We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 4

Fertility Results After Artificial Insemination with Bull Semen Frozen with Low Density Lipoprotein Extender

L. Briand-Amirat, D. Bencharif, S. Pineau and D. Tainturier

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/51868

1. Introduction

The freezing process exposes the spermatozoa to thermal shock, which results in damage to the plasma membrane and acrosome [1, 2]. Various extenders have been tested in an attempt to limit cellular injury. Egg yolk is the most widely used of these extenders by artificial insemination centres. Many demands were formulated to replace egg yolk in extenders by it's cryoprotector factor. In recent years, centrifugation techniques have enabled the isolation of the LDL(Low Density Lipoproteins) that are responsible for the cryopreservative effect of egg yolk [3,4,5]. The incorporation of LDL in bovine extenders has given improved motility results in comparison with extenders containing egg yolk [5,6,7]. However, to extend the use of this new LDL-based extender to insemination centres, a fertility study was essential. Fertility can either be assessed in the laboratory using *in vitro* fertilisation tests or by artificial insemination in the field [8]. The latter provides the most reliable means of assessing semen fertility following freezing and thawing. *In vitro* fertility results have already been published [6]; blastocysts were obtained after 7 days of *in vitro* culture from oocytes collected from abattoirs, matured, and then fertilised *in vitro* with spermatozoa that had been frozen-thawed in the LDL extender. An *in vitro* study is insufficient to assess the fertility of semen that has been frozen in the LDL extender, an *in vivo* field study was therefore necessary.

In vivo fertility of bull semen that had been frozen-thawed in the LDL extender was assessed. A widely available, standard extender (Tris-egg yolk) was used as a control. Cows were thus inseminated with semen that had been frozen-thawed in the LDL extender; pregnancy diagnoses were undertaken to assess the maintenance of fertility.

1.1. Collection of semen, dilution and processing

Two extenders were used; Tris-egg yolk extender (T-EY)): 20 ml of chicken egg yolk and LDL extender: 8% LDL (w/v) in accordance with the method described by Moussa et al. (2002) (patent n° 0100292) [5]. The extenders were thawed on the day of sampling and maintained at 37°C. Three bulls belonging to an artificial insemination centre and that had been approved for public use, were used. All three had a recorded progeny. Using the Laicophos® extender, the artificial insemination centre had a non-return rate at 60-90d of 64.7% for Bull 1, 57.2% for Bull 2, and 72.2% for Bull 3. The semen was collected using an artificial vagina. To excite the bulls, they were teased with a Normandy cow for thirty minutes prior to sampling. The semen was collected into a glass tube that had been previously warmed to 37°C. Following collection, the ejaculates were immediately placed in a water bath at 37°C. Each ejaculate was divided into two equal fractions. Each fraction was immediately diluted to 100 x 10^6 spz/ml with the two extenders that had been previously warmed to 37°C, and then subjected to progressive cooling from 37°C to +4°C over 1h and 30 in a refrigerated unit before being placed into straws. The semen was maintained in equilibrium for 4 hours at +4°C. The straws were held for 10 minutes at +4 cm from the surface of the liquid nitrogen (-120°C) before being immersed and then stored in liquid nitrogen (-196°C).

1.2. Semen evaluation before artificial insemination in the field

Before inseminating the cows, semen was evaluated on motility and plasma membrane integrity. The semen was analysed using the Hamilton-Thorne sperm analyser with the CEROS 12 software program, Hamilton-Thorne biosciences, Inc, Beverly, USA. The machine had been previously configured for the analysis of bovine semen. The following parameters were studied: motility (% mobile spermatozoa), straight line velocity: VSL (μm/sec.), curvilinear velocity: VCL (μm/sec.), the linearity index: LIN (= VSL/VCL x 100), amplitude of lateral head displacement: ALH (μm), and average path velocity: VAP (μm/sec). VAP, VSL, STR, and LIN provide information about the progressive movements of the spermatozoa, VCL and ALH characterise the lateral movements, and BCF (Beat Croix Frequency) provides information about the frequency of movements.

The post-thaw percentage of motile spermatozoa was greater in the LDL extender than in the Tris-egg yolk extender (table 1). The proportion of motile spermatozoa was nearly twice as high in the LDL extender, 58.3% vs. 46% in the Tris-egg yolk (table 1). To evaluate plasma membrane integrity, semen was added to an hypo-osmotic solution (100 mOsm/kg H_2O). The spermatozoa was observed under a phase-contrast microscope and classified as positive or negative. Positive spermatozoa (plasma membrane intact) tail swollen and / or curled:. Negative spermatozoa (plasma membrane damaged) tail not curled. No significant difference (table 2) was found between the semen that had been frozen-thawed in the LDL and Tris-egg yolk extenders. The results of the motility analysis and plasma membrane integrity demonstrated that the semen could be used by stock breeders for artificial insemination.

http://dx.doi.org/10.5772/51868

2. Assesment of in vivo fertility after AI of the cows

One hundred and ninety-three females from 83 different herds were inseminated by three inseminators with 25 years of experience. The females included in the study were from dairy or suckler herds with a Calving to First Insemination Interval (CFI) of more than 60 days, heifers over 18 months old, and first inseminations only. For each insemination, the following data was recorded: date of insemination, herd number, the animal's identification number, breed, lactation or calving index, date of the previous calving if relevant, condition score, the bull used, and the extender used. The pregnancy diagnoses were conducted by recording returns to oestrus and trans-rectal palpation between the 65th and 150th day of gestation. This data is summarised in table 3. Pregnancies can be obtained in the field following the artificial insemination of cows with semen that has been frozen and thawed in the LDL extender. However, no significant difference could be found between the LDL extender and the Tris egg yolk extender in terms of the success rates of insemination (Table 4).

	LDL 8%	**Triladyl**
Motile spermatozoa (%)	58.3 ± 16.7	46.0 ± 18.2
Rapid (%)	45.3 ± 14.2	27.0 ± 12.3
Average (%)	5.7 ± 3.1	7.7± 2.5
Slow (%)	7.3 ± 3.2	11.3±4.5
Static (%)	43.7 ± 5.5	54.0± 15.1
Hyperactive (%)	5.3 ± 2.1	6.3 ± 5.9
Progressive (%)	34.7 ± 4.0	16.0 ± 6.1
VAP (µm/sec.)	83.5 ± 7.7	71.0 ± 5.3
VSL (µm/sec.)	66.6 ± 9.2	57.3 ± 6.3
LIN (%)	60.7 ± 1.5	58.3± 5.5
STR (%)	82.0 ± 1.7	79.7± 3.2
VCL (µm/sec.)	104.1± 28.6	99.9 ± 5.8
ALH (µm)	4.3 ± 0.3	4.6 ± 1.1

Table 1. Results of the motility of bovine spermatozoa following freezing and thawing in the LDL extender and in the Tris-egg yolk extender obtained using the Hamilton Thorne image analyser (n=3).The results given are the means ± standard deviation of the motility characteristics recorded for the three bulls.

Extender	Total spermatozoa (n)	Swollen spermatozoa (intact) (n) (%)	
LDL 8%	1296	542	41.8
Tris-Egg yolk	1314	559	42.5

Table 2. The effect of LDL and Tris-egg yolk extenders on the integrity of spermatozoal plasma membranes according to the HOS test N = sum of spermatozoa taken from Bulls 1, 2, and 3.

Characteristics of the population		LDL n=98	Tris egg yolk n=95
Breeds	Holstein	n=67	n=81
	Normandy	n=22	n=5
	Charolais	n=7	n=9
	Charolais cross	n=1	
	Aubrac	n=1	
Lactation index	Mean ± standard dev.	1.6 ± 1.9	1.5 ± 1.5
	0	n=29	n=31
	1	n=28	n=24
	2	n=20	n=19
	3	n=9	n=12
	4	n=6	n=5
	"/>4	n=6	n=4
Condition score (mean ± standard deviation)		3.0 ± 0.2	3.0 ± 0.3
Calving to first insemination interval in days (mean ± standard deviation)		94 ± 25 (1 CFI not given)	95 ± 32 (1 CFI not given)

Table 3. Characteristics of the study population for each extender

Extender	Cows inseminated (N)	Positive pregnancy diagnosis	Not pregnant	Success rate at insemination (%)
LDL	98	58	40	59.2
Tris-Egg yolk	95	62	33	65.3

Table 4. Effect of the extender used for freezing the semen on the success rate at insemination (as a %)

3. Is there a correlation between motility and fertility?

Pregnancies were obtained following the artificial insemination of cows with semen that has been frozen-thawed in an LDL extender without any significant difference in the

http://dx.doi.org/10.5772/51868

success rate following insemination between the 2 extenders. The initial objective was not to demonstrate the superiority of the LDL extender, but to demonstrate its efficacy in the field in terms of percentage gestation. The success rates with artificial insemination are satisfactory (table 3): 59.2% for the LDL extender and 65.3% for the Tris-egg yolk extender. In a previous study, Amirat et al.2004 [6] demonstrated that fertility was maintained *in vitro*. The hypoosmotic test was chosen to assess plasma membrane integrity as a proven correlation has been found between the results of the HOS test and the *in vivo* fertility rate [9]; the HOS test can therefore be used to predict fertility. Plasma membrane integrity was maintained with both the LDL and Tris-egg yolk extenders (table 2). These results concur with previous studies undertaken in the bovine species [10,11]. Around 60% of the spermatozoa that were frozen-thawed in the LDL extender presented with an alteration of the plasma membrane, whilst around 40% of the spermatozoa lost their motility. The percentage of spermatozoa with an altered plasma membrane may be higher than to the percentage or spermatozoa presenting with a loss of motility. This implies that a certain number of spermatozoa may retain their motility with a damaged plasma membrane, this result agree with that reported by Salamon and Maxwell (1995) [11]. Nevertheless it is unlikely that such spermatozoa would be capable of crossing the zona pellucida.

Motility results demonstrate that the percentage of motile spermatozoa following thawing is superior in the LDL extender in comparison with the Tris-egg yolk extender. These results concur with the works of Moussa et al. (2002) [5] and Amirat et al. (2004) [6]. However, inter-individual variability on the motility performances following thawing has already been reported by Farrell et al. (1998) [12] and Holt (2000) [13]. The results obtained do not make it possible to relate the motility of the spermatozoa to fertility due to the insufficient number of measurements. No study has demonstrated a precise correlation between motility parameters and fertility in cows. In cattle, the percentage of mobile spermatozoa, linearity (LIN), and straight line velocity (VSL) seem to be correlated to fertility according to Budworth et al. (1988) [14], and Farrell et al. (1998)[12]. The average path velocity (VAP), curvilinear velocity (VCL), and the frequency of tail movements (FTM), also appear interesting [12]. According to Liu et al. (1991) [15], the most interesting motility parameters in human semen are linearity (LIN), straight line velocity (VSL), and the percentage of rapid spermatozoa.

4. What parameters could influence the AI success rate?

The confirmation of pregnancies were performed by rectal palpation on average at around the 100th day. However, embryonic mortality is recorded in the same way as failure of fertilisation; this reduces the fertility results observed. Ultrasonographic pregnancy diagnosis at 30 days would have been more accurate for measuring the fertility of the semen as the impact of embryonic mortality is lower between D0 and D30 than between D0 and D150. Descoteaux et al. (2006) [16] thus report that 10% of cows

that are given a positive pregnancy diagnosis at 28 days present with embryonic mortality at D60. Nevertheless, the cows included in the present study were selected as a function of various criteria that ensure satisfactory female fertility, which explains the difference in fertility recorded between the results of our study and those reported by Barbat et al. (2005) [17] and Freret et al. (2006) [18]. These studies are based on the results of inseminations conducted over a given period by insemination centres, without any selection criteria for the cows used. Female fertility was therefore inferior to that observed in our study. The observed fertility is a combination of the fertility of the male and female.

In addition to the many different diseases that can affect fertility, other parameters may influence the fertility of cows as breed [17] or lactaction index [19]. In the study described here, the lactation index did not have any significant effect on the overall AI success rate ($p<0.05$). However, the lactation index had an impact for the LDL extender. Superior fertility was observed in the heifers, followed by the primiparous cows. A reduction in fertility was seen in cows with a lactation index of 2 or 3. There were insufficient numbers of cows with high lactation indexes to reveal any trends (Table 6). Milk production [18], energy profile [20], post-partum pathologies [21], and the herd effect [19] are other parameters that interfere with fertility results. Amman and Pickett (1987) [22] show that to measure male fertility a significant number of inseminations are necessary to rule out variations caused by female fertility. Van Wagttendonk de Leeuw et al. (2000) [23] demonstrate that to detect a 2% difference in the non-return rate, with a confidence interval of 95% and a statistical power of 80%, 6,600 inseminations are needed per extender. A limited population of 193 cows was inseminated as it was impossible to undertake a larger scale study due to the difficulty of convincing the breeders to use semen that had been frozen and thawed in an extender that did not have proven *in vivo* efficacy. The population was divided into two relatively homogeneous groups (mean lactation index, condition score, CFI) to limit variations in female fertility. The inclusion criteria could be improved: it would have been judicious to use only one breed to limit interbreed fertility differences [17]. It would also have been preferable for all of the cows to have the same lactation index. The milk production of the animals affects their fertility [18]. The latter was not recorded as some of the farmers in this study did not keep individual milk production records. The use of condition scoring enables any animals that are in negative energy balance to be excluded [24]. The bulls were chosen on the basis of good individual fertility and on the presence of the bulls at the centre at the time of semen collection. The bull factor did not exert any significant difference ($p<0.05$) on the total insemination success rate (Table 5). The bull effect was observed in the sub-population of cows that had been inseminated with the semen that had been frozen-thawed in the LDL extender ($p=0.019$). The semen from bull 2 that had been frozen in Tris-egg yolk was significantly more fertile than that which had been frozen in the LDL ($p=0.046$). The variation in success for bull 3 was due to the small number of cows inseminated. The cows were taken from numerous farms and we did not al-

http://dx.doi.org/10.5772/51868

ways have intra-herd paired animals: the herd effect could not therefore be assessed. In this study, fewer constraints were voluntary imposed on the inclusion criteria as many animals as possible in order to facilitate the task of the inseminators.

The inseminator did not have a significant influence on the total Insemination Success Rate, with a threshold of significance of p=0.05 (Table 5). Inseminator 3 achieved higher insemination success rates for both extenders McKenna et al. (1990) [25] calculated the inseminator effect at ± 9.5 points. The animals that he inseminated were on average younger with a higher proportion of heifers. Barbat et al. (2005) [17] reported superior fertility in heifers in comparison with cows.

Percentage success at insemination (%)	**Inseminator 1**		**Inseminator 2**		**Inseminator 3**		**TOTAL**
extender	LDL	T.E.Y	LDL	T.E.Y	LDL	T.E.Y	
Bull 1	25.0(*). (**)	83.3(*)	72.2(**)	60.0	87.5(**)	62.5	82
	n=12	n=12	n=11	n=15	n=16	n=16	
Bull 2	38.5	73.3	36.4	41.7	50.0	90.0	71
	n=13	n=15	n=11	n=12	n=10	n=10	
Bull 3	88.2	50.0	50.0	25.0	50.0	100.0	40
	n=17	n=8	n=2	n=4	n=6	n=3	
TOTAL	54.8	71.4	54.2	48.4	68.7	75.8	**193**
	n=42	n=35	n=24	n=31	n=32	n=31	

*: significant difference between the two extenders for Bull 1 when the insemination was performed by inseminator 1 (p=0.002)

**: significant difference between the inseminators for Bull 1 with the LDL extender (p= 0.004)

Table 5. Insemination success rate (in %), details of the bulls and inseminators for each of the extenders, LDL and Tris Egg Yolk (TEY)

Lactation index	LDL	Tris egg yolk	Total AI success rates
0	82.8(*)	61.3	71.6
	n=29	n=31	n=60
1	53.6(*)	75.0	63.5
	n=28	n=24	n=52
2 and 3	37.9(*)	61.3	50.0
	n=29	n=31	n=60
≥4	66.7(*)	66.7	66.7
	n=12	n=9	n=21

*: significant difference in the lactation index for the 8% LDL extender (p=0.005)

Table 6. Insemination success rates (in %) as a function of the lactation index

5. Conclusion

Although semen fertility was difficult to measure due to the various parameters that intervene causing variations in the results, this preliminary study enabled us to demonstrate for the first time that bull semen that has been frozen then thawed in the LDL extender retains a good level of fertility since gestations were obtained following artificial insemination. The continuation of this study in a larger population would make it possible to specify the impact of the LDL extender on the success of artificial insemination.

Author details

L. Briand-Amirat*, D. Bencharif, S. Pineau and D. Tainturier

Laboratory of Biotechnology and Pathology of Reproduction, Nantes Atlantic College of Veterinary Medicine,
Food Science and Engineering, Nantes, France

References

[1] Woelders H, Matthijs A, Engel B. Effects of trehalose and sucrose, osmolality of the freezing medium, and cooling rate on viability and intactness of bull sperm after freezing and thawing, Cryobiology 1997;35:93-105.

[2] Celeghini, E.C.C.; Arruda, R.P.; Andrade, A.F.C.; Nascimento, J.; Raphael, C.F.; Rodrigues, P.H.M. Effects that bovine sperm cryopreservation using two different extenders has on sperm membranes and chromatin. Animal Reproduction Science 2007; 100: 1-13.

[3] Pace M M, Graham E F. Components in egg yolk which protect bovine spermatozoa during freezing. J Anim Sci 1974; 39: 1144-1149.

[4] Demianowicz W, Strezek J. The effect of lipoprotein fraction of egg yolk on some of the biological properties of boar spermatozoa during storage of the semen in liquid state. Reprod Dom Anim 1996; 31: 279-280

[5] Moussa M, Martinet V, Trimeche A, Tainturier D, Anton M. Low density lipoproteins extracted from hen egg yolk by an easy method: cryoprotective effect on frozen-thawed bull semen. Theriogenology 2002;57:1695-1706

[6] Amirat L, Tainturier D, Jeanneau L, Thorin C, Gérard O, Courtens JL, Anton M. Bull semen in vitro fertility after cryopreservation using egg yolk LDL: a comparison with Optidyl, a commercial egg yolk extender. Theriogenology 2004; 61:895-907.

[7] Vera Munoz O, Amirat-Briand L, Diaz T, Vasquez L, Schmidt E, Desherces S, Anton M, Bencharif D, Tainturier D. Effect of semen dilution to low-sperm number per dose on motility and functionality of cryopreserved bovine spermatozoa using low-density lipoproteins (LDL) extender : Comparison to Triladyl and Bioxcell. Theriogenology 2009, 71: 895-900

[8] Larsson B, Rodriguez-Martinez H. Can we use in vitro fertilization tests to predict fertility ? Anim reprod Sci 2000; 60/61:327-336.

[9] Brito LF, Barth AD, Bilodeau-Goeseeis S, Panich PL, Kastelic JP. Comparison of methods to evaluate the plasmalemma of bovine sperm and their relationship with *in vitro* fertilization rate. Theriogenology 2003;60:1539-51

[10] Correa JR, Rodriguez MC, Patterson DJ, Zavos PM. Thawing and processing of cryopreserved bovine spermatozoa at various temperatures and their effects on sperm viability, osmotic shock and sperm membrane functional integrity. *Theriogenology*, 1996; 46(3), 413-420

[11] Salamon S, Maxwell WMC. Frozen storage of ram semen. II. Causes of low fertility after cervical insemination and methods of improvement. Anim Reprod Sci 1995;381-36.

[12] Farrel PB, Presicce GA, Brockett CC, Foote RH. Quantification of bull sperm characteristics measured by computer-assisted sperm analysis (C.A.S.A.) and the relationship to fertility. *Theriogenology*, 1998, 49, 871-879

[13] Holt WV. Fundamental aspects of sperm cryobiology: the importance of the species and individual differences. *Theriogenology*, 2000; 53(1), 47-58

[14] Budworth PR, Amann RP, Chapman PL. Relationship between computerized measurements of motion of frozen-thawed bull spermatozoa and fertility. *Journal of Andrology* 1988, 9, 41-54.

[15] Liu DY, Clarke GN, Gordon Baker HW. Relationship between sperm motility assessed with the Hamilton-Thorn Motility Analyser and fertilisation rates in vitro. *Andrology.* 1991, 12, 231-239

[16] Descôteaux L, Carrière PD, Durochet J. Ultrasonography of the reproductive system of the cow : basic principles, practical uses and economic aspects of the diagnostic tool in dairy production. 24th World Buiatrics Congress, Nice, 2006, p303-310

[17] Barbat A, Druet T, Bonati B, Guillaume F, Colleau JJ, Boichard D. Bilan phénotypique de la fertilité à l'insémination artificielle dans les trois principales races françaises. Renc. Rech. Ruminants. 2005, 137-140

[18] Freret S, Ponsart C, Rai DB, Jeanguyot N, Paccard P, Humblot P. Facteurs de variation de la fertilité en première insémination et des taux de mortalités embryonnaires en élevages laitiers Prim'Holstein. Renc. Rech. Rum. 2006, 281-284

[19] Seegers H, Beaudeau F, Blosse A, Ponsart C, Humblot P. Performances de reproduction aux inséminations de rangs 1 et 2 dans les troupeaux Prim'holstein. Renc. Rech. Rum. 2005, 141-144

[20] Domecq JJ, Skidmore A L, Lloyd J W, Kaneene J B. Relationship between body condition scores and conception at first artificial insemination in a large dairy herd of high yielding Holstein cows. *J. Dairy Sci.* 1997, 80(1), 113-120

[21] Hanzen C, Pluvinage P. Stress et performances de reproduction. *Le Point Vétérinaire.* 2005, 36, 94-98

[22] Amman RP, Pickett BW. Principles of cryopreservation and a review of cryopreservation of stallion spermatozoa. Equine Vet. Sci. 1987, 7, 145-173

[23] Van Wagtendonk-de Leeuw A M, Haring R M, Kaal-Lansbergen L M T E, Den Daas J H G : Fertility results using bovine semen cryopreserved with extenders based on egg yolk and soy bean extract. *Theriogenology.* 2000, 54(1), 57-67

[24] Grimard B, Humblot P, Ponter A A, Mialot J P, Sauvant D, Thibier M. Influence of postpartum energy restriction on energy status, plasma LH and oestradiol secretion and follicular development in suckled beef cows. *J Reprod Fertil.* 1995, 104(1), 173-179

[25] McKenna T, Lenz R W, Fenton SE., Ax RL. : Nonreturn Rates of Dairy Cattle Following Uterine Body or Cornual Insemination. *J. Dairy Sci*. 1990, 73(7), 1779-1783