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PARAMETERS OF THE QUALITY OF BULL SPERM AND FERTILITY[1]

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Abstract: Main prerequisite for success of artificial insemination is production of high quality deep frozen bull semen. Centers for artificial insemination try to produce and deliver semen doses of as high quality possible. However, bull semen is not the only prerequisite for success of insemination and other important conditions must be satisfied in order to realize conception. For instance, very important factor is the owner who detects and reports estrus and insemination and inseminator – person who carries out the insemination. For any kind of analysis of great importance is also correct keeping of records. In this paper, our intention was to compare obtained results on fertility achieved by insemination in one private veterinary clinic over period of several years and using bull semen from three centers for artificial insemination and results obtained from other station where several persons were performing inseminations. These are correct, precise data with recorded observations which were than analyzed and present objective situation achieved in application of artificial insemination in private veterinary clinics on the territory of the Republic of Serbia.

During regular activities relating to production of semen and insemination of cows and heifers, we have analyzed results of work of single veterinary clinic over the period of several years. Total number of first inseminations in the period November 1998 – December 2002 was 8071 females, and number of repetitions after first insemination was 2.378 or 29,46%, second repetitions 487 or 6,0 % and third repetitions 41 or 0,5%. During the mentioned period semen from 18 bulls was used (in average 366 doses for first inseminations or 497 doses per bull for all inseminations). Most inseminations per single bull were 1.684, and the least 52 doses. Percentage of repetitions varied from 20,31% to 47,05%. Total percentage of repetitions for bulls in centers of their origin was approximately from 28,91% to 30,36%. Index of semen utilization for all inseminations was from 1,346 to 1,380 doses. Through analysis of data collected in 2002 in comparison to season of the year it was observed that the lowest percentage of repetitions was recorded in the fourth quarter 25,48%, ant the highest in the first quarter 29,91%. We would like to point out that total percentage of repetitions recorded in 2002 was 28,11%. Similar results were obtained by analysis of insemination results in other private veterinary clinic and we observed considerable differences in percentage of repetitions between certain individual inseminators. In centers for artificial insemination for evaluation of the fertility – bull fertility, routinely N/R method is used for periods (56) or 60 – 90 days and breeding value of bulls expressed through percentage of pregnant cows after first insemination.

For evaluation of bull fertility several laboratory methods are used which are still not completely reliable and cannot predict with higher certainty fertility of bull in field conditions.

Key words: insemination, fertility, repetition, N/R method

Introduction

Efficient production of doses of deep frozen bull semen of high quality is prerequisite for success of artificial insemination. Centers for artificial insemination try to deliver semen doses of high quality with maintained possibility of conception after first insemination. Breeding bulls intended for AI centers are selected according to their genotype and phenotype traits as well as semen quality after cryo preservation including the fertility. They should first of all be healthy and tested for presence of various specific causers of diseases as regulated by law.

Usual conventional methods for evaluation of semen which are performed prior and subsequent to cryo preservation include determination of volume, color, smell, pH value, motility and morphology. These parameters enable detection of clear cases of sub or infertility but they don't predict the bull fertility. Subjectively evaluated quality of native and processed semen doesn't show significant links with fertility determined in field conditions. For now, there are numerous laboratory methods used for evaluation of the

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level of fertility but they are still not completely reliable. Beside traditional methods of evaluation of morphology and motility other methods should be mentioned, such as: fluorescent colouring, hypoosmotic sperm test, comparative analysis of sperm motility and detection of specific proteins in semen plasma responsible for fertility - High Fertility Proteins and Low Fertility Proteins.

Main efforts in this field of research are directed towards basic study of the physiology of sperm and improvement of functional laboratory techniques measuring the fertilization ability of fresh and processed bull semen.

Fertility of bull semen seems to originate from heterogenic nature of the sperm population in ejaculate, qualitative and quantitative parameters of the ejaculate and complex nature of fertilization. In present conditions of application of cattle insemination we come across number of few tens of thousands cows inseminated annually by semen from single bull. This was possible not only by the selection of best bulls in regard to their performances in breeding and production but also due to improvement in techniques of semen collection and processing, also cryo preservation with the lowest possible number of sperms in doses guaranteeing acceptable fertility in field conditions.

Application of strict health and andrology control of bull with application of adequate order of semen collection depending on the age, breed, testicle size and seasonal variations in semen production, obtaining of high quality semen is ensured free of pathogenic presence, and with adequate dilution and improvement of cryo preservation process and addition of different combinations of antibiotics. Directive 90/429/EEC regulates number of spermatozoids per dose to 15.000.000, with use of adequate combination of antibiotics added to native semen per 1ml to provide health safety of semen doses.

Material and methods

Usual procedure of bull semen collection performed in our country as well as in majority of countries in the world is carried out twice a week with the use of semen collector from all bulls. After obtaining of native bull semen, volume, color, smell and concentration using Spectrophotometer (IMV) are recorded. Corrugated (wavy) movement, progressive motility and number of live and degenerated spermatozoids are evaluated under microscope with phase contrast using enhancement of 200 x. After such examinations semen with progressive motility above 70% is diluted to 20 - 25 x 10 -6 native spermatozoids in doses using Tris solvent with egg yolk and glycerin in recommended ratio. Diluted semen is later packaged in 0,5 ml. or 0,25 ml. pailletes Cassou (IMV). Filled pailletes are then cooled for period of 4 -5 h on + 4 degrees Celsius and subsequently frozen for 10 minutes on - 120 to -130 degrees Celsius and submerged directly into liquid nitrogen and stored until later control after freezing. Evaluation after thawing was carried out on 1-3 pailletes per ejaculate. Pailletes were thawed on + 38 degree Celsius and kept in water bath 30 - 60 seconds prior to evaluation of motility and percentage of live spermatozoids (phase contrast microscope, enhancement by 200 x). Semen with less than 60% of motile spermatozoids is not acceptable for artificial insemination and it is discarded as not fit for use. In our center for AI 4-11% of such discards are registered annually depending on many factors. Semen which was evaluated as ready for use can be distributed in quantities demanded without any limitations. 60 to 90 days after insemination, expert of the center for AI or person carrying out inseminations collects data on first insemination and repetitions according to N/R method and processes collected data in order to evaluate fertility for each single bull. This is usual practice in our Center from its founding until today.

Data on insemination are taken from the AI records according to principle of first insemination and repetition and processed according to N/R method for period of 60 to 90 days. Also data given as remarks – observations on inseminations and repetitions were considered. These data is important for explanation of reasons for repetitions presented in this paper.

Results and discussion

Insemination of cows and heifers in Republic of Serbia is traditionally carried out by veterinary doctors, technicians or educated/trained inseminators organized in veterinary stations, clinics or cooperatives. Recently number of cows and heifers inseminated is increasing and amounts to over 50% of all females. This is considerable increase, considering that number of inseminated females stagnated for long time on 35%. There are several reasons for such development: more executors of this measure, less females in total,

economical demands for high quality offspring, better quality of semen and executed services in insemination, etc.

Fertility of bulls is often taken as reason of repetitions of inseminations but that is not always the case. It is understood that semen from AI centers is delivered in standard quality. Possibility that reason for repetition of insemination is bad quality of bull semen exists but it is not always the main reason. There are differences in fertility of bulls which can be registered based on results collected in the field. It is still not possible to predict the quality of semen in laboratory although great progress in this area has been realized and many new laboratory methods developed.

Main reasons for repetitions, as stated by numerous authors, are: time of insemination and detection of estrus, inseminator as executor of this measure, etc. There are significant differences between individual inseminators in realized insemination results and this fact must also be accepted as objective factor. Also, detection of estrus and determination of time of insemination are equally important and need much attention from owner of the female animal, which is not always the case. These two reasons are in correlation and need to be closely studies in future evaluations of bull fertility (*Hadshow 1993*).

Collected and processed results of obtained over period of several years of engagement in insemination in two private veterinary clinics are presented in table 1.

Table 1. Insemination results according to years in AI centers and first veterinary clinic

	Inseminations					
	I	II	III	IV		
Center					% of repetitions	Year
002	116	37	12	1	31,89	1998
002	993	296	58	1	29,80	1999
001	596	194	49	3	32,55	"
003	163	50	14	1	30,67	"
001	1.076	310	55	10	28,81	2000
002	453	146	24	2	32,22	"
003	493	141	24	3	28,60	"
001	1.024	353	87	5	29,31	2001
002	413	125	35	3	30,26	"
003	302	90	18	2	29,80	"
001	1.694	466	73	7	27,50	2002
003	568	170	38	3	29,92	"

Table 2. Insemination results according to AI centers for period from 1998 to 2002

	I	II	III	IV		
Center	Number of inseminations				Percentage of rep.	Index of semen use
001	4.570	1.223	264	25	28,94	1,352
002	1.975	604	129	7	30,58	1,362
003	1.526	451	94	9	29,55	1,374
Total	8.071	2.378	487	41	29,46	1,359

Achieved results regarding bull fertility are presented in table 1 and relate to results obtained in AI centers in different years. From data presented in table 2 it is apparent that insemination results obtained with semen from bulls of AI center 001 show least repetitions and with the highest number of first inseminations 4570 and 1.223 repetitions or 28,94 %, also the best result was realized. Total result regarding (29,46%) is acceptable and within limits of normal results achieved in insemination using this method.

Analysis of insemination results in the first veterinary clinic for year 2002 shows 2.262 first inseminations and 757 total repetitions, of which 636 were first repetitions, 111 second and 10 third repetitions. Achieved fertility according to N/R method was 71,89 %.

Structure of repetitions, or established diagnosis in females coming for repeated inseminations, is following:

- repetitions in regular cycle (18 - 23 days) in 172 heads or 27,04 %,
- with diagnosed endometrite 121 heads were registered or 19,02 %,
- repetitions with irregular cycle (from 8 -180 days) in 274 heads or 43,08 %,
- repetitions in period from 36 to 44 days, 74 heads were determined or 27,00 %,
- other repetitions could not be classified in any of above diagnosis and have unknown aetilogy.

Insemination results achieved in the second veterinary clinic during 2003 are presented in table 3.

Table 3. Results of insemination according to inseminators achieved in second clinic during 2003

Inseminator (code)	Insemination I	II	III	% repetitions
232	347	111	26	31,98
237	442	127	21	28,73
235	646	160	33	24,76
204	328	73	11	22,25
227	606	174	20	28,71
Total	2.369	645	111	27,22

In table 3 we can observe results regarding repetitions in relation to inseminator, using bull semen from the same AI center and in the same time which contributes to the thesis that inseminator as factor is very important and that there are significant statistical differences in achieved results. Total insemination results achieved in the second veterinary clinic are overall better, with lower repetitions percentage compared to the first clinic and with the semen from the same AI center.

Jovicin et al. (1997) sates result obtained by *Kommisurd et al. (1996*) who compared insemination results obtained in two AI centers in regard to average percentage of pregnancy of 70,5 and 71,4% as statistically significant. *Christensen P. et al. (1999)* state results on fertility of bulls according to N/R method for Jersey (63,50 %) and Holstein breed (69,20 %) and indicate great variations for both breeds.

Wiltbank J.N. et al. (1982) have determined that level of pregnancy had increased by 5-6% when cows had been inseminated with semen with over 70% of normal spermatozoids compared to random group.

Conclusion

- Analyzed insemination results are acceptable and are within limits of optimum,
- Beside quality of bull semen results of insemination are under great influence of time of insemination as well as person performing the insemination,
- There is significant difference in fertility and quality of bull semen and therefore it is necessary to make selection based on fertility,
- There is still no completely accurate laboratory method to predict fertility of bull semen in field conditions.

PARAMETRI KVALITETA SPERME BIKA I FERTILNOST

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R e z i m e

Osnovni preduslov za uspeh vestackog osemenjavanja je proizvodnja kvalitetnog duboko zamrznutog semena bikova. Centri za vestacko osemenjavanje nastoje da proizvedu i isporuce doze semena, sto je moguce boljeg kvaliteta. Obzirom da seme bika nije jedini preduslov za uspeh osemenjavanja, jer postoje i drugi preduslovi koji se moraju ispuniti da bi doslo do oplodnje.Tako je vazan preduslov vlasnik koji vrsi otkrivanje i prijavljivanje estrusa i osemenjavanja i osemenitelj - osoba koja vrsi osemenjavanje. Za bilo kakvu analizu osnova je tacna evidencija. Mi smo u ovom radu zeleli da uporedimo dobijene rezultate plodnosti postignute osemenjavanjem u jednoj privatnoj ambulanti u toku visegodisnjeg osemenjavanja semenom bikova iz tri razlicita Centra za v.o. i rezultate rada u drugoj stanici, gde na poslovima osemenjavanja radi vise izvrsioca. Ovo su tacni i pedantno vodeni podaci sa evidentiranim mnogim zapazanjima koja su analizirana i daju realno stanje koje se postize u primeni vestackog osemenjavanja na privatnom sektoru Republike.

U toku redovnog rada na poslovima proizvodnje i osemenjavanja krava i junica analizirali smo rezultate visegodisnjeg rada u jednoj privatnoj veterinarskoj ambulanti.Ukupan broj prvih osemenjavanja u periodu novembar 1998 - decembar 2002 godine iznosio je 8071 plotkinja, a broj pova|anja posle prvog osemenjavanja 2.378 ili 29,46%, drugih pova|anja bilo je 487 ili 6,0 % i trecih 41 ili 0,5%. U pomenutom periodu korisceno je seme 19 bikova (prosecno po 366 doza za prva osemenjavanja ili 497 doza po biku za sva osemenjavanja). Najveci broj osemenjavanja po jednom biku iznosio je 1.684, a najmanji 52 doze. Procenat pova|anja kretao se od 20,31% do 47,05%. Ukupan procenat pova|anja za bikove po centrima iz kojih poticu je priblizan i kretao se od 28,91% do 30,36%. Indeks utroska semena za sva osemenjavanja bio je od 1,346 do 1,380 doza. Analizom prikupljenih podataka u 2002 godini u odnosu na godisnje doba zapazeno je da je najmanji procenat pova|anja zabelezen u cetvrtom kvartalu 25,48%, a najveci u prvom 29,91%. Napominjemo da je ukupan procenat pova|anja zabelezen u 2002 godini 28,11% . Do slicnih rezultata smo dosli analizom osemenjavanja u drugoj privatnoj stanici, i uocili znacajne razlike u procentu pova|anja izme|u pojedinih izvrsioca osemenjavanja. Od metoda za ocenu plodnosti - fertiliteta bikova kao rutinska metoda u centrima za v.o., koristi se N/R metoda, za periode (56) ili 60 - 90 dana, i izrazava priplodne vrednosti bika preko procenta steonih krava nakon prvog osemenjavanja.

Za procenu fertiliteta bikova postoji vise labaratorijskih metoda koje jos uvek nisu pouzdane i ne mogu sa sigurnoscu predvideti fertilitet bika na terenu.

Kljucne reci: osemenjavanje, plodnost, povaćanje, N/R metoda.

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