III) RESULTS

III.1) Experiment 1: Effect of embryo culture on development of bovine embryos *in vitro*

III.1. A) Early embryonic stages of development and the timing of their occurrence, based on examination at 12-hour intervals

Data for occurrence of the higher percentage of embryos at 3-4 cells (stage preferred for evaluation for its analogy to the human scenario on day 2) are presented in Table III. 1. All the embryos were incubated in SOFaaBSA medium, subject to the same experimental conditions, and examined every twelve hours. At 48 hours after fertilisation, 197 embryos were allocated to another treatment. The data show 3-4 cell embryos are most prevalent in SOFaaBSA between 44 and 56 hours post insemination.

Table III.1: Distribution of different embryo developmental stages found between 32 and 68 hours post fertilisation. Culture medium: SOFaaBSA

32 hrs post fertil.

Uncleaved	Cleaved	3-4 cells	Total embryos
213 (50.7%)	202 (48.1%)	5 (1.2%)	420

44 hrs post fertil.

Uncleaved	2 cell	3-4 cell	5-8 cell	Total embryos
91 (21.7%)	129 (30.7%)	181 (43.1%)	19 (4.5%)	420

56 hrs post-fertil

Uncleaved	2 cell	3-4 cell	5-8 cell	9-16 cells	Total embryos
9 (4%)	59 (26.5%)	72 (32.3%)	79 (35.4%)	4 (1.8%)	223

68 hrs post-fertil

Uncleaved	2 cell	3-4 cell	5-8 cell	9-16 cells	Total embryos
4 (1.8%)	40 (18%)	58 (26%)	101 (45.2%)	20 (9%)	223

III.1.B) Blastocyst formation in different media

The yields and the timings of emergence of blastocysts formed in three different media are illustrated in Figures III.1.A and III.1.B The treatments used to generate the blastocysts were:

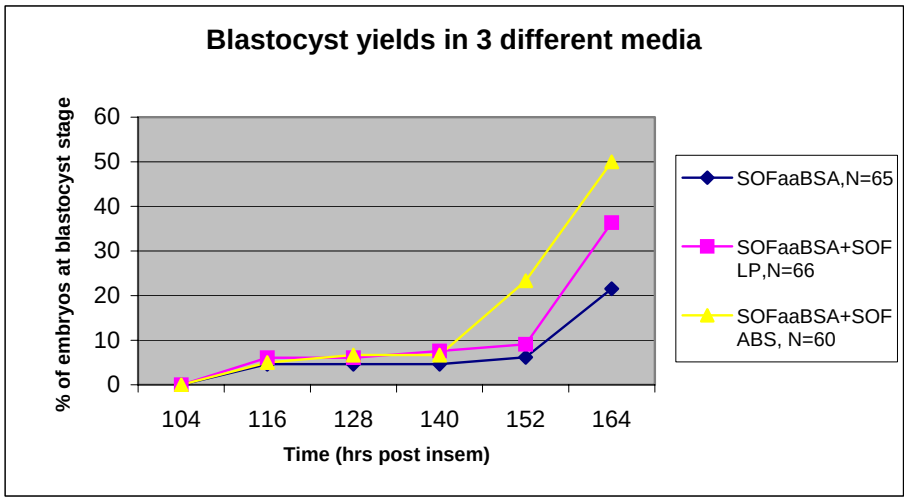
Treatment 1: Synthetic Oviduct Fluid (SOFaaBSA) until day 7

Treatment 2: Synthetic Oviduct Fluid (SOFaaBSA) until day 2 and then change to SOFaaBSA plus 2% lipoproteins (bovine plasma) (Sigma, cat No L - 3626)

Treatment 3: Synthetic Oviduct Fluid (SOFaaBSA) until day 4 and then change to SOF+ 10% v/v ABS

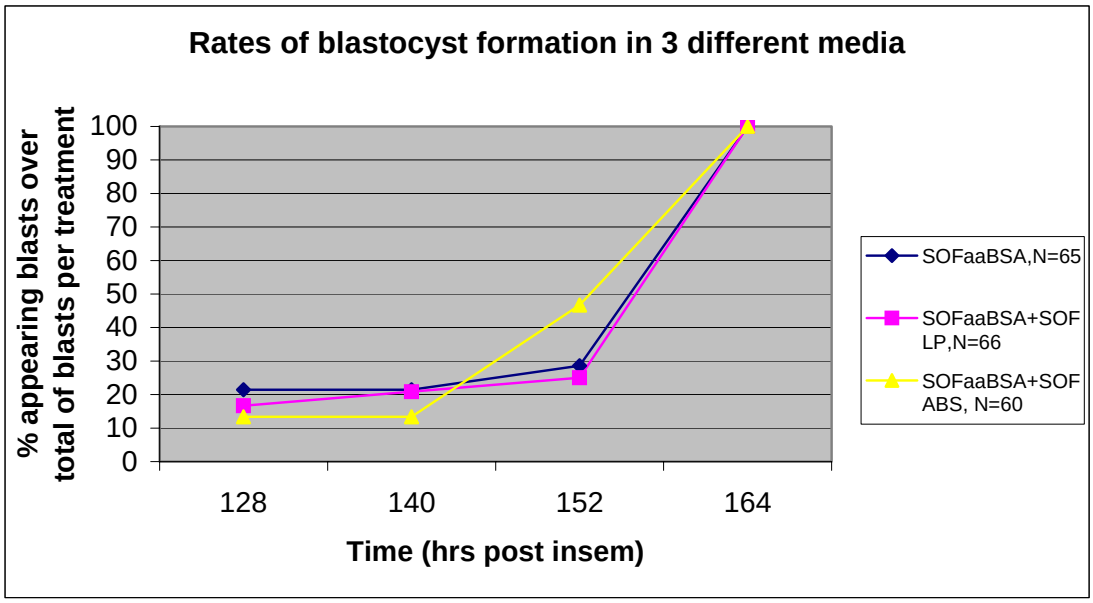
In Fig. III.1.A it can be appreciated that presence of serum increased blastocyst yields at 152 and 164 hrs post-insemination (23.3% and 50% of total embryos under that treatment respectively). Use of lipoprotein supplement in SOFaaBSA supported blastocyst formation, especially between 152 and 164 hours (9.09% and 36.6% respectively). Unsupplemented SOFBSA had the lowest yields of blastocysts (6.15% and 21.54% at 152 and 164 hours).

Figure III.1.A: Percentage of blastocyst yields in 3 contrasting media. Percentage of blastocysts is based on the total amount of embryos cultured. Observations took place every 12 hours between 104 and 164 hours post insemination. N is the total of embryos assigned for each treatment.



In Fig. III.1.B it can be appreciated that blastocysts yield in presence of serum is doubled between 152 and 164 hrs post insemination (46.67% and 100% of total blastocysts obtained on that treatment). In the same period, the yield (although lower in absolute terms) increased four-fold in the lipoprotein-supplemented medium (25% and 100%).

Figure III.1.B: Blastocyst evolution in 3 different media from 128 to 164 hours post insemination, based on the final blastocyst yield. The emergence of blastocysts is expressed as percentage of the total number of blastocysts at 164 hrs hours post insemination. N is the total of embryos assigned for each treatment.



III.2) Experiment 2: Comparison of development and viability of "selected" and "unselected" bovine embryos cultured *in vitro*

III.2.A) DAY 2 EMBRYOS

Table III.2.2a, III.2.2b, III.2.2c, III.2.2d and III.2.2e provide data for the different stages and grading of embryos on day 2 after insemination for 5 discrete replicates. Embryos were also categorised as two different cohorts (but continued under identical culture conditions): selected and unselected. Table III.2.2f is a summary of data from the 5 replicates (n=220 embryos), their totals, means, standard deviations and SEM (Standard Error of the Mean). Embryos were observed and classified on day 2, between 48 and 52 hours post insemination. All of the 5-8 cells (5-8 C) embryos were assigned to the "selected" cohort. See means in Table III.2.2 f. The percentage of grade 1 embryos was significantly higher in the selected group than in the unselected group ($P<0.001$). The differences between replicates were not significant among the three stage categories (2-C; 3-4C and 5-8C). The within stage category difference between selected and unselected embryos was significant ($P<0.001$ in all cases).

Table III.2.2a: Development and grading of embryos on day 2 after insemination. Replicate 1. n=50. Each dish represents a "patient" (10 embryos/dish or "patient"). Cleavage rate of replicate: 78%

Category:	Selected				Unselected			
Stage:	2 C	3-4 C	5-8 C	No Gr.1	2 C	3-4 C	5-8 C	No Gr.1
Dish 1	0	3	2	5	3	2	0	3
Dish 2	0	3	2	3	4	1	0	2
Dish 3	0	3	2	5	3	2	0	0
Dish 4	0	2	3	3	3	2	0	2
Dish 5	0	4	1	1	5	0	0	0
Subtotal	**0**	**15**	**10**	**17**	**18**	**7**	**0**	**7**
Mean		**3.00**	**2.00**	**3.40**	**3.60**	**1.40**		**1.40**
St. Dev.		**0.40**	**0.40**	**1.28**	**0.72**	**0.72**		**1.12**
SEM		**0.18**	**0.18**	**0.57**	**0.32**	**0.32**		**0.50**

Table III.2.2b: Development and grading of embryos on day 2 after insemination. Replicate 2. n=40. Each dish represents a "patient" (10 embryos/dish or "patient"). Cleavage rate of replicate: 78%

Category:	Selected				Unselected			
Stage:	**2 C**	**3-4 C**	**5-8 C**	**No Gr.1**	**2 C**	**3-4 C**	**5-8 C**	**No Gr.1**
Dish 1	0	4	1	4	3	2	0	3
Dish 2	0	4	1	4	2	3	0	1
Dish 3	0	1	4	2	2	3	0	0
Dish 4	0	1	4	4	1	4	0	1
Subtotal	**0**	**10**	**10**	**14**	**8**	**12**	**0**	**5**
Mean		**2.5**	**2.5**	**3.5**	**2**	**3**		**1.25**
St. Dev.		**1.5**	**1.5**	**0.75**	**0.5**	**0.5**		**0.875**
SEM		**0.75**	**0.75**	**0.38**	**0.25**	**0.25**		**0.44**

Table III.2.2c: Development and grading of embryos on day 2 after insemination. Replicate 3. n=60. Each dish represents a "patient" (10 embryos/dish or "patient"). Cleavage rate of replicate: 86%

Category:	Selected				Unselected			
Stage:	**2 C**	**3-4 C**	**5-8 C**	**No Gr.1**	**2 C**	**3-4 C**	**5-8 C**	**No Gr.1**
Dish 1	0	1	4	3	2	3	0	3
Dish 2	0	1	4	4	2	3	0	0
Dish 3	0	1	4	3	3	2	0	1
Dish 4	0	4	1	3	4	1	0	3
Dish 5	0	1	4	5	3	2	0	0
Dish 6	0	4	1	4	5	0	0	2
Subtotal	**0**	**12**	**18**	**22**	**19**	**11**	**0**	**9**
Mean		**2**	**3**	**3.67**	**3.17**	**1.83**		**1.5**
St. Dev.		**1.33**	**1.33**	**0.67**	**0.89**	**0.89**		**1.17**
SEM		**0.54**	**0.54**	**0.27**	**0.36**	**0.36**		**0.48**

Table III.2.2d: Development and grading of embryos on day 2 after insemination. Replicate 4. n=40. Each dish represents a "patient" (10 embryos/dish or "patient"). Cleavage rate of replicate: 74%

Category:	Selected				Unselected			
Stage:	**2 C**	**3-4 C**	**5-8 C**	**No Gr.1**	**2 C**	**3-4 C**	**5-8 C**	**No Gr.1**
Dish 1	0	3	2	4	3	2	0	0
Dish 2	0	3	2	4	3	2	0	1
Dish 3	0	2	3	5	1	4	0	0
Dish 4	1	3	1	2	5	0	0	0
Subtotal	**1**	**11**	**8**	**15**	**12**	**8**	**0**	**1**
Mean	**0.25**	**2.75**	**2**	**3.75**	**3**	**2**		**0.25**
St. Dev.	**0.375**	**0.38**	**0.5**	**0.88**	**1**	**1**		**0.37**
SEM	**0.13**	**0.19**	**0.25**	**0.44**	**0.50**	**0.50**		**0.19**

Table III.2.2e: Development and grading of embryos on day 2 after insemination. Replicate 5. n=30. Each dish represents a "patient" (10 embryos/dish or "patient"). Cleavage rate of replicate: 77%

Category:	Selected				Unselected			
Stage:	**2 C**	**3-4 C**	**5-8 C**	**No Gr.1**	**2 C**	**3-4 C**	**5-8 C**	**No Gr.1**
Dish 1	0	3	2	5	1	4	0	0
Dish 2	0	0	5	2	1	4	0	1
Dish 3	0	1	4	3	3	2	0	0
Subtotal	**0**	**4**	**11**	**10**	**5**	**10**	**0**	**1**
Mean		**1.33**	**3.67**	**3.33**	**1.67**	**3.33**		**0.33**
St. Dev.		**1.11**	**1.11**	**1.11**	**0.89**	**0.89**		**0.44**
SEM		**0.64**	**0.64**	**0.64**	**0.51**	**0.51**		**0.26**

Table III.2.2f: Summary of developmental stages and grading of embryos on day 2 after insemination. 5 replicates. n=220.

Category:	Selected				Unselected			
Stage:	**2 C**	**3-4 C**	**5-8 C**	**No Gr.1**	**2 C**	**3-4 C**	**5-8 C**	**No Gr.1**
Subtotal	1^{a}	52^{b}	57^{c}	78	62^{a}	48^{b}	0^{c}	23
Mean	0.045	2.36	2.59	3.55	2.82	2.18		1.05
St. Dev.	0.087	1.15	1.19	0.95	0.96	0.96		0.97
SEM	0.02	0.24	0.25	0.20	0.20	0.20		0.21
Mean (%)	0.9^{a}	47.3	51.8^{a}	70.9^{a}	56.4^{b}	43.6	0.0^{b}	20.9^{b}

Within each stage category (2C, 3-4C and 5-8C), values with different superscripts differ as follows:
$^{ab}P<0.001$, $^{bc}P<0.001$

Prior to assigning embryos to selected and unselected cohorts, overall day 2 data analysis had shown that there were significant differences among the three different stage categories in terms of abundance of embryos:

- 2 cell embryos (2C)
- 3 to 4 cell embryos (3-4 C)
- and 5 to 8 cell embryos (5-8C).

T-tests of pairwise differences showed that the numbers (expressed as a % of total embryos) of 3 to 4-cell embryos were significantly higher from those of 2 cell and 5 to 8 cell embryos ($P<0.001$), whereas 2-cell embryos and 5-8 cell embryos didn't have significant differences ($P=0.521$). Almost half of the 220 embryos cultured during the study were at the 3-4 cell stage (selected n=52, unselected n=48) on day 2 post insemination. See Table III.2.2g.

Table III.2.2g: Summary of number of embryos at different stages of development on day 2 after insemination irrespective of whether they had been selected or unselected. n=220.

	2 C	3-4 C	5-8 C	No > 2C	No > 4C	No Gr.1
Subtotal	63	100	57	157	57	100
Mean	2.86	4.55	2.59	7.14	2.59	4.55
St Dev	1.36	1.18	1.33	1.36	1.33	1.50
SEM	0.29	0.25	0.28	0.29	0.28	0.32
Mean (%)	28.6[a]	45.5[b]	25.9[a]	71.4	25.9	45.5

Values with different superscripts differ as follows: ab P<0.001

III.2. B) DAY 4 EMBRYOS

Tables III.2.3a, III.2.3b, III.2.3c, III.2.3d and III.2.3e provide data per replicate for the different stages of development achieved by embryos by day 4 after insemination. Embryos were in the same cohorts as on day 2: selected and unselected. Table III.2.3f is a summary of the 5 replicates (n=220), their totals, means, standard deviations and SEM (Standard Error of the Mean). Most embryos between 9 and 16 cells were in the selected group (see means in Table III.2.3f) and just one was in the unselected group. Embryos that had 8 or fewer cells were present in both groups but were more prevalent in the unselected group. The differences between replicates were significant ($P< 0.001$). The difference between cohorts (selected vs. unselected) was significant at the 9 to 16 cell stage ($P<0.001$), reflecting the higher incidence of advanced embryos in the selected group.

Table III.2.3a: Different developmental stages of embryos on day 4 after insemination. Replicate 1. n=50. Each dish represents a "patient" (10 embryos/dish or "patient").

Category:	Selected			Unselected		
Stage:	**< or = 4 C**	**5-8 C**	**9-16 C**	**< or = 4 C**	**5-8 C**	**9-16 C**
Dish 1	1	1	3	3	2	0
Dish 2	1	2	2	4	1	0
Dish 3	0	0	5	4	1	0
Dish 4	4	1	0	5	0	0
Dish 5	0	2	3	3	1	1
Subtotals	**6**	**6**	**13**	**19**	**5**	**1**
Mean	**1.20**	**1.20**	**2.60**	**3.80**	**1**	**0.20**
St. Dev.	**1.12**	**0.64**	**1.28**	**0.64**	**0.40**	**0.32**
SEM	**0.50**	**0.29**	**0.57**	**0.29**	**0.18**	**0.14**

Table III.2.3b: Different developmental stages of embryos on day 4 after insemination. Replicate 2. n=40. Each dish represents a "patient" (10 embryos/dish or "patient").

Category:	Selected			Unselected		
Stage:	**< or = 4 C**	**5-8 C**	**9-16 C**	**< or = 4 C**	**5-8 C**	**9-16 C**
Dish 1	4	1	0	3	2	0
Dish 2	2	3	0	3	2	0
Dish 3	1	4	0	3	2	0
Dish 4	1	4	0	2	3	0
Subtotals	**8**	**12**	**0**	**11**	**9**	**0**
Mean	**2**	**3**		**2.75**	**2.25**	
St. Dev.	**1**	**1**		**0.38**	**0.38**	
SEM	**0.50**	**0.50**		**0.19**	**0.19**	

Table III.2.3c: Different developmental stages of embryos on day 4 after insemination. Replicate 3. n=60. Each dish represents a "patient" (10 embryos/dish or "patient").

Category:	Selected			Unselected		
Stage:	**< or = 4 C**	**5-8 C**	**9-16 C**	**< or = 4 C**	**5-8 C**	**9-16 C**
Dish 1	0	3	2	4	1	0
Dish 2	0	3	2	5	0	0
Dish 3	1	4	0	5	0	0
Dish 4	4	1	0	5	0	0
Dish 5	0	3	2	5	0	0
Dish 6	2	2	1	4	1	0
Subtotals	**7**	**16**	**7**	**28**	**2**	**0**
Mean	**1.17**	**2.67**	**1.17**	**4.67**	**0.33**	
St. Dev.	**1.22**	**0.78**	**0.83**	**0.44**	**0.44**	
SEM	**0.50**	**0.32**	**0.34**	**0.18**	**0.18**	

Table III.2.3d: Different developmental stages of embryos on day 4 after insemination. Replicate 4. n=40. Each dish represents a "patient" (10 embryos/dish or "patient").

Category:	Selected			Unselected		
Stage:	**< or = 4 C**	**5-8 C**	**9-16 C**	**< or = 4 C**	**5-8 C**	**9-16 C**
Dish 1	2	1	2	4	1	0
Dish 2	3	2	0	4	1	0
Dish 3	1	1	3	4	1	0
Dish 4	3	2	0	5	0	0
Subtotals	**9**	**6**	**5**	**17**	**3**	**0**
Mean	**2.25**	**1.50**	**1.25**	**4.25**	**0.75**	
St. Dev.	**0.75**	**0.50**	**1.25**	**0.38**	**0.38**	
SEM	**0.38**	**0.25**	**0.63**	**0.19**	**0.19**	

Table III.2.3e: Different developmental stages of embryos on day 4 after insemination. Replicate 5. n=30. Each dish represents a "patient" (10 embryos/dish or "patient").

Category:	Selected			Unselected		
Stage:	**< or = 4 C**	**5-8 C**	**9-16 C**	**< or = 4 C**	**5-8 C**	**9-16 C**
Dish 1	3	2	0	4	1	0
Dish 2	2	3	0	5	0	0
Dish 3	1	3	1	4	1	0
Subtotals	**6**	**8**	**1**	**13**	**2**	**0**
Mean	**2.00**	**2.67**	**0.33**	**4.33**	**0.67**	
St. Dev.	**0.67**	**0.44**	**0.44**	**0.44**	**0.44**	
SEM	**0.39**	**0.26**	**0.26**	**0.26**	**0.26**	

Table III.2.3 f: Summary of different developmental stages of embryos on day 4 after insemination. n=220.

Category:	Selected			Unselected		
Stage:	**< or = 4 C**	**5-8 C**	**9-16 C**	**< or = 4 C**	**5-8 C**	**9-16 C**
Subtotals	36	48	26	88	21	1
Mean	1.64	2.18	1.18	4.00	0.95	0.05
St. Dev.	1.15	0.94	1.21	0.64	0.61	0.09
SEM	0.24	0.20	0.26	0.14	0.13	0.02
Mean (%)	32.7	43.6	23.6^{a}	80.0	19.1	0.9^{b}

Within each stage category, values with different superscripts differ as follows: ^{ab} $P<0.001$

III.2.C) DAY 6 EMBRYOS

Tables III.2.4a, III.2.4b, III.2.4c, III.2.4d and III.2.4e provide data per replicate for the different stages in development achieved by embryos on day 6 after insemination. Embryos remained in the same categories as on day 2: selected and unselected. Table III.2.4f is a summary of the 5 replicates (n=220), their totals, means, standard deviations and SEM (Standard Error of the Mean). Embryos at the morula (Mor) stage were present both in the selected and in the unselected group, with a slightly higher number in the selected group (see total means in Table III.2.4f). The difference is equally evident in terms of embryos that had reached at least the morula or blastocyst stage, the more advanced embryos being in the selected group (see Table III.2.4f). The embryos that had reached the late morula-early blastocyst (LM/EB) stage also showed a difference, favouring the selected group. The differences between replicates were significant ($P< 0.001$) for the comparisons of morulas and of morulas and blastocysts ($P<0.001$) but not significant for the late morula-early blastocyst stage ($P=0.68$). Replicate number 5 was poorer than the others: very low amount of morulas (see Table III.2.4e). The difference between cohorts (selected vs. unselected) was significant ($P<0.005$ in all cases), showing higher percentage of more advanced developmental stages in the selected group.

Table III.2.4a: Different developmental stages of embryos on day 6 after insemination. Replicate 1. n=50. Each dish represents a "patient" (10 embryos/dish or "patient").

Category:	Selected			Unselected		
Stage:	**< or = 16**	**Mor**	**LM/EB**	**< or = 16**	**Mor**	**LM/EB**
Dish 1	3	1	1	5	0	0
Dish 2	2	1	2	5	0	0
Dish 3	2	3	0	4	0	1
Dish 4	3	1	1	4	1	0
Dish 5	0	4	1	0	3	2
Subtotal	**10**	**10**	**5**	**18**	**4**	**3**
Mean	**2**	**2**	**1**	**3.60**	**0.80**	**0.60**
St. Dev.	**0.80**	**1.20**	**0.40**	**1.44**	**0.96**	**0.72**
SEM	**0.36**	**0.54**	**0.18**	**0.65**	**0.43**	**0.32**

Table III.2.4b: Different developmental stages of embryos on day 6 after insemination. Replicate 2. n=40. Each dish represents a "patient" (10 embryos/dish or "patient").

Category:	Selected			Unselected		
Stage:	**< or = 16**	**Mor**	**LM/EB**	**< or = 16**	**Mor**	**LM/EB**
Dish 1	2	1	2	3	1	1
Dish 2	2	1	2	2	2	1
Dish 3	3	2	0	3	2	0
Dish 4	0	5	0	2	3	0
Subtotal	**7**	**9**	**4**	**10**	**8**	**2**
Mean	**1.75**	**2.25**	**1**	**2.50**	**2**	**0.50**
St. Dev.	**0.88**	**1.38**	**1**	**0.50**	**0.50**	**0.50**
SEM	**0.44**	**0.69**	**0.50**	**0.25**	**0.25**	**0.25**

Table III.2.4c: Different developmental stages of embryos on day 6 after insemination. Replicate 3. n=60. Each dish represents a "patient" (10 embryos/dish or "patient").

Category:	Selected			Unselected		
Stage:	**< or = 16**	**Mor**	**LM/EB**	**< or = 16**	**Mor**	**LM/EB**
Dish 1	3	1	1	4	1	0
Dish 2	1	3	1	5	0	0
Dish 3	4	0	1	5	0	0
Dish 4	5	0	0	5	0	0
Dish 5	3	0	2	5	0	0
Dish 6	3	1	1	5	0	0
Subtotal	**19**	**5**	**6**	**29**	**1**	**0**
Mean	**3.17**	**0.83**	**1**	**4.83**	**0.17**	
St. Dev.	**0.89**	**0.83**	**0.33**	**0.28**	**0.28**	
SEM	**0.36**	**0.34**	**0.14**	**0.11**	**0.11**	

Table III.2.4d: Different developmental stages of embryos on day 6 after insemination. Replicate 4. n=40. Each dish represents a "patient" (10 embryos/dish or "patient").

Category:	Selected			Unselected		
Stage:	**< or = 16**	**Mor**	**LM/EB**	**< or = 16**	**Mor**	**LM/EB**
Dish 1	3	1	1	5	0	0
Dish 2	5	0	0	4	0	1
Dish 3	2	3	0	4	1	0
Dish 4	2	2	1	5	0	0
Subtotal	**12**	**6**	**2**	**18**	**1**	**1**
Mean	**3**	**1.50**	**0.50**	**4.50**	**0.25**	**0.25**
St. Dev.	**1**	**1**	**0.50**	**0.50**	**0.38**	**0.38**
SEM	**0.50**	**0.50**	**0.25**	**0.25**	**0.19**	**0.19**

Table III.2.4e: Different developmental stages of embryos on day 6 after insemination. Replicate 5. n=30. Each dish represents a "patient" (10 embryos/dish or "patient").

Category:	Selected			Unselected		
Stage:	**< or = 16**	**Mor**	**LM/EB**	**< or = 16**	**Mor**	**LM/EB**
Dish 1	5	0	0	5	0	0
Dish 2	4	0	1	5	0	0
Dish 3	4	1	0	4	0	1
Subtotal	**13**	**1**	**1**	**14**	**0**	**1**
Mean	**4.33**	**0.33**	**0.33**	**4.67**		**0.33**
St. Dev.	**0.44**	**0.44**	**0.44**	**0.44**		**0.44**
SEM	**0.26**	**0.26**	**0.26**	**0.26**		**0.26**

Table III.2.4 f: Summary of different developmental stages of embryos on day 6 after insemination. n=220. Selected embryos: n=110 embryos/22 dishes. Unselected embryos: n=110 embryos/22 dishes

	Selected			Unselected		
	< or = 16	**Mor**	**LM/EB**	**< or = 16**	**Mor**	**LM/EB**
Subtotal	61	31 [a]	18 [b]	89	14 [a]	7 [b]
Mean	2.77	1.41	0.82	4.05	0.64	0.32
St. Dev.	1.09	1.10	0.60	0.95	0.81	0.46
SEM	0.23	0.23	0.13	0.20	0.17	0.10
Mean (%)	55.5[c]	28.2[c]	16.4[a]	80.9[d]	12.7[d]	6.4[b]

Within each stage category, values with different superscripts differ as follows: [ab] $P<0.05$; [cd] $P<0.01$

III.2.D) DAY 7 EMBRYOS

Tables III.2.5a, III.2.5b, III.2.5c, III.2.5d and III.2.5e provide data per replicate for the different stages in development achieved by embryos on day 7 after insemination. Embryos are shown in the same categories as on day 2: selected and unselected. Table III.2.5f is a summary of the 5 replicates (n=220), their totals, means, standard deviations and SEM (Standard Error of the Mean). The percentage of embryos that had reached at least the late morula stage: late morula/early blastocyst (LM/EB), mid-blastocyst (mid-blast) or expanding blastocyst (Exg blast) at day 7 was higher for the selected group than the unselected group ($P<0.001$). The percentage of embryos that had reached the full blastocyst stage (mid- plus expanding blastocysts) was higher for the selected group than for the unselected group ($P<0.001$). See means in Table II.2.5f. The differences between replicates were always non significant ($P> 0.05$).

The grading of the best embryos (those blastocysts that reached a grade 1 or 2) was also analysed (see Tables III.2.5g and III.2.5h). The percentages of total embryos that yielded grade 1 and 2 blastocysts (mid- and expanding blastocyst stages) were higher for the selected group than for the unselected group ($P<0.05$). However, the percentages of blastocysts classified as grade 1 or 2 did not differ between selection cohorts ($P=0.691$). Between-replicate differences were not significant in either case (grade 1 and 2/total, $P=0.651$; grade 1 and 2/blastocysts, $P=0.394$).

Figure III.2.2 shows groups of cohorts of embryos on day 7: selected vs. unselected. The selected group has a higher percentage of more developed embryos.

Table III.2.5a: Developmental stage of embryos on day 7 after insemination. Replicate 1. n=50. Each dish represents a "patient" (10 embryos/dish or "patient").

Categ:	Selected					Unselected				
Stage:	<or = 16	Mor	LM/EB	Mid-blast	Exg blast	< or= 16	Mor	LM/EB	Mid-blast	Exg blast
Dish 1	2	0	2	1	0	5	0	0	0	0
Dish 2	2	0	3	0	0	4	1	0	0	0
Dish 3	2	0	0	3	0	4	0	1	0	0
Dish 4	3	0	1	0	1	5	0	0	0	0
Dish 5	0	4	0	1	0	0	2	1	1	1
Total	**9**	**4**	**6**	**5**	**1**	**18**	**3**	**2**	**1**	**1**
Mean	**1.8**	**0.8**	**1.2**	**1**	**0.2**	**3.6**	**0.6**	**0.4**	**0.2**	**0.2**
St Dev	**1.10**		**1.30**	**1.22**	**0.45**	**2.07**	**0.89**	**0.55**	**0.45**	**0.45**
SEM	**0.49**		**0.58**	**0.55**	**0.20**	**0.93**	**0.40**	**0.25**	**0.20**	**0.20**

Table III.2.5b: Developmental stage of embryos on day 7 after insemination. Replicate 2. n=40. Each dish represents a "patient" (10 embryos/dish or "patient").

Categ:	Selected					Unselected				
Stage:	< or = 16	Mor	LM/EB	Mid-blast	Exg blast	< or = 16	Mor	LM/EB	Mid-blast	Exg blast
Dish 1	2	2	0	1	0	1	3	0	1	0
Dish 2	4	0	0	1	0	4	1	0	0	0
Dish 3	1	3	0	1	0	2	2	1	0	0
Dish 4	1	3	1	0	0	2	2	0	1	0
Total	**8**	**8**	**1**	**3**	**0**	**9**	**8**	**1**	**2**	**0**
Mean	**2**	**2**	**0.25**	**0.75**		**2.25**	**2**	**0.25**	**0.5**	
St Dev	**1.41**	**1.41**	**0.50**	**0.50**		**1.26**	**0.82**	**0.50**	**0.58**	
SEM	**0.71**	**0.71**	**0.25**	**0.25**		**0.63**	**0.41**	**0.25**	**0.29**	

Table III.2.5c: Developmental stage of embryos on day 7 after insemination. Replicate 3. n=60. Each dish represents a "patient" (10 embryos/dish or "patient").

Categ:	Selected					Unselected				
Stage:	< or = 16	Mor	LM/EB	Mid-blast	Exg blast	< or = 16	Mor	LM/EB	Mid-blast	Exg blast
Dish 1	1	1	2	1	0	4	1	0	0	0
Dish 2	1	0	0	4	0	5	0	0	0	0
Dish 3	1	2	0	1	1	5	0	0	0	0
Dish 4	5	0	0	0	0	5	0	0	0	0
Dish 5	2	1	0	1	1	5	0	0	0	0
Dish 6	1	3	0	1	0	5	0	0	0	0
Total	**11**	**7**	**2**	**8**	**2**	**29**	**1**	**0**	**0**	**0**
Mean	**1.83**	**1.17**	**0.33**	**1.33**	**0.33**	**4.83**	**0.17**			
St Dev	**1.60**	**1.17**	**0.82**	**1.37**	**0.52**	**0.41**	**0.41**			
SEM	**0.65**	**0.48**	**0.33**	**0.56**	**0.21**	**0.17**	**0.17**			

Table III.2.5d: Developmental stage of embryos on day 7 after insemination. Replicate 4. n=40. Each dish represents a "patient" (10 embryos/dish or "patient").

Categ:	Selected					Unselected				
Stage:	< or= 16	Mor	LM/EB	Mid-blast	Exg blast	< or= 16	Mor	LM/EB	Mid-blast	Exg blast
Dish 1	3	0	0	2	0	5	0	0	0	0
Dish 2	5	0	0	0	0	4	0	0	1	0
Dish 3	2	2	0	1	0	2	2	0	1	0
Dish 4	2	2	0	1	0	5	0	0	0	0
Total	**12**	**4**	**0**	**4**	**0**	**16**	**2**	**0**	**2**	**0**
Mean	**3**	**1**		**1**		**4**	**0.5**		**0.5**	
St Dev	**1.41**	**1.15**		**0.82**		**1.41**	**1.00**		**0.58**	
SEM	**0.71**	**0.58**		**0.41**		**0.71**	**0.50**		**0.29**	

Table III.2.5e: Developmental stage of embryos on day 7 after insemination. Replicate 5. n=30. Each dish represents a "patient" (10 embryos/dish or "patient").

Categ:	Selected					Unselected				
Stage:	< or= 16	Mor	LM/EB	Mid-blast	Exg blast	< or= 16	Mor	LM/EB	Mid-blast	Exg blast
Dish 1	4	1	0	0	0	5	0	0	0	0
Dish 2	4	0	0	1	0	4	1	0	0	0
Dish 3	4	0	0	1	0	4	0	0	1	0
Total	**12**	**1**	**0**	**2**	**0**	**13**	**1**	**0**	**1**	**0**
Mean	**4**	**0.33**		**0.67**		**4.33**	**0.33**		**0.33**	
St Dev	**0.00**			**0.58**		**0.58**				
SEM	**0.00**			**0.33**		**0.33**				

Table III.2.5f: Summary of totals, means, standard deviation and SEM (Standard Error of the Mean) for different developmental stages of embryos on day 7 after insemination. n=220. Selected embryos: n=110 embryos/22 dishes. Unselected embryos: n=110 embryos/22 dishes

Categ:	Selected					Unselected				
Stage:	< or= 16	Mor	LM/EB	Mid-blast	Exg blast	< or= 16	Mor	LM/EB	Mid-blast	Exg blast
Total	**52**	**24**	**9**	**22**	**3**	**85**	**15**	**3**	**6**	**1**
Mean	**2.36**	**1.09**	**0.41**	**1**	**0.14**	**3.86**	**0.68**	**0.14**	**0.27**	**0.05**
St Dev	**4.53**	**2.39**	**1.33**	**2.14**	**0.47**	**8.00**	**1.81**	**0.47**	**0.63**	**0.21**
SEM	**0.96**	**0.51**	**0.28**	**0.45**	**0.10**	**1.70**	**0.39**	**0.10**	**0.13**	**0.05**
Mean (%)	**47.3**	**30.9**	**8.2**	**20.0**	**10.0**	**77.3**	**0.9**	**2.7**	**5.5**	**2.7**
Mean (%)			**30.9[a]**					**9.1[b]**		
Mean (%)				**22.7[a]**					**6.4[b]**	

Within rows, values with different superscripts differ as follows: [ab] P<0.001

Table III.2.5g: Summary and percentages per replicate of mid and expanding blastocysts (denoted as M+Ex) on day 7 and of those classified as grades 1 and 2

	Selected		Unselected	
Replic 1	**M+ExBcyst**	**Gr. 1-2M+Ex**	**M+ExBcyst**	**Gr. 1-2M+Ex**
Total	6 (24%)	3 (50%)	2 (8%)	1 (50%)
Mean	1.2	0.6	0.4	0.2
St Dev	1.10	0.55	0.89	0.45
SEM	0.49	0.25	0.40	0.20
Replic 2	**M+ExBcyst**	**Gr. 1-2M+Ex**	**M+ExBcyst**	**Gr. 1-2M+Ex**
Total	3 (12%)	0	2 (8%)	1 (50%)
Mean	0.75		0.5	0.25
St Dev	0.50		0.58	0.50
SEM	0.25		0.29	0.25
Replic 3	**M+ExBcyst**	**Gr. 1-2M+Ex**	**M+ExBcyst**	**Gr. 1-2M+Ex**
Total	10 (40%)	3 (30%)	0	0
Mean	1.67	0.5		
St Dev	1.37	0.55		
SEM	0.56	0.22		
Replic 4	**M+ExBcyst**	**Gr. 1-2M+Ex**	**M+ExBcyst**	**Gr. 1-2M+Ex**
Total	4 (16%)	3 (75%)	2 (8%)	1 (50%)
Mean	1	0.75	0.5	0.25
St Dev	0.82	0.5	0.58	0.50
SEM	0.41	0.25	0.29	0.25
Replic 5	**M+ExBcyst**	**Gr. 1-2M+Ex**	**M+ExBcyst**	**Gr. 1-2M+Ex**
Total	2 (8%)	2 (100%)	1 (4%)	0
Mean	0.67	0.67	0.33	0
St Dev	0.58	0.58	0.58	0.00
SEM	0.33	0.33	0.33	0.00

Table III.2.5h: Summary of mid and expanding blastocysts on day 7 (denoted as M+Ex) and of those classified as grades 1 and 2

	Selected		Unselected	
	M+ExBcyst	**Gr. 1-2M+Ex**	**M+ExBcyst**	**Gr. 1-2M+Ex**
Total	25	11	7	3
Mean	1.14	0.50	0.32	0.14
St Dev	2.55	1.10	0.72	0.35
SEM	0.54	0.23	0.15	0.07
Mean % Gr.1-2/total		10.0^{a}		2.7^{b}

Values with different superscripts differ as follows: ^{ab} $P<0.05$

III.2.E) DAY 7 BLASTOCYST CELL COUNTS AND PYRUVATE UPTAKE DATA

Data for cell counts (see Table III.2.6A and III.2.6.B) didn't present a normal distribution, so they required a log transformation prior to statistical analysis. The mean for the cell counts was 72.9 ± 6.23 for the selected group and 69.7± 14.4 for the unselected group of embryos. No significant difference in cell counts was found for either replicates (P=0.577) or groups of selected and unselected (P=0.376). Photos of blastocysts' cell counts can be seen in Fig. III.2.1

The mean for blastocysts' diameters was 171.58 ± 2.65 μm for the selected groups and 170.42 ± 5.41 μm for the unselected group. No significant difference in diameters was found for either replicates or groups of selected and unselected embryos (P>0.05). Replicate results are presented in Tables III.2.6.A and III.2.6.B

Data for embryo viability by assessment of pyruvate metabolism ($^{14}CO_2$ production) are also shown in Tables III.2.6.A and III.2.6.B. Since the expected result for a viable ruminant blastocyst would be at least 10 pmol per embryo $3h^{-1}$ (Reis *et al.*, 2003) and most of the present data were negative, pyruvate uptake measurements were not taken into account for further analysis of the embryos' viability. They are presented here as indicative of an alternative evaluation criterion.

Table III.2.6.A: Grading, measurement, cell counts and pyruvate uptake metabolism results of Day 7 blastocysts for selected (S) embryos.

REPLIC.	STAGE OF DEV.	GRADE	DIAM (µm)	CELL COUNT (Average)	PYRUVATE METAB INDEX *
1	Blast	2	176.228	63	
1	Blast	2	183.006	98	
1	Blast	2.5	166.061	88	
1	Blast	3	166.061	41	
1	Exg blast	2	196.562	133	
1	Blast	3	169.45	39	
2	Blast	2.5	155.894	83	-17.2
2	Blast	2.5	169.45	115	5.5
2	Blast	3	155.894	57	-15.7
3	Early Blast	2.5	169.45	52	-5.6
3	Early Blast	3	159.283	22	-1.4
3	Blast	3	169.45	73	-0.5
3	Blast	2	176.228	63	-1.2
3	Blast	3	166.061	31	0
3	Blast	3	169.45	51	-5.9
3	Blast	2.5	162.672	15	-5.1
3	Exg Blast	2	203.34	63	-21.3
3	Blast	3	155.894	38	-56.4
3	Exg Blast	2	199.951	131	20.2
3	Blast	3	172.839	119	38
3	Blast	3	166.061	72	18.2
4	Blast	2	179.617	93	10.4
4	Blast	3	145.727	68	3.9
4	Blast	2	166.061	75	1
4	Blast	2	183.006	84	7
5	Blast	2	162.672	82	16.7
5	Blast	2	186.395	120	-2.8
	Mean	**2.5**	**171.58**	**72.93**	**-0.58**
	St Dev	**0.46**	**13.75**	**32.37**	**18.63**
	SEM	**0.09**	**2.65**	**6.23**	**3.58**

*Based on $^{14}CO_2$ production (pmol per embryo $3h^{-1}$)

Note: 2 early blastocysts in replic 3 laso had their cells counted and underwent pyruvate uptake tests.

Table III.2.6.B: Grading, measurement, cell counts and pyruvate uptake metabolism results of Day 7 blastocysts for unselected (U) embryos.

REPLIC.	STAGE OF DEV.	GRADE	DIAM (µm)	CELL COUNT (Average)	PYRUVATE METAB INDEX *
1	Exg blast	2	193.173	129	
1	Blast	3	155.894	34	
2	Blast	2	179.617	102	-4.6
2	Blast	2.5	176.228	52	9.3
4	Blast	2	169.45	89	13.5
4	Blast	2.5	166.061	29	-5.7
5	Blast	2.5	152.505	53	3.8
	Mean	**2.36**	**170.42**	**69.71**	**3.26**
	St Dev	**0.38**	**14.07**	**37.54**	**8.42**
	SEM	**0.15**	**5.41**	**14.44**	**3.24**

* Based on $^{14}CO_2$ production (pmol per embryo $3h^{-1}$)

Figure III.2.1 – Photographs of blastocysts' cell counts (selected "S" embryos). Blastocysts were stained with Hoechst 33342 stain and then cells counted (twice) under a binocular fluorescence microscope (400x magnification).

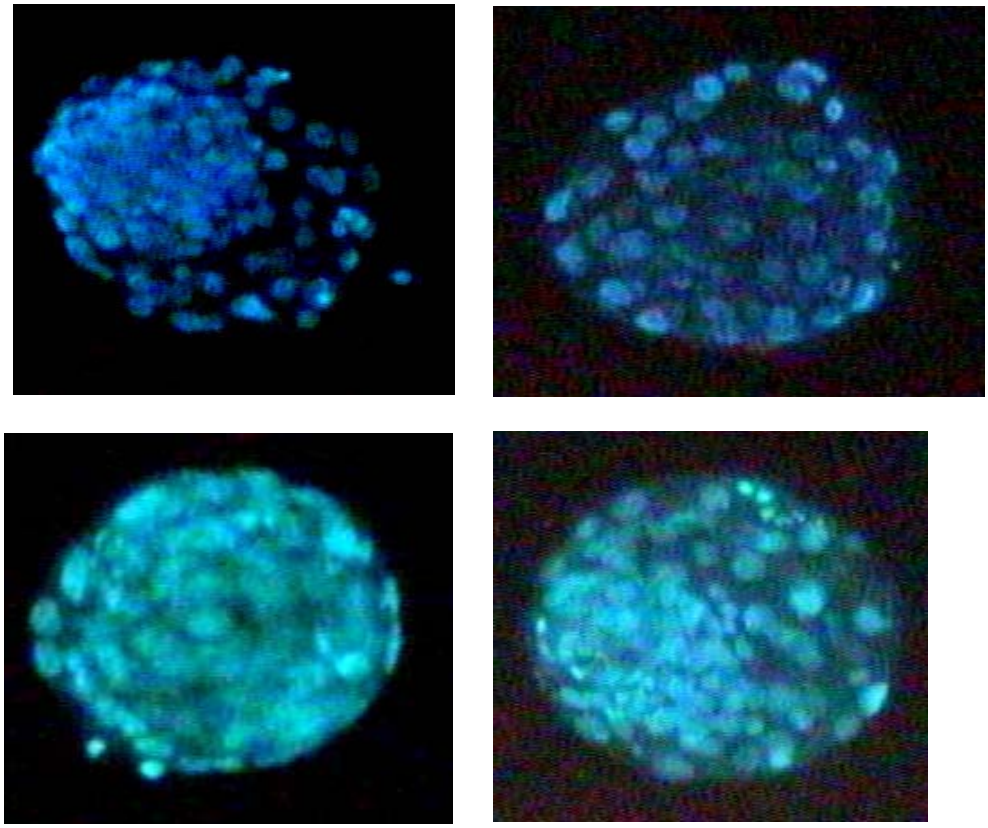
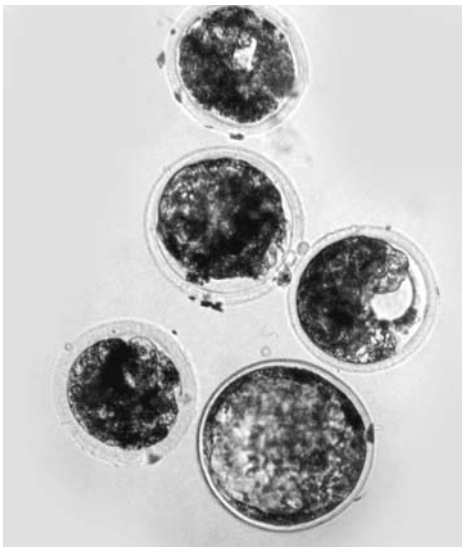
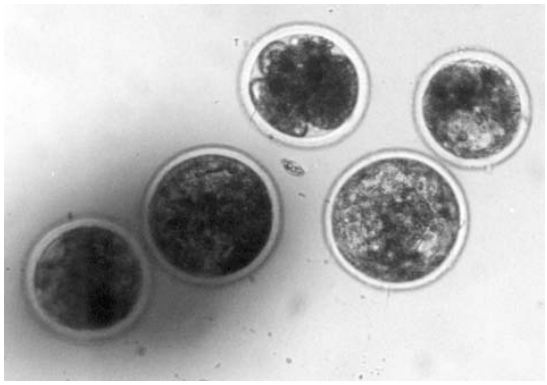


Figure III.2.2 – Photographs of embryo cohorts. Selected versus unselected groups of embryos on day 7 (x320 magnification, inverted microscope). The selected groups had a higher number of blastocysts and more advanced embryos.



Selected "S" cohort



Selected S" cohort

Unselected "U" cohort