SUMMARY

Keywords: bull, fertility, non-return rate, CASA.

Thesis entitled "**Correlation between the biological value of semen and fertility, in beef bulls breed**" contains 176 pages and its made according to the regulations currently in force; consists of two main parts. To achieve this, it was used, as source of information and documentation, 181 number of bibliographic titles from romanian and abroad literature regarding the subject of the thesis, information was subsequently used to interpret the data obtained in the second part.

The first part entitled "**The current study of knowledge**" expands on a number of 72 pages and is divided into 3 chapters that present data from the literature regarding reproductive morphology and physiology of the bull, management of the bulls, methods of semen collection, evaluation of the biological value of semen, dilution and preservation of semen and artificial insemination.

The second part, entitled "**Personal contributions**" includes a number of 100 pages and is structured in 7 chapters, showing the purpose and importance of the research, materials and methods, results of analyzes and related discussions and ends with general conclusions.

In the first chapter of the second part entitled "The aim and objectives of the thesis' research objectives are presented as follows:

- the significance of the using computer-assisted analysis techniques to obtain as much objective information about the biological value of fresh or frozen / thawed semen;
- fertility evaluation of beef breeds bulls based on data collected from the field about reproductive performance of cows;
- assessing bull fertility according to some parameters of semen and non-return rates of cows;
- determining correlations between different semen parameters (mobility, volume, concentration, etc..) and non-return rates of cows.

Chapter V -,,**Methods of bull semen processing in two frozen semen producing units**"- presents the methodology of assessing the biological value of bull semen from two semen production units in Romania and influence of methodology over semen quality.

In **chapter VI**, entitled "**Analysis of fertility of beef breeds bulls from unit B, expressed through non-return rates of cows**", are made correlations between semen fertility

of 10 bulls of different beef breeds for a period of four years (2008 - 2011) and the value of non-return rate of females, according to: year, month, age of cows and bulls breed. The analyze of non-return rate from each year revealed that: 2011 recorded the lowest non-return rate (62.32 ± 9.72%) and the highest value (67.22 ± 7.77%) was recorded in 2009. Average non-return rate in the 4 year period was 64.84 ± 5.83%.

Regarding the non-return rate value according to the month in which artificial inseminations were performed, the highest value was obtained in April-May-June, a period coinciding with taking out the animals to pasture, grass-based diet plus concentrate feeding. Berry et al. (2011) following a study conducted in Ireland regarding non-return paths for each month of the year, observed an increase in fertility in April and May.

These non-return rate variations can be caused by many factors, including: weather conditions prevailing in each month, the inexperience of technicians who have performed artificial insemination, inaccurate estrus detection and a lower or a higher number of inseminated females in each month.

During the study, the largest non-return rate was recorded at Aberdeen Angus breed bulls (66.64 ± 4.4%), the following positions being placed Limousin bulls (64.95 ± 2, 44%), Blue Blanc Belgian (64.09 ± 1.92%) and Charolaise (63.81 ± 1.68%). Low fertility in Limousin and Charolaise bulls was reported also by Berry et al., in a study published in 2011 (Berry et al., 2011).

In the following chapters it was analyzed the semen from Flechvieh bulls (Dual Purpose Simmental), from a semen production unit from Bavaria, Germany. Bulls were fed and had identical maintenance conditions, they are high value bulls and are used as donors of high quality semen.

Chapter VII entitled **"Time resistance of fresh semen while diluted with caprogen 5% and kept at different storage temperatures"** followed analysis of some parameters of semen stored for up to 9 days at three different temperatures: 4^0C, 12^0C and 22^0C, in order to observe the changes that may occur after storage at the three temperatures. In the present experiment it was found that the values were higher for the semen stored at 4^0C compared to the other two categories of temperature. Results that are not consistent with the results obtained by Shannon and Curson in 1984, which demonstrated that the survival of spermatozoa (from semen diluted with Caprogen) at 37^0C in vitro, was significantly higher after storage at 16^0C - 20^0C than after storage at 5^0C and was also significantly higher after storage at 15.6^0C - 21.1^0C than after storage at 26.7^0C - 32.2^0C. Spermatozoa trajectory parameters (DSL, DCL and DAP) and velocity parameters (VAP, VCL and VSL) showed

higher values for 4^0C storage temperature during the 9 days of the study, those values decreasing progressively for the other two storage temperatures, 12^0C and 22^0C respectively. Both parameters describing the straightness movements (STR, LIN and WOB) and those that describe the types of sperm movement (ALH and BCF) were higher for 4^0Cstorage temperature. The unit in which were carried out the studies, recommends insemination with the semen diluted with Caprogen 5% and stored at 4^0C only for a period of up to 4 days, although in the present study, the motility is maintained within acceptable limits at least 9 days, just as in the case of semen diluted with Caprogen.and stored at 12^0C.

Artificial insemination centers need accurate methods to determine sperm concentration and membrane integrity, essential determination to maximize the number and quality of insemination doses prepared from fresh semen and optimize fertility of thawed semen. The lack of precision regarding the right estimation of the semen concentration, directly affect the units efficiency to produce semen, semen quality and fertility (Anzari et al., 2009).

Thus, Chapter VIII, entitled **"Determination of raw semen concentrations using three methods: SQA, SDM 5 and Thoma counting chamber and comparing the results"** aimed to evaluate the accuracy of three methods for determining the concentration of semen: two new methods SQA and SDM5 and classical method with Thoma counting chamber.

Average semen concentration was 1.11 ± 0.22 measured with Thoma counting chamber, 1.13 ± 0.16 with SQA and 1.26 ± 0.19 with SDM5. Concentration measured with Thoma counting chamber and SQA were similar and the concentration measured SDM5 was higher than the first two methods.

ANOVA - *one way* demonstrates that there were no statistically significant differences between the three compared methods ($p = 0.683$). Tukey's test also showed that the semen concentration measured with Thoma counting chamber, SQA and SDM5 are statistically similar ($p > 0.05$).

Correlation relationship between the three methods showed that there is a strong linear correlation, positive and statistically significant ($p < 0.001$). The coefficient of determination (R2) between SQA and SDM5 was 0.937, very close to the value 1. In the other two cases of correlation Thoma vs SQA and Thoma vs. SDM5 coefficient of determination was also high: 0.897 and 0.863. Determination of semen with SDM5 is the fastest and cheapest method. The long time needed for concentration determination with Thoma counting chamber and errors that may occur due to the human factor represent disadvantages and analysis with SQA has a big disadvantage due to the high costs of consumables.

Artificial insemination in cows are mainly performed with frozen / thawed semen. However, the cryopreservation induces loss of sperm viability and causes functions destruction of sperm that survive, which results in a lower fertilizing capacity of frozen / thawed semen (Watson, 2000). Several studies (Dham et al., 1992; Dham and Sahni, 1993 and Kaproth Foote, 2002; Gao et al., 1997; Muiño et al., 2007) question the need of semen equilibration and its actual benefit over sperm viability.

Thus, the minimum equilibration time required to be successful, in semen cryopreservation, remains controversial (Dham et al., 1992; Dham and Sahni, 1993).

Chapter IX entitled, "Analysis of frozen-thawed bull semen subject to different equilibration times by three methods" aimed to analyze sperm mobility subjected to three different equilibration times: 4, 24 and 72 hours, using three methods. The higher was obtained at 24 hours equilibration time analyzed by IFN Schonow (56.5 ± 10.75) and SQA (64.44 ± 10.21). Also a good percentage of sperm mobility for the two methods was obtained at 72 hours equilibration time, 48.08 ± 6.73 - IFN Schonow and 57.16 ± 18.59 - SQA. When analyzed with SpermVison highest mobility rate was recorded for the 4 hours equilibration time (43.19 ± 7.17) respectively 72 hours (41.41 ± 5.78). The results are consistent with studies performed by Almquist and Gilbert (1978) and by Frijters (2003), which are to an extended equilibration time: between 9 to 16 hours, because they have noted that after this period a better percentage of acrosomal integrity (membrane integrity) than in shorter equilibration periods.

Chapter X - "**Correlations between some parameters of raw semen and non return rate at 60-90 days in Fleckvieh bulls"** aimed semen quality assessment, retrospective measurement of fertility rate regarding non-return rate and also establish the relationship between some semen parameters determined using standard laboratory techniques, equilibration time, the type of semen (frozen or liquid) and non-return rates. A total of 1,275 with 1,184 non-return rates associated to inseminated cows and 449 non-return rates associated to inseminated heifers were analyzed. Those ejaculates were obtained between 2nd November, 2009 and 28th October, 2011. A total of 77 394 cows and 40 250 heifers were inseminated. Of these 47 992 cows and 28 284 heifers were declared pregnant.

The average ejaculate volume was 6.03 ml ± 1.96, with a range between 3.97 ± 0.98 ml and 8.18 ± 1.92 ml, differences between bulls were statistically significant ($p < 0.001$). Responsible for volume differences of semen between bulls can be: individual variations of genetic potential and genetically superior bulls produce higher volumes of semen. Average mobility from the 8 bulls was 68.88 ± 5.51%, range between 62.67 ± 5.37% and 71.63 ±

5.14%, differences between bulls were statistically significant ($p < 0.001$). Sperm motility is one of the most important parameters associated with fertilizing ability of semen and for many years recognized as essential for the transport of spermatozoa in the female reproductive tract (Januskauskas et al., 1999 Verstegen et al., 2002).

Average concentration values was $(1.49 \pm 0.39) \times 10^9$ sperms / ml, range between $(1.86 \pm 0.29) \times 10^9$ sperm / ml and $(0.87 \pm 0.25) \times 10^9$ sperm / ml, differences between bulls were statistically significant ($p < 0.001$).

In cows the average non-return rate value was 63.11 ± 8.24%. In heifers the average non-return rate was 69.72 ± 7.88%, its lowest value being 66.47 ±, 9.69%, while the highest value was of 71.45 ± 2.95%. Differences between bulls regarding non-return rate in heifers were not significant, $p > 0.01$ ($p = 0.210$). Regarding the non-return rate differences between cows and heifers, Malham et al. (2007) suggest that low developmental competence of oocytes from older cows may explain the decreased fertility observed in them. Also, multiple calving are responsible for the delayed uterine involution and diminishing uterine conditions and represents a major risk for postpartum fertility (Berglund, 2008).

Analysis of the relationship between volume and non-return rate. The value of the correlation coefficient (r) - 0783 shows that between the two variable, volume and non-return rate, there is a strong negative correlation significantly different from zero ($p < 0.05$) and the coefficient of determination (R2) has a value of 0,613.

Analysis of the relationship between mobility and non-return rate. Estimated correlation coefficient is 0.880, showing that between the two variables, non-return rate and mobility, there is a strong positive correlation, as mobility increases, non-return rate also increases. Correlation is significantly different from zero, where $p < 0.01$ and the coefficient of determination is 0.775.

Analysis of the relationship between concentration and non-return rate. Estimated correlation coefficient of 0.822 shows that between the two variables, concentration and non-return rate, there is a strong positive correlation. As the concentration increases, non-return rate increases. Correlation is significantly different from zero, where $p < 0.05$ and the coefficient of determination (R2) has a value of 0.675 which means that 67.5% of the variation of non-return rate is explained by the concentration value. The aim was also to compare non-return rates (as a witness of in vivo fertility) of two different methods of preserving semen: a method of preserving semen with Caprogen 5% (fresh/liquid semen) and a method of preserving semen with Tryladil (frozen semen). Non-return rate of semen diluted

with Caprogen 5% was higher compared with non-return rate obtained from semen diluted with Tryladil.

Sperm mobility related to the 24 hours vs. 4 hours equilibration time was approximately equal: 24 hours equilibration time - 69.29 ± 2, 10%; 4 hours equilibration time - 69.11 ± 2.83%.

Results are not consistent with the results obtained in Chapter IX, where the values regarding sperm mobility from IFN Schonow (56.5%) and SQA (64.44%) were higher during 24 hour equilibration than during 4 hours equilibration time (IFN Schonow: 47.46% and SQA 46.43%) respectively 72 h (IFN Schonow: 48.08% and SQA: 57.16%).

After analyzing mobility of semen collected on Monday, Wednesday and Friday on Friday showed the highest value of mobility (69.07 ± 3.24%), values from Mondays and Wednesdays being approximately equal: 68.74 ± 2.95% 68.70 ± 3.03%.

Depending on who performed the semen collection (veterinarians vs. technicians) the value of non-return rate was higher for the group represented by the veterinarians (66.12 ± 4.54%) compared to non-return rate value recorded for the group of technicians (64.98 ± 5.11%). Differences between the two groups can probably be put on the lack of experience of the technicians or incorrect semen collection.