CREATION OF A GERMPLASM BANK (FROZEN SPERM AND EMBRYOS) IN THE MORENAS GALLEGAS CATTLE BREEDS PRESERVATION PROGRAM

FORMACION DE UN BANCO DE GERMOPLASMA (SEMEN Y EMBRIONES CONGELADOS) EN EL PROGRAMA DE PRESERVACION DE LAS RAZAS BOVINAS MORENAS GALLEGAS

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Palabras clave adicionales

Razas bovinas autóctonas. Preservación recursos genéticos. Parámetros seminales. Factores de variación semen. Factores de variación embriones.

SUMMARY

The best conditions to obtain a germplasm bank are evaluated to develop an *ex situ* method to preserve Morenas Gallegas cattle breeds. The spermatic characteristics of these populations were studied, using 2286 ejaculates obtained in 1143 collections proceeding from 50 bulls: 6 Cachena, 5 Caldelana, 5 Limiana, 5 Vianesa and 29 Alistano-Sanabresa (including those of Frieiresa breed). All the bulls were kept in the Centre of Animal Reproduction and Selection of Fontefiz (Ourense) to obtain frozen semen, between 1985 and 1989. We have analyzed and evaluated seminal parameters and observed that season, year of collection, age and bull breed have a significant influence on spermatic characteristics.

Some factors of embryo transfer technology were studied in 31 cows kept in the same Centre of Fontefiz. Superovulatory response, evaluated as number of *corpora lutea* and follicles, embryos and ova, and viable embryos, is highly specific for each breed. Other factors wich have an influence on superovulatory response in Galician cattle breeds are hormonal treatment and dose of gonadotropic hormones. However, the season of year, repeated superovulations, age and number of calvings of donors, and the day of oestral cycle in which superovulatory treatments were initiated, have had no influence on superovulatory response in these breeds, considered as a whole.

RESUMEN

Para el desarrollo de un método *ex situ* de

preservación de las razas vacunas Morenas Gallegas, se valoran las mejores condiciones para la formación del banco de germoplasma (semen y embriones congelados). Se estudian las características espermáticas de estas poblaciones a partir de 2286 eyaculados obtenidos en 1143 recogidas, procedentes de 50 toros de las razas Cachena (6), Caldelana (5), Limiana (5), Vianesa (5) y Alistano-Sanabresa (29, incluida la Frieiresa), empleados en el Centro de Escolma e Reproducción de Fontefiz (Ourense) para la producción de semen congelado durante los años comprendidos entre 1985 y 1989. Se analizan y valoran los parámetros seminales y se comprueba que la estación, año de recogida, edad y raza del toro, influyen significativamente sobre las características espermáticas.

Se estudian también algunas técnicas de transferencia embrionaria, a partir de 31 hembras adscritas al mismo Centro de Fontefiz. La respuesta superovulatoria medida como número de cuerpos lúteos y folículos, de embriones y óvulos y de embriones viables, ha sido específica según la raza. También, el tratamiento superovulatorio, y la dosis de hormonas gonadotropas han influído en la respuesta superovulatoria. Las superovulaciones repetidas, la estación, edad y número de partos de las donantes, y día, del ciclo estral, que comenzó el tratamiento, no han afectado a la respuesta superovulatoria de estas razas.

INTRODUCTION

Current animal production is determining the decrease, and even extinction, of many of farm breeds, which causes the loss of their genes, ignoring productivity, response to genetic improvement or heterotic power. From these statements some reasons for preservation can be deduced:

- Loss of genetic variation will limit man's capacity to respond to future necessities (changes in economic forces for the exploitation of animal production in tomorro's world).
- Loss of many breeds specially adapted to hard climates and able to use poor quality feed and resist diseases.
- There is a lack of scientific and economic evaluation of purebred indigenous breeds in their environment to know the real value in an integrated animal production system.
- The autochthonous breeds are of major sociologic importance, because they are the result of a process of human civilization, animal domestication, and isolation in different environments, over thousands of years, having special features and adaptations (animal diversity). They are an important aspect of human cultural heritage.
- By preserving autochthonous (indigenous) livestock breeds, we are preserving traditional animal breeding systems in some areas with difficult environmental conditions, avoiding depopulation and obtaining natural, homemade and high quality products.

These and other considerations show the necessity for the preservation of galician bovine genetic resources by means of an *ex situ* method or germplasm bank (cryopreservation of semen and embryos), due to the constraints of the preservation through living animals (maintenance costs, disease and accident risks, genetic drift, inbreeding depression...).

Today, it is possible to store a wide variety of living cells for long periods. In fact, the maximum length of storage time has not yet been experimentally measured, as it appears to be indefinite when cells are stored at the temperature of liquid nitrogen (-196 °C). Outstanding progress has been made with semen of domestic species

and the techniques are now a routine. Also, embryos of several mammalian species may now be frozen and subsequently used to produce normal offspring. We have used cryoconservation of sperms and embryos for the preservation of animal germplasm of Morenas Gallegas cattle breeds, but in the next years other effective techniques could be used, such as cryopreservation of oocytes, cells and nuclei, and storage of genes (isolated chromosomes, DNA, and isolated genes).

MATERIAL AND METHODS

Bovine frozen semen preparations can be stored in liquid nitrogen for a long time (it appears to be indefinite), with a slight decrease in fertility. While all the genetic information of a cattle breed is contained in semen from a prescribed number of males, a relatively complex breeding system over several generations is needed to regenerate a purebred population from semen alone, avoiding inbreeding (if there is only deep frozen semen one can restore the breed in the future only by upgrading procedure over 5 generations, which results virtually in the original breed or strain, but even then there are still about 3% of foreign genes).

However, when this method is used in a preservation program, freezing and storage of semen from the major number of bulls is the aim. As with closed nuclei of preservation of living animals, with frozen semen the maintainance of an adequate size of population is necessary, to avoid the loss of genetic variability by inbreeding; and this must be the principal criterion to calculate the number of individuals and cells in the cryoconservation program. The most important factors are:

- Effective population size of the preservation program.
- Freezing and thawing losses.
- Losses before introduction in the breeding nucleus.

Some experiments have been carried out to determine the optimal mating system which produces the minimum inbreeding. After Smith (1984), 25 sires (not related) per breed are needed to obtain an acceptable maximum level of 2% of inbreeding. This level of inbreeding is obtained in a standard bovine farm with a normal mating system in four generations.

We have carried out an analysis of different physiologic parameters of reproduction which can influence or conditionate the improvement of seminal doses production for the germplasm banks in the *ex situ* preservation program of the endangered galician native cattle breeds. These are: sexual behaviour, study and valoration of seminal characteristics of bulls, and fertility.

For this study we have used records of quality and production from 2286 ejaculates and of sexual behaviour from 50 Northwest brown sires: 6 Cachena, 5 Caldelana, 5 Limiana, 5 Vianesa and 29 Alistano-Sanabresa (including those of Frieiresa breed), kept in the Centre of Animal Reproduction and Selection of Fontefiz (Ourense), for frozen semen production between 1985 and 1989.

Semen was collected weekly, obtaining 2 ejaculates (n=2286), the second one ten minutes after the first one; for sexual behaviour different collections

were performed. The spermatic analysis of all ejaculates was based on volume (V), concentration (C), mass motility (M) and individual progressive motility, at the moment of collection (IPM), after a period of equilibration about 4-6 hours (IPME), and after freezing/thawing (IPMFT).

In the formation of the bank of frozen embryos, some factors which could influence the results of embryo transfer technology were evaluated. The donors of embryos were cows (19 Cachena, 7 Caldelana, 5 Vianesa), minimum of 2 years aged, with regular oestral cycles and optimum sanitary state. Superovulation was carried out using three gonadotropic preparations: FSH, HAP and PMSG. The product commercial FSH-P, is a FSH from porcine pituitary, using doses of 20-44 mg (Armour units), two daily intramuscular injections in decreasing doses, administered for four days. The HAP is a horse anterior pituitary or equine FSH, used with 21-33 mg of dose, in three intramuscular decreasing injections, for three days (one injection a day). The PMSG was used in doses of 2000-3000 I.U. in a single injection. The superovulatory treatments started from day 8 up to day 13 of the oestrous cycle, followed 48 hours later by an injection of a prostaglandin F_2 natural or structural analogue (500g). The donors were inseminated 12, 24 and 36 hours after the onset of superovulatory estrus, with one dose of frozen semen each time. In coincidence with the first insemination, a monoclonal anti-PMSG was adminestered intravenously (5ml into the jugular vein) to donors superovulated with PMSG.

The *corpora lutea* and follicles for each donor were counted on the day before collection by rectal palpation. The animals were flushed when at least one *corpus luteum* had been detected. Embryos were collected nonsurgically 6 to 8 days after estrus, and washed, in PBS + 0.2% BSA, for their evaluation, using IETS system (1 to 4). Embryos of category 1 and 2 (excellent, good or fair) were transferred into glycerol and PBS solutions of 0.47 M, 0.94 M and 1.4 M at 10 minute intervals. The embryos were then loaded into 0.25 cc plastic straws and frozen in a programmable freezer (Planner R204), by the conventional method of cooling (1°C/min from 20°C to -7°C, seeding, equilibration to -7°C for 10 minutes, 0.3°C/min. from -7°C to -35°C, 0.1°C/min from -35°C to -38°C) and immersion in liquid nitrogen (-196°C). After months of storage, some samples of frozen embryos were thawed (warm water at 35°C for 20 seconds) and cryoprotectant removed by six-step dillution, 5 minutes equilibration at each step (decreasing dillutions of glycerol of 1.40, 1.17, 0.94, 0.70, 0.47 and 0.23M). Embryos were washed in a holding medium and graded again. Viable embryos were transferred nonsurgically into recipients, which were palpated to determine pregnancy between 60 and 90 days after transfer. The post-thaw pregnancy rates were evaluated to verify survival after freezing.

The data were studied using analysis of variance (General Linear Models), and the correlation coefficients were calculated according to the method of least squares regression (SAS system).

RESULTS AND DISCUSSION

Seminal parameters of these populations, such as volume, concentration, and mass motility, are summarized in **table I**. There are significant differences by breeds and number of ejaculate. The average volume and spermatic concentration are higher, in general, than those of the most studied breeds.

The results obtained with the number of ejaculate show clear differences

Table I. *Statistics of the two ejaculates obtained from the same session (Sánchez García y Vallejo, 1990).* (Comparación de los dos eyaculados en cada sesión de recogida)

Breed	Parameteres	Volume (ml)		Motility (1)		Concentration (2)	
		EJ-1	EJ-2	EJ-1	EJ-2	EJ-1	EJ-2
	N	322		264		284	
Alistano-	x	5.07	4.83	1.96	2.80	1.22	1.07
Sanabresa	SD	2.12	1.85	0.88	0.56	0.47	0.38
	SE	0.12	0.10	0.05	0.03	0.03	0.02
	t	2.247*		-14.872		6.110***	
	N	70		60		64	
Cachena	x	4.47	4.90	1.87	2.63	1.23	1.11
	SD	1.74	1.53	0.57	0.58	0.50	0.24
	SE	0.21	0.18	0.07	0.08	0.06	0.03
	t	-2.442*		-9.172***		1.790*	
	N	260		228		224	
Caldelana	x	4.59	5.13	2.02	2.88	1.44	1.29
	SD	1.56	1.40	0.90	0.41	0.55	0.40
	SE	0.10	0.09	0.06	0.03	0.04	0.03
	t	-4.334***		-14.762***		4.300***	
	N	230		204		216	
Vianesa	x	5.86	5.40	2.17	2.87	1.37	1.00
	SD	2.38	2.13	0.80	0.42	1.34	0.26
	SE	0.23	0.20	0.08	0.04	0.03	0.03
	t	1.975+		-8.918***		10.974***	
	N	150		144		146	
Sayaguesa	x	5.48	5.48	2.28	2.90	1.41	1.07
	SD	1.84	1.61	0.84	0.39	0.43	0.34
	SE	0.15	0.13	0.07	0.03	0.04	0.03
	t	-0.031+		-9.349***		10.530***	

(1): Range 1 to 5; (2): (Espermatozoa/mm^3) x 106; N: number; x: Arithmetic average; SD: Standard deviation; SE: Standard Error of the mean; t: t test; + $p>0.05$; * $p<0.05$; *** $p<0.001$.

with results obtained in other breeds with similar environmental variance (Fuente *et al.*, 1984 and Gutiérrez *et al.*, 1989). In the second ejaculate no significant lower quantity (volume) was detected. Excepted Cachena breed, significant higher quality, mass motility ($p<0.01$) and lower concentration ($p<0.001$), have been observed.

In relation with sexual behaviour in all cases and breeds, the best global results were obtained with one or two collections per week regimes, carried out after 2.6 false mountings and with 7.5 minutes between both ejaculates.

Spermatic quality, as a predictor of fertility, was estimated using subjective methods and the averages of live and dead sperms percents (Bloom's classic method). We have considered the observations by Graham *et al.* (1980), who obtained correlations between 0 and close to 60p.c.

In **table II** we have summarized the statistical analysis of some factors of variation which can have an influence on seminal production and quality, and concluded that all effects have a significant influence on the seminal and spermatic characteristics studied, as data published by other authors show (Gutiérrez *et al.*, 1989).

Bull and year effects have explained the major variance percentages (66.72 p.c. and 89.68 p.c. respectively), the first, being more important for seminal characteristics of quantity, and the second, for quality factors. Breed has not been the most important component of the explained variance of the mathematic model, but it has been significantly higher ($p<0.05$) in the spring-summer seasons than in the autumn-winter seasons, when the minimum photoperiod occurs. The influence of year on the characteristics analyzed has been very significant. We must emphasize the very significant and rising curve of seminal dose quality, which indicates an improvement in the laboratorial methodology. Other tendencies shown in the data are the ratios age of bull/ seminal volume and age of bull/ number of sperms, which increased up to 4 years of age.

The results obtained for individual progressively motility (**table III**) show a high level of fiability and a significant differences between breeds, as in other parameters related with motility, IPME and IPMFT.

If we analyze the average losses (18.48 p.c.±3.73) for an average semen quality, it would result that the subjective data considered in this work are highly positive, because they are between 14.14 p.c. for Limiana breed and 13.39 p.c. for Vianesa breed.

At present 338566 doses belonging to 78 bulls of these endangered Morenas Gallegas cattle breeds, are stored in the bank of frozen semen: 26 Cachena (54275 doses), 19 Caldelana (105387 doses), 13 Frieiresa (58931 doses), 7 Limiana (64640 doses) and 13 Vianesa (55333 doses).

The results of superovulations carried out in females of Morenas Gallegas cattle breeds are shown in **table IV**. These results have a great informative value, in spite of the scarce number of animals used, because they belong to a high percentage of the population of living animals and they are the first data known for these breeds.

Table II. *Analysis of variance of different effects on semen characteristics of Morenas Gallegas bulls in artificial insemination (n:1143 observations). (Sánchez García and Vallejo, 1990).* Análisis de factores de variación que influyen en características seminales de toros de las razas Morenas del Noroeste (n: 1.143 observaciones).

Effects	V (ml) DF	R^2 (1)	F	C(2) DF	R^2(1)	F	NE (3) DF	R^2 (1)	F
Season	3	0.09	0.44+	3	0.08	0.34+	3	0.20	0.81+
Year	4	16.30	59.06***	4	50.76	163.85***	4	40.91	123.87***
Age	3	11.41	55.13***	3	2.60	11.18***	3	8.26	33.34***
Breed	5	21.78	63.11***	5	14.36	37.08***	5	12.28	29.77***
Bull	63	50.42	11.60***	62	32.20	6.71	62	38.35	7.49***
Error	1064			958			958		
m ± SD		5.3822 ± 0.0328			1.2308 ± 0.0079			6.7984 ± 0.0638	
R2		0.5766			0.5741			0.5584	
CV		20.6140			20.8437			30.1897	

Effects	M (4) DF	R^2 (1)	F	IPM (5) DF	R^2(1)	F	IPME (5) DF	R^2(1)	F	IPMFT (5) DF	R^2(1)	F
Season	3	10.01	9.38***	3	6.61	24.80***	3	4.59	11.56***	3	2.85	35.93***
Year	4	12.26	8.61***	4	61.00	171.70***	4	36.21	68.36***	4	72.73	688.13***
Age	3	1.30	1.22+	3	2.21	8.29***	3	6.73	16.93***	3	2.37	29.95***
Breed	5	9.18	5.16***	5	7.13	16.05***	5	10.71	16.17***	5	5.10	38.58***
Bull	62	67.25	3.05***	63	23.05	4.12***	63	41.76	5.01***	63	16.95	10.18***
Error	992			962			928				925	
m ± SD		2.4420 ± 0.01543			71.7839 ± 0.1732			71.3871 ± 0.1333			63.7829 ± 0.2083	
R^2		0.2336			0.5392			0.4486			0.8036	
CV		19.9357			7.7835			5.8869			10.3491	

Average values/ejaculate: V= volume; C= sperm concentration; NE: number espermatozoa; M: motile sperm; Progressively motile sperm : IPM: in fresh inmediatly; IPME: after 4-6 hours; IPMFT: after freezing-thawing; DF: Degrees of freedom; R^2: Variation in the dependent variable accounted for by the model; CV: Coefficient of variation; (1): Relative values to the % of variation accounted for by the mathematical model; (2): (Espermatozoa/mm^3) x 10^6; (3) Espermatozoa x 10^9; (4) Range 1 to 5; (5): Percentage of live espermatozoa; + p >0.05; ***p<0.005; Include Morenas Gallegas. Alistano-Sanabresa and Sayaguesa.

Table III. *Least-squares means on semen characteristics of different breeds bulls in artificial insemination, in relation of different effects (n:1143 observations). (Sánchez García and Vallejo, 1990).* Medias de mínimos cuadrados de parámetros seminales de toros de diversas razas autóctonas españolas, en función de diversos factores de variación (n: 1143 observaciones).

Effects		V(ml)		C(1)		NE(2)		M(3)		IPM(4)		IPME(4)		IPMFT(4)	
Classes	Levels	n	m	n	m	n	m	n	m	n	m	n	m	n	m
	Spring	352	5.33 a	313	1.18 a	313	6.48 a	302	2.53 a	322	78.64 b	309	73.71 a	309	61.07 b
Season	Summer	132	5.39 a	127	1.15 a	127	6.21 a	126	2.57 a	127	79.58 c	125	74.59 a	125	63.07 c
	Autumn	336	5.45 a	313	1.16 a	313	6.36 a	292	2.36 b	295	74.90 a	286	70.20 b	283	58.86 a
	Winter	323	5.34 a	283	1.11 a	283	6.05	280	2.40 b	297	74.90 a	286	70.20 b	283	58.86 a
	1985	170	5.98 a	156	0.97 a	156	5.99 b	163	2.56 a	165	79.58 a	165	74.59 a	165	52.35 a
	1986	175	5.67 a	167	0.95 a	167	5.42 b	168	2.55 a	169	79.58 b	169	75.48 b	168	54.51 b
Year	1987	286	4.67 b	247	1.01 a	247	4.84 a	225	2.40 b	251	74.98 c	245	70.20 d	245	63.21 d
	1988	215	4.88 b	184	1.34 b	184	6.72 c	174	2.45 b	179	75.84 c	172	71.08 c	172	63.14 c
	1989	297	5.69 a	282	1.49 c	282	8.54 d	270	2.35 b	277	73.96 c	256	69.32 bc	254	63.30 c
	≤ 1 year	112	4.60 a	100	1.11 a	100	5.15 a	92	2.42 a	104	76.12 a	104	73.01 a	104	62.01 a
Age	2 year	482	5.16 b	440	1.21 c	440	6.34 b	426	2.50 a	436	77.71 b	423	72.84 b	421	62.33 a
	3 year	178	5.86 c	262	1.12 a	262	6.59 c	249	2.51 a	261	76.12 b	253	69.59 b	252	58.74 b
	≥ 4 year	271	5.90 c	234	1.17 b	234	7.13 d	233	2.42 a	240	75.25 c	227	72.30 c	227	63.72 a
	A.Sanabresa	323	5.19 b	284	1.12 b	284	5.80 b	264	2.38 ab	275	74.20 bc	268	70.81 a	266	60.00 b
	Vianesa	110	5.49 bc	102	1.19 bc	102	6.43 c	100	2.57 d	98	79.67 a	94	74.59 b	94	60.28 bc
Breed	Cachena	70	4.95 ab	64	0.87 a	64	4.47 a	60	2.34 a	63	74.20 b	59	70.81 b	59	58.38 ab
	Caldelana	260	4.62 a	224	1.35 d	224	6.43 c	228	2.46 bc	242	76.77 d	229	71.96 bc	228	61.94 a
	Limiana	230	6.34 d	216	1.14 b	216	7.50 d	204	2.44 b	215	75.84 c	209	71.08 c	209	61.70 d
	Sayaguesa	150	5.68 c	146	1.25 c	146	7.19 d	144	2.60 d	148	80.52 c	148	75.47 c	148	61.92 c

Average values/ejaculate: V= volume; C= sperm concentration; NE: number espermatozoa; M: motile sperm; Progressively motile sperm : IPM: in fresh inmediatly; IPME: after 4-6 hours; IPMFT: after freezing-thawing; (1): (Espermatozoa/mm ^{3}) x 10 ^{6}; (2) Espermatozoa x 10 ^{9}; (3) Range 1 to 5; (4): Percentage of live espermatozoa; Within a column means with different letters are significantly differents

Table IV. *Superovulations carried out in Morenas Gallegas cattle breeds cows.* (Superovulaciones realizadas en vacas de las razas Morenas Gallegas).

Breeds	Number of superovulations	Response n	Response %	No response n	No response %
Cachena	51	37	72.55	14	27.45
Caldelana	17	12	70.59	5	29.41
Vianesa	10	6	60.00	4	40.00
Total	78	55	70.51	23	29.49

Superovulatory response was measured by the 8 variables shown in **table V**, and the results are lower than in other authors who do not consider non-response superovulations and collections with no ova.

The influence of breed can be detected in the most important variables of the superovulatory response, such as corpora lutea and follicles, total embryos and ova collected and viable embryos (with a high correlation). Other authors (Sreenan and Beehan, 1976; Greve, 1982; Donaldson, 1984 and Breuel *et al.*, 1991) also obtained variations between breeds, but in different variables.

Other factors such as number of superovulations, age of donors, number of parturitions and day of oestrous cycle when superovulatory treatment started did not have a significant influence on embryo variables, but some tendencies have been observed.

So, with the season of year (**table VI**) the results agree with other authors (Critser *et al.*, 1980; Greve, 1982; Lerner *et al.*, 1986 and Nibart, 1991),

Table V. *Superovulatory response (x±SD) in Morenas Gallegas cattle breeds.* (Caracterización de la respuesta superovulatoria (x±SD) en razas Morenas Gallegas).

Variables	Cachena (19)	Caldelana (7)	Vianesa (5)
No. of corpora lutea and follicles	7.02±4.79	4.71±4.17	4.20±4.39
No. of embryos and ova	5.16±4.89	3.94±4.05	3.50±4.65
No. of retarded and degenerated	2.06±2.57	1.59±1.87	1.30±1.83
No. of unfertilized ova	2.67±2.98	2.12±2.29	2.20±3.36
No. of viable embryos	0.43±1.10	0.24±0.56	0.00±0.00
Recovery rate	65.64±38.23	77.64±32.40	71.43±39.34
Viability percentage	55.46±31.70	49.34±30.29	55.14±35.24
Unfertilization rate	4.98±9.47	3.94±7.72	0.00±0.00

(): Number of donors; x ± SD: Mean ± Standard deviation

Table VI. *Effect of the season on the superovulatory response of Galician cattle breeds. F values and signification rate estimated in the analysis of variance.* (Valores de F y significación estimados en los análisis de varianza para estudiar el efecto de la estación en relación con las variables reproductivas caracterizadoras de la respuesta al tratamiento superovulatorio).

Variables	Gallegas (174)[1]	Cachena (51)	Caldelana (17)	Vianesa (10)
Nº of corpora lutea and follicles	0.763	0.911	1.214	1.133
Nº of embryos and ova	0.679	1.180	1.633	1.315
Nº of retarded and degenerated	1.053	1.680	0.874	13.050***
Nº of unfertilized ova	0.006	0.802	0.234	-
Nº of viable embryos	0.269	0.227	4.420*	0.381
Recovery rate	1.337	0.577	2.402	0.857
Viability percentage	0.715	1.059	1.053	4.452
Unfertilization rate	0.495	0.779	1.051	-

() Total number of superovulatory treatments;
[1)] Included Rubia Gallega (32). Cachena (19). Caldelana (7) and Vianesa (5) breeds.
* $p < 0.05$; *** $p < 0.005$

because season did not have any significant influence ($p>0.05$) on all variables of superovulatory response of all breeds as a whole. However, Ourense's climatic conditions where the Centre of Fontefiz is located and the experiments were carried out, are of continental type. The effects of season have been detected by some authors in extreme environmental conditions, very cold winters (Holm *et al.*, 1987) or very hot summers (Almeida, 1987).

Therefore, only these very extreme conditions could have a bad influence on the bovine superovulatory response. Nevertheless, with Caldelana breed a higher mean of viable embryos is obtained in summer than in other seasons ($p<0.05$). And with Vianesa breed the mean of retarded and degenerated embryos is significantly higher in spring than in the other seasons. But in the last case, the number of superovulations was low; and therefore a higher number of experiments will be necessary to obtain better fiability in the results.

An effect of hormonal treatment was observed in Cachena (**table VII**), in which HAP originated a higher viability percentage than FSH and PMSG ($p<0.05$); PMSG produced a superior unfertilization rate than FSH and HAP ($p<0.05$). Nevertheless, the number of cases was low.

In Vianesa breed dose of FSH is correlated significantly with the number of *corpora lutea* and follicles, and the total number of embryos and ova. The highest values are obtained between 32 and 35 mg. Dose of PMSG resulted in positive and significant correlation with the number of viable embryos and the viability percentage, and the best results were obtained with

Table VII. *Effect of the superovulatory hormone on the superovulatory response of galician cattle breeds. F values and signification rate estimated in the analysis of variance.* (Valores de F y significación estimados en los análisis de varianza para estudiar el efecto de la hormona superovulatoria, en relación con las variables reproductivas caracterizadoras de la respuesta al tratamiento superovulatorio. en razas bovinas gallegas).

Variables	Gallegas (174)[1]	Cachena (51)	Caldelana (17)	Vianesa (10)
Nº of corpora lutea and follicles	1.472	0.483	0.970	2.036
Nº of embryos and ova	2.333	1.511	1.507	1.719
Nº of retarded and degenerated	0.741	2.134	1.418	1.228
Nº of unfertilized ova	4.474***	4.921**	0.425	-
Nº of viable embryos	1.111	1.443	1.007	1.378
Recovery rate	2.957*	2.700	4.568	2.667
Viability percentage	0.329	4.278*	0.001	0.021
Unfertilization rate	1.613	6.011***	0.601	-

(): Total number of superovulatory treatments
1) Included Rubia Gallega (32). Cachena (19). Caldelana (7) and Vianesa (5) breeds
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$

2500 I.U. and 3000 I.U., all breeds considered as a whole.

Thirty five embryos from different freezings and donors were thawed, of which 29 embryos were transferred, and yielded 11 pregnancies. This represents a post-thawing pregnancy rate of 37.93%. The post-thawing survival rate was of 82.86% (29/35). These are the first embryo transfer results in these breeds and therefore they could be improved in the future.

At present 325 embryos of galician autochthonous cattle breeds are stored in the Bank of frozen embryos. We expect to increase the number and variety of frozen embryos stored in the embryo bank, to obtain the aims proposed for the germplasm bank of the preservation program.

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