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„DOCTORAL (PHD) THESES”

**THE EXAMINATION OF REPRODUCTIVE PERFORMANCE OF THE ROE
DEER (CAPREOLUS CAPREOLUS, L.) IN VARIOUS FIELD HABITATS**

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THE BACKGROUNDS AND THE OBJECTIVE OF THE RESEARCH

Roe deer (*Capreolus capreolus*, L.) is the most common big game in Hungary, their number is estimated above threehundred thousand. They have high population growth potential, indisputable hunting significance and they are considered to be „everyone’s big game”.

Considering their dynamics and harvest, the most significant parameter is reproduction. At the same time there are a lot of unanswered questions, delusions and contradictions in this topic. We should know the potential and the grown-up offspring survival rate to be aware of the possibilities and problems, since the analysis of the statistics indicates that even within the field roe deer stocks we can find significantly different performances in different regions and years. In practice, the population management is based mostly up on no realistic biological parameters; as these are unknown in many cases. The reproductive performance of a population is realised in the ratio of the grown-up offspring survival rate, which is greatly influenced by the correlation between the animal and its environment and this must be the base of the planning and effectuation of the harvest. Resulting from the complex approach of this topic, my research objective deals with several fields, as follows:

- The examination of the timing of the reproductive parameters on the sampling areas: In this field my purpose is to make a statistically correct analysis on the data collected during several years from different quality roe deer populations on different habitats and possibly from a large number of reliable samples. I wish to examine the quantitative differences in the reproductive performance among certain Great-Plain-habitat populations and years in the successive phases of the reproduction process (number of corpora lutea, number of embryos/fetuses and reared fawns). Where did losses happen and to what extent. Which are the separately measurable parameters, the knowledge of which enables us to forecast the expected number of the offspring and to create a suitable population management and harvest strategy.

- The sex ratio examination of the offspring:

Within the frame of this research I could not undertake the task of a complex exploration of the factors affecting the sex ratio of the fetuses and the newborn offspring. What I intend to do is to examine if there are any differences in this regard between the certain years within the sampling areas, and also the differences between the areas. The possibility for influencing the sex ratio even before the birth is well-known (selective conception, different implantation according to the sex, competition in the uterus). During the embryonic diapause and around the time of the implantation the possibility of the sexual selection comes up. To examine this we need a process to determine the sex of the early embryos. This can help us to reveal if the intrauterine losses have any connection with the sex of the embryo. Within the frame of this research I undertook to work out the PCR (Polymerase Chain Reaction) process for roe deer, which enables to identify the Y-chromosome specific sequence (SRY gene), which determines the male sex.

- To prepare a population management and harvest model using the results:

The scientific research reaches its goal if the results can be used in practice.

This is why one of my goals is to prepare a practical computer-run roe deer population planning and controlling model for game managers. It will be based on the data-collection methods, data-types and results of my experiments. Population management is a cyclical activity repeated every year, which has to be built on regular data collection and control, so a mistake made in one year (may it be the consequence of a badly assessed parameter) can be corrected and compensated in the following year, or at least the negative effects can be reduced. The model can provide an effective help for this.

THE METHOD OF THE RESEARCH

My experiments were carried out between 2002 and 2005. To create the database I gathered organ-samples and collected data from does and female fawns shot in their hunting season between 1st October to 31st January (from the year of 2004 their hunting season is from 1st October to 28th February). I counted the grown up offspring while observing in the fields. I made the laboratory examinations and gathered data from 366 does (in Nagyszénás: 41 in 2002, 91 in 2003, 21 in 2004; in Székkutas: 21 in 2002, 22 in 2003, 16 in 2004; in Hódmezővásárhely: 13 in 2002, 61 in 2003, 47 in 2004; in Ruzsa: 15 in 2002, 9 in 2003, 9 in 2004) and 34 female fawns altogether (2 in Székkutas, 7 in Hódmezővásárhely, 25 in Ruzsa), while I registered 1930 roe deer counted in the fields.

• The description of the examined areas and roe deer populations

When describing the areas I use the data from the wildlife management plans of the concerned game management units provided by the National Wildlife Management Database (Országos Vadgazdálkodási Adattár = OVA). The examined areas are:

- Petőfi Hunting Society, Nagyszénás (04-953600-1-4-1): an intensively cultivated farming area with fields of various sizes, 1,3 % forest cover, the quality of the roe deer population is „excellent”, the population density (statistical) is 4,6 roe/100 ha.
- Petőfi Hunting Society, Székkutas (06-803200-1-4-1): steppe and cultivated area with fields of various sizes, 0,8 % forest cover, the quality of the roe deer population is „good”, the population density (statistical) is 3,0 roe/100 ha.
- Szakszervezeti Hunting Society, Hódmezővásárhely (06-803300-1-4-1): an intensively cultivated farming area, dominantly with extensive, 0,9 % forest cover, the quality of the roe deer population is „excellent”, the population density (statistical) is 3,4 roe/100 ha.
- Landowners in Ruzsa and Neighbourhood Hunting Society, Ruzsa (06-801501-1-3-1): low fertility cultivated fields and woods, 26,6 % forest cover, the quality of the roe deer population is „moderate”, population density (statistical) is 4,6 roe/100 ha.

- **Gathering organ samples and recording the individual data:**

After eviscerating the sex organs (ovary, oviduct, uterus and cervix), the left kidney with the fat tissue and the jaw-bone (mandible) were stored in 3 % formaline solution, and we took the clean (eviscerated) body weight (with head and legs).

- **Laboratory examination:**

I carried out the examination of the sex organs and embryos/fetuses, the measurement of the kidney fat and kidney weight as well as the cementum layer counts in the laboratory of the University of Szeged, College of Agriculture. The PCR research was done in the Agricultural Biotechnological Research Centre in Gödöllő.

- **Age estimation by tooth wear and cementum layer counting:**

The age estimation of the does was done on the basis of tooth wear and cementum layer counting with the method of AITKEN (1975) on M1 molar tooth. In my following examinations I did not always need the exact distinction between the years, therefore, groups based on the results of HEWISON (1996), I made four age groups: fawn, 1-year-old, between 2-7-year-old, 8 and above-8-year-old.

- **Weighing and body condition scoring:**

The clean body weight (BW) was taken and registered by the game wardens. The body condition scoring was carried out by counting the kidney-fat index (KFI) (SUGÁR, 1983). I measured the weight of the left kidney and the surrounding fat tissues separately. I counted the kidney fat index with the method of CAUGHLEY and SINCLAIR (1994): $KFI = \frac{\text{the weight of the fat around the kidney}}{\text{the weight of the kidney}}$.

- **Corpora lutea and embryo/fetus counting:**

I got the number of the corpora lutea (CL) by cutting the oviducts at several sections and at the same time I also counted the follicles. From the third part of December when the reactivation of the blastocysts started, and the elongation and implantation occurred I could count some embryos/fetuses as well. From the end of January when the fetuses were as long as 4-5 cm I could identify their sex, too.

- **Sex identification:**

I had no difficulty in the sex identification of the adequately developed fetuses. As for the early embryos, first the circumstances of a DNS-based PCR-reaction (Polymerase Chain Reaction, MULLIS and FALOONA, 1987) had to be established. The examination was based on the skin and skeletal muscle samples taken from bucks and does shot in the hunting season. We also started as an attempt to wash the blastocysts out of the uterus.

- **The examination of the reared offspring:**

The estimation of the reared offspring was done in the areas concerned between November and January. I was able to observe them only when the visibility of the roe deer was suitable due to the lack of cover, but larger group had not been formed yet, so the association of the fawn and their mother could be determined more precisely.

- **The estimation of the reared offspring per doe:**

To estimate the reared offspring per doe I had to estimate the number of the mature does in the autumn population, which had not had kids yet. Since they could not be distinguished by phenotype in this period (1,5-year-old) I estimated the number of the yearling does from the national data with a calculation method of my own.

- **Sex ratio examination in the reared offspring:**

Together with the game wardens we went near the roe deer by car and with the help of binoculars and spectacles we registered the animals grouping them in buck–doe–male-fawn–female-fawn classes. When the sex of the fawn was uncertain I registered it as unknown sex.

- **Calculation of the Reproductive loss:**

I consider the CL number as potential offspring per doe. This is the basis of the calculation of the loss that happens during the reproduction process. I consider the fetus number the same as the number of the newborn kids (fecundity, secondary natality). As for the perinatal loss it is not possible to examine on open territories therefore they are recorded in the rearing loss. The reared litter means the 5-7-month-old fawns.

- Total loss (TL, kid/doe) = average number of CL – average number of reared litter
- Fetal loss (FL, kid/doe) = average number of CL – average number of fetuses

- Rearing loss (RL, kid/doe) = average number fetuses – average number of reared litter
- $TL (kid/doe) = FL + RL$
- $FL (\%) = (FL / TL) \times 100$
- $RL (\%) = (RL / TL) \times 100$

- **The method of the statistical process:**

I processed the data with **SPSS** for Windows (11.0.0. Standard Version) statistics programme. With **extreme value control** I spotted and excluded the extremely high and low body weight and kidney fat index values from the further examination. I made the **descriptive statistical** examination of the variables on each area in each year. I completed the **normality examination** with Kolmogorov-Szmirnov test. I used **one-way ANOVA** to compare variables between territories by years. Prior to the variance analysis I made homogeneity examination with **Levene** test. In case of homogeneous variance I made **LSD**-test, while in case of non-homogeneous variances **Tamhane**- test. It happened in some areas that there were no data about one of the three years. Then I compared the data lines of the two years that I had with **two-sample t-test**. I used a **univariate analysis of variance** to compare all the examined variables within a territory, annually. To find out if there is a correlation between the examined variables and, if any, how strong it is, I used **correlation and regression** analysis. Since the number of corpora lutea and the number of embryos do not show normal distribution in any of the examined areas, I used the nonparametric **Spearman**-correlation method.

THE MAIN ESTABLISHMENTS OF THE ESSAY

1. **The variability of the examined characteristics of the does:** When estimating the four areas together during the three years, out of the examined characteristics the KFI showed the highest variability, that is 75 % (the variability is 100 % when the given parameter statistically different in each area and in each year).

Table 1.

The variability of the examined parameters on the different areas (%)

	Age	BW	KFI	Number of CL	Number of Fetuses	Total
Nagyszénás	67	33	100	67	33	60
Székkutas	0	33	33	0	0	15
Hódmezővásárhely	33	67	67	67	67	60
Ruzsa	0	67	100	0	0	38
Total	25	50	75	33	38	-

The variability of the BW was 50 %, while that of the age was only 25 %. Considering the reproductive parameters, the number of CL changed 33 %, and the number of fetuses 38 % (Table 1.). There is a significant difference in the variability of the parameters of the examined years as well: out of the possible 100 %, 33 % change occurred in 2002, 48 % in 2003 and only 13 % in 2004 (Table 2.).

Table 2.

The variability of the examined parameters by years (%)

	Age	BW	KFI	Number of CL	Number of Fetuses	Total
2002.	0	50	33	50	33	33
2003.	33	67	100	33	67	48
2004.	0	33	33	0	0	13

These results prove that the year of 2004 was the most balanced probably because the balanced climatic and nutritional conditions. When comparing the areas and considering all the examined parameters the highest variability can be seen in Nagyszénás and in Hódmezővásárhely (60-60 %), while in Ruzsa it is 38 % and in Székkutas it is only 15 %.

From these I can conclude that within the three years the most changes happened on the former two areas, which is indicated by the variability of the examined parameters of the roe population.

To sum up we can state that the examined characteristics of the does do not follow evenly the changes of the environmental conditions. The condition (KFI) is the most changeable one, probably this indicates most sensitively the changes of the habitat conditions, but there can be significant differences individually, or among the areas and years. It is remarkable that the reproductive parameters (primer and secunder natality) do not reflect such variance, but there are significant differences in these parameters between areas and between years.

2. **Fertility, number of CL, and number of fetuses:** The fertility of the does is 99,45 % on all the four areas, which is proved by the presence of the CL. The pregnancy rate is 92,38 % altogether; there is no statistically provable difference contrary to the fact that within the areas the annual pregnancy parameters are different. The highest pregnancy rate can be observed in 2004, which probably has a connection with the above mentioned environmental conditions.

In KÖNIG's (1988) previous examinations based on 1975/76 data the pregnancy rate was 95,8 %, at the same time FODOR (1978) found 94,5 % and later FARKAS (2004) 89,7 %, in his examinations between 1974-82 (Table 3.). During the last 20 years this parameter did not decrease permanently.

The distribution of the CL in the examined areas show differences mainly in the presence of does carrying one CL (between 1,69-15,15 %) or 3 CL (between 3,03-11,86 %). The most typical is they're having 2 CL, which is between 81,82-86,45 % on the areas.

Table 3.

Pregnancy rate and distribution of fetuses in the roe populations on the Great Hungarian Plain

	Pregnancy (%)	Distribution of Fetuses (%)					Average number of Fetuses
		0	1	2	3	4	
König, 1988.	95,80	4,20	20,83	52,76	16,66	5,55	1,97
Fodor, 1978.	94,50	5,50	10,00	64,00	17,00	3,50	-
Sugár, 1979.		-	-	-	16,00	-	2,04
Farkas, 2004.	89,70	10,30	10,30	79,40	0,00	0,00	1,70
Own, 2004.	92,38	7,62	10,48	75,24	5,71	0,95	1,82

There were only 2 does which had no CL, at the same time I did not find 4 CL in any of the 366 does!

Considering the average of three years, the number of CL in Székkutas is the highest (2,10), which is the consequence of the fact that here is the lowest ratio of does with 1 CL (1,69 %) and the highest ratio of does with 3 (11,86 %). The lowest average number of CL was found in Ruzsa (1,88), where the presence of 3 CL in the does was extremely rare (3,03 %), while the presence of 1 CL was the most frequent (15,15 %). We can conclude, that the average number of CL is determined by the relative incidence of does with CL numbers other than 2. In addition, the number of CL in our conditions here in Hungary is 2 per doe mostly, and we cannot expect significant changes in this respect between the populations of the given areas, except where the environmental conditions are extremely unfavourable (pl. extreme weather conditions, lack of food/high population density). The distribution of the fetus number is similar to that of the CL, the difference between them presumably equals the fetal-losses, which is not too high in does. The rank of the areas in this respect is different from the one that was established by the number of CL. The highest fetus number was in Székkutas as well, while the lowest was not in Ruzsa (the area with the lowest CL number) but in Nagyszénás. Altogether the average of the four areas is 1,82 fetuses per doe; that is the fetal-loss was approximately 8,08 % (in the percentage of average CL number).

In all areas the 2 fetuses are the most common (72,73-85,72 %), but the scatter is higher than in case of the CL. As for the average of a certain area, it is also the incidence of 1 fetus (0-18,18 %) and 3 fetuses (0-8,0 %) that determines the average value. There was only one doe with four fetuses (0,95 %). During his above mentioned examinations in 1975/76 KÖNIG (1988) found 20,83 % with 1 fetus, 52,76 % with 2 fetuses, 16,66 % with 3 fetuses and 5,55 % with 4, so the average was 1,97 fetuses in 72 does. FODOR (1978) found 1 fetus in 10 %, 2 fetuses in 64 %, 3 fetuses in 17 % and 4 fetuses in 3,5 %. SUGÁR (1979) found an average fetus number of 2,04. FARKAS (2004) found 1 fetus in 10,3 %, 2 fetuses in 79,4 %, while he did not find 3 or 4; the average fetus number in case of 161 does was 1,70 (Table 3).

When considering the past thirty years, based on these data, it turns out that in the 1970s the average fetus number in the examined roe populations was higher (1,97 and 2,04) than ten years later (1,70), and today it is higher (1,82) than in the mid-1980s but it does not reach the level of the 1970s.

The distribution pattern of the fetuses shows a considerable change. The incidence of 2 fetuses that is typical for the roe chronologically was 52,76-64,00-79,40-75,24 % and the number of non-pregnant changed to 4,2-5,5-10,3-7,62 %. The number of does with 1 fetus fell back to 20,83-10,00-10,30-10,48 %, those with 3 fetuses to 16,66-17,00-0,00-5,71 %, while the does with 4 embryos practically disappeared.

It seems that in the past three decades the slight decrease of the reproductive performance occurred with no change in the pregnancy rate and the definite dominance of 2 fetuses. The incidence of the deviation from this (1-3-4 embryos) decreased significantly. Out of the 34 doe fawns examined on three areas only one had CL proving that it conceived but since it was shot before implantation (XII. 17.) no pregnancy could be stated. It is not sure either if it conceived in the normal rutting or in the „post-rutting season”. Out of the 25 does shot after 1st January none bore , which means none conceived. On the Ruzsa area 9 fawns had various numbers of follicle (1-2 mm), which denotes puberty. The follicle number indicates correlation between the condition, because here the average KFI (0,68) was the double of the values measured in the female fawns with inactive ovary (0,32).

3. The correlation between the age, the number of CL and the number of fetuses: It is characteristic of both the number of CL and the number of fetuses that they show higher variation in the 1-year-old and in the above-the-8-year-old age group than in the prime-aged group (2-7-year-old). The greatest difference among the average number of CL of the different areas was 0,28 in the prime-aged, 0,67 in the 1-year-old and 0,47 in the age group above-the-8-year-old. The average number of fetuses was 0,2, 1,5, 1,5, which means that both parameters are significantly more changeable in the young and old age groups and the differences are also considerable between the regions. It is not true that the potential reproductive performance of the older does decreases on each area. Presumably the growing age in itself does not result decrease in the reproductive performance. The fetal losses are very different on the areas: it is 10 % (0,2/doe) in Nagyszénás, 17,9 % (0,39/doe) in Székkutas, 7,5 % (0,15/doe) in Hódmezővásárhely and 13,7 % (0,26/doe) in Ruzsa. Because of the variance decrease of the CL number and fetus number in the past two or three decades no spectacular results can be expected in the correlation examinations, except for, perhaps, under the above mentioned extreme conditions. However, as the population density is only 3,0-4,6 roe/100ha (even if it is somewhat higher) on the examined areas, and intensive feeding

is carried out everywhere in the winter months, which can decrease the negative effects of the occasionally extremely severe weather, the roe deer population does not have to face real extreme environmental impacts. This fact is backed up by the CL and fetus number as well as the correlation analysis results of the individual characteristics of the does (age, BW, and condition). On those areas where the connection was significant, there was weak or medium correlation between the number of CL and the age-class of the does ($r = 0,244, P < 0,01$; $r = 0,512, P < 0,01$) and the number of fetuses and the age-class of the does ($r = 0,236, P < 0,1$).

4. The connection between the BW, the number of CL and the number of fetuses: It was only in Hódmezővásárhely that weak positive correlation was found between the BW of the does and the number of CL ($r = 0,279, P < 0,01$). The lowest eviscerated BW was 13,0 kg, which means approximately 17,5 kg live weight at the shot and which can be the minimum threshold BW of the rut. The eviscerated BW of the two does without any CL were 13,4 kg (Hódmezővásárhely) and 14,4 kg (Nagyszénás) respectively, and the KFI of the latter was 0,00! The eviscerated BW of the only rutting female fawn was 10,3 kg (approx. 13,5 kg live weight), while the KFI was 2,03. Presumably the threshold BW necessary for rutting is somewhat different in each area and the quality of the populations and this, together with the condition, have an effect on mating. Out of the surely pregnant does (having an embryo/fetus) the lowest eviscerated BW was 14,5 kg, which means about 19,3 kg live weight and which can be the threshold BW of the successful conception. The correlation of the BW and the fetus number was weak-medium positive in Nagyszénás ($r = 0,321, P = 0,145$ NS), in Hódmezővásárhely ($r = 0,358, P < 0,05$) and in Ruzsa ($r = 0,310, P = 0,197$ NS). Despite the fact that significant correlation cannot not always be proved, it appears that the incidence of the does with two fetuses is more common among the does with a greater BW.

5. The correlation between the condition, the number of CL and the number of fetuses: Despite the fact that the variance of the KFI (condition) is the highest among the examined parameters, it indicates a weak positive correlation both with the number of CL (Nagyszénás $r = 0,145, P < 0,1$; Hódmezővásárhely $r = 0,222, P < 0,05$) and the number of fetuses (Hódmezővásárhely $r = 0,324, P < 0,05$). We can conclude, however, that the improvement in the condition of the doe results in a slight increase in

the number of CL and the number of fetuses, even under consolidated environmental conditions.

6. **The reared offspring and the reproductive losses:** These parameters show considerable differences between the areas and within the areas in different years (Table 4 and 5). In the examined period the reproductive losses (total loss = fetal loss + rearing loss) expressed in percentage of the CL number (potential offspring) were 44,7-64,3 % in Nagyszénás, 21,5-49,1 % in Székkutas, 14,7-43,2 % in Hódmezővásárhely and 20,8-45,0 % in Ruzsa. When evaluating the whole period altogether reproductive losses (in the percentage of CL number) were 57,6 % in Nagyszénás, 30,0 % in Székkutas, 30,3 % in Hódmezővásárhely and 31,4 % in Ruzsa. The majority of the losses is rearing loss, that is the mortality of the fawns (Table 5). The ratio of fetal loss was between 23,7-28,3 %, except for Hódmezővásárhely, where it was 13,3 %. At the same time the rearing loss was the greatest in Hódmezővásárhely (86,7 %), while on the other areas it changed between 71,7-76,3 %.

Table 4.

The quantitative changes of reproduction between 2002-2005

	Number of CL		Number of fetuses		Reared offspring	
	N	\bar{x}	N	\bar{x}	N	\bar{x}
Nagyszénás	153	1,96	22	1,64	255	0,83
Székkutas	59	2,10	14	1,93	145	1,47
Hódmezővásárhely	121	1,98	50	1,90	134	1,38
Ruzsa	33	1,88	19	1,74	74	1,29

Table 5.

Reproductive losses between 2002-2005

	Fetal loss		Rearing loss		Total loss	
		%		%		%
Nagyszénás	0,32	28,3	0,81	71,7	1,13	100
Székkutas	0,17	26,9	0,46	73,1	0,63	100
Hódmezővásárhely	0,08	13,3	0,52	86,7	0,60	100
Ruzsa	0,14	23,7	0,45	76,3	0,59	100

The losses of reproduction are usually greater and so the number of the reared offspring is lower where the number of the potential offspring is higher and as it is especially the result of the rearing losses, we have to look for the causes within the environmental system. One important factor might be the differences between the habitat types. The better quality roe deer populations can be found on the intensively cultivated agricultural areas; here the number of the potential offspring is the highest (number of CL). At the same time the rearing losses are also the greatest here. I think it can be related, on the one hand, to the quantity and quality of the available foodstuff in the vegetation period (positive factor) and, on the other hand, to the lack of cover (negative factor) that could provide shelter in winter, which is the highest in the area with a weaker quality roe deer population (Ruzsa, forest cover approx. 30 %), this is why the fawn survival is the best here. Besides, the prey of the fawns by foxes is probably higher in open areas.

7. The correlation between the number of reared offspring and the current condition of does: There is a medium positive correlation between the two parameters ($r = 0,500$, $P = 0,207$ NS). Although it is not a significant correlation, the better condition resulted in the higher number of the reared offspring on three areas. The reason for this might be that the autumn-winter condition (KFI) of the does indicate the quality of the habitat (home range), which is basically important with regard to the survival (and also the birth-weight) and growth of the fawns. On the fourth area (in Ruzsa) the survival of the fawns primarily does not have a connection with the condition (feed supply) of the does.

8. The sex of fetuses: It was in 2004 that there were enough fetuses in Hódmezővásárhely for evaluation the sex ratio of which showed female dominance (41:59 %). If we accept the observation that in a habitat with limited conditions the does have mainly male kids, then we can say that this territory with the existing population density does not limit the potential reproductive performance. In this very same place I found correlation between the age of the does and the sex of the fawns. There is a medium positive correlation between the age of does and the number of male fawns ($r = 0,479$, $P < 0,05$), while in case of the female fawns there is a weak-medium negative correlation ($r = - 0,316$, $P = 0,175$ NS). The prime-aged (2-7-year-old) does have a higher ratio of female fetuses, while in case of the older ones (older than 8 years) the

number of male fawns is one and a half times higher. With regard to the number of reared offspring there is more or less a female dominance. The significantly higher number of does in the reared offspring in Hódmezővásárhely and Ruzsa probably cannot be the result of such a high deviation from the fetal sex ratio, it is more likely to happen because of some mortality factor affecting the male fawns more strongly.

9. **The size of the fetuses:** When comparing the 26 fetuses of 11 does shot after 20th January the males were 1,7-26,7 % (average 10,77 %) significantly bigger ($P < 0,05$). There is possibly a similar size difference between the kids at birth as well. It is questionable if it has any effect on the survival chances of the fawns, if there is a sexual selection, which could be at least partly an explanation to the considerable difference of the sex ratio of the reared offspring from the 50 – 50 %.

10. **Early embryonal sex-identification with PCR-method:** We made the first step towards this as we managed to set reaction parameters and conditions which help us to select the fragment on the roe deer DNA chain surely containing the SRY-sequence. The early sex-identification enables us to verify the sex of the blastocysts and early embryos (January pregnancy) washed out of the uterus of the does, then, based on this, we can examine the quantitative and qualitative (sex) conditions (differences) between the embryonal diapause and the implantation. My further objective is to make the PCR-reaction more specific, that is why the first step is to identify the basis rank order of the SRY-gene and then synthesise a specific primer for this.

THE NEW AND NOVEL SCIENTIFIC RESULTS OF THE ESSAY

1. Using the results of the research of FODOR (1978), SUGÁR (1979), KÖNIG (1988) and FARKAS (2004) as a basis, I found that the fertility of the does did not change in a statistically definable way and there is no difference between the areas in this respect. **However, the individual distribution of the fetus number changed significantly**, which means the dominance of the does with 2 fetuses, while the ratio of the does with 1 and 3 (4) decreased. In the subsequent phases of the reproduction process I presented **the numeral change of the characteristic parameters (number of CL, number of fetuses, reared offspring) in space and time** and within that the compensational connection between the potential and the realised offspring, where, according to my results, the current condition of the does have a role, which indicates the quality of the habitat as well.
2. I revealed the numerical correlation between the age, the BW, the condition (KFI) of the does and the reproductive parameters. **Among the does with greater BW the incidence of more than two fetuses is higher, while the improvement of the condition results in a slight increase of the number of CL and the number of fetuses.** Furthermore I pointed out the difficulties in the examinations of the above-mentioned correlation especially due to the low variance of the CL number.
3. With the help of the available **data I tried to determine the threshold BW of the rut and pregnancy of the does.** At the time of the puberty of the female fawns I found signs of the correlation between the number of the follicles and the condition, as the average KFI (0,68) in the female fawns with active ovary was the double of the values measured in the ones with inactive ovary (KFI = 0,32).
4. During my examinations concerning the sex of the fetuses and the above-mentioned parameters of the does I found correlation between the sex of the fetus and the age of the doe. Accordingly, **the prime-aged (2-7-year-old) does had more female fetuses, while the ratio of the male fetuses were higher in the ones that were more than 8 years old.** I also highlighted that there were differences in the sex ratio of the reared offspring on each area, especially in Ruzsa where the ratio of the male fawns was very

low, which, I presume, can be the result of some environmental factor strongly deteriorating the survival of the male fawns.

5. To examine the suspected correlation between the quantitative changes (fetal loss) during the embryonic diapause and the fetal sex ratio in the uterus we used a method not applied in the examinations of the roe deer before. **The first step was to work out and prove the possibility of the early sex identification with PCR method.**

6. Based on the sampling methods and the examination methods as well as the results of the **examination I worked out a roe deer population regulation model.** Using the number, composition, reproduction and mortality data of the given population we can plan the target population and the implementation of the plan can be followed day by day.

THE PRACTICAL USABILITY OF THE RESULTS

The environmental factors and characteristics affecting the reproductive performance of the roe deer populations **change differently by the areas and by the periods.** Knowing the correlation between them, collecting and using relevant data we can make the foundation of the rational population management and harvest. This job cannot be built on the „old approved methods” of the recent years, on data based on supposition; **we always have to consider the changes that have taken place and their impact on the roe deer population.** During the experiment I pointed out the changeability, within a region and also between the regions, of the most essential parameters having effect on reproduction as well as the basic correlation between them, **which must be used in practice.**

The population regulation model that I worked out can be run on computer (in MS Excel environment) and can be used in practice; the basic data and bio-indicators necessary for its operation can be taken up in a way described in the material and method part. The described model is suitable for basic evaluation, which certainly can be expanded. To make professional evaluation and draw the conclusions are the tasks of the local game managers.

The essence of the operation of the model is that the programme calculates the result after loading the basic data and parameters listed below. **In this way it is possible to get from an existing population to a planned target population while knowing the details of the way leading there.**

The parts of the model:

Table I.: Roe deer population regulation model (to reach the planned target population). Its essence is (after loading the basic start data and the target data and setting the parameters) how to get from an existing population to a planned (imagined, optimal) target population. The programme calculates the number of animals that have to be shot to reach this objective.

Table II.: Roe deer population regulation model (to complete the plan and control it). Table II offers the possibility to control the completion of the plan after transferring the necessary data from Table I. and finally it gives the population (from the loaded data and the number of the shot animals) at the end of the hunting year.

Basic data:

- Spring population: After the doe-hunting season it can be determined by counting, grouping them as buck–doe–male-fawn–female-fawn.
- The age distribution of the bucks: The ratio of the one-year-old and adult bucks can be calculated on the basis of observations on the area. Within the adult group the ratio of the young, middle-aged and the old must also be given. The ageing will happen automatically according to the parameters I have set. Accordingly the 2-4-year-old belong to the young, the 5-7 -year-old belong to the middle-aged, while the ones more than 8 years old belong to the old age group. Certainly it can vary by regions and the programme can be modified.
- Number of CL, number of embryos/fetuses and the number of reared offspring: The CL number and embryo/fetus number counts can be done easily from the shot animals; at the end of the hunting season (from mid-January) the sex of the fetuses can be identified as well. We can estimate the reared offspring by counting, then it is necessary to distinguish the fawns by sex.
- Mortality: It is a parameter most difficult to estimate. We must estimate the mortality percentage (summer population percentage) of the young and the adult separately, by sex. At the fawn mortality we must consider that the survival of the buck fawns are usually worse than that of the doe fawns. The mortality of the young bucks is probably higher than that of the adults. When we have learnt the average number of the born fawns from the CL examinations, we can estimate the average number of the reared offspring with an autumn count and a representative sample. The difference between the two adds up to a significant part of the fawn mortality.
- The examination of the shot animals: takes place by the bag. When examining the does by their teeth we can distinguish the young (subadult), the ones that have not had kids yet. All the others belong to the adult group. The registration of the male and female fawns is not a problem. To draw a distinction between the young bucks is simple, all the others are adult bucks.

After the planned and executed shots the final population (which is, theoretically, the same as the population of next spring) can be and must be checked with the spring count. There is a possibility of the population being different from the optimal so much we can reach our goal in several steps that is in a few years only. It is particularly so

when we wish to increase the population number at the same time. The planned implementation can be modified in the meantime (e.g. the extent of mortality changes) and of course, the parameters can be altered (actualised) accurately, any time. The programme will calculate the result according to this.

It is important to compare the planned and realised shots to be able to learn the current number of the population.

The model is suitable for calculating the different variations and for the demonstration of the expected changes in the parameters. This enables us to carry out an incomparably faster evaluation, control and feedback than the manual calculation method; moreover, we can see the possible effects of the changes in the characteristic parameters immediately. It is obvious, however, that the use of the model needs expertise and control, we cannot trust the results blindly. Nevertheless I believe that with **conscientious data collection and control it can be a usable tool in planning and realising roe deer population regulation.**

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