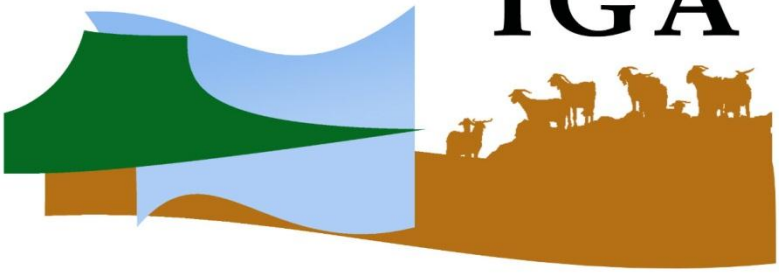
BOOK OF ABSTRACTS

XI INTERNATIONAL CONFERENCE ON GOATS

Gran Canaria, Spain, 23-27 September 2012

XI International Conference on Goats

IGA



Gran Canaria, Spain 2012

Supervised by

IGA

International Goat Association

R-38

Effect of the type of egg yolk–based extenders and the removal of seminal plasma on sperm cryopreservation of goat from Blanca de Rasquera breed

Tabarez, A.[1], W. Garcia[1], A. Ferrando[2], J. Jordana[2], M. Terre[3], M.J. Palomo[1]

Universitat Autonoma de Barcelona. (1) Animal Medicine and Surgery; (2) Animal and Food Science; (3) Ruminant Unit, Institut de Recerca de Tecnologia Agroalimentaria, Spain.

In order to accelerate the preservation of this Catalonian goat breed in extinction danger, we proposed the constitution of a sperm bank from breeders previously selected in early ages in function of their genetic variability. First, the main objective was to reduce the heterogeneity of the cryopreservation method and the potential risk of microbiological contamination by replacement of fresh egg yolk by pasteurized powered egg yolk. Simultaneously, we studied the effect of the fresh clarified egg yolk, obtained by centrifugation of fresh egg yolk twice at 10,000 x g for 45' at 4ºC. In addition, we also assessed the effect of the seminal plasma on sperm freezability of young males. Briefly, fresh ejaculates from 6 bucks (1 year old) were collected by artificial vagina and immediately mixed in equal quantities. Then the pooled semen was split into two samples. One sperm sample was washed by centrifugation and then the pellet was split into three equal aliquots and re-suspended in an extender containing 15% (v/v) of different type of egg yolk (powered, fresh or fresh clarified) supplemented with 5% glycerol in a Tris-based medium. The other semen sample was directly split into three equal aliquots and re-suspended in the same extenders, but containing 2% (v/v) of different type of egg yolk (powered, fresh or fresh clarified). The sperm cryosurvival after thawing, determined by eosine-nigrosine stain (mean ± SE, n = 6), was significant higher ($P < 0.0001$) when the spermatozoa were washed and preserved in extenders containing 15% of egg yolk, showing not significant differences between powered (44.0 ± 7.5), fresh (48.1 ± 5.9) and fresh clarified egg yolk (42.3 ± 2.8) based media. Likewise, when whole semen was preserved in extenders containing only 2% of egg yolk, even the sperm survival was quite lower in the three tested extenders, not differences either were found between powered (4.4 ± 1.8), fresh (6.0 ± 1.2) and fresh clarified (3.0 ± 0.4) egg yolk based media samples. Nevertheless, the sperm quality motion characteristics, analysed by a computer-assisted sperm analysis system (ISAS®), were similar between all the treatments($P > 0.05$), except the total motility ($P < 0.0001$), suggesting that the pasteurized powered egg yolk is effective for freezing goat sperm, but the removal of seminal plasma on goat sperm cryopreservation is still recommended. Supported by INIA (RZ2009-00008-00-00), Generalitat de Catalunya (2009SGR0621 and CUR-DIUE) and FSE and Fundacion Carolina.

Effect of the type of egg yolk–based extenders and the removal of seminal plasma on sperm cryopreservation of goat from Blanca de Rasquera breed

A Tabarez[1], W García[1], A. Ferrando[2], J. Jordana[2], M. Terre[3], MJ Palomo[1]

Universitat Autònoma de Barcelona. Animal Medicine and Surgery [1].
Animal and Food Science [2]. Ruminant Unit. Institut de Recerca de Tecnologia Agroalimentaria 3. Spain

AIMS

- To reduce the heterogeneity of the cryopreservation medium and the potential risk of microbiological contamination by replacement of fresh egg yolk by pasteurized powdered egg yolk.
- To assess the effect of the seminal plasma on sperm freezability of young males.

MATERIALS AND METHODS

Semen collection

Semen collected by an artificial vagina from six Blanca of Rasquera bucks (1 year old)

Pooled semen was or not washed by centrifugation (twice at 600xg for 10 min)

Washed and non-washed sperm were re-suspended in two steps in the Tris-based medium to 400 x 10^6 sperm/mL

Fresh clarified egg yolk was obtained by centrifugation (twice at 10 000 xg for 45 min)

Composition extenders

T1: Fresh egg yolk 15% + washed (FEY-W)
T2: Powdered egg yolk 15% + washed (PEY-W)
T3: Clarified egg yolk 15% + washed (CEY-W)
T4: Fresh egg yolk 2% + non-washed (FEY)
T5: Powdered egg yolk 2% + non-washed (PEY)
T6: Clarified egg yolk 2% + non-washed (CEY)

5 % glycerol final concentration

Freezing and thawing protocol

Cooled to 5 °C for 2 h + 2 h for equilibration

Packaged in 0.25 mL straws

Frozen in liquid nitrogen vapors for 10 min (5 cm above level)

Thawing by immersing in 37 °C water for 60 s

Semen analysis

Motion parameters determined by a CASA system *Integrated Semen Analysis System V. 1.0; ProiSer SL.*

Motion variable	Definition
VCL	Curvilinear Velocity (µm/s)
VSL	Linear Velocity (µm/s)
VAP	Mean Velocity (µm/s)
LIN	Linearity coefficient. VSL/VCL (%)
STR	Straightness coefficient. VSL/VAP (%)
WOB	Wooble coefficient. VAP/VCL (%)
ALH	Lateral Head Displacement (µm)
BCF	Frequency of head Displacement (Hz)
DNC	Dance. VCL x ALH (µm²/seg)

Viability assessment by eosine-nigrosine stain

Statistical Analysis

A general linear models multivariate and univariate were used to analyze the sperm motility parameters and viability respectively. Tukey test was used for multiple comparisons.

RESULTS

Table 1: Effect of the type of egg yolk–based extenders and the removal of seminal plasma on sperm viability and motion parameters (mean + SE, n=6) after the freezing-thawing process.

TX	VIABILITY	VCL	VSL	VAP	LIN	STR	WOB	ALH	BCF	DNC
FEY-W	48.1±5.9 [a]	86.0±1.9	32.0±1.1	46.7±1.2	38.9±1.2	67.9±1.4	55.0±0.9	3.7±0.1	8.3±0.3	369.1±18.0
PEY-W	44.0±7.5 [a]	79.3±2.6	32.6±1.6	45.5±1.8	42.2±1.6	69.2±1.8	57.4±1.2	3.3±0.1	8.5±0.3	322.6±22.0
CEY-W	42.3±2.8 [a]	86.6±2.0	32.4±1.3	46.8±1.3	39.0±1.3	64.8±1.5	55.3±0.9	3.7±0.1	8.5±0.3	379.1±18.1
FEY	6.0±1.2 [b]	73.8±7.4	30.6±3.6	41.2±3.8	47.3±4.4	74.5±4.6	59.5±3.2	3.2±0.4	8.3±0.8	307.0±67.2
PEY	4.4±1.8 [b]	74.1±8.8	23.3±3.7	38.2±4.4	33.6±4.4	62.1±6.7	53.4±3.2	3.8±0.5	5.3±0.8	336.4±70.8
CEY	3.0±0.4 [b]	71.7±9.0	23.0±3.5	39.9±4.3	36.7±5.8	61.0±7.2	59.5±4.4	3.2±0.5	6.8±1.2	287.5±74.5

Different letters in the same column show significant differences (P< 0.0001) among subpopulation mean values.

CONCLUSIONS

The sperm quality motion characteristics, analysed by a computer-assisted sperm analysis system, were similar between all the treatments, except the total motility, suggesting that the pasteurized powdered egg yolk is effective for freezing goat sperm, although the removal of seminal plasma is still recommended on goat sperm cryopreservation.

INIA · Generalitat de Catalunya · Fundación Carolina