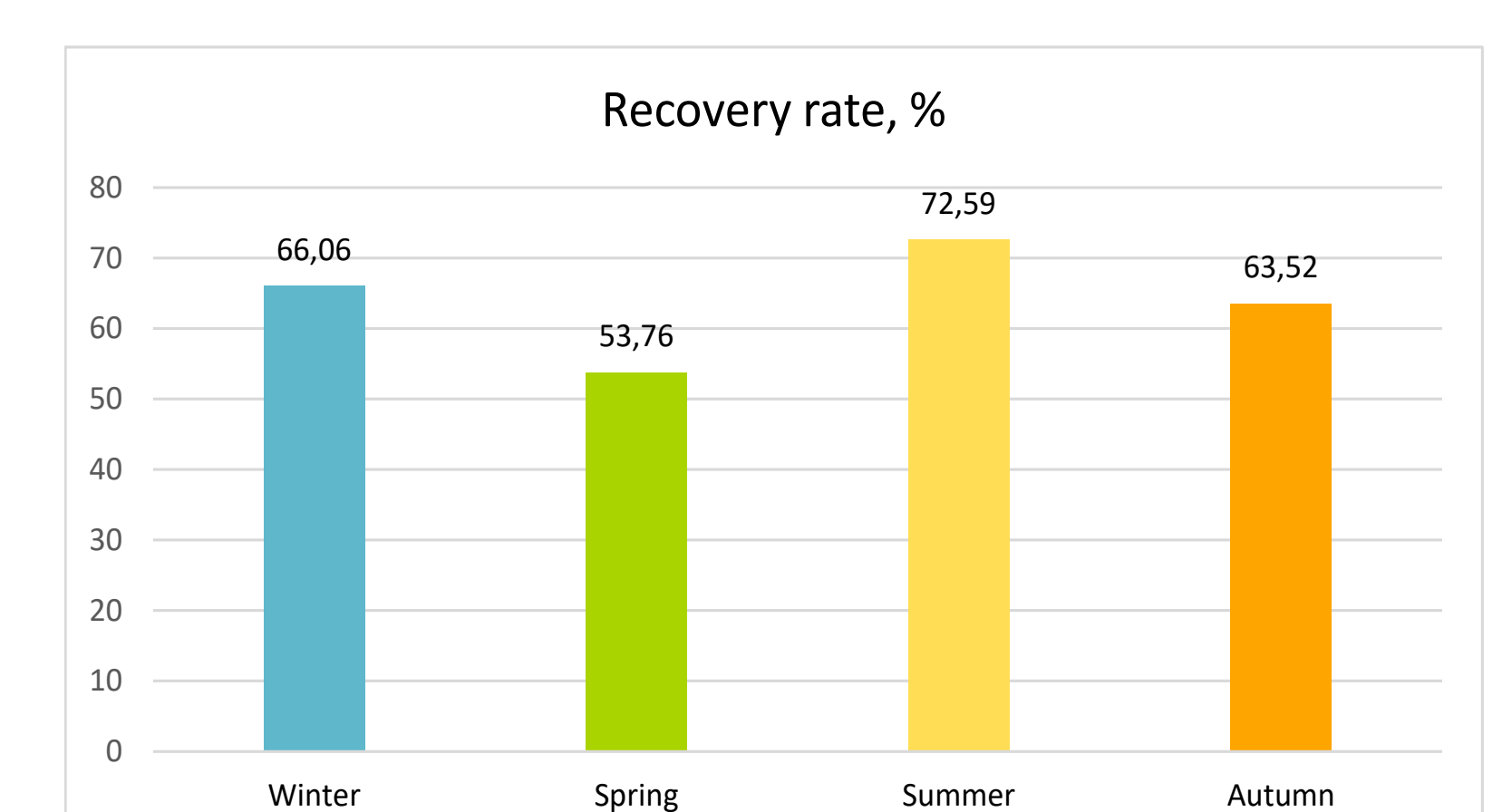


Figure 1. Oocyte recovery rates based on the time of year.

## CONCLUSIONS

In conclusion, oocyte recovery and maturation rates in the Estonian Sport Horse breed are suitable for a commercial OPU-ICSI program. Moreover, overnight holding of oocytes in IH, EM, and ST holding media support good oocyte maturation rates in this breed. Despite the extreme weather conditions in Estonia, OPU efficiency remains stable across seasons.