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IS IT NECESSARY TO CONTROL SEASONAL QUALITY OF THE RABBIT EJACULATE?

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ABSTRACT

The aim of our study was to compare the rabbit ejaculate quality during the year in term of the seasonal effect on the rabbit fertility. Semen samples from 25 bucks were collected using an artificial vagina throughout the year (5 collections in each season). Each sample of fresh ejaculate was evaluated for concentration and motility parameters using CASA system. The sperm concentration showed decreasing tendency from spring to winter, when it fell at the lowest value in comparison to the other seasons ($p<0.001$). The percentage of motile and progressively motile spermatozoa reached the highest value in summer in comparison to other seasons. The sperm motility ($p<0.001$) and also the progressive motility ($p<0.05$) were higher in summer than in winter season. The motility ($p<0.05$) as well as the progressive motility ($p<0.01$) of spermatozoa in spring were lower than in summer. In conclusion, our observed results clearly show the seasonal effect on the fertility traits of rabbit ejaculates. For that reason, it is really necessary to evaluate the semen quality of bucks throughout the year in order to choose appropriate season and animals for planned experiments.

Key words: rabbit; fertility; seasonal effect; CASA

INTRODUCTION

One of the most immediate benefits of artificial insemination (AI) has been the reduction in the number of males used for breeding. Thus, the bucks employed in AI must have good genetic characteristics and provide a good semen yield both in terms of quality and quantity (Panella and Castellini 1990; Battaglini 1992; Castellini and Dal Bosco 1998).

There are many factors influencing the quality and quantity of rabbit semen such as breed (Amin et al., 1987), male (Castellini, 1996), age (Gogol *et al*., 2002), season (Bodnar *et al*., 2000), photoperiod (Theau-Clement *et al*., 1995), nutrition (Fodor *et al*., 2003), collection rhythms (Nizza *et al*., 2003) and transgenesis (Chrenek *et al*., 2007b, c).

Under our experimental conditions the rabbit breeding is carried out using batch management system ("cycled production") for the purpose of meat production. This intensive breeding technique requires the use of AI at regular intervals. Moreover, artificial insemination by the use of health and quality animals allows realization of various experiments based on the rabbit as a biological model, such as insemination of does for the purpose of oocyte or embryo recovery (genetic manipulation; Chrenek *et al*., 2006; 2007a), sex-detection in newborn rabbits by X-chromatin and PCR-SRY (Parkányi *et al*., 2008), fertilizing capacity of cryopreserved spermatozoa (Makarevich *et al*., 2008), elimination of apoptotic spermatozoa using magnetic-activated cell sorting (Vasicek *et al*., 2011) etc.

When AI is applied in a rabbitry, it is estimated that one single buck may affect the fertility and prolificacy of about one hundred does (Seleem, 2005). Consequently,

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the reliable evaluation of both semen and the fertilizing ability of bucks are of vital importance to the success of the AI technique (Safaa *et al*., 2008).

The aim of our study was to compare the rabbit ejaculate quality during the year in term of the seasonal effect on the rabbit fertility for the purpose of permanent use of animals in experiments.

MATERIALS AND METHODS

Animals

Sexually mature (4 – 5 month old) and clinically health males of New Zealand White (NZW) line (n = 25) reared in a partially air-conditioned hall of a local rabbit farm at APRC Nitra were used in the experiment. The males were housed in individual cages, under a constant photoperiod of 14 h of light day. Temperature and humidity in the building were recorded continuously by means of a thermograph positioned at the same level as the cages (average relative humidity and temperature during the year was maintained at 60±5 % and 17±3°C). The rabbits were fed *ad libitum* with a commercial diet (KV; TEKRO Nitra, s.r.o.) and water was provided *ad libitum* with nipple drinkers.

Semen collection and analysis

Semen samples from each buck were collected throughout the year using an artificial vagina (5 collections per each season). Each sample of fresh ejaculate was evaluated using CASA (Computer Assisted Semen Analysis; MiniTüb, Tiefenbach, Germany) system combined with Olympus BX 51 microscope (Olympus, Japan) and following parameters were evaluated: concentration (10^9 cells per ml); percentage of motile spermatozoa (motility > 5 μm/s), percentage of progressively motile spermatozoa (motility > 20μm/s), DAP (distance average path, μm), DCL (distance curved line; μm), DSL (distance straight line, μm), VAP (velocity average path, μm/s), VCL (velocity curved line, μm/s), VSL (velocity straight line, μm/s), STR (straightness – VSL:VAP), LIN (linearity – VSL:VCL), WOB (wobble – VAP:VCL), ALH (amplitude of lateral head displacement, μm) and BCF (beat cross frequency, Hz).

Statistics

The ejaculate quality in each season was monitored. Obtained results were statistically evaluated using SAS 6.02 statistical software (SAS Institute Inc., U.S.A.). The data are expressed as mean ± SD (standard deviation). P-values at $p<0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

The basic parameters of the rabbit ejaculate collected in each season throughout the year are shown in Table 1. The highest ejaculate volume was recorded in winter and the lowest in summer; however the differences between all seasons were not statistically significant. Similarly, Safaa *et al.* (2008) noted higher semen volume in winter than in summer. On the other hand, another authors reported lower semen volume in winter season compared to summer (El-Masry *et al.*, 1994; Nizza *et al.*, 2003). Despite contradictory results, there was no significant seasonal effect on the rabbit ejaculate volume.

In contrast to ejaculate volume, the sperm concentration showed decreasing tendency from spring to winter, when it fell at the lowest value in comparison to the other seasons. There was a highly significant difference ($p<0.001$). Nizza *et al.* (2003) presented similar results at temperatures close to our climate, where the concentration was significantly higher ($p<0.01$) in summer season than in winter. On the contrary, El-Masry *et al.* (1994) observed significantly lower ($p<0.05$) spermatozoa concentration in summer than in winter, that may be explained by relatively higher air temperature.

Nizza *et al.* (2003) noted slightly higher motility of rabbit spermatozoa collected in summer compared to winter, that correlates with our observations, since the percentage of motile and progressively motile spermatozoa reached the highest value in summer in comparison to other seasons. The sperm motility ($p<0.001$) and also the progressive motility ($p<0.05$) were higher in summer than in winter season. The motility of spermatozoa ($p<0.05$) noted in spring was lower than in summer as well as the progressive motility ($p<0.01$). The lowest sperm motility was measured in the ejaculates collected in winter, whereas the lowest progressive motility was observed in spring. Contrary to our data, El-Masry *et al.* (1994) reported decrease in sperm motility during summer season, which is probably also due to the hot climate of the region where the experiment was carried out.

Under our experimental conditions, the fact that motility values were highest during summer suggests that the seasonal variations could be associated with changes in the environmental temperature. Similarly as in the experiments of Nizza *et al.* (2003), also our average temperature did not exceed 25°C. Alvarino (2000) observed increasing morphological alterations with temperatures higher than 27°C. Marai *et al*. (2002) reported that the increase in temperature adversely affects semen quality and quantity. Therefore, due to the big temperature fluctuation in recent years in our area, the evaluation of ejaculate quality of rabbit in each season is really required.

Table 1: Seasonal changes in volume and CASA parameters of the rabbit ejaculate

PARAMETER	SEASON			
	Spring	Summer	Autumn	Winter
Ejaculate volume (ml)	0.68±0.25	0.62±0.14	0.66±0.26	0.75±0.24
Sperm concentration (x 10^9 cells. ml^{-1})	1.18±0.38^{e}	1.11±0.43^{e}	1.10±0.54^{e}	0.70±0.47^{f}
Motility (%)	69.94±16.64^{a}	79.42±12.50^{b,e}	72.66±18.71	67.91±23.17^{f}
Progressive motility (%)	52.23±20.63^{c}	66.39±20.03^{a,d}	56.79±29.21	54.24±27.80^{b}
DAP (µm)	17.53±4.78^{e,d}	21.64±5.51^{c}	20.09±7.66^{e}	25.55±9.03^{d,f}
DCL (µm)	34.87±10.88^{e,c}	42.10±9.36^{d}	39.86±13.51^{a}	45.48±14.47^{b,f}
DSL (µm)	12.73±3.65^{a,e}	15.96±4.61^{b,e}	15.35±6.59^{e}	21.11±8.18^{f}
VAP (µm.s^{-1})	41.35±10.56^{d,e}	50.74±12.77^{c}	47.59±17.35^{e}	59.02±21.01^{d,f}
VCL (µm.s^{-1})	81.44±24.21^{c,e}	97.99±21.84^{d}	93.28±30.30	104.21±33.10^{f}
VSL (µm.s^{-1})	30.22±8.15^{a,e}	37.59±10.61^{b,e}	36.52±14.95^{e}	48.91±18.97^{f}
STR	0.71±0.13^{e}	0.73±0.05^{a}	0.76±0.07	0.79±0.18^{b,f}
LIN	0.37±0.09^{e}	0.38±0.04^{e}	0.39±0.10^{c}	0.45±0.15^{d,f}
WOB	0.50±0.10	0.51±0.03	0.51±0.10	0.54±0.15
ALH (µm)	4.03±0.82	4.38±0.70	4.00±0.75	3.62±1.12
BCF (Hz)	22.81±5.75^{a,e}	25.88±3.56^{c,e}	26.22±7.67^{b,d}	30.17±8.10^{f}

a vs. b – statistically significant at $p<0.05$
c vs. d – statistically significant at $p<0.01$
e vs. f – statistically significant at $p<0.001$

The other motility parameters clearly proved the effect of season on the fertility traits of rabbits (Table 1), since there were highly significant differences ($p<0.001$) in following parameters: DAP and VAP between winter and spring as well as autumn season; DCL, VCL and STR between winter and spring season; DSL and VSL in winter comparing to all other seasons; and LIN and BCF between winter and spring as well as summer season. The rest of motility parameters (WOB and ALH) did not statistically differ within the year.

In recent years, a number of techniques for objective assessment of movement characteristics of human and animal spermatozoa using computer–assisted (automated) semen analysis (CASA) systems have been introduced. The use of computer-assisted (automated) semen analyzer – CASA is a promising and objective alternative to the traditional approach of microscopic visualization of spermatozoa motility and heamocytometric evaluation of spermatozoa concentration (Massanyi *et al*., 2008).

CONCLUSIONS

Our observed results clearly show the seasonal effect on the fertility traits of rabbits. Since the rabbits are reared in a partially air-conditioned hall of a local rabbit farm, it is not possible to fully prevent the temperature effect of the external environment, especially in case of big temperature fluctuation. Furthermore, there is a big variability in the quality of each rabbit ejaculate. For these reasons, it is really necessary to evaluate the semen quality of bucks throughout the year, in order to choose appropriate season and animals for planned experiments.

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